Environmental and Aquatic Sciences

Nutrient Dynamics in an Integrated Prawn (*Penaeus latisulcatus* Kishouye 1896) and Macroalgae (*Sargassum* sp.) culture system

Huong Mai

This thesis is presented for the Degree of
Master of Science (Aquaculture)
of
Curtin University of Technology

January 2009

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree of diploma in any university.

Huong Mai **30th January 2009**

STATEMENT OF ACCESS TO THESIS

I, the undersigned, the author of this thesis, understand that Curtin University of Technology, Perth, Western Australia, will make it available for use within university library and allow access to users in other approved libraries. All users consulting this thesis have to sign the following statement:

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author, and no make proper written ackowledgement for any assistance which I have obtain from it"

Beyond this I do not wish to place any restrictions on this thesis.

Huong Mai **30th January 2009**

ABSTRACT

Rapid global expansion of the aquaculture industry has prompted the need for development of techniques for effective environmental management. In intensive aquaculture, effluents have resulted in environmental degradation of surrounding ecosystems. As a result, wastewater treatment techniques using biological means is growing worldwide. The present research was conducted to investigate the nutrient flows in a system where seaweed *Sargassum* sp. was integrated into western king prawn (*Penaeus latisulcatus)* culture.

Three laboratory experiments were conducted to determine the nutrient load discharged from western king prawn culture and, the capacity of *Sargassum* sp. to reduce nutrient loads in the integrated culture system. The effects of changes in stocking biomass of prawns and seaweed on the growth rates of both species and nutrient flows in an integrated culture system were also investigated. Prawn and seaweed growth, prawn survival, water quality parameters, nitrogen and phosphorus contents in tissue of prawns, seaweed and feed were analysed.

These experiments demonstrated that by integrating seaweed into prawn culture, the concentrations of total ammonium nitrogen (TAN), nitrite-nitrogen (NO_2^-) and nitrate-nitrogen (NO_3^-), dissolved inorganic nitrogen (DIN), total nitrogen (TN), phosphate (PO_4^{3-}) and total phosphorus (TP) were significantly reduced ($p \leq 0.05$) than in the prawn monoculture and remained within non-toxic limits for the duration of the experiment. Overall, *Sargassum* sp. removed a greater percentage of DIN (35.8-52.6%) and phosphate (5.62-65.9%) than other nutrient forms.

The mean nutrient uptake rates of *Sargassum* sp. were 0.33-0.69 mg g^{-1} dry wt day^{-1} for nitrogen and 0.13-0.25 mg g^{-1} dry wt day^{-1} for phosphorus. The integrated culture systems effectively retained nutrients into harvested products in comparison to monoculture system. The rates of nutrient conversion into waste were significantly lower in the integrated culture systems (52.46-70.05% for nitrogen and 49.09-69.41% for phosphorus) than in prawn monoculture (82.31% for nitrogen and 85.53% for phosphorus).

Integrating *Sargassum* sp. with prawn culture did not alter the specific growth rate (SGR) and survival rate of the prawns. The SGR of *Sargassum* sp. in integrated culture increased at the rate of 3.16 ± 0.74% day^{-1}, while was 5.70 ± 0.82 % day^{-1} in seaweed monoculture. Decreasing the stocking biomass of *Sargassum* sp. negatively affected its growth rate and capacity in uptaking the nutrients.

The results of this study suggest that integrating *Sargassum* sp. into prawn culture can benefit prawn farming by assisting in the maintenance of optimum water quality and thereby, reduce environmental impacts on surrounding ecosystems. However, the lower growth rate of seaweed cultured with prawns than those cultured in isolation suggests the presence of several limiting factors for the growth of the seaweed in integrated seaweed and prawn culture.

ACKNOWLEDGEMENTS

There are many people who have contributed to the completion of this thesis. I hope to acknowledge their support here. First of all, thanks to Australian Agency for International Development (AusAID) for granting me a scholarship to pursue my MSc study. Thanks to which I grew up in the perception about and living abroad.

I am grateful to my daily supervisor, A/Prof. Ravi Fotedar for his support, valuable guidance and critical advice. His enthusiasm and insight in seeking answers for many questions arising from my research has strongly stimulated me to get through my job. He has taught me many things and opened up opportunities I may not otherwise have had.

I would like to thank my co-supervisor Ms. Jane Fewtrell for her friendly assistance and special efforts in planning and execution of the laboratorial works. I appreciate very much the technical assistances.

Thanks also to Mr. Simon Longbottom for his support during the early stages of the research. He spent the time with me to set up experiments and to collect seaweed for all experiments.

My fellow post-graduate students at Aquatic Science have been a great help and encouragement along the way. In addition, my life in Curtin would have been so boring without my colleagues. I especially thank Pham Thi Minh Tam, La Ngoc Quang, Nguyen Van Chinh, Nguyen Van Tuan, Le Van Khoi, Huynh Minh Sang, Phan Thuong Huy, Ngo Van Hai, Julieta Muñoz, Vivek Kumar and Jane McNeil for their considerate help during the early time of my study. I am very grateful to some of my friends in/from Vietnam who now and then sent me cordial words of consideration and encouragement. Thanks also go to my former classmates and long lasting friendship for their supports, kind help and sharing experience.

Thanks to my family for their support, encouragement and interest. Thank to Mr. Nguyen Ngoc Tuan, my wonderful husband, for putting up with many encouragements and untiring care all this while. Especially, thank to my lovely son,

his words “mommy, you try to finish your works as soon as possible and go back with me” that help me to be able to have come this far.

I owe a dept of deepest gratitude to my parents for their untiring care and unconditional love to me. A special acknowledge is made to my parents-in-law for taking care of my son in Vietnam while I was studying in Australia.

Thank you!!!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATE

LIST OF ABBREVISTIONS

DIN	Dissolved inorganic nitrogen
DM	Dry matter
ISP	Integrated seaweed-prawn culture
LSD	Latin Square Design
N	Nitrogen
P	Phosphorus
PM	Prawn monoculture
SGR	Specific growth rate
SM	Seaweed monoculture
TAN	Total ammonium nitrogen
TN	Total nitrogen
TP	Total phosphorus

LISTS OF SCIENTIFIC AND COMMON NAMES USED IN THE THESIS

SCIENTIFIC NAME	COMMON NAME
Asparagopsis armata Harvey	Red seaweed
Ceramium rubrum Hudson	Red seaweed
Chaetomorpha linum Muller	Spaghetti algae
Chanos chanos Forsskal	Milkfish
Chondrus crispus Linnaeus	Irish moss
Cladophora vagabunda Linnaeus C.Hoek	Green seaweed
Codium fragile Suringar	Dark green macroalga
Dicentrarchus labrax Linnaeus	Sea bass
Enteromorpha intestinalis Linnaeus	Gut weed
Eucheuma denticulatum (N.L. Burman) Collins and Hervey	Red seaweed
Gelidium amansii Lamouroux	Red algae
Gelidium latifolium (Grev.) Bornet & Thur. Gelidium	Dark red alga
Gracialria lemaneiformis (Bory) Daws	Red algae
Gracilaria caudate J. Agardh	Red algae
Gracilaria cervicornis (Turner) J. Agardh	Red algae
Gracilaria chilensis Greville	Red algae
Gracilaria cornea J. Agardh	Red algae
Gracilaria crassa Harvey ex J. Agardh	Red algae
Gracilaria edulis (Gmelin) Silva	Red seaweed
Gracilaria gracilis (Stackhouse) Steentoft, L Irvine & Farnham	Red alga
Gracilaria longissima (S.G.Gmelin) Steentoft, L. Irvine & Farnham	Red algae
Gracilaria parvispora Abbott	Edible Red Seaweed
Gracilaria tikvahiae McLachlan	Red seaweed
Gracilaria verrucosa (Hudson) Paperfuss	Red algae
Gracilaria. bursa pastoris (S Gmelin) P Silva	Red algae
Haliotis discus hannai Ino	Pacific abalone
Hynea musciformis (Wulf.) Lamx	Red algae

Hynea spinella Kg. L c	Red algae
Kappaphycus alvarezii (Doty) Doty ex Silva	Tropical red algae
Kappaphycus striatum (Schmitz) Doty ex Silva	Red algae
Laminaria groenlandica Rosenvinge	Brown seaweed/Kelp
Laminaria japonica J.E. Areschoug	Brown seaweed/Kelp
Laminaria saccharina (Linnaeus) Lamouroux	Brown seaweed/Kelp
Marone saxatitis Walbaum	Striped bass
Nereocystis luetkeana (Mertens) Postels & Ruprecht	Kelp/Seatron
Oncorhynchus kisutch Walbaum	Coho salmon
Oncorhynchus myskiss Walbaum	Rainbow trout
Oreochromis aureus Steindachner	Blue/ Israeli tilapia
Oreochromis niloticus Linnaeus	Nile tilapia
Palmaria palmate (L.) Kuntze	Red algae
Paracentrotus lividus Lamarck	Purple sea urchin
Penaeus chinensis Osbeck	Chinese prawn
Penaeus indicus H. Milne Edwards	Indian white prawn
Penaeus japonicas Bate	Kuruma prawn
Penaeus latisulcatus Kishinouye	Western king prawn
Penaeus monodon Fabricius	Black tiger/giant tiger prawn
Porphyra amplissima (Kjellman) Setchell & Hus ex Hus	Red algae
Porphyra haitanensis T.J. Chang & B.F. Zheng Baofu	Red seaweed
Porphyra katadai Tang, X.-R.; Fei, X.-G.	Red seaweed
Porphyra leucosticte d. Lagos, Màlaga (E)	Red seaweed
Porphyra purpurea (Roth) C. Agardh	Red algae
Porphyra tenera Kjellman	Red algae
Porphyra umbilicalis (L.) Kütz. var. laciniata (Lightf.) J. Ag.	Red algae
Porphyra yezoensis Ueda	Red/purple algae
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	Red algae
Saccostrea commercialis Iredale & Roughly	Rock oyster
Saparus aurata Linnaeus	Gilthead bream

Sargassum echinocarpum J. Agardh	Limu Kala/Brown seaweed
Sargassum everve	Brown seaweed
Sargassum flutans (L.) J. Meyen	Brown seaweed
Sargassum fusiforme (Harvey) Setchell	Brown seaweed
Sargassum muticum (Yendo) Fensholt	Brown seaweed/Wireweed
Sargassum natans (Linn.) J Meyen	Sargasso weed
Scophthalmus maximus Linnaeus	Turbot
Sebastodes fuscescens Houttuyn	Black rock fish
Ulva clathrata (Roth) C. Agardh	Green alga
Ulva intestinalis Linnaeus	Gut algae/Green algae
Ulva lactuca Linnaeus	Sea Lettuce
Ulva pertusa Kjellman	Green algae
Ulva reticulate Forsskal	Green algae
Ulva rigida C. Agardh	Green algae /Sea lettuce
Ulva rotundata Blid. Planta.	Green algae
Undaria pinnatifida (Harvey) Suringar	Japanese seaweed/Wakame

LIST OF PUBLICATION

Mai, H., Fotedar, R. & Fewtrell, J 2008, 'Removal of inorganic nitrogen by integrating seaweed *Sargassum* sp. into western king prawn (*Penaeus latisulcatus*, Kishinouye 1896*)* culture'. in Proceeding of Tropentag 2008: *Conference on International Research on Food Security, Natural Resource Management and Rural Development*, University of Hohenheim, Stuttgart, Germany, http://www.tropentag.de/2008/abstracts/full/313.pdf.

PREAMBLE

This thesis presents an investigation into the nutrient dynamics in an integrated prawn (*Penaeus latisulcatus* Kishinouye 1896) and macroalgal (*Sargassum* sp.). The thesis includes 6 chapters.

Chapter 1 is starts with a review of the available literature on the general aquaculture aspects. This is followed by a review of environmental problems due to intensive aquaculture, especially prawn culture. The western king prawn culture also forms the section in Chapter 1. The chapter also reviews the suggested solutions in order to overcome those problems. There is a section in Chapter 1 which includes the review on seaweed species used in integrated aquaculture system. The effects of integrated aquaculture system in terms of nutrient retention and removal are also reviewed in this chapter.

Chapter 2 begins with an introduction, which includes the rationale, aim and objectives of the research.

The materials and methodology applied in current research is described in Chapter 3.

The results and discussion of the Experiment 1 are in the Chapter 4 whereas Experiment 2 and 3 form the Chapter 5. The Chapter 4 and 5 answers the questions related to the impact of *Sargassum* sp. when they are integrated into the western king prawn culture. The nutrient dynamics (Nitrogen and Phosphorus) in the systems is also discussed. The limiting factors for growth and survival of *Sargassum* sp. are explained and compared with the results of the previous studies.

The general discussion and conclusions are presented in chapter 6. This chapter gives the reviews of the discussion in terms of nutrient flows in both monoculture and integrated culture systems and the capacity of nutrient uptake of *Sargassum* in comparison to other seaweed species. It summarises the discussion of all three experiments and offers the recommendations on future research.

CHAPTER 1: LITERATURE REVIEW

1.1 Aquaculture

Aquaculture is the fastest growing seafood production sector in comparison to other animal food producing sectors, accounting for 59.4 million tons, the equivalent of US$ 70.3 billion, in 2004 (FAO 2006b; Subasinghe 2006). Growth in global aquaculture averaged 7.3% annually (Lowther 2006) and now accounts for nearly 45% of the world's consumption of seafood (FAO 2006b). The development of aquaculture industries has been seen as the means to supply the future demand for seafood, in particular, providing a major world-wide protein source (Shpigel et al. 1993a). It is predicted that aquaculture will provide more than 50% of the total demand of seafood products (Tidwell & Allan 2001) and reach at least 100 million tons per annum by 2030 to maintain the current per capita consumption (FAO 2006b). Thus, the development of the aquaculture industry has focused towards more intensive practices to increase aquaculture efficiency and production (Gutierrez-Wing & Malone 2006).

Traditional aquaculture systems (e.g. extensive pond farming) dominate many regions, but such systems are not able to cope with the effects of urbanisation, industrialisation and chemical runoff from agriculture. Thus, these are now being replaced by semi-intensive or intensive systems to obtain a higher production per unit area than extensive systems (New & Wijkstrom 1990; Shpigel et al. 1993a). These systems are presently being used in many regions of the world for both grow-out and juvenile production of aquatic species.

Semi-intensive and intensive systems often involve high rearing densities (e.g. above 15-20 prawns m^{-2}) to enhance production (Bratvold et al. 2004). In order to sustain high yields in intensive systems, high quality feed inputs are used to ensure satisfactory survival, growth rates and feed conversion efficiency of the cultured species (Brzeski & Newkirk 1997). However, only a minor part of these nutrients is taken up by the cultured species, with the remainder being released as wastes where they can have negative impacts on water quality and for the environment (Folke & Kautsky 1992; Buschmann et al. 1994). Successful aquatic culture requires maintenance of pond water quality at acceptable levels so, from an economical and

ecological point of view, the management of water quality is of particular concern (Shpigel et al. 1993b; Bratvold et al. 2004).

In many countries, aquaculture practices have resulted in the destruction of coastal areas, salinisation of land, pollution of waterways and massive crop losses (Phillips et al. 1993). Thus, there is a demand for developing new techniques of aquaculture practices that require less space (Brzeski & Newkirk 1997) and have minimal adverse environmental impacts (Troell et al. 1999). Techniques that contribute to the diversification of cultured organisms in aquaculture and, therefore provide potential economical benefits, are of particular interest (Buschmann et al. 1994).

1.2 Prawn farming

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. In 2005, more than 2 million tonnes of commercial shrimp/prawns were farmed worldwide, a contribution of 5.5% of world total aquaculture production of aquatic animals. FAO (2006b) reported that penaeid shrimps/prawns farming contributed about 63.1% to brackishwater aquaculture production. The contribution of aquaculture to global prawn production has more than doubled from 1999 to 2004 and, in 2005, was valued at over US$ 10 billion (FAO 2006a). In Australia, the prawn farming industry is small, contributing an annual production of 3,541 MT in 2005-2006 (ABARE 2008), accounting for 0.18% of world production of farmed prawn. Penaeid species that exhibit commercial potential have been cultured in some forms belong mainly to five subgenera of the genus *Penaeus*, including *Penaeus, Fenneropenaeus, Marsupenaeus, Melicertus* and *Farfantepenaeus* (Primavera 1985).

1.3. Western king prawn

1.3.1 Distribution and Taxonomy

The western king prawn (*Penaeus latisulcatus* Kishinouye 1896) belongs to the subgenera of *Melicertus*. It occurs predominantly on intertidal sand and mud-flats, generally located between shallow subtidal/intertidal seagrass beds and mangroves higher on the shoreline (Tanner & Deakin 2001) and is widely distributed throughout

the Indo-West Pacific region (Dore & Frimodt 1987). In Australia, western king prawns inhabit most of the coastal areas off Western Australia, South Australia, Northern Territory, Queensland and northern New South Wales (Grey et al. 1983) and is exploited commercially in the northern Great Barrier Reef (Gribble et al. 2007).

A taxonomic classification of *Penaeus latisulcatus* is as follows (Integrated Taxonomic Information System 2005):

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

1.3.2 Western king prawn aquaculture

The western king prawn is one of the candidate species for commercial culture in many Asian countries. At present, the commercial culture of this prawn is being practiced in some countries such as Japan, Thailand, India and China (Prangnell 2007). Research into improving the culture of western king prawn has focused on monoculture systems with few studies assessing the growth and survival rates of the western king prawn in different environmental conditions (Sang & Fotedar 2004; Prangnell & Fotedar 2006ab).

1.4 Environmental impacts of aquaculture

Aquaculture is known to have several impacts on the environment (Troell et al. 1999; Neori et al. 2000; Matos et al. 2006; Zhou et al. 2006) including; physical

habitat destruction, introduction of diseases and alien species and the release of large amount of wastes into the environment (Figure 1.1). Wastes usually contain elevated concentration of dissolved nutrients, suspended solids and chemicals (Ziemann et al. 1992). The actual environmental impacts of aquaculture depend on the surrounding environment and the choice of species, culture method, stocking density, food composition and feeding techniques and management (Iwama 1991).

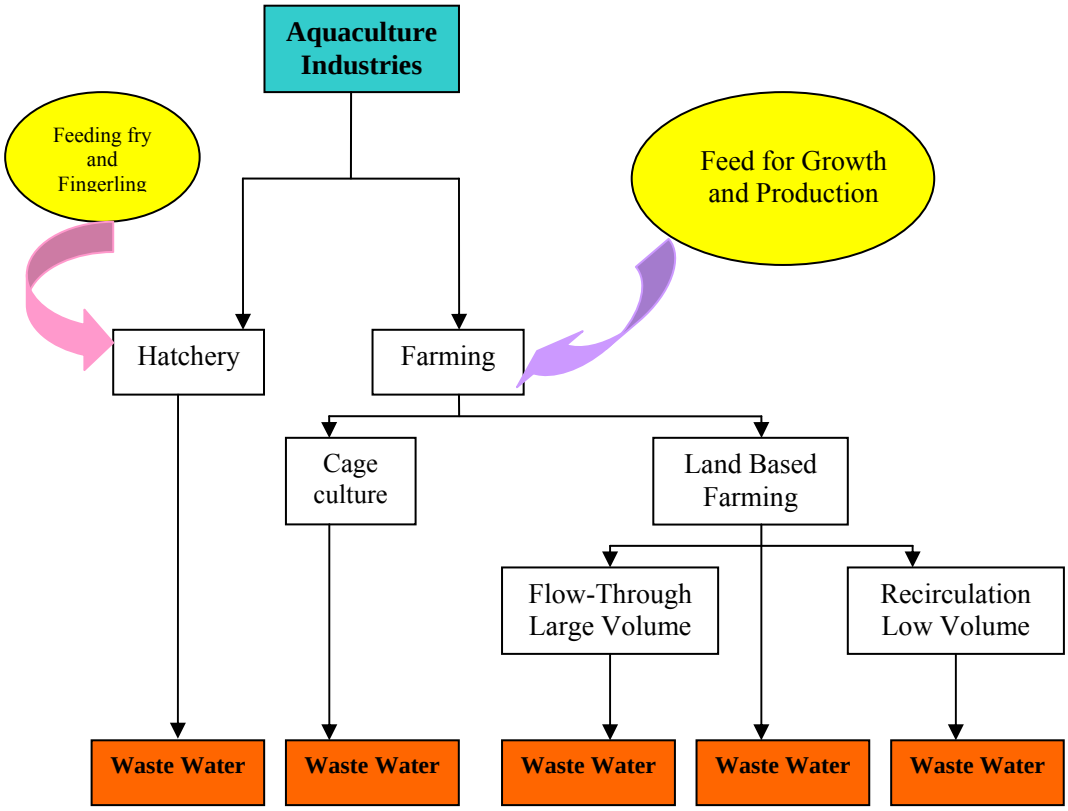


Figure 1.1 Production of wastewater from aquaculture industries (Siddiqui 2003)

Amongst the most significant negative effects of the aquaculture industry are those related with high nutrient loading, which are characterised by high organic matters, dissolved and particulate nutrient concentrations and producing large amount of gases (Porter et al. 1987; Krom & Neori 1989; Mendiguchia et al. 2006). The high nutrient levels are primarily due to the unused feed in the forms of unconsumed feed or undigested feed residue and inorganic nutrient excretions from the cultured species (Troell et al. 1997; Msuya et al. 2006). The dissolved nutrients may be sufficient to stimulate the rapid growth of 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). As a result, the outbreak of infectious disease such as pathogenic bacteria can occur (Lin et al. 1993; Primavera 1997). These wastes also cause increased biological oxygen demand and solid accumulation (Tovar et al. 2000a) 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 & Montaño 2007).

The most significant polluting components of effluents in aquaculture systems are nitrogen (N) and phosphorous (P) (Troell et al. 1997; Tovar et al. 2000b; Burford et al. 2003; Matos et al. 2006; Zhou et al. 2006). Effluents containing nitrogen and phosphorus have been reported to have caused eutrophication of receiving waters (Foy & Rosell 1991). Previous studies have shown that of the total amount of feed applied to a pond, harvested fish recover approximately 11-27% nitrogen (N), and 30-32% phosphorus (P) of the total nutrient inputs in intensive, and 5-25% N, and 5-18% P in semi-intensive ponds (Acosta-Nassar et al. 1994; Green & Boyd 1995; Troell et al. 1997; Troell et al. 1999; Neori et al. 2000). Particularly, in prawn culture, less than one third of the nitrogen-budget in the diet is converted into prawn biomass (Primavera 1993; Briggs & Funge-Smith 1994). Depending on the species and culture technologies, the various unused portions of the nutrients may be lost into the environment in the form of dissolved or particular wastes through feed wastage, excretion, faeces production and respiration (Lin et al. 1993; Wu 1995; Pagand et al. 2000; Piedrahita 2003). For example, the effluent from prawn farms discharging into Moreton Bay, Queensland often has concentrations of dissolved nitrogen and phosphorus which are 60 fold higher than receiving water (Jones et al. 2001b). The Gulf of California in Mexico annually receives about 112 and 32 kg ha^{-1} for N and P, respectively, from prawn farms (Páez-Osuna et al. 1999; Páez-Osuna et al. 2003).

Thus, in order to maintain water quality at an acceptable level, the prawn farming industry has promoted the need to develop appropriate ways of treating the effluent waters from prawn ponds before they are released back to the receiving water.

Moreover, the effluents may have many potential uses and should not necessarily be regarded as a waste product (Troell et al. 2003; Neori 2007).

1.5 Waste water management

In order to reduce environmental pollution from aquaculture effluents, cost-effective methods of effluent treatment have been developed to control nutrient loads from aquaculture farms and prevent eutrophication (Ryther et al. 1975; Shpigel et al. 1993b). There are a number of methods and commercially available systems currently used to improve the water quality of aquaculture farm discharges (Table 1.1).

The wastewater from aquaculture can be treated by applying methods that are based on mechanical and/or biological processes. Methods such as denitrification, nitrification, mechanical filtration, sedimentation and releasing effluents to wetlands have proven capable of reducing suspended solids, organic matter, ammonia, nitrite, nitrate and phosphorus from effluents and contaminated environment. For example, filtering effluents by wetlands can remove 82-90% total phosphorus, 92-93% dissolved phosphate and 86-89% total nitrogen from a fish farm (Summerfelt et al., 1999), and 30% total phosphorus in prawn culture (Tilley et al. 2002). By using sedimentation, between 18-26% of nitrogen and phosphorus can be removed from shrimp ponds (Teichert-Coddington et al. 1999). The denitrification process control ammonium nitrogen and nitrite nitrogen within acceptable ranges (<0.5 and <0.2 mg/l respectively) for culture of black tiger prawn (*Penaeus monodon*) broodstock (Menasveta et al. 2001). Combinations of nitrification and denitrification for treating effluents removed approximately 70% of the phosphorus added with the feed in tilapia (*Oreochromis niloticus* x *aureus*) culture (Shnel et al. 2002). Although these methods have proven to reduce nutrient loading in aquaculture, in order to gain more economical efficiency and ecological balance in the aquaculture industry, alternative solutions that utilise the nutrients have been proposed, such as the integration of macroalgae with aquatic animals (Chopin et al. 2001).

Table 1.1 Common methods for the treatment aquaculture effluents.

Methods	**References**
Water exchange	Primavera (1993)
Mechanical filtration	Tseng et al. (1998), Palacios & Timmons (2001), Siddqui (2003)
Biomechanical filtration by filter feeding animal	Shpigel et al. (1997), Jones et al. (2001a)
Sedimentation	Wang (1990), Tseng et al. (1998), Teichert-Coddington et al. (1999), Jackson et al. (2003a), Siddqui (2003)
Wetland treatment to filter effluents	Summerfelt et al. (1999), Negroni (2000), Palacios & Timmons (2001), Tilley et al. (2002)
Bioengineering (recirculation)	Siddqui (2003), Piedrahita (2003)
Denitrification	Aboutboul et al. (1995), van Rijn et al. (1995), Menasveta et al. (2001), Erler et al. (2004)
Nitrification	Hagopian & Riley (1998), Grommen et al. (2002), Chuntapa et al. (2003), Lyseenko & Wheaton (2006)
Combination of denitrification and nitrification	Arbiv & van Rijn (1995), Abeysinghe et al. (1996), Shnel et al. (2002), Siddqui (2003)
Integrated aquaculture-hydroponics	Seawright et al. (1998), Brown et al. (1999), Lin & Yi (2003)
Microbial agents (bacteria)	van Rijn (1996), Chuntapa et al. (2003), Liu & Han (2004)
Fungi	Hwang et al. (2004)
Using microalga	Chuntapa et al. (2001), Borges et al. (2005), Sreesai & Pakpain (2007)
Using macroalgae as biofilter	Ryther et al. (1975), Harlin et al. (1978), Neori et al., (1996), Brzeski & Newkirk (1997), Neori et al. (1998), Lyngby et al. (1999), Chopin et al. (2001), Mwandya et al. (2001), Martínez-Aragón et al. (2002a), Hernández et al. (2002), Troell et al. (2003), Erler et al. (2004), Torres et al. (2004), Bartoli et al. (2005), Hernández et al. (2005), Troell et al. (2005), Marinho-Soriano (2007), Neori (2007), Rees et al. (2007)

Although the nutrients can be waste products from aquaculture farm, they could meet the nutrient requirements for the culture of many macroalgal species (Edwards et al. 1988; Ruddle 1991; Troell et al. 2003; Carmona et al. 2006). Integrating macroalgae into monoculture systems to assimilate the remaining dissolved nutrients has shown to be a viable alternative solution to improve water quality and increase overall profits, provide social benefits and diversify production (Troell et al. 1997; Chopin et al. 2001; Neori et al. 2007).

1.5.1 Macroalgae

The use of macroalgae to absorb waste nutrients from aquaculture and, at the same time, reduce the risk of eutrophication of the environment was first suggested by Ryther et al. (1975). In addition, macroalgae do not compete with other uses of the aquatic environment but rather complement them by helping to close nutrient cycles (FAO 2006b). The potential to use macroalgae (Table 1.2) for wastewater biofiltering in integrated polyculture systems has been successfully demonstrated on a laboratory scale (Troell et al. 2003) to a large field scale (Neori et al. 2004). The common macroalgae genera *Ulva, Porphyra* and *Gracilaria* have been used in integrated systems for removing nutrients from waste water in fish, prawn and scallop aquaculture prior to disposal (Ryther et al. 1975; Vandermeulen & Gordin 1990; Buschmann et al. 1994; Krom et al. 1995; Troell et al. 1999; Nelson et al. 2001; Martínez-Aragón et al. 2002b; Neori et al. 2004). These macroalgal species have proven to effectively reduce the nutrient load in effluents and maintain water quality at acceptable levels (Marinho-Soriano 2007; Neori et al. 2007). In addition, these seaweeds can be utilized in various ways for example, nutritional purposes (human consumption as food) and fodder for other high-valued aquaculture organisms, such as abalone (Chopin et al. 2001; Neori et al. 2004).

The genus *Ulva* has been successfully integrated into mid- or large-scale mariculture systems (Neori et al. 1996; Neori et al. 2003). These authors stated that the use of *Ulva* sp. significantly reduced the concentration of dissolved nitrogen in effluents from seabream farm ponds with the low stocking density in Eilat (Aqaba), Israel. Neori et al. (2000) and Schneider et al. (2005) also reported a high growth rate of *Ulva* when co-cultured with gilthead seabream species (*Sparus aurata)* and abalone (*Haliotis discus hannai)*, but harvested biomass was limited to low-profit

agar extraction (Neori et al. 2000; Carmona et al. 2006). Seaweed from the genus *Porphyra* not only has the potential to use nutrients in aquaculture effluent for growth, but also produces biomass with high market value (Carmona et al. 2006). However, the life cycle of *Porphyra* is not well understood (Neori et al. 2004) and may not integrate well with aquatic species.

Table 1.2 Macroalgal species used as biofilters in treating effluents from aquaculture.

Species	**Countries**	**References**
1. Rhodophyta (red seaweed)		
Chondrus crispus	Portugal	Matos et al. (2006)
Eucheuma denticulatum	Zanzibar (Tanzania)	Mwandya et al. (2001)
Gracilaria bursa pastoris	Portugal	Matos et al. (2006)
Gracilaria. chilensis	Chile,	Buschman et al. (1994), Buschman et al. (1996b), Troell et al. (1997)
Gracilaria cornea	Spain	Viera et al. (2005)
Gracilaria conferta	Israel	Neori et al. (1998)
Gracilaria crassa	Tanzania,	Mwandya et al. (2001), Msuya & Neori (2002)
Gracilaria edulis	Australia,	Jones et al. (2001a), Jones et al. (2002)
Gracilaria gracilis	Spain	Martínez-Aragón et al. (2002a)
Gracilaria longissima	Spain	Hernández et al. (2005)
Gracilaria parvispora	Hawaii	Nelson et al. (2001)
Gracilaria verrucosa		Brambilla et al. (2007)
Hynea spinella	Spain	Viera et al. (2005)
Hynea musciformis	Spain	Viera et al. (2005)
Palmaria palmate	Portugal	Matos et al. (2006)
Porphyra amplissima	Stamford (USA)	Kraemer et al. (2004), Carmona et al. (2006)
Porphyra haitanensis	Stamford (USA)	Carmona et al. (2006)
Porphyra katadai	Stamford (USA)	Carmona et al. (2006)
Porphyra leucosticte	Korea	NFRDI (1994)
Porphyra purpurea	Canada, Stamford (USA)	Chopin et al. (1999), Kraemer et al. (2004), Carmona et al. (2006)
Porphyra tenera	Korea	NFRDI (1994)
Porphyra umbilicalis	Stamford (USA)	Kraemer et al. (2004), Carmona et al. (2006)
Porphyra yezoensis	Canada, Stamford (USA)	Chopin et al. (1999), Carmona et al. (2006)
Gelidium amansii	China	Liu et al. (2004)
Gelidium latifolium	Sevastopol Bay (Ukraine)	Silkin & Chubchikova (2007)

Species	Countries	References
Ceramium rubrum	Denmark	Lyngby et al. (1999)
Kappaphycus alvarezi,	Philippines	Lombardi et al. (2006)
Kappaphycus striatum	Philippines	Rodrigueza & Montaño (2007)
Pterocladiella capillacea	New Zealand	Rees et al. (2007)
Asparagopsis armata	Portugal	Schuenhoff et al. (2006)
2.Chlorophyta (green seaweed)		
Chaetomorpha linum	Spain	Menénder et al. (2002)
Codium fragile	Korea	Kang et al. (2008)
Enteromorpha sp.	Australia	Erler et al. (2004)
Enteromorpha intestinalis	Spain	Martínez-Aragón et al. (2002a)
Ulva clathrata	China	Lu et al. (2008)
Ulva intestinalis	New Zealand,	Rees et al. (2007)
Ulva lactuca	Chile, Israel, Denmark	Harlin et al. (1978), Vandermeulen & Gordin (1990), Shpigel et al. (1993a), Krom et al. (1995), Ellner et al. (1996), Neori et al. (1996), Neori et al. (1998), Lyngby et al. (1999), Neori et al. (2000), Neori et al. (2003), Msuya & Neori (2008)
Ulva reticulate	Tanzania	Mwandya et al. (2001), Msuya & Neori (2002), Msuya et al. (2006),
Ulva pertusa	China	Liu et al. (2004)
Ulva rigida	Spain, Italia	Jiménez del Río et al. (1994), Porrello et al. (2003)
Ulva rotundata	Spain	Martínez-Aragón et al. (2002a), Hernández et al. (2005)
3.Phaeophyta (brown seaweeds)		
Laminaria japonica	Korea	NFRDI (1994)
Laminaria saccharina	Canada	Ahn et al. (1998)
Nereocystis luetkeana	Canada	Ahn et al. (1998)
Sargassum everve	China	Liu et al. (2004)
Sargassum fusiforme1	Korea	NFRDI (1994)
Undaria pinnatifida	Argentina	Torres et al. (2004)
Xiphophora chondrophylla	New Zealand	Rees et al. (2007)

Due to the commercially valuable products produced from the species of genus *Gracilaria,* such as agar, agarose and food, it is one of the most desirable seaweeds to be incorporated into integrated systems (Santelices & Doty 1989). This has led to

much interest in *Gracilaria* farming and therefore, a diverse range of farming methods has been developed. The integration of *Gracilaria* with several finfish species in mariculture systems has been studied. For example, *Gracilaria* was co-cultivated with salmon (*Oncorhynchus mykiss* and *O. kisutch*) grown in cages to reduce environmental impact from nutrient loading and increase economic output (Troell et al. 1997); with turbot (*Scophthalmus maximus* Linnaeus) and sea bass (*Dicentrarchus labrax* Linnaeus) for evaluating the use of the nutrient-rich effluents from farms (Matos et al. 2006), and with the rockfish (*Sebastodes fuscescens)* in tanks for investigation into growth and bioremediation potential (Zhou et al. 2006).

1.5.2 *Sargassum species*

The genus *Sargassum* belongs to the brown seaweeds and is a common macroalgae occurring worldwide. They are found in subtidal areas in both tropical and temperate water, especially in the Indo-west Pacific region and Australia (Tseng et al. 1985). *Sargassum* has been used to monitor metal concentrations in the coastal waters (Favero & Frigo 2002). Al-Shwafi & Rushdi (in press) reported that *Sargassum* sp. plays a significant role in the uptake of heavy metals in seawater. Recently, Liu et al. (2004) indicated that *Sargassum* has potential to be used as a biofilter for removing redundant nutrients in aquaculture. Similarly, other reports stated that *Sargssum* sp. has the potential to remove metabolic nutrients in the ocean environment (Hanson 1977; Lee 1980; Phlips et al. 1986).

1.6 Integrated aquaculture

1.6.1 Integrated aquaculture systems

Methods using seaweeds or molluscs for treating effluents from fish or shrimp farming began in the mid-1970s and principally originated in China, Japan and South Korea (Goldman et al. 1974; Ryther et al. 1975). In order to use resources more efficiently, increase production and profit as well as remove nutrients from pond effluents, prawns/fish are grown in combination with biofilters such as molluscs and/or seaweed. For example, in order to create an "environmentally clean" system, an integrated fish-molluscs-macroalgae system was proposed by Gordin et al. (1981) in Israel. Also, salmon species are farmed in integration with the red alga *Gracilaria chilensis* in Chile (Troell et al. 1997), thereby reducing the potential for negative environmental impacts and increasing economic diversification.

Integrated systems can be land-based or open-water systems and may include several combination of species (Neori et al. 2004). The appropriate organisms are chosen based on the roles and functions they play in the ecosystem, their economic value and/or their acceptance by consumers. 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).

In an open-water system, seaweed culture takes place near the fish nets/pens in order to take up dissolved inorganic nutrients produced from within the fish nets/pens (Troell et al. 1999). However, the continuous tidal exchange of water makes waste disposal difficult to control, making it difficult to design experiments and to collect the data in coastal areas (Zhou et al. 2006). Therefore, research into open-water integrated systems is limited and has not yet spread or reached commercial reality in the coastal mariculture farms. Only a few studies have investigated the possibilities of integrating seaweed or bivalves as biofilters into open-water farming (Troell et al. 1999).

However, for land-based systems, the integration of seaweed into fish or shrimp ponds/tanks is recognised as a promising form of sustainable mariculture (Neori et al. 2004) and as a result, the combination of species from different tropic or nutritional levels in the same system has been researched and developed more comprehensively (Troell et al. 2003). Studies integrating macroalgae into land-based aquaculture systems include fish-macroalgae-bivalves (Shpigel et al. 1993b), fish-macroalgae; and fish-macroalgae-shellfish (Neori et al. 2000).

1.6.2 Prawns in integrated systems

In many countries, including Australia, a large amount of waste water from shrimps/prawns ponds is released into receiving water-ways without treatment (Jones et al. 2001b; Marinho-Soriano et al. 2002). With the expansion of prawn pond sites, both prawn farmers and resource managers are becoming increasingly aware that the development of effective methods of effluent treatment such as integrated culture is crucial for sustainable aquaculture development (Troell et al. 1999; Nelson et al.

2001). Indeed, effluents from prawn ponds consist mainly of nitrogen and phosphorus which have recently been shown as a good nutrient source for seaweed culture (Jones et al. 2001b; Nelson et al. 2001; Jones et al. 2002; Marinho-Soriano et al. 2002).

Systems that integrate prawns and seaweed have been studied in several countries. Nelson et al. (2001) studied the cultivation of *Gracilaria parvispora* in shrimp-farm effluent ditches and floating cages in Hawaii. In Australia, Jones et al. (2002) reported that the use of macroalgae *Gracilaria edulis* (Gmelin) Silva has the potential to effectively improve effluent water quality from a commercial kuruma shrimp farm *(Penaeus japonicas)*. The filamentous macroalgae *Enteromorpha* sp. has been cultivated in *Penaeus monodon* ponds to decrease nitrogen levels (Erler et al. 2004). Other small-scale studies on the integration of red seaweed (Gracilaria) with prawn farming have been done by Phang et al. (1996) in Malyasia, Liu et al. (1997) in China, and Kinne et al. (2001) in USA. Green seaweed (*Ulva* sp.) has been used in laboratory-scale integrated culture with prawns in Japan (Ali et al. 1994).

1.6.3 Nutrient dynamics in integrated aquaculture

1.6.3.1 Nutrient retention efficiencies in integrated systems

Seaweeds have a significant capacity to convert a large quantity of waste nutrients into valuable products (Troell & Norberg 1998; Troell et al. 2003; Neori 2007). Several studies reported that the waste products from aquaculture support most of the nutrient requirements for seaweed growth (Neori et al. 2000; Viera et al. 2005). 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 fish species (Schneider et al. 2005). Overall, at least 60% of the nutrient input can reach commercial products when fish or prawns are cultured with macroalgae (Shpigel et al. 1993b). 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 or macrophytes is integrated with fish culture, the total nutrient retention of feed increases by 20-50% in N and up to 53% in P. This agrees with the finding of Shpigel et al. (1993a) and Neori et al. (2000) that, *Ulva* is able to retain between 20-30% feed N in an integrated aquaculture system. Viera et al. (2005) also reported that *Ulva* sp. could

increase in protein content from 11% to over 32% when cultured in the nutrient-rich waters of intensive fish/shrimp aquaculture.

1.6.3.2 Nutrient removal efficiency of macroalgae

Removing redundant nutrients is one of the important aspects of water quality control and plays a crucial role in aquaculture. Several studies have reported that the dissolved nutrients in aquatic animal compartments can be removed by macroalgae (Troell et al. 1999; Neori et al. 2000; Neori et al. 2004). Thus, nutrient levels in the discharged effluent can be significantly reduced when compared to effluents from conventional mariculture technology (Krom et al. 1995). However, different macroalgae species vary in their capability to remove nutrients from aquaculture effluents (Table 1.3).

Table 1.3 Efficiency of some seaweed in removing nitrogen and phosphorus from aquaculture effluents.

Species	**Nutrient**	**Rate (%)**	**References**
Asparagopsis armata	TAN	18.86	Schuenhoff et al. (2006)
Chondrus crispus	TN	41.4	Matos et al. (2006)
Codium fragile	TAN	Up to 99.5	Kang et al. (2008)
Enteromorpha sp.	TN	43-46	Erler et al. (2004)
Enteromorpha intestinalis	PO_4^{3+}	85.3-99.6	Martínez-Aragón et al. (2002b)
Gracilaria sp.	TAN	50-95	Shpigel et al. (1993b)
	PO_4^{3+}	27	Buschman et al. (1994),
	TN	41	Buschman et al. (1996b),
	TP	52	Troell et al. (1999)
Gracilaria bursa pastoris	TN	76.7	Matos et al. (2006)
Gracilaria crassa	NO_3^-	56	Mwandya et al. (2001)
Gracilaria conferta	TN	3-88	Neori et al. (1998)
Gracilaria verrucosa	TAN (in spring)	65.38	Brambilla et al. (2007)
	TAN (in summer)	32.43	
Gracilaria chilensis	DIN	5	Troell et al. (1997)
	PO_4^{3+}	27	
Gracilaria caudate	TAN	60	Marinho-Soriano (2007)
	NO_3^-	50	
	PO_4^{3+}	12	

Species	Nutrient	Rate (%)	References
Gracilaria cervicornis	NO_3^-	90	Review in Marinho-Soriano (2007)
Gracilaria gracilis	PO_4^{3+}	71.4-98	Martínez-Aragón et al. (2002b)
Gracilaria edulis	TAN	76	Jones et al. (2001a)
	NO_3^-	30	
	PO_4^{3-}	35	Jones et al. (2002)
	TN	66	
	TP	56	
Gracilaria longissima	TAN	19.1	Hernández et al. (2005)
	DIN	17	
	PO_4^{3+}	3.2	
Kappaphycus alvarezii and K. striatum	TAN	41-66	Qian et al. (1996), Rodrigueza & Montaño (2007)
Laminaria saccharina	DIN	26-40	Subandar et al. (1993)
Palmaria palmate	NUF	41	Matos et al. (2006)
Paracentrotus lividus	TAN	66-70	Schuenhoff et al. (2003)
	PO_4^{3+}	20	
Porphyra amplissima	TP	70-100	Carmona et al. (2006)
	PO_4^{3+}	35-91	
Ulva sp.	TAN	85	Vandermeulen & Gordin (1990)
Ulva intestinalis	TAN	40	Rees et al. (2007)
Ulva lactuca	TAN	67-90	Krom et al. (1995),
	PO_4^{3+}	9-21	Schuenhoff et al. (2003),
	TN	73-80	Neori et al. (2000), Neori et
	TN	42-66	al. (2003)
	NUF	16-50	Neori et al. (1998) Msuya and Neori (2008)
Ulva reticulate	TAN (outflow)	63 – 65	Mwandya et al. (2001),
	TAN (inflow)	44	Msuya et al. (2006)
	PO_4^{3+}	33 – 58	
Ulva rigida	TAN	76	Jiménez del Río et al. (1994)

Species	Nutrient	Rate (%)	References
Ulva rotundata	TAN	24.4	Hernández et al. (2005)
	DIN	54	
	PO_4^{3+}	8.9	Martínez-Aragón et al.
	PO_4^{3+}	60.7-96.2	(2002b)

TAN = total ammonia nitrogen, TN = total nitrogen, NO_3^- = nitrate-nitrogen, DIN = dissolved inorganic nitrogen, NUF = nitrogen uptake efficiency, TP = total phosphorus, PO_4^{3-} = orthophosphate

1.6.4 Benefits of integrated approach

The economic value of integrated/polyculture systems has been investigated since 1985 (Neori et al. 2004). 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). Recycling or recirculating aquaculture systems are practical and functional in most cases and usually lead to reduced effluent being discharged into open water bodies.

Several fish farms use bacteria to effectively treat waste water which is discharged from intensive fish or shrimp ponds (van Rijn 1996). However, these systems can be complex (Troell et al. 1999; Neori et al. 2004) and expensive (Matos et al. 2006). Furthermore, such systems only convert the nutrients into less toxic forms and, generally do not reduce overall nutrient levels (Troell et al. 2003; Matos et al. 2006). A method of effluent treatment that lowers overall nutrient levels have the potential to reduce farm water consumption which would be beneficial, both economically and environmentally (Pagand et al. 2000).

In addition to addressing the waste-disposal environmental problems from aquaculture, integrated 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. 1999; 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; Neori et al. 2004). For example, by integrating *Gracilaria* with salmon, approximately 48.9 kg m^{-2} $year^{-1}$ of *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 sea bream-shellfish-seaweed can harvest 25 tons of fish, 50 tons of bivalves and 30 tons of seaweed

annually (Neori et al. 2004). Hence, using macroalgal/seaweeds for biofiltration in aquaculture systems has both ecological and economic benefits.

However, the optimisation of an integrated aquaculture system depends on the objectives to achieve, i.e. biofitration versus biomass production (Neori et al. 2003; Schuenhoff et al. 2003). It is known that the maximising both macroalgae production and nutrient removal efficiency in an integrated aquaculture system could be difficult. Schuenhoff et al. (2006) reported that an integrated aquaculture system has a high biomass yield of macroalgae, 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.

Overall, it can be seen that both economical and environmental advantages could be achieved when integrating macroalgae with aquaculture. By integrating macroalgae with aquaculture, the wastes from species can become a resource for others species. Therefore, integration could serve as an effective approach to reduce the cost to treat the effluents from aquaculture farms. Thus, integrated aquaculture systems are bound to play a major role in the sustainable development of world aquaculture. However, each organism fed externally in an integrated system has different characteristics with respect to the quality and quantity of wastes released to the surrounding environment. The development of techniques where macroalgae are used as biofilters need further exploration as the could be species depandent.

CHAPTER 2: INTRODUCTION

2.1 Introduction

In recent years, superfluous nutrients from aquaculture operations have become a world-wide environmental problem in the coastal areas, with the rapid development of intensive aquaculture becoming a major pollution source. Waste products from aquaculture consist mainly of nitrogen and phosphorus (Porter et al. 1987; Boaventura et al. 1997; Coloso et al. 2003). These nutrients harm the surrounding aquatic ecosystem in several ways, by degrading the quality of water and reducing diversification of aquatic species (Chin & Ong 1997; Troell et al. 2003; Bratvold et al. 2004). Therefore, the integration of macroalgae and fish or prawn aquaculture has been proposed because of their capacity in reutilising waste products from aquaculture (Chopin et al. 1999; Chopin et al. 2001; Neori et al. 2004). Various species of macroalgae have been studied for their suitability as nutrient sinks in the integrated aquaculture systems. *Gracilaria* sp. in integrated aquaculture system are well recognised to remove inorganic wastes (Buschmann et al. 1994; Buschmann et al. 1996a; Troell et al. 1997; Jones et al. 2001a; Jones et al. 2002; Martínez-Aragón et al. 2002b; Hernández, I. et al. 2005; Matos et al. 2006). *Ulva* sp. (Harlin et al. 1978; Msuya et al. 2006; Rees et al. 2007; Lu et al. 2008), *Porphyra* sp. (Chopin et al. 1999; Kraemer et al. 2004; Carmona et al. 2006) and *Laminaria* sp. (Ahn et al. 1998) perform the similar function of nutrient removal from aquaculture.

However, no attempts are made in integrating *Sargassum* sp. with western king prawn cultures in Australia and information on the role of *Sargassum* sp. in nutrient dynamics in any prawn integrated culture system is unknown. It is expected that by integrating *Sargasum* sp. with western king prawns will improve the productivity of the aquaculture system by improving the water quality. Introduction of seaweed in intensive aquaculture will also improve the utilisation of nutrients and will assist in mitigating the problems associated with release of waste water to the surrounding environment. This integrated farming approach can provide knowledge on the nutrient uptake in the integrated aquaculture system. The outcomes of any such research undertaken can provide the baseline data for the development of *Sargassum* as a biofilter in intensive aquaculture. This study aims to investigate the efficacy of *Sargassum* sp. in removing nutrients from the western king prawn (*Penaeus*

latisulcatus, Kishinuoye 1896) culture when both species co-exist in the same environment.

2.2 Aim and Objectives

The aim of this study is to investigate the effects of integrating *Sargassum* sp. into western king prawn (*Penaeus latisulcatus* Kishinouye 1896) culture on nutrient dynamics.

The above aim is achieved by meeting the following specific objectives:

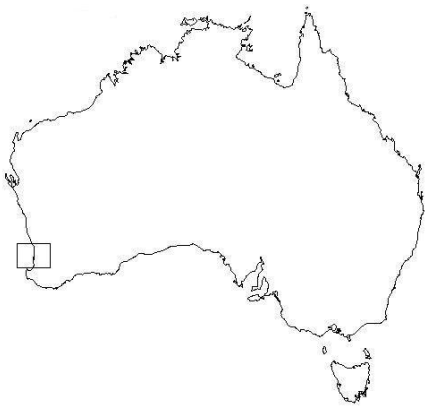
1. To quantify the nutrients discharged from western king prawn culture when prawns are fed formulated feeds under laboratory conditions.
2. To calculate the amount of nutrients removed by *Sargassum* sp. when integrated with western king prawn culture.
3. To determine the relationship between growth rates and conversion rates of nitrogen and phosphorus from formulated feed into prawn and seaweed in integrated culture system
4. To evaluate the changes in stocking biomass of prawns and seaweed on growth rates of those species and nutrient flows in an integrated culture system.
5. To determine the relationships between the seaweed stocking density (i) and nutrient uptake and (ii) seaweed growth.

CHAPTER 3: MATERIALS AND GENERAL METHODS

3.1. Source of prawns and seaweed

Juvenile western king prawns were collected in the mouth of the Swan River (near Point Water Reserve) in Bicton, Western Australia (32^0 40"S 115^0 13"E) (Figure 3.1). Prawns for all experiments were caught between 19:00-21:00 (post sunset), using drag-nets in water up to 1.5m in depth. The prawns were transported to the Curtin Aquatic Research Laboratory, Curtin University of Technology and were held in ocean water under laboratory conditions until the commencement of experiments. *Sargassum* sp. was collected from the Cottesloe coast in Western Australia (31^0 57"S 115^0 05"E) (Figure 3.1).

A.



B.

Figure 3.1 Seaweed (*Sargassum* sp.) and prawn (*Penaeus latisulcatus*) collection sites.
Note: **A.** Region of collection sites in Australia, **B.** Specific collection sites for prawns and seaweed

3.2. Experimental approach

3.2.1 Experimental setup

Three experiments were conducted indoors in 100L plastic tanks. Each tank was provided with aeration and a heater. A diagram of this experimental tank is shown in Plate 3.1.

Plate 3.1 Experimental tank used for experiments.

3.2.2 Morphometric measurement

Prior to being weighed, prawns were dried with paper towel to remove excess moisture. A digital balance was used to measure the total biomass (g) of each prawn to three decimal places. All prawns were weighed before the commencement of the experiment. Prawns were re-weighed weekly to obtain the data required for determining the total growth rate, biomass gain and biomass increment.

Specific growth rates (SGR % g per day) and weight gain (WG g) were calculated by using the formula:

$$SGR = 100 \times (\ln W_t - \ln W_0)/t$$

$$WG = W_t - W_{0;}$$

where W_0 = initial biomass; W_t = biomass at time (t); t = day of trial.

Biomass increment (%) = (final biomass – initial biomass) x 100/initial biomass

3.2.3 Survival

In each experiment, prawn survival was monitored daily, with any mortality removed promptly. Mortalities showing signs of cannibalism and those with a soft exoskeleton (recently moulted) were noted. The survival rate (St_n) of the prawns in each tank was calculated using the formula:

$$S_{tn} = N_{tn}*100/N_i;$$

where, N_{tn} = number of prawns surviving at the time n; N_i = number of prawns at the beginning of each experiment.

3.2.4 Feed

Commercial western king prawn diets were supplied by Ridley Aqua-Feed Company, Queensland. Prawns were fed twice daily at a feeding rate of 2.5% of prawn biomass. Any uneaten feed and faeces were removed at least twice daily.

3.2.5 Nutrient analysis

3.2.5.1 Nitrogen

Feed, prawns and seaweed, and wastes (faeces and uneaten feed) were oven-dried at 105^0C overnight to a constant weight. Nitrogen content was analysed by the Kjeldahl method (AOAC 1995) using a Tecator 1015 heater block operated by a Tecator autostep 1012 controller, and a Tecator Kjeltec 1030 Auto Analyser. Approximately 100 -1000 mg of dried sample was transferred into a 250 ml digestion tube, placed in the heating block and digested with 10 ml of digestion acid (H_2SO_4 + H_2PO_4) in the presence of a catalyst at 420^oC until the sample became clear (approximately 60 – 80 minutes). The digested sample was then analysed using the Kjeltec auto analyser. Nitrogen content of the sample was calculated using the following formula:

$$N\ (\%) = [14.01 \times M \times 100 \times (\text{ml } HCl_{sample} - \text{ml } HCl_{blank})] / \text{mg sample}$$

where 14.01 = the atomic weight of nitrogen

M = the molarity of the hydrochloric acid used in Kjeltec auto analyser to titrate ammonia (0.5M)

3.2.5.2 Phosphorus

Dried samples were analysed for phosphorus by using the spectrophotometric molybdovanadate method (AOAC 1995). Approximately 0.5 -1.0 g of dried prawns, seaweed and feed samples were transferred into pre-weighed (W_0) crucibles. The weight of the crucible with the sample was recorded as W_1. The crucible with sample was ignited in the muffle furnace (CarboliteTM) at 550^{o}C overnight. The crucible was removed from the furnace, allowed to cool in the desiccator and reweighed (W_2). Dry-ashed samples were then dissolved in a HCl:HNO3 (4:1 v/v) solution and were heated to dryness (Cheng et al. 2006). The resulting residue was dissolved with 10 ml of 1% HCl in a steam bath and then diluted to 100 ml volumetric flask. If any insoluble matter was present the solution was filtered through filter paper (Whatman Ø 90 mm). 5 ml of molybdovanadate reagent (see Appendix 1) was then added into each sample and the absorbance of the sample was recorded using a spectrophotometric set a wave length of 400 nm. Phosphorus content of the sample was calculated using the following formulas:

mg P_2O_5/100 g test sample = 100 x (mg P_2O_5/10 ml from standard curve*)/g test sample in 10 ml ash solution (* see Appendix 1 for methodology for preparation of standard curve.)

$$\% P_2O_5 = (\text{mg } P_2O_5/100 \text{ g test sample})/1000$$

$$\text{Conversion rate (\%)} = \text{nutrient gained/total nutrient fed x } 100$$

where: nutrient gained (N or P) = final nutrient – initial nutrient; total nutrient fed = total N or P input to the tanks

3.2.6 Water quality parameters

Aeration was provided to all tanks in every experiment and was monitored using a DO meter (YSI-550A). The temperature and pH of the tanks were measured using a pH meter (TPS-WP-80) during the experimental period. The salinity of systems were measured with a handheld refractometer and was maintained at 35-36‰, except for experiment described in Chapter 4 (28-29‰). During the experiments, no water exchanges were performed. Evaporation losses were compensated for by the addition

of distilled water to maintain the salinity at 35-36‰. The light cycle was controlled by using an automatic timer switch and was set up at 12:12h light:dark.

Nutrient parameters in water were measured weekly in all treatments. All samples were analysed in triplicate.

3.2.6.1 Metabolite nutrients

The metabolite nutrients measured in this study were; total ammonia nitrogen (TAN = NH_3 + NH_4^+), nitrite nitrogen (NO_2^-), nitrate nitrogen (NO_3^-) and orthophosphate (PO_4^{3-}). Filtered water samples were analysed for TAN, NO_2^- and PO_4^{3-} using the indophenol blue method, colorimetric and ascorbic acid methods, respectively (APHA 1998) (see Appendix 1). Nitrate nitrogen was analysed by using a DR/890 Colorimeter (HACH 2005). Dissolved inorganic nitrogen (DIN) was calculated as total of TAN, NO_2^- and NO_3^-.

3.2.6.2 Total nitrogen

Unfiltered water samples (50 ml) were digested with a potassium persulphate solution in an autoclave for one hour. Subsequently, nitrates and nitrites were reduced to ammonium with Devarda alloy (Raveh & Avnimelech 1979). After adding Devarda alloy to the autoclaved samples, they were left overnight. Aliquots of 100ml were then taken by decantation or filtration of the samples. Aliquots were neutralized to pH 5 with NaOH solution. Ammonium concentration was determined by the indophenol blue method (APHA 1998) (see Appendix 1).

3.2.6.3 Total phosphorus

Approximately 50 ml of unfiltered water samples were pre-digested using persulfate in an autoclave at 121^0C for 30 minutes (Hansen & Koroleff 1999). The samples were taken out the autoclave and cooled. The concentration of total phosphorus was determined by using the ascorbic acid method (APHA 1998) (see Appendix 1).

Nitrogen removal (NR %) in the integrated systems was estimated according to the following equation:

$$NR = 100 \times (C_{cnl} - C_p)/C_{cnl}$$

where C_{cnl} = nutrient concentration in the prawn monoculture treatment (mg l^{-1})

C_p = nutrient concentration in the integrated culture treatment (mg l^{-1}).

3.2.7 Nutrient budget and uptake

In order to find the nutrients (nitrogen and phosphorus) in the inputs and outputs in each treatment and/or experimental unit, total nutrients of inputs, outputs, uptakes and accumulations in the culture system during the rearing cycle were measured. The nutrient budget of N and P were calculated based on inputs and outputs as follows:

Nutrient inputs = Nutrient outputs + Nutrient loss

In which: Nutrient inputs = nutrients in water + nutrients in stocked prawn and/or seaweed + nutrients in feed

Nutrient outputs = nutrients in harvested prawn and/or seaweed + nutrients in drained water + nutrients in sediment (faeces, uneaten feed and dead thallus of seaweed)

Nutrient inputs and outputs in the form of water represents nutrient contained in water on the day of prawn stocking and on the harvest-day, respectively and was calculated by multiplying the nutrient concentration with total water volume.

Nutrient concentrations (nitrogen and phosphorus) in the initial and final tissue samples of prawn and seaweed were determined to calculate the total nutrient contained in the tissue. Nutrient inputs and outputs in the form of prawns and seaweed were calculated as follows:

Nutrient in prawn/seaweed (mg) = nutrient concentrations in prawn/seaweed X total dry biomass.

Nutrient (N and P) inputs in the form of prawn feed were calculated as follows:

Nutrient in feed (mg) = nutrient concentration in feed x total amount of feed supplied.

Mean nutrient uptake rate (U_{mean}; mg g dry wt^{-1} day^{-1}) of the seaweed was estimated based on nutrient content in the thalli according to the following equation

$$U_{mean} = SGR \times N_{tissue}/100$$

where; SGR = specific growth rate (% g day^{-1}) for thalli; N_{tissue} = nutrient content in thalli tissue (mg g dry wt^{-1})

3.3 Data analysis

Data was stored on the hard disc at "I" drive for research students. Results were presented as means ± S.E. (standard error). The SPSS statistical program (versions 12 and 15) and Microsoft Excel were used for data analysis in all the trials. Analysis of Variance (ANOVA), independent sample T-test and LSD post hoc tests were used to determine any significant differences among treatment means. Regression analysis was used to assess relationships between variables. All statistical tests were performed at a significance level of $p < 0.05$.

CHAPTER 4

Sargassum sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) aquaculture system

4.1. Introduction

Prawn farming has been developing steadily over the last decade in response to an increasing world market demand. Since the 1970s, the western king prawn (*Penaeus latisulcatus,* Kishinouye 1896*)* has been considered as one of the candidate species for culture (Kathirvel & Selvaraj 1987) and has been widely cultured in several Asian countries. In recent years, the culture systems for prawns have intensified (Gutierrez-Wing & Malone 2006) by using high quality feed (Shepherd & Bromage 1988; Seymour & Bergheim 1991; Brzeski & Newkirk 1997) which accounted for more than 95% of the nutrient input in aquaculture ponds (Krom & Neori 1989). However, only less than one third of these nutrients are assimilated into the prawn biomass (Briggs & Funge-Smith 1994) and the remaining portion is lost to the system (Wu 1995; Piedrahita 2003).

In order to improve effluent water quality, assist in maintaining the sustainable development of prawn farming and to mitigate the environmental impacts of prawn farming, various methods have been proposed to address the issue of nutrients discharged from intensive king prawn aquaculture (Troell et al. 2003; Neori et al. 2004). One viable approach is to integrate macroalgae with prawn aquaculture where macroalgae is expected to assimilate the nutrients from prawn effluents.

Integrating macroalgae with fish or prawn aquaculture is a biologically and technically feasible method to reduce the environmental impacts of aquaculture effluents. This approach is based on the use of macroalgae to remove the dissolved nutrients from aquaculture pond effluents. The concept of developing an "environmentally clean" aquaculture system based on an integrated fish-mollusc and macroalgae system was first proposed by Gordin et al. (1981). The system was further tested by Gordin et al. (1990) and Shpigel et al. (1991). Other authors have also developed systems integrating fish or prawn and macroalgae culture (Liu et al. 1997; Neori et al. 1998; Troell et al. 1999; Jones et al. 2001a).

Several macroalgae species such as *Ulva, Porphyra* and *Gracilaria* have been proven to effectively reduce the nutrient load in effluents both under laboratory and field conditions (Troell et al. 2003; Neori et al. 2004). However, this study is the first to integrate *Sargassum* sp. into western king prawn culture. *Sargassum* sp. are common macroalgae occurring worldwide and are distributed in subtidal areas in both warm and temperate water, especially in the Indo-west Pacific region and Australia (Tseng et al. 1985). *Sargassum* communities are considered to metabolise nutrients in the pelagic environment (Hanson 1977; Phlips et al. 1986). The aim of this experiment was to evaluate the efficacy of *Sargrassum* sp. in assimilating nitrogen (N) and phosphorus (P) in effluents from western king prawns and to calculate the nutrient budget of N and P in western king prawn (*Penaeus latisulcatus*) aquaculture.

4.2. Materials and Methods

Three treatments were used, *viz*. prawn monoculture (PM), seaweed monoculture (SM) and integrated seaweed and prawn culture (ISP). Each treatment consisted of four replicates in the form of tanks and these tanks were arranged in a completely randomised design (Fig. 4.1). The experiment was conducted for 42 days under laboratory conditions. The initial biomass of the prawns in each tank in PM and ISP was the same (i.e. 96 g m^{-2}). Seaweed was stocked at the initial biomass of 0.5 kg m^{-2} (140 g per tank) in SM and ISP. The feeding rate for the prawns at the commencement of the experiment was 2.5% of the prawn biomass per day. The feed contained 8.12% of N and 1.29% of P. Prawn mortalities in each tank were removed immediately and weighed and any sign of cannibalism was recorded.

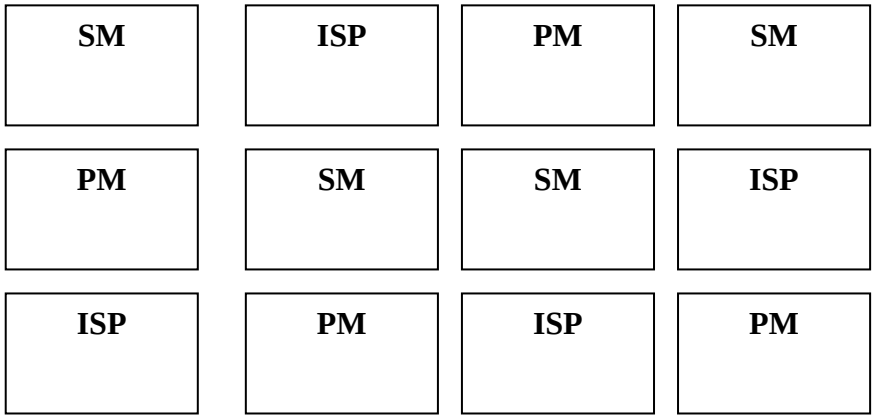


Figure 4.1 Experimental tank layout (front view).
Note: PM = prawn monoculture; SM = seaweed monoculture; ISP = integrated seaweed-prawn.

Sixty western king prawns (5.48 ± 0.29g each) were acclimated for 28 days in two 250-L tank containing ocean water. Five prawns were then placed into each PM and ISP tank and total tank biomass was recorded. Prawns were re-weighed weekly to obtain the data required to calculate growth. The survival (S_{tn}) of the prawns in each tank was also recorded over the period of the experiment. The seaweed *Sargassum* sp. was rinsed with ocean water to remove any epiphytes and was then placed into the SM and ISP culture.

Salinity levels of the systems were maintained at 28.96-30.19‰ over the experiment period, which is within the optimum range for prawn culture (Sang & Fotedar 2004; Prangnell 2007). During the experiment, evaporation losses of water were compensated by the addition of distilled water to maintain the salinity level around 29-30‰ (Appendix 2).

Data collection method and analysis procedures were conducted as described in Chapter 3 of Materials and Methods.

Nutrient removal (NR %) in the integrated culture systems was estimated according to the following equation:

$$NR = 100*(C_{cnl} - C_p)/C_{cnl}$$

where C_{cnl} (mg/L) = nutrient concentration in the prawn monoculture treatment

C_p (mg/L) = nutrient concentration in integrated seaweed-prawn treatment

4.3. Results

4.3.1 Water quality

Mean temperature and dissolved oxygen (DO) was 23.60-25.08^{0}C and 5.81-6.16 mg l^{-1}, respectively over the experimental period (Appendix 2, Table I). The pH of water ranged from 7.91 to 8.17, which is within the optimum range for prawn culture (Allan & Maguire 1992a; Wang et al. 2002).

Overall, the mean concentration of nutrients over time was significantly lower ($p < 0.05$) in the ISP and SM than in the PM (Figure 4.2). The concentration of total nitrogen and DIN in the ISP was significantly lower ($p < 0.05$) than the PM, even when no seaweed was present in ISP for the last 14 days of the experiment. Similarly, the total phosphorus concentration of ISP was significantly lower ($p < 0.05$) than the PM while seaweed was present in the tanks. However, the concentration of total phosphorus in ISP and PM was the same ($p > 0.05$) until day 35 of the experiment when seaweed did not present in ISP. At the end of the experiment, ISP was recorded the significant lower concentration of total phosphorus than PM. However, the concentration of PO_4^{3-} in the ISP was lower than that in PM during the first 28 days of the experiment and then the concentration of PO_4^{3-} was the same ($p > 0.05$) at both ISP and PM when all seaweed was removed from the tanks at day 28 until the conclusion of the experiment. The DIN, total nitrogen, PO_4^{3-} and total phosphorus concentrations in SM were significantly lower ($p < 0.05$) than PM and ISP for the duration of the experiment.

In all treatments, NO^-_3 and total ammonium nitrogen (TAN) were the predominant dissolved inorganic nitrogen forms. While the NO^-_3 seems to be stable, the TAN has deceased significantly during the period of day 28-42 (Figure 4.2). Overall, the most significant nutrient dynamics occurred during day 21-35. It is worthy noting that during day 21 to 28, all Tan, DIN and NO^-_3 increased by almost 3-4 times the previous sampling times. On the other hand, the NO_2^- concentrations peaked at the end of the experiment and no significant difference ($p > 0.05$) between PM and ISP was observed.

After 28 days of the experiment, seaweed was removed from the tanks and faeces and uneaten feed were siphoned out. Following this, the concentration of TAN

decreased significantly ($p < 0.05$) in all treatments and was not significantly different ($p > 0.05$) between ISP and PM. The concentration of NO_3^- decreased slightly in PM, while the NO_3^- concentration in ISP continued to increase and reached 3.13 mg l^{-1} at the end of the experiment. In contrast, NO_2^- concentration was generally at the lowest concentration and was always lower in ISP than in PM (Figure 4.2). NO_2^- concentration did not significantly alter ($p > 0.05$) over the experimental period in all treatments and ranged from 0.003 mg l^{-1} to 1.35 mg l^{-1} in PM, 0.36 mg l^{-1} in SM and 1.42 mg l^{-1} in ISP.

During the experimental period, nitrogen was the more abundant nutrient when compared to phosphorus (Figure 4.2). Dissolved inorganic nitrogen (DIN) and total nitrogen varied from the beginning until the end of the experiment for all treatments. The concentration of DIN and total nitrogen at the beginning of the experiment was the same for all treatments, with 0.13 mg $l^{-1}$and 0.17 mg l^{-1}, respectively. At the end of the experiment, the DIN and total nitrogen had increased significantly ($p < 0.05$) in all treatments. In ISP the concentrations of DIN and total nitrogen were 6.06 mg $l^{-1}$and to 8.17 mg l^{-1}, respectively, while in the PM was 10.99 mg l^{-1} for DIN and 14.66 mg $l^{-1}$for total nitrogen. In SM, the concentration of DIN (1.03 mg l^{-1}) and total nitrogen (1.58 mg l^{-1}) was lower than in PM and ISP.

For the duration of the experiment, the concentration of orthophosphate (PO_4^{3-}) was less than 1 mg l^{-1} and total phosphorus was less than 2 mg l^{-1} in all treatments (Figure 4.2). Over the period of the experiment, the concentration of PO_4^{3-} and total phosphorus increased gradually in all treatments, from the initial sample of 0.014 mg l^{-1} to 0.93 mg l^{-1} for PM, 0.92 mg l^{-1} for ISP and 0.25 mg l^{-1} for SM. The maximum concentration of total phosphorus was recorded at the end of experiment in PM and SM (1.64 mg l^{-1} and 0.60 mg l^{-1} respectively), while the highest concentration of total phosphorus in ISP was 1.13 mg l^{-1} on day 35 of the experiment.

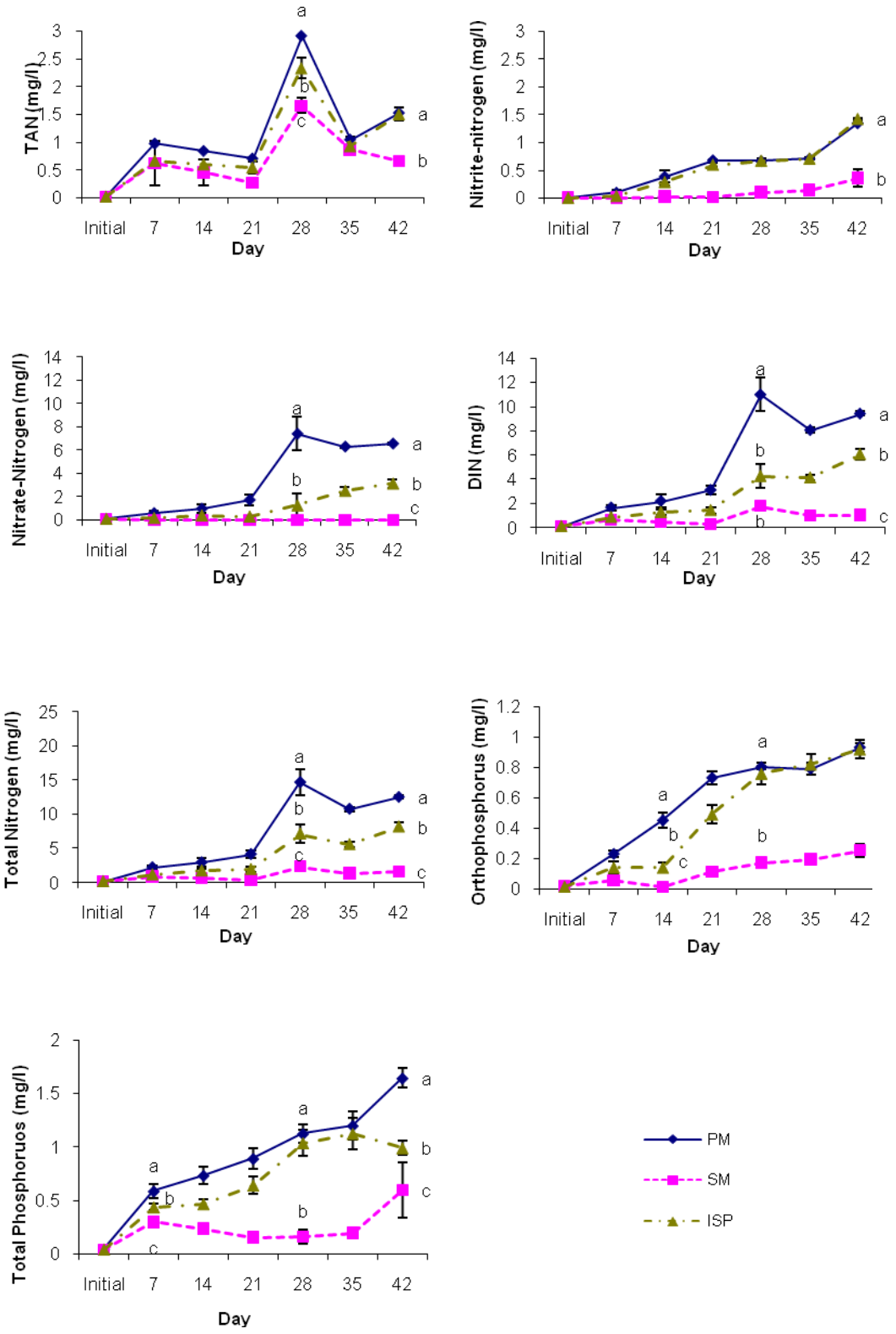


Figure 4.2 Water quality parameters in treatments recorded over the experimental period (mean ± SE).
Note: PM = prawn monoculture; SM = seaweed monoculture; ISP = integrated seaweed-prawn culture. *Different letters denote a significant difference between treatments ($p < 0.05$).*

4.3.2 Nutrient removal

The removal rates of nitrogen metabolites by *Sargassum* sp. remained constant over the experimental period, except for NO_2^- which showed a significant decrease ($p < 0.05$) and was almost zero during the last 21 days of the experiment (Figure 4.3). The removal rate of NO_3^- generally increased with an increasing NO_3^- concentration, while the removal rate of TAN decreased with an increasing TAN concentration (Figure 4.2 and 4.3). At the end of the experiment, the removal rate of TAN was 1.31%. Overall, the present of *Sargassum* sp. in prawn culture tanks resulted in more efficient removal of NO_3^- than TAN, with a removal rate ranging from 52.5 to 75.04% and 1.31-29.21%, respectively.

No significant difference ($p > 0.05$) was found between the DIN removal rates over the period of the experiment. The maximum removal rate was 52.6% for DIN and 61.9% for total nitrogen on day 28, but the differences were not statistically significant ($p > 0.05$) for DIN over time, whereas a significant difference ($p < 0.05$) was only found for total nitrogen at day 28 of the experiment. The nutrient removal rate varied over the experimental period, but generally, the removal rate of DIN and total nitrogen were higher than those of PO_4^{3-} and total phosphorus (Figure 4.3 and 4.4). Mean removal rates of DIN and total nitrogen ranged from 35.8 to 52.6% and from 34.7to 61.9%, respectively.

There was a significant difference between the removal rates of PO_4^{3-} over the experimental period. The highest PO_4^{3-} removal rate was observed on day 14 of the experiment with a 65.9% removal efficiency, but deceased significantly ($p < 0.05$) thereafter to 5.6% at day 21 and was almost zero from day 28 onward (Figure 4.3). Total phosphorus was removed at an efficiency of 14.5% to 37.0%. The removal rate of total phosphorus did not significantly change ($p > 0.05$) until day 35 of the experiment, when significantly higher total phosphorus removal was observed ($p < 0.05$).

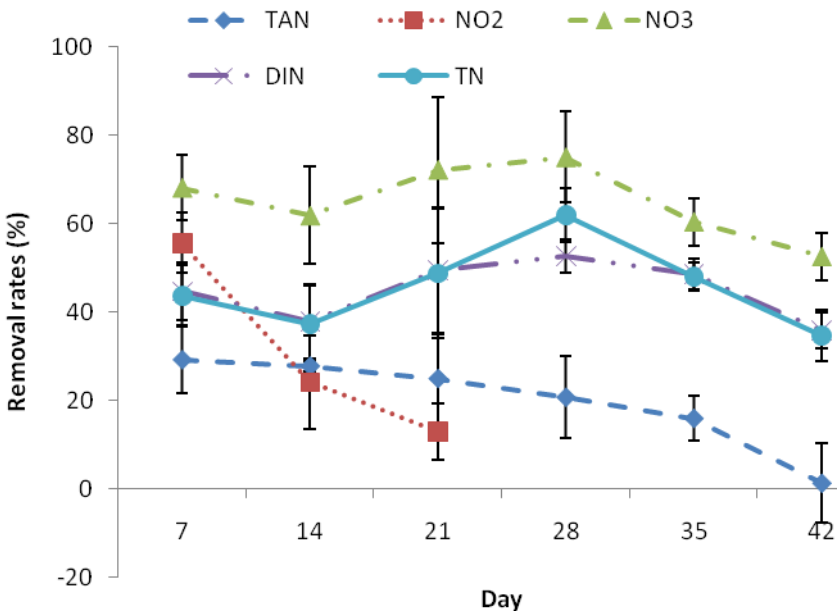


Figure 4.3 Percentage nitrogen removal in integrated western king prawn and *Sargassum* sp. culture tanks (mean ± S.E.).
Note: TAN = total ammonium nitrogen, DIN = dissolved inorganic nitrogen, TN = total nitrogen

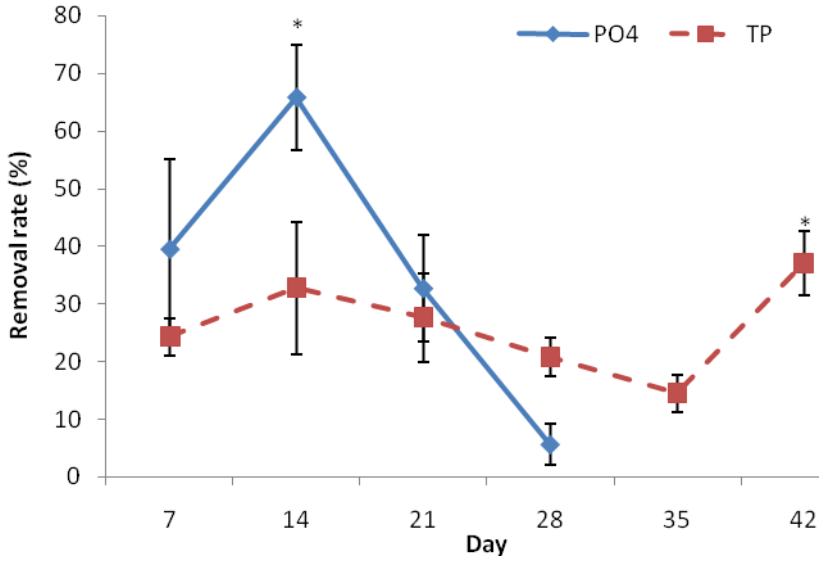


Figure 4.4 Percentage phosphorus removal in integrated western king prawn and *Sargassum* sp. culture tanks (mean ± S.E.).
Note: TP = total phosphorus. *The asterisk (*) represents the point from which the concentration of nutrients was significant higher ($p < 0.05$) within a treatment.*

4.3.3 Nutrient budget

4.3.3.1 Nitrogen

Most of the nitrogen entered to the tanks containing prawns came from the formulated prawn feed, with 62.27% in PM and 47.53% in ISP, whereas nitrogen input in SM was primarily from the intake water (59.26%). In PM and ISP, prawn accounted for 25.63% and 19.53% of the nitrogen inputs, respectively. Nitrogen from seaweed contributed about 40.74% in SM, but only 22.84% in ISP. The intake water accounted for the remainder of the N input in PM and ISP (Table 4.1). Within the tank, 17.69% in PM and 18.99% in ISP of the input N from feed was converted to harvested prawns (Figure 4.5). Wastes, including uneaten/wasted feed, faeces and/or dead seaweed, accounted for 27.81% of the nitrogen in PM and 24.42% in ISP and only 8.35% in SM. In the present study, unaccounted nitrogen occurred from systems with 5.00% in PM and 9.12% in ISP, whereas, up to 50.30% was recorded in SM.

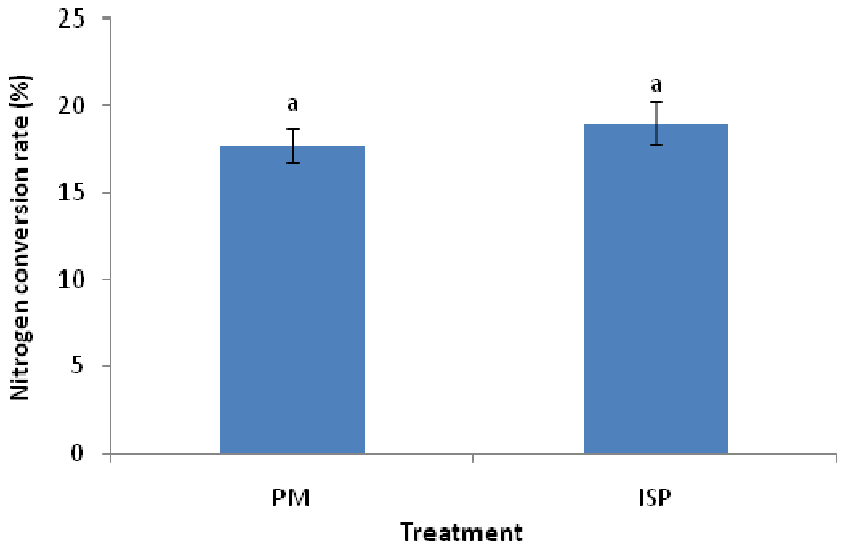


Figure 4.5 Nitrogen conversion rate from feed into western king prawn (mean ± S.E.).
Note: PM = prawn monoculture. ISP = integrated seaweed-prawn culture. *Same letters denote no significant difference (p > 0.05).*

Table 4.1 Nitrogen budget for prawns and seaweed monoculture and integrated seaweed-prawn culture (mg per 100 L) (means ± S.E.).

Treatments	**PM**	**SM**	**ISP**
Inputs			
Water	223.04±0.09	223.04 ± 0.09	223.04 ± 0.09
(%)	(12.09±0.57)	(59.26 ± 2.90)	(10.11 ± 0.62)
Prawns	475.46±38.20		436.25 ± 32.79
(%)	(25.63±1.81)		(19.53 ± 0.80)
Seaweed		156.37 ± 20.59	499.78 ± 43.01
(%)		(40.74 ± 2.90)	(22.84 ± 2.91)
Feed	1158.67±76.75		1074.87 ± 147.12
(%)	(62.27±1.99)		(47.53 ± 0.82)
Total	1857.16	379.41	2233.93
(%)	(100)	(100)	(100)
Outputs			
Water	570.67±70.26	113.07 ± 13.46	463.80 ± 15.24
(%)	(30.52 ±2.95)	(28.99 ± 6.08)	(20.93 ± 1.01)
Prawns	681.64±50.07		642.27 ± 63.28
(%)	(36.66 ±1.64)		(28.60 ± 1.12)
Seaweed		49.42 ± 20.88	388.35 ± 74.32
(%)		(12.36 ± 4.51)	(17.93 ± 3.77)
Waste	513.61 ± 22.60	31.72 ± 4.48	531.16 ± 90.59
(%)	(27.81 ±1.65)	(8.35 ± 1.13)	(23.42 ± 2.64)
Unaccounted	91.24 ±12.53	185.20 ± 31.89	208.34 ± 45.43
(%)	(5.00 ±0.79)	(50.30 ± 10.66)	(9.12 ± 1.38)
Total	1857.16	379.41	2233.93
(%)	(100)	(100)	(100)

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed and prawn culture.

4.3.3.2 Phosphorus

Phosphorus input in PM and ISP was approximately 66.55% and 50.10% respectively and occurred mostly from the prawn feed, while seaweed contributed the largest source of P in SM (95.53%). Prawns contributed about 32.05% in PM and 24.16% in ISP. In the ISP, there was about 24.57% of P input coming from seaweeds. The intake water accounted for the remainder, which was 1.39%, 4.47%

and 1.15% in PM, SM and ISP treatments, respectively (Table 4.2). After 42 days of the experiment, the intake water represented 14.02% in PM, 30.54% in SM and 22.91% in ISP of the total input P.

The phosphorus budget indicated that prawn converted about 14.47% and 13.79% of the input P as feed to prawn biomass (Figure 4.6). The contribution of phosphorus from wastes that is, uneaten feed, faeces and/or dead seaweed mostly accumulated in the tanks during the rearing, averaged 42.63% in PM, in 35.67% ISP and 50.92% in SM. Due to weight loss of the seaweed, the amount of P accounted for only 5.79% in ISP and 13.45% in SM. There was input P that could not be accounted for in the sum of recorded input P in all treatments, that is, only 1.62 % in PM, 5.09% in SM and 4.81% in ISP.

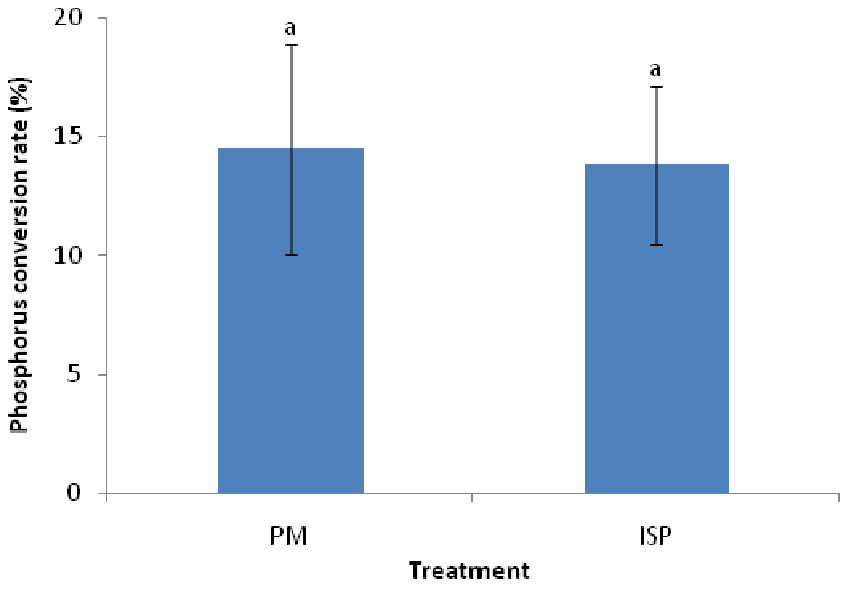


Figure 4.6 Phosphorus conversion rate from feed into western king prawn (mean ± S.E.).
Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *Same letter denote no significant difference ($p > 0.05$).*

Table 4.2 Phosphorus budget for prawns and seaweed monoculture and integrated seaweed-prawn culture (mg per 100 L) (means ± S.E.).

Treatments	**PM**	**SM**	**ISP**
Inputs			
Water	3.60 ± 0.03	3.60 ± 0.03	3.60 ± 0.03
(%)	(1.39 ± 0.07)	(4.47 ± 0.45)	(1.15 ± 0.07)
Prawns	83.27 ±6.69		76.40 ± 5.74
(%)	(32.05 ±2.21)		(24.16 ± 1.31)
Seaweed		80.12 ± 10.55	76.24 ± 5.74
(%)		(95.53 ± 0.45)	(24.59 ± 3.54)
Feed	173.33 ± 11.48		160.80 ± 22.01
(%)	(66.55 ± 2.23)		(50.10 ± 3.13)
Total	260.20	83.72	317.03
(%)	(100)	(100)	(100)
Outputs			
Water	36.13 ± 0.88	24.85 ± 3.07	72.42 ± 5.78
(%)	(14.02 ±0.88)	(30.54 ± 4.63)	(22.91 ± 1.44)
Prawns	108.14 ± 7.83		97.18 ± 4.65
(%)	(41.74 ±3.07)		(30.82 ± 1.07)
Seaweed		12.43 ± 5.30	18.59 ± 2.99
(%)		(13.45 ± 3.75)	(5.79 ± 0.63)
Waste	111.61 ± 11.52	42.21 ± 4.67	113.14 ± 8.98
(%)	(42.63 ± 2.86)	(50.92 ± 3.57)	(35.67 ± 0.94)
Unaccounted	4.32 ± 1.16	4.23 ± 0.50	15.72 ± 4.75
(%)	(1.62 ± 0.40)	(5.09 ± 0.41)	(4.81 ± 1.21)
Total	260.20	83.72	317.03
(%)	(100)	(100)	(100)

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed and prawn culture.

4.3.4 Nitrogen and Phosphorus in tissues

4.3.4.1 Nitrogen and Phosphorus in prawn tissue

Changes in N and P concentrations in the prawn tissue are shown in Table 4.3. There was no significant difference in the N and P concentrations between the initial samples and the final sample of prawn. However, due to the increment in the prawn biomass, the net N and P biomass increased in both PM and ISP (Appendix 3, Table II). When data from PM and ISP treatments were pooled, there was no relationship between SGR and the net N biomass gained in tanks of the prawns (r^2 = 0.35, p = 0.38; Fig. 4.7). A higher correlation was recorded between the SGR and the net P biomass gained (r^2 = 0.60, p = 0.02; Fig. 4.8). Over the period of the experiment, the N:P ratio of prawns in both the PM and ISP treatments did not alter significantly (p > 0.05) .

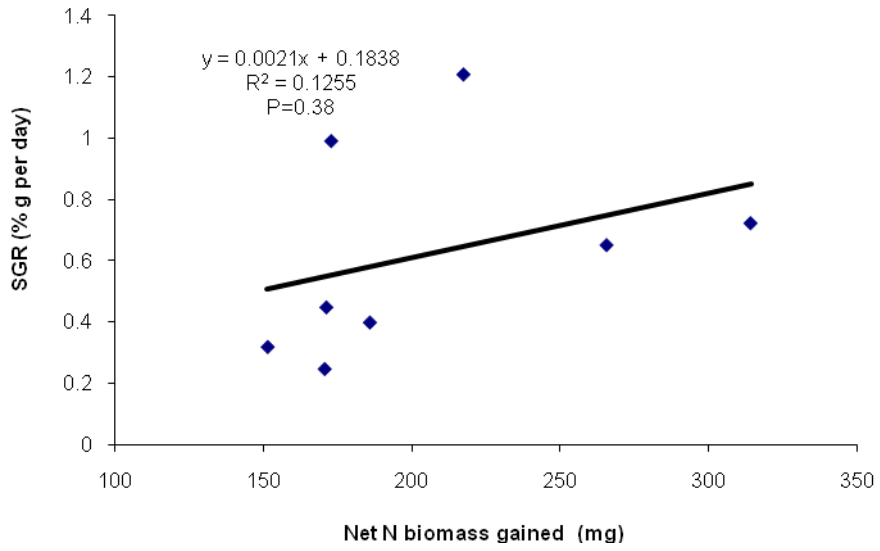


Figure 4.7 Relationship between specific growth rate and net N biomass gained in culture during the experiment.

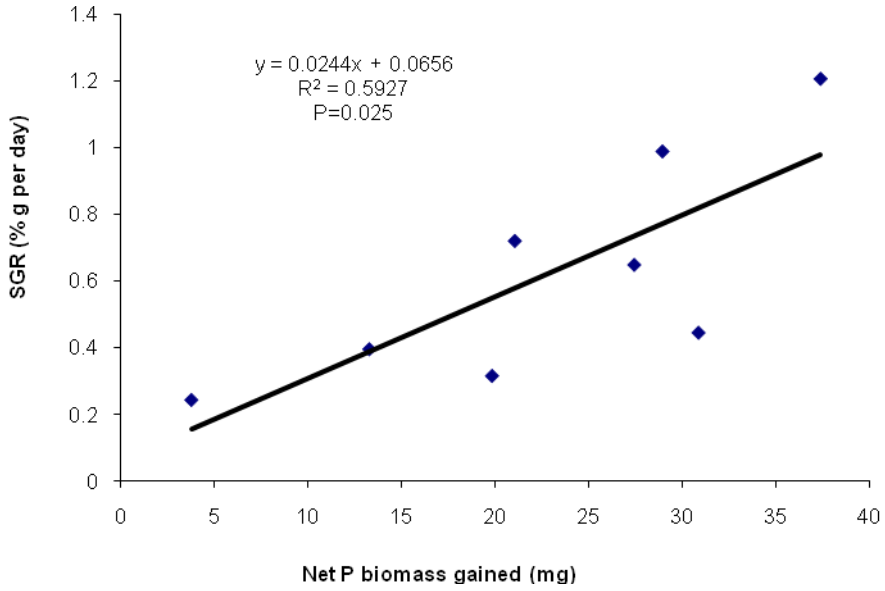


Figure 4.8 Relationship between specific growth rate and net P biomass gained in culture during the experiment.

4.3.4.2 Nitrogen and Phosphorus in seaweed tissue

At the end of the experiment, the seaweed under both monoculture and integrated culture conditions showed a decrease in the N and P content from the initial concentrations (Table 4.3). Due to losses of seaweed biomass, the amount of N and P gained in SM and ISP had negative values (Appendix 3, Table II). After day 7 onward, the thallus of the seaweed began to deteriorate and 100% mortality was observed by the day 28. Therefore, neither N and P contents nor N:P ratio could be determined at the end of the experiment (Table 4.3).

Table 4.3 N and P concentrations in prawn and seaweed tissue and N:P ratios at the initial and the end of the experiment (mean ± S.E.).

Species	**Treatments**	**Tissue N (% DM)**		**Tissue P (% DM)**		**N:P ratio**	
		Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
Prawn	PM	10.39±0.09[a]	10.51±0.14[a]	1.82±0.01[a]	1.72±0.20[a]	13.31±0.13[a]	14.45±0.89[a]
	ISP	10.39±0.09[a]	10.89±0.46[a]	1.82±0.01[a]	1.63±0.16[a]	13.31±0.13[a]	14.13±0.81[a]
Seaweed	SM	1.40±0.03[a]	1.33±0.07[a]	0.33±0.08[a]	0.27±0.03[a]	9.87±0.25[a]	-
	ISP	1.40±0.03[a]	1.39±0.08[a]	0.33±0.08[a]	0.36±0.07[a]	9.87±0.25[a]	-

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed and prawn culture. *Values in any one row not followed by the same superscript letters are significantly different at p<0.05.*

4.3.5 Survival, growth and biomass increment of prawns

The mean growth rate of the prawns was not significantly different ($p > 0.05$) between PM and ISP, (i.e. 0.64 % g day^{-1} and 0.61 % g day^{-1}, respectively) (Figure 4.9). Similarly, the increase in prawn biomass in ISP did not significantly differ from the PM, reaching 3.99 g and 3.31 g, respectively. However, the prawn biomass gain at day 35 and day 42 of the experiment was significantly lower ($p < 0.05$) than day 7 of the experiment in the ISP, while there was no significant difference ($p > 0.05$) in the biomass gain of the prawns in the PM treatment over the period of the experiment (Figure 4.10).

The presence of the seaweed in prawn culture did not affect the survival of the prawns ($p > 0.05$). The survival rate of the prawns was 55% in the PM and 60% in the ISP (Figure 4.11).

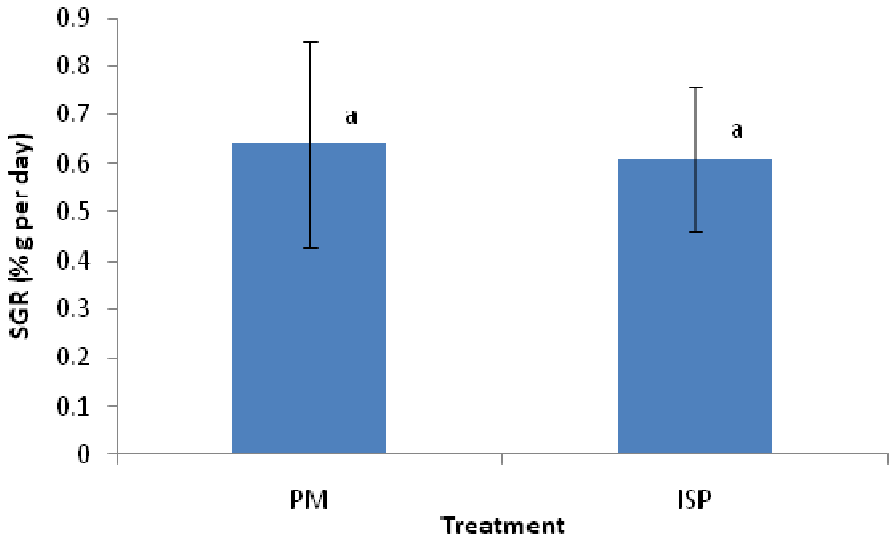


Figure 4.9 Specific growth rate of prawn biomass in treatments (mean ± S.E.).
Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture.
Same letters denote no significant difference (p>0.05).

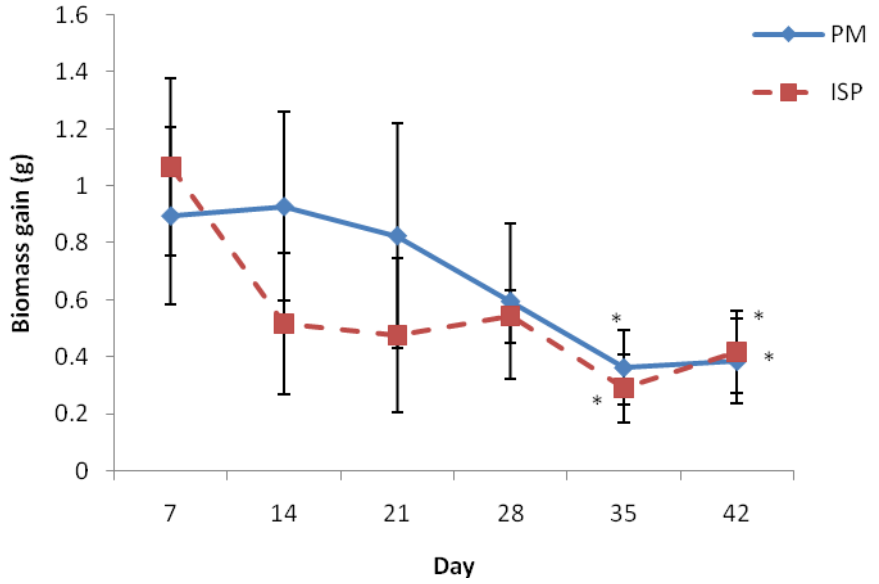


Figure 4.10 Prawn biomass gain in treatments over the period of experiment (mean ± S.E.).
Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *The asterisk (*) represents the point from which the concentration of nutrients was significant lower ($p < 0.05$) within a treatment.*

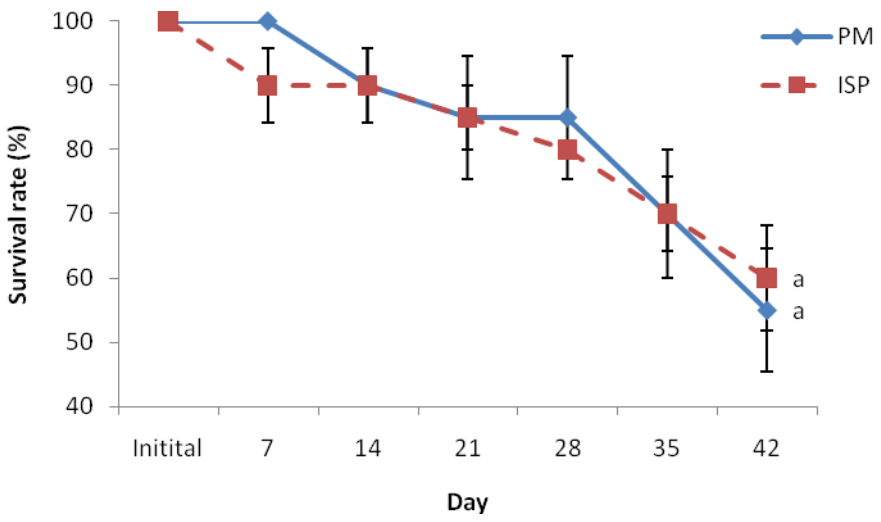


Figure 4.11 Survival rate of prawn in treatments (means ± S.E.).
Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *Same letters denote no significant difference ($p > 0.05$).*

4.3.6 Growth of seaweed

The mean specific growth rate (SGR) of the *Sargassum* is shown in the Figure 4.10. Culturing *Sargassum* with prawns resulted in a significantly lower ($p < 0.05$) growth rate than the monoculture of the seaweed. In SM, the SGR was approximately 5.7 % g day^{-1}, while SGR of the seaweed in the ISP was 3.16% g day^{-1}. However, the *Sargassum* began to die after 7 days of the experiment and consequently, the production loss in the subsequent days was seen. One hundred percent mortality of seaweed was recorded by the day 28 of the experiment (thallus deterioration and disintegration) and then dead seaweed was removed from the tanks.

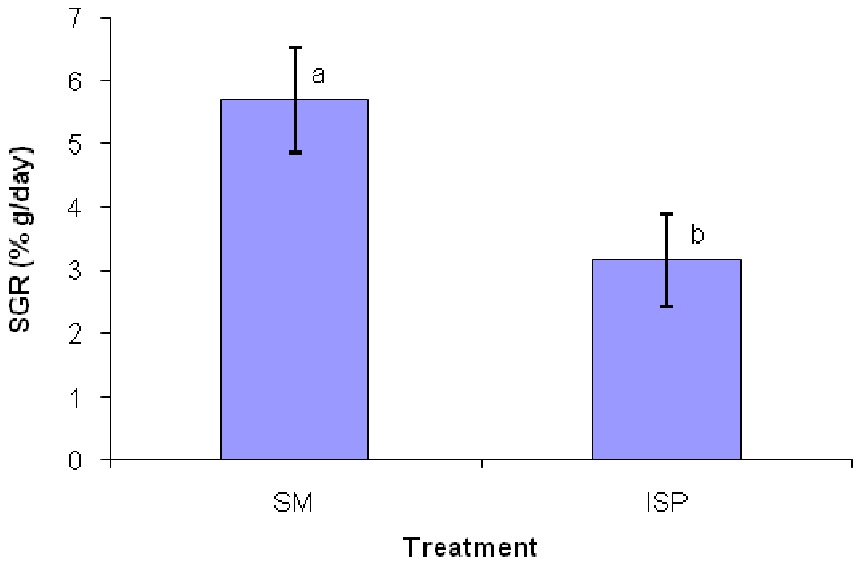


Figure 4.12 Specific growth rate of seaweed in the treatments (mean ± S.E.). Note: SM = seaweed monoculture; ISP = integrated seaweed-prawn culture. *Different letters (a,b…) denote significant difference ($p < 0.05$).*
(Note: Biomass of seaweed in the first week of the study).

4.4 Discussion

Integrating seaweeds into prawn culture has proven to solve several of the environmental problems faced by modern aquaculture (Neori 2007). Different studies have pointed out that species of the *Ulva* and *Gracilaria* genus are ideal candidates for the development of integrated culture. For instance, Jiménez del Río et al. (1996) designed a system in which *Ulva rigida* removed more than 90% of DIN in the waste water from gilthead seabream (*Sparus aurata*) cultivation tank. Recently, in an integrated treatment of shrimp effluent with oyster (*Saccostrea commercialis*) and macroalgae (*Gracilaria edulis*), Jones et al. (2001a) found that the presence of *Gracilaria eduli* reduced more than 95% of the ammonium concentration in two hours. However, using *Sargassum* species to treat waste water from aquaculture has not been widely applied. For instance, Liu et al. (2004) designed a system in which *Sargassum enerve* reduced the TAN and NO_3^- concentrations of the water by 4-fold in five days (from 80 μM l^{-1} to 20 μM l^{-1}) under laboratory conditions. Therefore, the present study is designed to have a better understanding of the effects of the integration of *Sargassum* sp. species with western king prawn culture in terms of nutrient flows, nutrient removal and the performances of the cultured species.

4.4.1 Water Quality and Nutrient Removal

In the present experiment, there was a significant increase in the concentrations of the metabolised nitrogen and the total nitrogen at day 28 of the experiment, except for NO_2^- which showed no significant difference over the period of the experiment in all treatments (Figure 4.1). In addition, NO_2^- was generally in the form of DIN at the lowest concentration. This suggests that NO_2^- could have been accumulating in the culture system probably due to incomplete nitrification and the kinetic reaction could have been controlled by ammonia oxidation over the experimental period (Timmons et al. 2002).

The increase in the offloading of the metabolite nitrogen at day 28 of the experiment was probably caused by the decaying thallus of the seaweed (Jones 1999). The thallus of *Sargassum* began to deteriorate and disintegrate after 7 days of the experiment and completely died by day 28 of the experiment. Consequently the high concentration of DIN and TN were observed from day 28 until the end of the

experiment. Similarly, the DIN was greater than 14 mg l^{-1} after red seaweed (*Gracilaria)* died when cultivated in black tiger prawn (*P. monodon)* effluents (Marinho-Soriano et al. 2002). However, the TAN and NO_2^- concentrations remained within safe limits for prawn (3.0 mg l^{-1} and 1.0 mg l^{-1}, respectively) in both the prawn monoculture and integrated culture systems (Timmons et al. 2002).

Although phosphorus does not constitute a danger to fish or prawn culture, it contributes to the eutrophication process. In the absence of *Sargassum*, the PO_4^{3-} and TP concentration remained relatively high. The high concentration of PO_4^{3-} and TP observed in the prawn monoculture was probably caused from the uneaten feed and excretion by the prawns (Buschmann et al. 1996a). However, integrating *Sargassum* with prawn culture significantly reduced the concentration of phosphorus in the tanks. Even though all of the *Sargassum* died at day 28 of the experiment, the concentration of PO_4^{3-} and TP in the integrated culture still remained relatively lower than in prawn monoculture.

Other studies on macroalgae (*Ulva pertusa*, *Gelidum amansii* and *Sargassum enerve)* have shown that these species to be more useful in the removal of TAN than NO_3^- from the water (Krom et al. 1995; Naldi & Wheeler 2002; Liu et al. 2004). In contrast, *Sargassum* sp. in the present study was more efficient at removing NO_3^- than TAN. Similarly, green seaweed *Codium fragile* (Hanisak & Harlin 1978) and *Chaetomorpha linum* (Menéndez et al. 2002), brown seaweed *Laminaria groenlandica* (Harrison et al. 1986), *Laminaria saccharina* (Ahn et al. 1998) and red seaweed *Porphyra yezoensis* (Hafting 1999) removed NO_3^- more efficiently than TAN. There is an evidence that under conditions of high NO_3^- and low salinity, seaweed is able to metabolise NO_3^- more rapidly (Lartigue & Sherman 2006). Karmer and Fong (2000) found that low salinity resulted in a decline in growth of seaweed, but the effects of low salinity are mitigated if inorganic nitrogen availability is high (Karmer & Fong 2000; Karmer & Fong 2001). As a consequence, seaweed is able to metabolize NO_3^- more rapidly during high NO_3^- and low salinity conditions (Lartigue & Sherman 2005). In addition, for some macroalgal species, ammonium is a less favourable source of nutrients than nitrate because during daylight periods the pH inside the photosynthetic algae increases and thereby reduces the pH of the external medium which lead to higher level of volatilise ammonium

(Menéndez et al. 2002). Alternatively, in the presence of aeration, ammonium may be converted into nitrate by nitrification with high concentrations of oxygen (Timmons et al. 2002). In this study, the tanks were provided oxygen at the optimal level for the growth of the prawns and hence conversion of ammonium to nitrate form.

The removal efficiency of both DIN and TN by *Sargassum* in the present study were generally higher (35.82-52.57% and 34.68-61.94%, respectively) than the values previously reported in literature. For instance, *Gracialaria longissima* removed only 17% of DIN when integrated with fish (*Sparus auratus)* culture (Hernández et al. 2005). *Gracilaria tikvahiae* removed around 10-14% of the nitrogen in the effluent pond which was used for the intensive culture of the Pacific white prawns (*Litopenaeus vannamei)* (Kinne et al. 2001). Therefore, *Sargassum* sp. has a great potential to act as a nitrogen sink when integrated with western king prawn culture.

Various studies have shown that several seaweed species have high biofiltering capacities and thus can contribute to efficient removal of dissolved N and P waste from fish or prawn ponds (Ryther et al. 1981; Neori et al. 1991; Troell et al. 1999; Jones et al. 2001a; Nelson et al. 2001; Neori et al. 2004). Most of the studies are focused on dissolved N removal (especially ammonium) (Neori et al. 1996). In contrast, few studies have addressed the efficiency of P removal. Recently, Jones et al. (2001a) reported that *G. edulis* was able to remove up to 95% of PO_4^{3-} when cultivated in prawn effluents. In the present experiment, *Sargassum* was able to remove approximately 65.85% of the PO_4^{3-} and 37.04% of TP during the experiment. However, compared with the majority of other seaweeds, the performance of *Sargassum* in phosphate removal in this study was relatively high. For instance, integrating *Gracilaria chilensis* and salmon culture resulted in the removal of 32% of the PO_4^{3-} from the fish farm (Buschmann et al. 1996b). Studies on other seaweed species have also shown relatively low removal efficiency for PO_4^{3-} (DeBoer et al. 1978; Neori et al. 1996). Neori et al. (1998) reported that *Ulva lactuca* and *Gracilaria conferta* removed less than 25% of the PO_4^{3-} from an integrated system. Troell et al. (1997) showed that *G. chinensis* would be capable of removing 27% of the phosphate from salmon cages. The finding in the present study therefore shows

the potential ability of *Sargassum* to effectively reduce the phosphorus concentration when integrated with prawn culture, and thus the quality of water for prawn culture. This has significant management implication for water quality, especially in relation to the possibilities for improving the discharged water into the receiving water.

Overall, integrating seaweed with prawn culture resulted in relatively lower concentrations of DIN, TN, PO_4^{3-} and TP than in the prawn monoculture. This indicates that *Sargassum* probably absorbed dissolved nutrients in the integrated system and thereby significantly reduced the concentrations of nutrients (N and P) in the waste water. Previous studies by Neori et al. (1996) and Troell et al. (1999) proved the role of seaweeds in reducing the risks of deterioration in water quality and of nutrient release to the surrounding environment when integrated with mariculture. The results of the present experiment emphasises the role of *Sargassum* sp. seaweeds to improve the water quality in prawn culture.

4.4.2 Nutrient budget

The determination of the nutrient budget in fish or prawn ponds enables one to determine the major sources and sinks of nutrients in the system, and how they vary with different conditions in aquaculture systems (Funge-Smith & Briggs 1998). Nutrient budgets have been constructed for coldwater marine fish farms (Beveridge 1984), warm brackish water and marine fish ponds (Krom et al. 1985ab; Krom & Neori 1989), but the dynamics and quantification of nutrient flows in integrated seaweed and prawn culture system have yet to receive any attention. Therefore, this experiment aimed to analyse the sources and sinks of nitrogen and phosphorus in an integrated seaweed and prawn system in order to: (a) further understand and quantify the nutrient cycling processes operating under integrated culture system, (b) derive data to help define effective methods for reducing water and sediment quality problems within prawn culture, and (c) quantify the pathways of nutrient loss to the environment to enable the definition of the most appropriate methods for reducing these losses from prawn culture.

Based on the calculation of nutrient budgets, these results obtained from the present study suggest that integrating seaweed with prawn culture could generally have no effect on the efficiency of converting nutrients (N and P) into prawn bodies.

Nitrogen

Feed is normally the greatest source of nitrogen in fish or prawn ponds. For example, feed accounted for 88% of nitrogen input in striped bass (*Morone saxatitis)* ponds (Daniels & Boyd 1989) and 92% of N as feed in intensive prawn culture (Briggs & Funge-Smith 1994). In this experiment, feed was also the major source of nitrogen, contributing up to 62.27% in PM and 47.53% in ISP. These rates were low when compared with previous studies (Funge-Smith & Briggs 1998; Teichert-Coddington et al. 2000). The amount of nitrogen assimilated into prawn biomass is a minor fraction of the total N applied as feed. In the present study, 17.69% and 18.99% of N applied as feed was converted to prawn flesh in PM and ISP, respectively. This result is in close agreement with the findings of Funge-Smith & Briggs (1998) in Thailand, Jackson et al. (2003b) in Australia and Lemonnier & Faninoz (2006) in New Caledonia who reported that about 18-27% of input nitrogen was assimilated into prawn in an intensive prawn system.

Intake water had a low concentration of N (0.17 mg/l of total nitrogen in all treatments) and therefore, accounted for a minor source of nitrogen input in both PM and ISP (12.09 and 10.11%, respectively). These results are in contrast to the reports by Teichert-Coddington et al. (2000) and Shahidul Islam et al. (2004) which showed that approximatrly 55- 63% of nitrogen input came from intake water. Differences in experimental conditions or techniques may explain the differences in contribution of nitrogen sources. Teichert-Coddington et al. (2000) and Shahidul Islam et al. (2004) conducted their studies in ponds, while the present study was conducted in the laboratory. However, at the end of the experiment, the proportion of nitrogen in waste water in ISP was less than a third of those of PM. The water contributed 30.52% of nitrogen added to the PM, whereas only 20.93% in ISP. This resulted in the high concentration of nitrogen (approximately 13 mg l^{-1}) in the water of PM at the end of the experiment. Based on these results, it can be assumed that integrating *Sargassum* with prawn culture assisted in improving environmental conditions. On the other hand, intake water accounting for the majority of nitrogen input in SM (59.26%) because there were two sources contributed nitrogen to SM, including intake water and seaweed. During the experiment, although no nutrient was added to the SM, the high proportion (28.99%) of nitrogen lost through the water was recorded at the end of the study, probably due to the nitrogen leaching from

decomposing dead seaweed. This resulted in a decline in water quality (Qian et al. 1996). Eventually, nitrogen could escape to the atmosphere in gaseous forms after denitrification process was completed.

It is also important to determine the role of wastes in nutrient dynamics in prawn production systems. In this experiment, wastes accounted for nitrogen input of 23.42% in ISP and 27.81% in PM. According to Briggs & Funge-Smith (1994), a significant proportion of the nitrogen output is found in the wastes after each culture cycle. Better waste management practices within culture cycles can substantially reduce the cumulative accumulation of organic matter in the wastes, resulting in better water quality and reducing wastes in prawn ponds.

When calculating nitrogen budgets for fish or prawn ponds, denitrification and diffusion process are two potential losses of N in the atmosphere and are rarely measured directly (Briggs & Funge-Smith 1994; Hopkins et al. 1995; Jackson et al. 2003b). Denitrification is the reduction of nitrate (NO_3^-) to gaseous N_2. Volatilisation is transmission of NH_3 from the water column to the atmosphere. Therefore in most studies, including the present one, these factors are estimated indirectly as the difference between the nitrogen inputs and outputs. In the present study, 50.30% of nitrogen in the SM was unaccounted for, probably due to nitrogen lost to the atmosphere as N_2 or ammonia (Funge-Smith & Briggs 1998). On the other hand, only 5% of the input nitrogen in the PM and 9.12% in ISP treatments was unaccounted for in this experiment. Varying results have been obtained in other studies, ranging from 3% to 66% of total nitrogen input (Funge-Smith & Briggs 1998; Páez-Osuna et al. 1999; Lemonnier & Faninoz 2006). However, most estimated less than 15% of nitrogen was lost to the atmosphere (Briggs & Funge-Smith 1994; Martin et al. 1998). A lower value of 6.5% was reported by Teichert-Coddington et al. (2000) and 3% by Jackson et al. (2003b) for nitrogen loss via denitrification or volatilization of ammonia in an intensive prawn farm.

Phosphorus

The contribution of feed to phosphorus input in the present study was observed to be the greatest source of phosphorus to the prawn tanks, contributing 66.55% in PM and 50.10% in ISP. This is in close agreement with Funge-Smith & Briggs (1998) and Teichert-Coddington et al. (2000) who reported feed as the principal source of phosphorus in prawn culture. It has been reported that, over time, an average of about 20-35% of the phosphorus added to the system can be retained in fish (Green & Boyd 1995; Boyd & Tucker 1998). However, lower conversion rates of phosphorus input were recorded in both PM and ISP in the present study. At the conclusion of the experiment, 14.14% of the phosphorus input as feed in PM and 13.79% in ISP were incorporated into prawn biomass. These results were comparable with those of Briggs & Funge-Smith (1994) and Shimoda et al. (2005). They indicated that only 13% of the feed input of phosphorus was incorporated into the prawn bodies at harvest.

According to Munsiri et al. (1995), phosphorus accumulates mostly in the wastes over time. In this experiment, the budget figures have shown that the wastes accounted for a major proportion of P loss in the prawn tanks, with 42.63% in PM and 35.67 in ISP. Similarly, Briggs & Funge-Smith (1994) indicated that up to 84% of phosphorus was retained in the wastes and water. In this study, a small proportion of phosphorus in waste water was observed in the PM (14.02%) and ISP (22.91%). This was expected, as waste water from pond always contains much less phosphorus than the amount added in feeds because most of the phosphorus is lost to the solid wastes (Boyd & Tucker 1998).

A minor proportion of input phosphorus was unaccounted for in the budget (1-5%). This discrepancy may be explained by phosphorus loss through leaching when drained off the water at the end of the experiment.

4.4.3 Survival and growth of prawn

Survival, growth and biomass increment are the main concerns in the operation of a commercial aquaculture farm. Survival of the prawn in all of the experimental tanks was higher than 55%. This rate is higher than another study which was 13.64 – 40.91% after 30 days of the experiment (Sang 2003). No significant difference in the

survival rates of prawn in PM and ISP is evidence that the incorporation of *Sargassum* into prawn culture has no effect on the survival of the prawns.

No significant difference in either the mean weight or the SGR of the prawns was found between PM and ISP. This indicates that *Sargassum* did not alter the growth performance of the prawns. Similarly, Lombardi et al. (2006) reported no significant differences in weight gain between monoculture and integrated culture systems when seaweed (*Kappaphycus alvarezii*) was integrated into Pacific white prawn (*Litopenaeus vanamei*) culture. Compared with studies on *P. monodon* (Chen et al. 1989; Thakur & Lin 2003), the SGR of western king prawns in both the monoculture and integrated culture systems in this experiment was high, possibly as a result of lower stocking densities. In the present study, the stocking density of the western king prawns was 18 prawns per m^2, while *P. monodon* were stocked at approximately 70 postlarvae per m^2 (PL_{25-27}) by Chen et al. (1989) and 20-25 juveniles per m^2 by Thakur & Lin (2003).

4.4.4 Growth of seaweed

The growth rate of *Sargassum* is different under different environmental conditions (Gellenbeck 1984; Guimaraens 1999). By integrating seaweed with prawn culture, the growth rate of seaweed in ISP was lower than in the SM. This was probably due to the excessive increase of dissolved inorganic nutrient concentrations observed during the last days of the experiment. There was an increase in the nutrient concentration during the last days of experiment due to decaying and dying seaweed. Tacon & Forster (2003) reported that aquatic animal culture releases large amount of nutrient in the form of metabolite wastes which led to nutrient enrichment of the water column. There is evidence from previous studies, using different species of seaweed (*Ulva* and *Gracialria*), that high nutrient levels can result in an inhibition in the growth rate of seaweed (Waite & Mitchell 1972; Parker 1982; Lignell & Pedersén 1987; Marinho-Soriano et al. 2002). High nutrient levels have also been shown to inhibit the growth of *Sargassum* (Schaffelke & Klimpp 1998; Diaz-Pulido & McCook 2005). Liu et al. (2004) also reported that the increase in fresh weight gain of *Sargassum enerve* was slower at high nitrogen concentration. This is in agreement with the finding by Larned (1998) who reported that *Sargassum echinocarpum* could maintain a positive growth rate without nitrogen enrichment.

Furthermore, the nutrient thresholds for the optimum growth rate of *Sargassum* species are low, ranging from 3 to 5 μM (equivalent to 0.03-0.05 mg l^{-1}) for TAN, 6-15 μM (equivalent to 0.06-0.17 mg l^{-1}) for (NO_3^- + NH_4^+) and 0.3-0.75 μM (equivalent to 0.014-0.035 mg l^{-1}) for PO_4^{3-} (Schaffelke & Klimpp 1998; Ray-Lien Hwang et al. 2004). Lower and unexpected higher nutrient concentrations resulted in reduced growth rates (Schaffelke & Klimpp 1998). However, on day 7 of the present experiment, concentrations on excess of these values were recorded, specifically, 0.67 mg l^{-1} for TAN, 0.845 mg l^{-1} for (NO_3^- + NH_4^+) and 0.14 mg l^{-1} for PO_4^{3-}. High nutrient levels have also been shown to inhibit the growth of the brown seaweed, but the mechanisms behind that inhibition remain unclear (Ogawa 1984; Burridge & Bidwell 2002). It seems that the mechanism(s) may involve a toxic effect due to higher levels of nutrients. Elevated nutrient concentrations, particularly of ammonium, may inhibit the capacity to assimilate nutrients by altering the electron transport chain, and may affect enzyme and membrane functions (Peckol & Rivers 1995; Kevekordes 2001).

Realistic growth data of seaweed in the present experiment could be collected only during the first seven days of the experiment. Subsequent, growth rate could not be determined due to the deterioration and eventual death of the seaweed at day 28, as all thalli began to lose weight. The deterioration of thalli was probably due to changes in physical environmental conditions during the experiment. The physical environmental factors including temperature, salinity and light can play an important role in the growth of marine macroalgae (Lobban et al. 1985). Jones (1999) assumed that the temperature, water flow rate or light availability under laboratory conditions are not adequate for macroalgae growth, resulting in a higher rate of biomass decaying than biomass production. The current experiment was conducted at a temperature of 25-26^{0}C, 12:12 h light:dark cycle and salinity level of 28-29 ‰. As optimum temperature was maintained in this experiment, it is assumed that the temperature in this experiment did not affect the growth performance of *Sargassum* species (Hanisak & Samuel 1987; Ray-Lien Hwang et al. 2004). Therefore, it seems that other factor (e.g. light and salinity) was involved in the performance of *Sargassum* when it was cultivated together with the prawns. The low light density in the laboratory conditions may cause the loss of seaweed biomass (Appendix 4). If the light density is not adequate for *Sargassum* growth, eventually there will more

organic nutrients decaying than being produced. Hanisak & Samuel (1987) reported that the optimal salinity range for growth of several *Sargassum* species such as *S. flutans* and *S. natans* is 36-42‰. However, in this study, salinity ranged from 28 to 29 ‰ and could have affected the performance of the seaweed. Moreover, *Sargassum* sp. used in this study was collected from Cottesloe beach, Western Australia where the salinity is at 35-36 ‰.

4.4.5 N, P and N:P ratio in prawn and seaweed tissue

The results of this study indicate that the presence of *Sargassum* in prawn culture does not alter the assimilation of N and P into prawn biomass. After 42 days of the experiment, the nitrogen and phosphorus contents of the king prawns were with 10.51% and 1.72% in PM, 10.89% and 1.81% in ISP, respectively. These results are similar to previous studies by Briggs & Funge-Smith (1994) and Teichert-Coddington et al. (2000) who found that the nitrogen and phosphorus contents were 11.5% and 1.19% in black tiger prawns (*P. monodon)*, and 11.2% and 1.25% in whiteleg prawns (*P. vannamei)*, respectively. The final N:P ratio in prawn tissue in both PM and ISP did not differ from the initial values.

It is expected that, most of the nitrogen and phosphorus striped from the water within an integrated culture system be accounted for by subsequent gains in macroalgae biomass. The N:P ratio for optimal seaweed growth is within the range of 13-15. The N:P ratio < 13-15 indicates N limitation and N:P > 13-15 indicates P limitation. The N:P ratio is also an indicator used to assess the efficiency in the removal of nutrients from the aquaculture system. The initial N:P ratio of *Sargassum* used in this experiment was 9.87, thereby indicating N limitation. Presumably, this explains the rapid uptake of P observed after day 7 of the experiment. Unfortunately, in the present study, the tissue N and P content was lost during the experiment. Due to the dealth of the seaweed at day 28, the tissue nitrogen and phosphorus contents were released to the tanks water and the toal nitrogen and phosphorus biomass of seaweed produced was not determined at the end of the experiment. Therefore, neither tissue N and P nor molar ratio of N:P in seaweed could be determined. Consequently, there was more organic P decaying from dead seaweed than being produced.

CHAPTER 5

The effect of stocking density of prawns at two seaweed densities

5.1 Introduction

Past research has demonstrated that a number of macroalgae species have the capacity to improve water quality when integrated into prawn culture (Liu et al. 1997; Chuntapa et al. 2001; Nelson et al. 2001; Chuntapa et al. 2003). Chapter 4 showed that the seaweed *Sargassum* has potential to remove nutrients when they were cultured together with western king prawns. Prawn performance in terns of growth and survival remained unaffected in the integrated culture system. In contrast, the performance of seaweed suffered and the death of seaweed occurred in the last days of the experiment. Whether the performance of prawns and seaweed can be improved through changes in the stocking density of those species is not known. According to Troell et al. (2003) and Neori et al. (2004), the stocking biomass of species in an integrated system has the potential to influence the performance of the cultured species. For example, the growth and survival of several prawn species such as *Litopenaeus vannamei*, *Penaeus indicus* and *Penaeus monodon* were significantly affected by stocking density (Nga et al. 2005; Arnold et al. 2006). Similarly, Vandermeulen & Gordin (1990) reported that the growth rate of seaweed was linked to stocking density. Therefore, the experiments described in this chapter aim to evaluate the effect of changes in stocking biomass of either prawns or seaweed on the performances of those species and nutrient flow in an integrated seaweed prawn (ISP) aquaculture system.

5.2 Materials and methods

5.2.1 Materials and experimental design

Ninety western king prawns (5.08 g ± 0.39) were acclimated in two 250-L tank containing ocean water for 21 days. They were then weighed to obtain total initial biomass. During the experimental period, prawns were weighed weekly to obtain the data required for growth determination. The survival of the prawns in each tank was also recorded over the period of experiment.

Two experiments were carried out, Experiment 1 and Experiment 2. In both experiments, the stocking biomass of the prawns was increased by 10% (ISP1), 20%

(ISP2) and 30% (ISP3) of the stocking density to that of experiment described in Chapter 4 (Table 5.1). The Experiment 2 had a half of seaweed biomass than Experiment 1 in all its treatments.

In Experiment 1, The *Sargassum* was cultivated at 0.5 kg m^{-2} (equivalent to 140 g per tank) for all treatments (ISP1-1, ISP2-1 and ISP3-1). In Experiment 2, the seaweed was cultivated at a stocking density of 0.25 kg m^{-2} (equivalent to 70 g per tank) for all treatments (ISP1-2, ISP2-2 and ISP3-2) (Table 5.1). Seaweed was collected from the tanks every 10 days, cleaned, weighed and replaced after readjusting to starting biomass. When necessary, thalli of separately cultivated plants were used to replace any loss of fond that occurred in any tanks.

Table 5.1 Prawns (*Penaeus latisulcatus*) and seaweed (*Sargassum* sp.) stocking density in Experiment 1 and 2.

Experiment	Treatment	Prawn stocking density (g m^{-2})	Seaweed stocking density (g m^{-2})	Prawn:Seaweed ratio
Experiment1	ISP1-1	106	500	1:4.72
	ISP2-1	116	500	1:4.31
	ISP3-1	126	500	1:3.97
Experiment 2	ISP1-2	106	250	1:2.36
	ISP2-2	116	250	1:2.16
	ISP3-2	126	250	1:1.98

Note: ISP – integrated seaweed-prawn culture

Both experiments were conducted for 21 days each under laboratory conditions. All treatments had 3 replicates each which were arranged in a LSD design (Figure 5.1 and 5.2). The N and P contents from prawns feed were 5.34% and 1.78%, respectively.

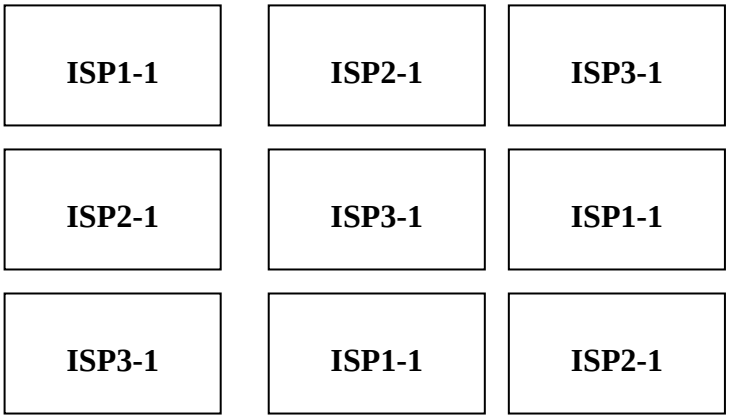


Figure 5.1 Experiment 1 tank layout (front view). Note: ISP = integrated seaweed-prawn culture.

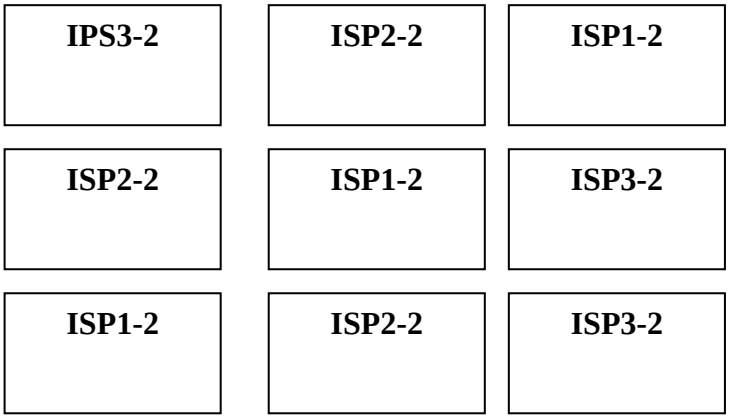


Figure 5.2 Experiment 2 tank layout (front view). Note: ISP = integrated seaweed-prawn culture.

Analysis procedures and data collection method used are described in Chapter 3 of Materials and Methods.

5.3 Results

5.3.1 Water quality

Mean temperature and dissolved oxygen (DO) were 21.60-22.48^{0}C and 5.81-6.16 mg l^{-1}, respectively over the experimental period. The pH of water ranged from 7.43 to 8.07, which was within the optimum range of prawn culture (Allan & Maguire 1992a; Wang, Wei-Na et al. 2002). The salinity of the water was maintained at 35.14-36.19‰ over the experimental period because *Sargassum* sp. was colleccted from Cottesloe beach where salinity level is at 35-36‰. This salinity was still within the optimum range for both prawn culture (Sang 2003; Prangnell 2007). During the experiment, evaporation losses were compensated by the addition of distilled water to maintain the salinity approximately 35-36‰ (Appendix 2, Table II).

Changes in water quality parameters in the integrated culture systems of the two experiments are shown in Figure 5.3 and 5.4.

In Experiment 1 (Figure 5.3), no significant differences ($p > 0.05$) were observed in the TAN, nitrite (NO_2^-), nitrate (NO_3^-), DIN, total nitrogen, PO_4^{3-} and total phosphorus among the treatments over the experimental duration (Figure 5.3). However, the concentration of TAN, DIN, total nitrogen, and PO_4^{3-} in all treatments increased significantly ($p < 0.05$) by day 14 and 21 of the experiment. Within each treatment, the NO_2^- concentration increased significantly ($p < 0.05$) by day 14 of the

experiment and was stable until the end of the experiment. However, the NO_2^- concentration remained below 1 mg l^{-1} throughout the experiment with the highest values in ISP3 (0.57 mg l^{-1}). The concentration of NO_3^- did not change significantly ($p > 0.05$) in any treatment until day 14 of the experiment. However, significantly higher values ($p < 0.05$) were recorded at the end of the experiment. The total phosphorus increased significantly ($p < 0.05$) in all treatments over time.

In Experiment 2, there were no significant differences ($p > 0.05$) in the concentration of TAN, NO_2^-, NO_3^-, DIN, total nitrogen, PO_4^{3-} and total phosphorus among treatments (Figure 5.4). However, from day 7 onwards, the concentrations of TAN, DIN, total nitrogen, PO_4^{3-} and total phosphorus within each treatment, were significantly higher ($p < 0.05$) when compared to the previous sampling time and the highest values were obtained at the end of the experiment. NO_2^- concentration did not significantly change ($p > 0.05$) until day 14 of the experiment. NO_2^- concentration was significantly higher ($p < 0.05$) during the last sampling time, but remained below 1.0 mg l^{-1}, except in ISP2. After 7 days of the experiment, the concentration of NO_3^- was higher ($p < 0.05$) than the beginning of the experiment, but then remained stable until the end of the experiment.

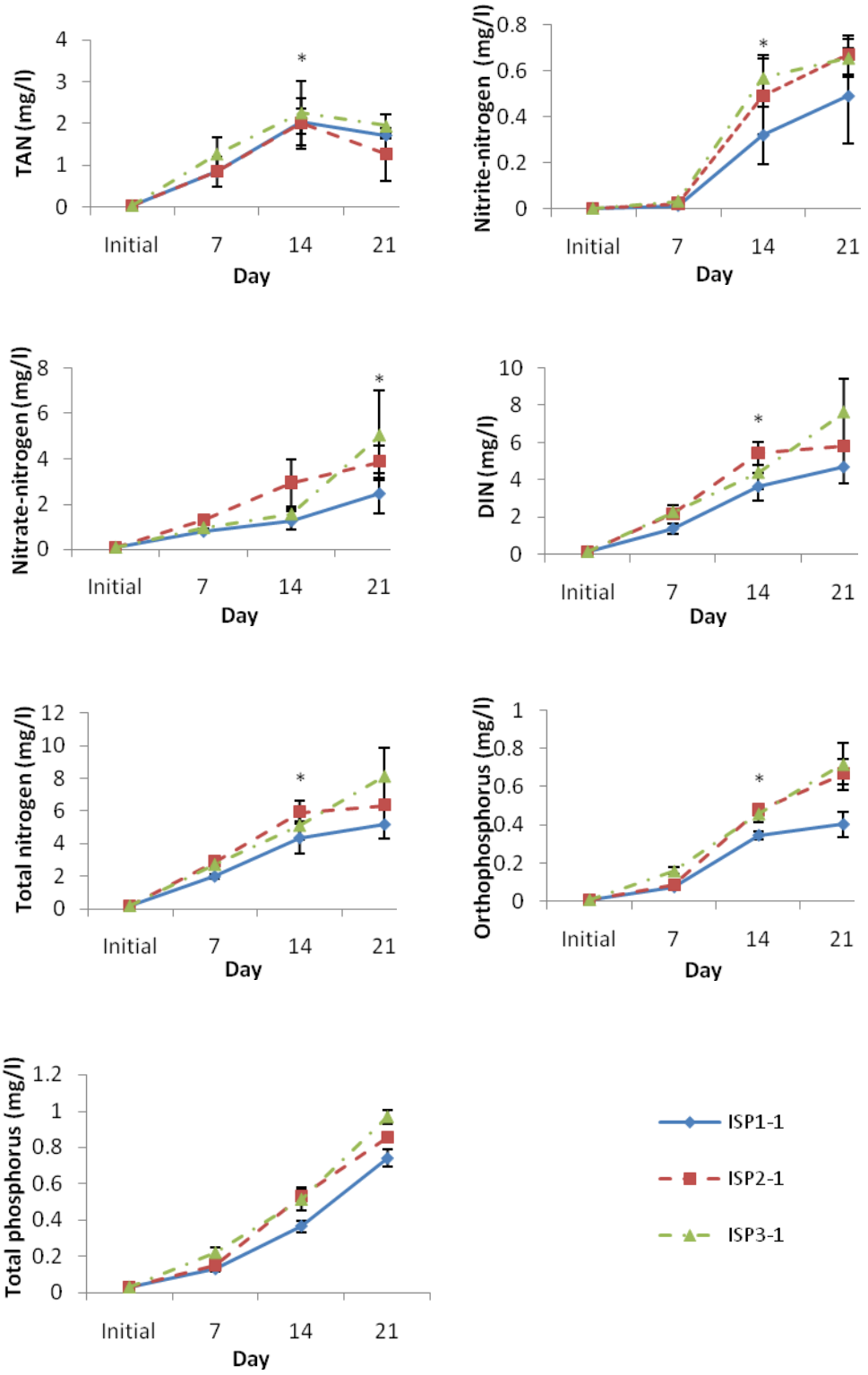


Figure 5.3 Water quality in Experiment 1 over 21 days (mean ± S.E.). Note: ISP = integrated seaweed-prawn culture.
The asterisk (*) represents the point from which the concentration of water quality parameters was significantly higher ($p < 0.05$).

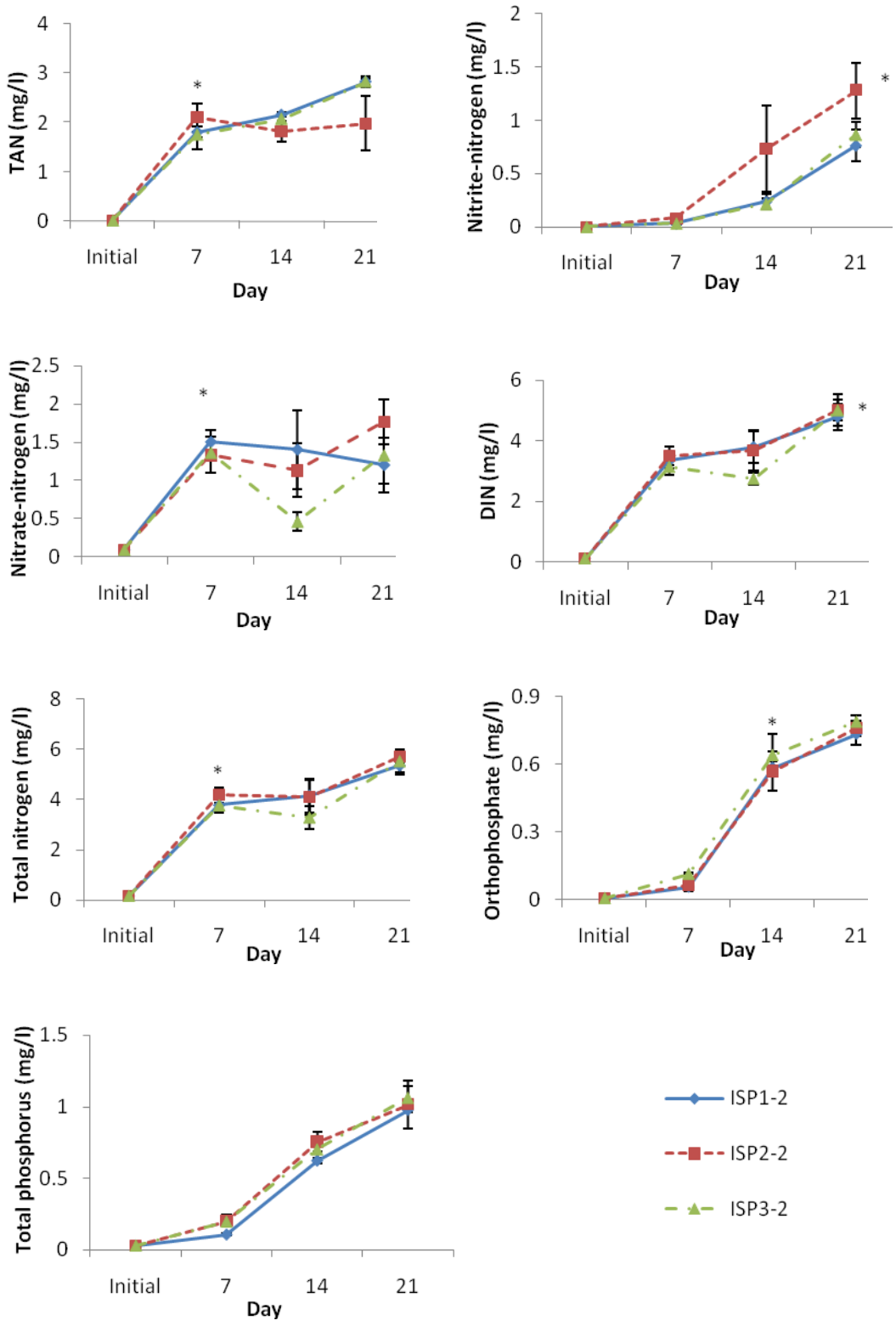


Figure 5.4 Water quality in Experiment 2 over 21 days (mean ± S.E.). Note: ISP = integrated seaweed-prawn culture.
The asterisk (*) represents the point from which the concentration of water quality parameters was significantly lower or higher ($p < 0.05$).

5.3.2 Growth and survival of prawns

At the end of the experiments, no significant differences ($p > 0.05$) were observed in the survival rates of the prawns among all treatments in either experiment (Figure 5.3). The survival of prawns in all treatments of Experiment 1 was approximately 93%, while the survival rates of prawns in Experiment 2 were 83% in ISP1-2, 66% in ISP2-2 and 82% in ISP3-2.

The specific growth rate (SGR) of prawns was significantly higher ($p < 0.05$) SGR at the stocking density of 106 g m^{-2} (ISP1-1) than other densities in both experiments (Figure 5.4). After 21 days, the mean SGR of prawns in ISP1-1 was 0.69 % g day^{-1} (ranging from 0.59-0.84 % g day^{-1}), while mean SGR of prawns ranged between 0.46-0.55 % g day^{-1} in other treatments in both experiments.

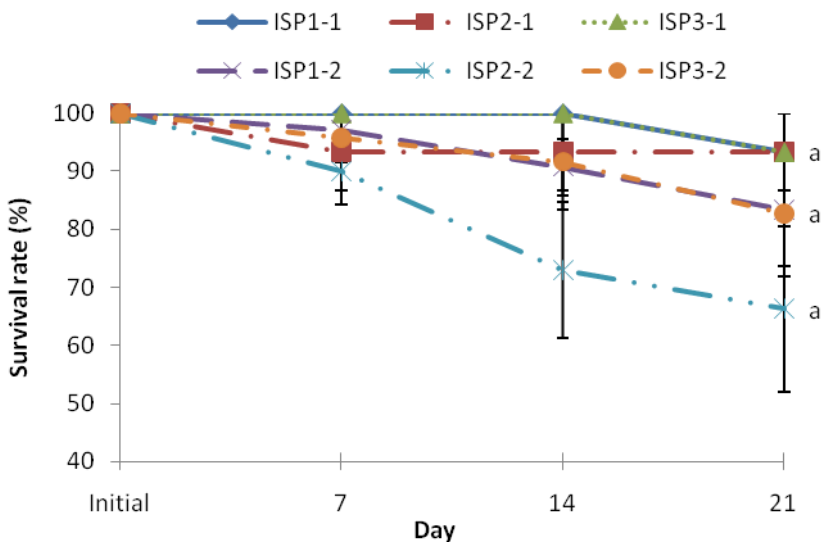


Figure 5.5 Survival rate of prawns in different treatments (mean ± S.E.).
Note: ISP = integrated seaweed-prawn culture. *Same letter denotes no significant difference ($p < 0.05$).*

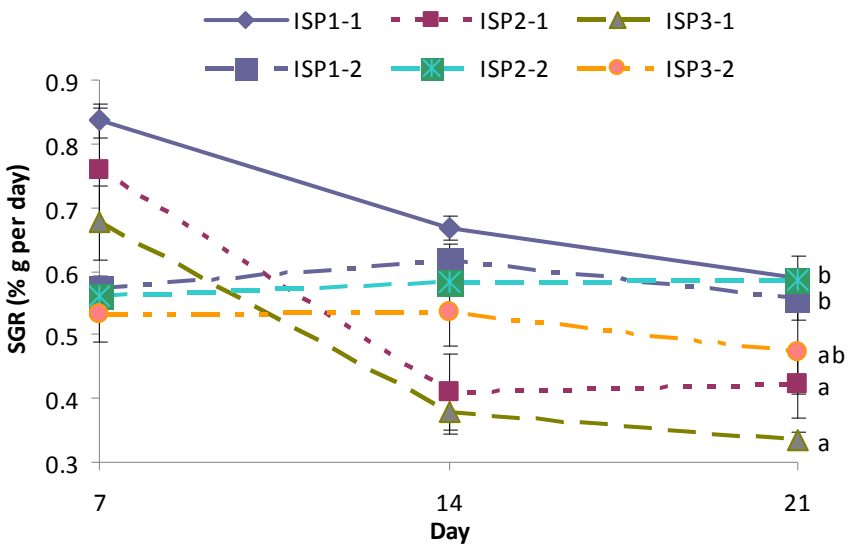


Figure 5.6 Specific growth rate (SGR) of prawns over the study period (mean ± S.E.).
Note: ISP = integrated seaweed-prawn culture. *Different letters (a,b) denote significant differences ($p < 0.05$) between treatments.*

5.3.3 Growth of seaweed

The specific growth rate of the *Sargassum* is presented in Figure 5.5. Results showed the growth rates of *Sargassum* varied between treatments, ranging from 2.98% to 3.62 % g day^{-1} over the period of the Experiment 1 and 1.79% to 2.83% g day^{-1} in Experiment 2. After 10 days, there were significant differences ($p < 0.05$) in the SGR of *Sargassum* among treatments in both experiments. The highest SGR was observed in the ISP1-1, with approximately 3.5 % g day^{-1}, while the ISP3-2 presented the lowest SGR with only 1.84 % g day^{-1}.

At day 21 of each experiment, no significant differences ($p > 0.05$) in seaweed were found among the treatments within each experiment. However, the overall values of the SGR obtained in Experiment 1 were significantly higher ($p < 0.05$) than the treatments in Experiment 2. The range of the SGR in Experiment 1 was 3.20 - 3.62 % g day^{-1}, while the SGR of seaweed in Experiment 2 was 1.79 – 2.32 % g day^{-1}.

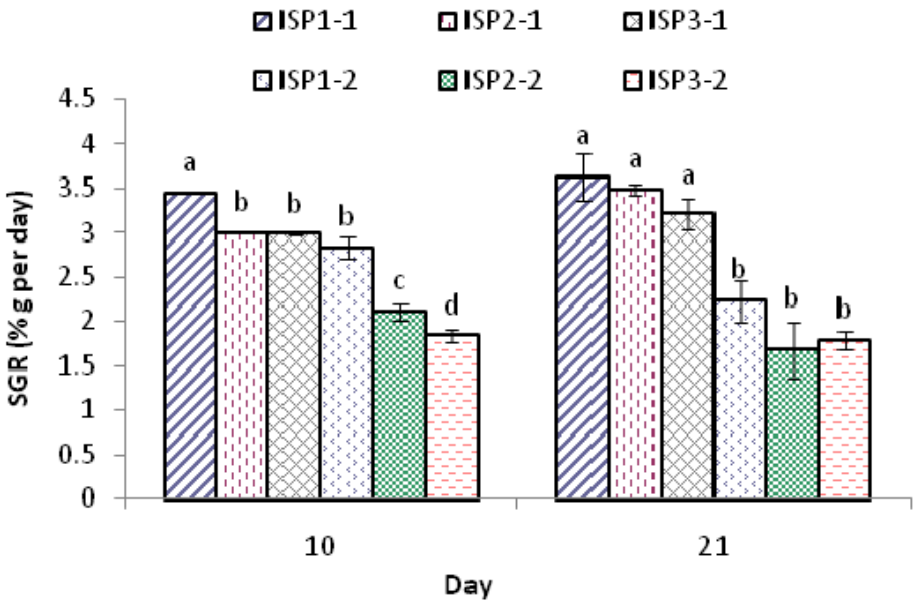


Figure 5.7 Specific growth rate of seaweed in treatments (mean ± S.E.).
Note: ISP = integrated seaweed-prawn culture. *Different letters (a,b,…) denote significant differences ($p < 0.05$).*

5.3.4 Conversion rates of nitrogen and phosphorus

The nitrogen content of the commercial feed pellets for prawns was 5.34% (dry weight). The concentration of nitrogen in the prawn tissue at the commencement of the experimental period was 9.43% and this did not change significantly ($p > 0.05$) within each treatment over the experimental period, ranging from 9.22 to 9.54% (Table 5.2). No significant difference ($p > 0.05$) in the conversion rates of feed nitrogen into prawn biomass was found between treatments within each experiment (Figure 5.8). However, the conversion rate of nitrogen in the ISP1-1 (23.70%) was significantly higher ($p < 0.05$) than in the ISP3-2 (17.80%).

The concentration of nitrogen in seaweed tissue did not change significantly ($p > 0.05$) over the experimental period (Table 5.2). The conversion rate of feed nitrogen into seaweed biomass showed a decreasing trend ($p < 0.05$) with decreasing seaweed stocking density, with the lowest value (12.15%) observed in ISP3-2 which had the highest prawn stocking density (Figure 5.9). However, no significant differences ($p > 0.05$) in nitrogen conversion were observed in the treatments within each experiment, which ranged from 18.86 to 23.84% in Experiment 1 and 12.15-14.03% in Experiment 2.

Table 5.2 Initial and final concentrations of nitrogen (%) in prawns (*Penaeus latisulcatus*) and seaweed (*Sargassum* sp.) tissues in Experiment 1 and 2 (mean ±S.E.).

Treatments	**Prawns**		**Seaweed**	
	Initial	**Final**	**Initial**	**Final**
ISP1-1	9.43±0.03[a]	9.47±0.10 [a]	1.72±0.05 [a]	1.87±0.03[a]
ISP2-1	9.43±0.03 [a]	9.51±0.10 [a]	1.72±0.05[a]	1.90±0.05[a]
ISP3-1	9.43±0.03[a]	9.54±0.10[a]	1.72±0.05[a]	1.89±0.04[a]
ISP1-2	9.22±0.02[a]	9.54±0.12[a]	1.68±0.03[a]	1.75±0.02[a]
ISP2-2	9.22±0.02[a]	9.43±0.13[a]	1.68±0.03[a]	1.68±0.08[a]
ISP3-2	9.22±0.02[a]	9.40±0.05[a]	1.68±0.03[a]	1.70±0.01[a]

Note: ISP = integrated seaweed-prawn culture. *Values in one column followed by the same letters are not significant differences (p > 0.05)*

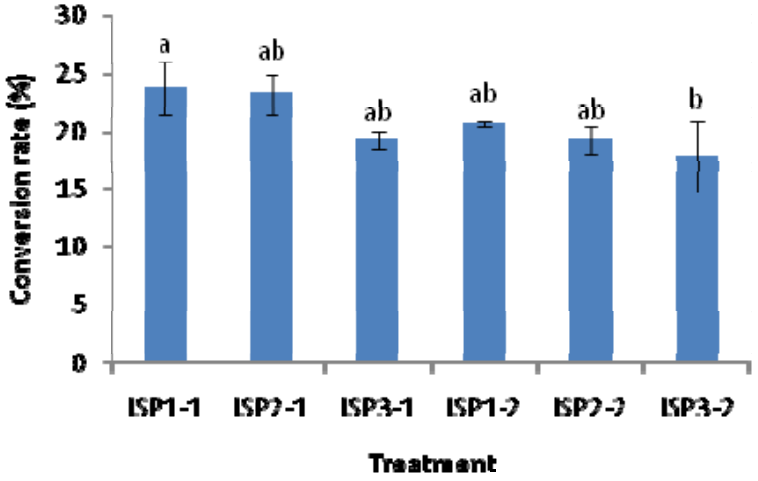


Figure 5.8 Conversion rates of nitrogen into prawn biomass (mean ± S.E.).
Note: ISP = integrated seaweed-prawn culture. *Different letters (a,b…) denote significant differences (p < 0.05).*

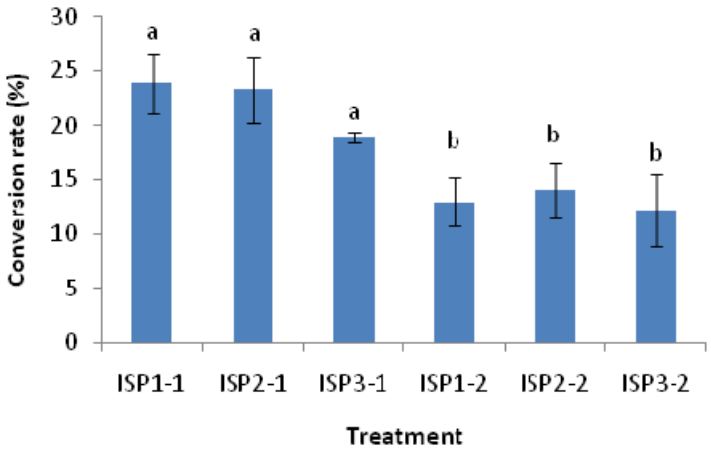


Figure 5.9 Conversion rates of nitrogen into seaweed tissue (mean ± S.E.).
Note: ISP = integrated seaweed-prawn culture. *Different letters (a,b…) denote significant differences (p < 0.05).*

The phosphorus content of the feed pellets was 1.78% dry weight. The phosphorus content in the initial samples of prawns was 1.14 (dry weight). At the end of the experiments, no significant differences between treatments were found in the phosphorus content of prawn tissue, which ranged from 1.27 to 1.42% in prawn tissue (Table 5.2). The phosphorus conversion rates ranged from 13.98 to 17.37% in the prawns and from 25.64 to 30.32% in the seaweed, without significant differences ($p > 0.05$) between treatments within an experiment. However, a significantly higher ($p < 0.05$) phosphorus concentration in prawn tissue was observed in Experiment 1 than in Experiment 2 (Figure 5.10).

The phosphorus content in the initial seaweed samples was not significantly different between the two experiments (0.51% dry weights in Experiment 1 and 0.46% in Experiment 2). At the end of the experiments, there were no significant differences ($p > 0.05$) in the concentration of phosphorus among the treatments in both experiments (Table 5.3). Consequently, the conversion rate of phosphorus into seaweed biomass was not significantly different ($p > 0.05$) among the treatments (Figure 5.11).

Table 5.3: Initial and final concentrations of phosphorus (%) in prawns (*Penaeus latisulcatus*) and seaweed (*Sargassum* sp.) tissues in Experiment 1 and 2 (mean ±S.E.).

Treatments	**Prawns**		**Seaweed**	
	Initial	**Final**	**Initial**	**Final**
ISP1-1	1.14±0.02 [a]	1.27±0.10 [a]	0.51±0.03 [a]	0.64±0.10 [a]
ISP2-1	1.14±0.02 [a]	1.30±0.02 [a]	0.51±0.03[a]	0.71±0.10[a]
ISP3-1	1.14±0.02[a]	1.42±0.03 [a]	0.51±0.03[a]	0.59±0.60[a]
ISP1-2	1.13±0.01[a]	1.30±0.06[a]	0.46±0.01[a]	0.73±0.06[a]
ISP2-2	1.13±0.01[a]	1.43±0.22[a]	0.46±0.01[a]	0.65±0.05[a]
ISP3-2	1.13±0.01[a]	1.22±0.08[a]	0.46±0.01[a]	0.69±0.06[a]

Note: ISP = integrated seaweed –prawn culture. *Values in one column followed by the same letters are not significant differences (p > 0.05).*

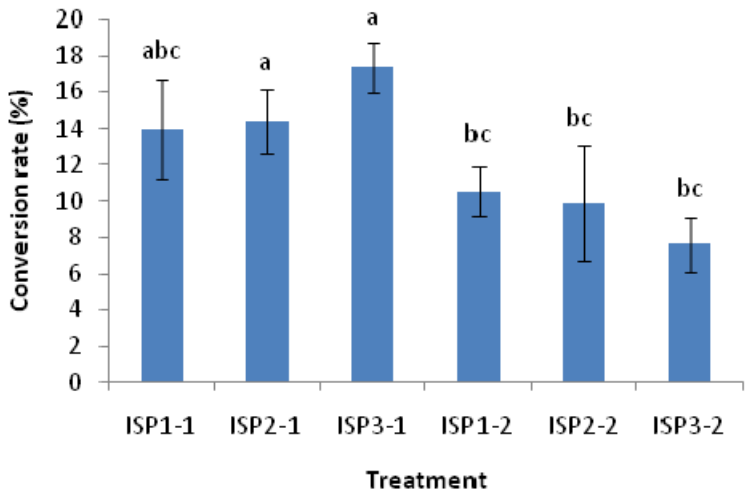


Figure 5.10 Conversion rates of phosphorus into prawn biomass (mean ± S.E.). Note: ISP = integrated seaweed-prawn culture. *Different letters (a,b…) denote significant differences ($p < 0.05$).*

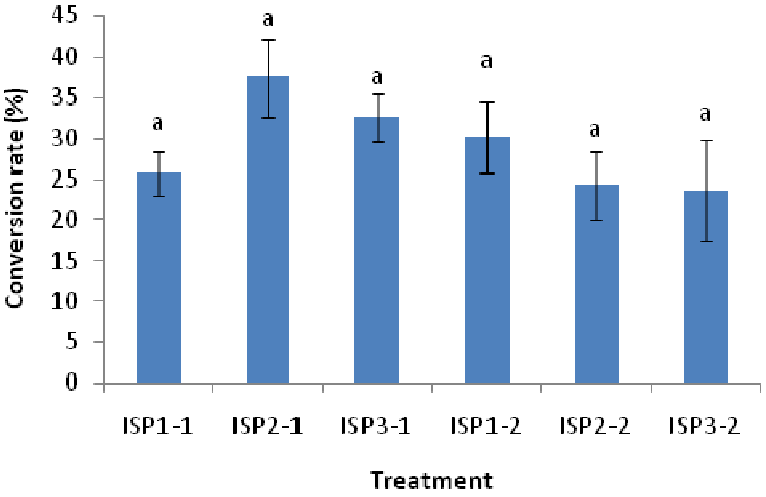


Figure 5.11 Conversion rates of phosphorus into seaweed tissue (mean ± S.E.). Note: ISP = integrated seaweed-prawn culture. *Same letter denotes no significant difference ($p > 0.05$).*

5.3.5 Nutrient uptake rates

There were significant differences ($p < 0.05$) in the nitrogen and phosphorus uptake rates among treatments in both experiments (Table 5.4). Mean nitrogen uptake rates based on N content in the thalli were estimated at 0.60, 0.59 and 0.55 mg N g dry wt^{-1} day^{-1} in ISP1-1, ISP2-1 and ISP3-1, respectively, whereas 0.49, 0.32 and 0.33 mg N g dry wt^{-1} day^{-1} were recorded in ISP1-2, ISP2-2 and ISP3-2, respectively. Higher phosphorus uptake rates were observed in Experiment1 (0.21-0.25 mg N g dry wt^{-1} day^{-1}) than in Experiment 2 (0.13-0.16 mg N g dry wt^{-1} day^{-1}).

When data from the two experiments were pooled together, a strong positive correlations between stocking density of seaweed and the mean uptake rates of nitrogen (r^2=0.70, p<0.001; Figure 5.10) and phosphorus (r^2=0.84, p < 0.001; Table 5.5) was observed. At the higher stocking density of seaweed (0.5 kg m^{-2}), the mean nitrogen and phosphorus uptake rates were greater than at the lower stocking density (0.25 kg m^{-2}). Similarly, the mean uptake rates of nitrogen and phosphorus had the strong positive effects on the SGR of seaweed in the integrated culture (Table 5.5). The treatment groups with the higher biomass of seaweed were found to have the highest mean nitrogen and phosphorus uptake rates (p < 0.001).

Table 5.4 Nutrient uptake rates of seaweed in two experiments.

Experiments	**Treatments**	**Nitrogen (mg N g dry wt^{-1} day^{-1})**	**Phosphorus (mg P g dry wt^{-1} day^{-1})**
Experiment 1	ISP1-1	0.66 ± 0.03^{a}	0.21 ± 0.02^{a}
	ISP2-1	0.59 ± 0.03^{b}	0.24 ± 0.01^{ab}
	ISP3-1	0.55 ± 0.02^{bc}	0.25 ± 0.01^{b}
Experiment 2	ISP1-2	0.49 ± 0.01^{c}	0.16 ± 0.002^{c}
	ISP2-2	0.32 ± 0.07^{d}	0.14 ± 0.01^{c}
	ISP3-2	0.33 ± 0.03^{d}	0.13 ± 0.002^{c}

Note: ISP = integrated seaweed-prawn culture. *Values in one column followed by the different letters are significant differences (p < 0.05).*

Table 5.5 Regression equations and coefficients (R^2) for the correlations between variables from data in two experiments combined. Data shown is pooled results from Experiment 1 and Experiment 2 (p < 0.05).

Factor (x,y)	**Regression equation**	**R^2**
SD of seaweed, $U_{mean}N$	y = 0.8811x + 0.161	0.700
SD of seaweed, $U_{mean}P$	y = 0.378x + 0.0468	0.839
SGR of seaweed, $U_{mean}N$	y = 0.1921x - 0.0244	0.962
SGR of seaweed, $U_{mean}P$	y = 0.0679x + 0.0061	0.784

SD = stocking density (kg m^{-2}), SGR = specific growth rate (% g day^{-1}), $U_{mean}N$ = mean nitrogen uptake rate (mg g^{-1} dry wt day^{-1}), $U_{mean}P$ = mean phosphorus uptake rate (mg g^{-1} dry wt day^{-1}).

5.4 Discussion

5.4.1 Growth and survival of prawns

The survival rate of prawns was not significantly different in either experiment, which suggests that the changes in stocking density of the prawns and seaweed had no effect on the survival of the prawns. The increasing stocking density of prawns by 10, 20 and 30% for these experiments compared to the previous study (as described in the materials and methods section of chapter 4) had not affected prawn survival too. This contention is in agreement with the observation of Allan & Maguire (1992b) who reported that increasing stocking densities from 5 to 40 prawns m^{-2} had no effect on survival of black tiger prawn (*P. monodon)*. The main cause of prawn mortality in all treatments may have been due to cannibalism as prawns were particularly vulnerable while moulting and thus reduced survival rate of prawns.

A significantly higher growth rate of prawns was observed in the ISP1-1. Measured water quality parameters (TAN, NO_2^-, NO_3^-, DIN, PO_4^{3-}, total nitrogen and total phosphorus) remained within a safe range for prawn culture and did not vary between treatments or experiments therefore, it is unlikely that water quality resulted in the difference in SGR (Chen et al. 1990). The variation in the SGR of prawns is likely to be due to the variation in their stocking densities (Haque et al. 2003). According to Forster & Beard (1974) and Sandifer & Smith (1975), a high density of prawns can have a detrimental effect on the growth rate in a closed system which supports the present findings. However, when the stocking density of seaweed decreased to 0.25 kg m^{-2} for all treatments in Experiment 2, the SGR of prawns was lower than in the ISP1 of Experiment 1 and did not change over time. This indicates that the decreasing the stocking density of seaweed had a negative effect on the growth of prawns. The highest SGR of prawns was observed when prawns were cultured at the stocking density of 106 g m^{-2} and stocking density of seaweed was 0.5 kg m^{-2} (i.e. ISP1-1 with the prawn-seaweed ratio of 1:4.72). This finding is supported, and can be explained by, the higher conversion rates of nitrogen and phosphorus from feed into prawn biomass in all treatments of Experiment 1 when compared to Experiment 2 (Figure 5.8 and 5.10). This is in agreement with the findings of Muangkeow et al. (2007) who reported that the growth rate of Pacifiv white prawns (*Litopenaeus vannamei*) increased with the increasing nutrient conversion.

5.4.2 Growth of seaweed

Comparison of the results of the two experiments in this study indicates that the growth of *Sargassum* was higher when the stocking density was 0.5 kg m^{-2} in comparison with the stocking density of 0.25 kg m^{-2}. The observed specific growth rates for *Sargassum* in all treatments in Experiment 1 were similar to those described by previous chapter (chapter 4), whereas, the SGR in Experiment 2 was lower, ranging from 1.84 to 2.83% g day^{-1}. These results are in agreement with the findings by Mwandya et al. (2001) who reported that the daily growth rate of *Ulva reticulate*, *Eucheuma denticulatum* and *Gracilaria crassa* increased with an increase in stocking density of seaweed. According to Troell et al. (2003) and Neori et al. (2004), there are several factors that affect to growth of seaweed and seaweed stocking density is one of the key factors. In this study, the decrease in seaweed stocking density in Experiment 2 probably reduced the growth of *Sargassum*. Reported optimum densities for maximum growth of seaweed in tanks cultures vary, depending on the species. For example, Lapointe & Tenore (1981) found that the stocking density of 0.8 kg fresh weight m^{-2} gave the maximum growth of *Ulva*. Similarly, Hurtado-Ponce et al. (1992) found that the appropriate stocking density of a number of *Gracilaria* species is 0.5-1 kg m^{-2}. Westermeier et al. (1993) found that a stocking density of 1.2 kg m^{-2} in a *Gracilaria* rope culture could increase annual production by up to 29% when compared to a 0.6 kg m^{-2} stocking density. However, the growth rate of *Sargassum* in Experiment 2 was similar to those reported by other studies for a number of seaweed species. For example, growth rates for red seaweed (*Gracilaria bailinae),* which was integrated with milkfish (*Chanos chanos*), range, from 2.27 to 2.54% g day^{-1} (Guanzon et al. 2004).

Another factor that could explain the higher SGR of seaweed in Experiment 1 when compared to Experiment 2, is higher nitrogen conversion rates and nutrient uptake rates into seaweed in Experiment 1, which would presumably be used by the seaweed for growth (Figure 5.9) (Peckol et al. 1994). This result is supported by Subandar et al. (1993) who reported that the SGR of seaweed decreased as nutrient uptake rates decreased.

5.4.3 N and P in seaweed tissue

The nutrient levels reached in *Sargassum* tissue are a consequence of the assimilation the nutrients that are based on the macroalgae demand and biomass dilution for the growth. Over the period of two experiments, the nitrogen content of *Sargassum* ranged from 1.70 to 1.92%, similar to the values reported by Hanson (1977) who reported that the nitrogen content of *Sargassum* was 1.69% when investigating its capacity in nitrogen fixation.

The results indicate that *Sargassum*'s capacity in nitrogen assimilation is comparable to, and some cases higher than other seaweed species such as *Gracilaria amansii* (Liu et al. 2004). Similarly, *Sargassum* also has a high capacity for phosphorus absorption. Results showed that the mean phosphorus content also increased from 0.54% to 0.59-0.71% in Experiment 1 and from 0.46% to 0.65-0.73% in Experiment 2. These values are also comparable to values reported for other seaweed species. Hernández et al. (2005) reported that the mean tissue phosphorus was 3.0 mg g^{-1} dry weight (0.3%) in *U. rotundata* and 3.49 mg g^{-1} dry weight (equivalent to 0.35%) in *G. longissima* when they were cultured in effluents from gilthead seabream (*Sparus aurata*) culture. Thus, it can be assumed that *Sargassum* has potential to assimilate superfluous nutrients in marine environment or in marine aquaculture situation.

Results also indicated that the nitrogen and phosphorus concentrations in *Sargassum* were always higher than critical values (that is, the tissue nitrogen and phosphorus concentration needed to sustain maximum growth) as reported for seaweed species. According to Hernández et al. (1997) and Lyngby et al. (1999), the values of the critical phosphorus concentration for different macroalgae are between 0.13 to 0.24% (dry weight). Ray-Lien Hwang et al. (2004) reported that the critical nitrogen content in some *Sargassum* species was 0.5 to 2% dry weight. Thus, it can be seen that nitrogen and phosphorus levels in the culture systems were not limiting the growth of *Sargassum* when integrated with western king prawn culture.

5.4.4 Nutrient uptake rates

Nutrient uptake rate is defined as the amount of nutrients removed per unit area, expressed in terms of, surface area, wet weight or dry weight (Lobban et al. 1985;

Troell et al. 2003). Differences in experimental techniques used in various studies make comparisons of nutrient uptake rates difficult. This majority of investigations on uptake of nutrients involved a continuous flow culture system, whereas the experiments in this study used a batch culture system. Furthermore, the nutrient uptake rate varies depending on culture conditions seaweed species and stocking density (Lobban et al. 1985; Troell et al. 2003). In this study, based on the N and P contents and growth rates of *Sargassum* integrated with prawn culture, the mean N and P uptake rates were estimated for the two experiments. Results showed that the mean N and P uptake rates were higher when the stocking density of seaweed was at 0.5 kg m^{-2} than at 0.25 kg m^{-2}. Recently, in an integrated culture of finfish and macroalgae, Mwandya et al. (2001) found that the nutrient uptake rate of seaweeds such as *Ulva reticulate*, *Eucheuma denticulatum* and *Gracilaria crassa* increased with increasing stocking density of seaweed. This may be attributed to a high surface area when seaweed cultured at high stocking density. According to Rosenberg & Ramus (1984) and Cohen & Neori (1991), the differences in the ratio of surface area to volume may lead to differences in nutrient uptake rates.

CHAPTER 6: GENERAL DISCUSSION

6.1 Introduction

The study analysed the benefits of integrating seaweed (*Sargassum* sp.) into western king prawn (*Penaeus latisulcatus*) culture system. The benefits were measured in terms of any improvements in growth and survival of respective species. However, main focus of the research was on the shift in the nutrient dynamics due to the integration. The shift was measured in terms of nitrogen and phosphorus absorption by the respective species, the role of seaweed in removing N and P in integrated seaweed prawns (ISP) systems and overall impacts on the nutrient budget due to this integration. During the first experiment of the study, nutrient dynamics in prawn monoculture (PM) and integrated seaweed-prawn (ISP) culture systems was analysed (Chapter 4). During the following two experiments (Chapter 5), impacts of the changes in the ratio of stocking densities of prawns and at two different levels of seaweed biomass were evaluated on the above mentioned benefits

Water quality and Nutrient removal

It has been known that seaweed effectively reduced the concentration of nitrogen and phosphorus metabolites from aquaculture systems (Jones 1999). In this study, analysis of water quality could detect differences in the concentration of nutrients between PM and ISP systems. The concentrations of DIN, total nitrogen, total phosphorus, TAN, NO_2^-, NO_3^- and PO_4^{3-} were lower in ISP systems than in PM system. Even though all of the *Sargassum* died at day 28 in Experiment 1, the efficiency of removal nutrients was still able to find in ISP system as indicated by the drop in the concentration of nutrients in water.

The nitrogen removal capacity by macroalgae depends on the concentration of nitrogen source(s), and in this study nitrogen from NO_3^- was shown preference over other nitrogen sources. Further, the increase in prawn stocking density did not affect the water quality variables, whereas the decrease of seaweed biomass influenced the nitrite concentration (Chapter 5). The increase of nitrite concentration with lower density of seaweed is probably as a consequence of the uncompleted nitrification process (Ray & Chien 1992). However, this concentration of nitrite is below the concentration of 6 mg l^{-1} reported to affect growth in penaeid prawns (Wickins

1976). Hence, no detrimental effect of nitrite on prawn growth is expected in this study.

Nutrient flow

Integration of seaweed with prawns did not alter the nitrogen and phosphorus conversion rates to any aquatic biomass. However, when data of three experiments were pooled, ISP systems had higher overall nutrient conversion rates than those in PM (Table 6.1 and 6.2). Thus, the rates of nitrogen and phosphorus conversion into waste, which is calculated as the harvest residuals, were lower in ISP than in MP (Chapter 4). This suggests that the ISP systems effectively retained nutrients into harvested aquatic products as seaweed could assimilate those nutrients which leached out from uneaten feed and faeces. Similarly, Troell et al. (2003) showed that integrated approach aquaculture systems can increase overall utilisation of the feed and, hence water and fewer nutrients are discharged into the waste (Schneider et al. 2005).

Different stocking density of either prawns or seaweed and culture techniques did not influence the nutrient recovery efficiencies into the prawn flesh. The nitrogen and phosphorus conversion rates by prawns in ISP systems of the present study ranged from 18.99-23.69% and 7.62-17.37%, respectively (Table 6.1 and 6.2). These numbers were close to that reported by Tian et al. (2001) for Chinese prawns *(Penaeus chinensis)* (N: 12.6-17.8%) and Pan et al. (2005) for Pacific white prawns *(Litopenaeus vannamei)* (P: 15.1-18.6%). However, the feed nitrogen conversion rates of seaweed were significant higher ($p < 0.05$) in high seaweed stocking density than in low stocking density, but no significant difference was observed in phosphorus conversion rates (Chapter 5). As a result, the nitrogen and phosphorus conversion rates to total biomass (that is, prawns plus seaweed) were higher with higher seaweed stocking density (0.5 kg m^{-2}). This demonstrated that by reducing the biomass of seaweed to 50% has a negative effect on the nutrient conversion rates into harvested biomass.

Table 6.1 Comparison of conversion rates (%) of feed nitrogen into prawns, seaweed and wastes of all systems (treatment) (mean ± S.E.).

Treatments	Prawns	Seaweed	Prawns and Seaweed	Waste
PM	17.69±1.01[a]	-	-	82.31±1.01
ISP	18.99±1.25[a]	-	-	-
ISP1-1	23.69±2.28[a]	23.84±2.72[a]	47.54±1.79[b]	52.46±1.79
ISP2-1	23.17±1.68[a]	20.32±3.03[a]	43.49±3.94[ba]	56.51±3.94
ISP3-1	19.19±0.78[a]	18.86±0.52[a]	38.05±1.23[a]	61.95±1.23
ISP1-2	20.66±0.23[a]	12.92±2.22[b]	33.59±2.16[c]	66.41±2.16
ISP2-2	19.22±1.22[a]	14.03±2.56[b]	33.25±3.74[c]	66.65±3.74
ISP3-2	17.80±3.06[a]	12.15±3.31[b]	29.95±5.73[c]	70.05±5.73

Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *Values followed by different letters (a,b…) of the same column are significantly different at $p < 0.05$.*

Table 6.2 Comparison of conversion rates (%) of feed phosphorus into prawns, seaweed and wastes of all systems (treatment) (mean ± S.E.).

Treatments	Prawns	Seaweed	Prawns and Seaweed	Waste
PM	14.47±4.40[ab]	-	-	85.53±4.40[a]
ISP	13.79±3.31[ab]	-	-	-
ISP1-1	13.98±2.72[ab]	25.64±2.68	39.63±0.75[ab]	60.38±0.75[b]
ISP2-1	14.40±1.75[ab]	37.51±4.84	51.91±3.25[a]	49.09±3.25[b]
ISP3-1	17.37±1.39[a]	32.45±2.92	49.82±2.71[a]	50.18±2.71[b]
ISP1-2	10.55±1.34[ab]	30.07±4.29	38.79±5.74[ab]	61.21±5.74[b]
ISP2-2	9.88±3.18[ab]	24.21±4.21	35.05±5.18[ab]	64.95±5.18[b]
ISP3-2	7.62±1.51[b]	23.56±6.27	30.59±6.83[b]	69.41±6.83[b]

Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *Values followed by different letters (a, b…) of the same column are significantly different at $p < 0.05$.*

Seaweed and nutrient uptake rates

As *Sargassum* sp. grew slower in ISP than SM (Chapter 4). reduced growth is a consequence from a range of factors, including physical water quality parameters (Ray-Lien Hwang et al. 2004) and nutrient metabolites (Peckol et al. 1994). Though temperature and pH were maintained within the narrow ranges as recommended for optimal growth of seaweed (DeBusk & Ryther 1984; Hanisak & Samuel 1987; Ray-Lien Hwang et al. 2004) however, the concentration of metabolic nitrogen and

phosphorus were higher in ISP than in SM, which probably would have inhibited the growth of seaweed.

A higher growth exhibited by *Sargassum* sp. at higher biomass (Table 6.3) is similar to *Gracialria chinesis* which showed the higher growth rates at higher stocking (Ugarte & Santelices 1992). The lower growth can be contributed to the large amount of suspended particles (faeces and uneaten feed from prawn culture) in water when the stocking density of seaweed was low (Hurtado-Ponce et al. 1992; Jones et al. 2002), though this ratio was not quantified in the research and was observed. The suspended particles resulted in a deposition of a thin layer of silt over thalli of seaweed and blocked the incident light and thus affected the growth due to reduced photosynthetic activity (Hurtado-Ponce et al. 1992; Nelson et al. 2001; Marinho-Soriano et al. 2002).

Table 6.3 Comparison of final SGR of *Saragssum* sp. under the conditions of different nutrient uptake rates, salinities and different stocking densities of seaweed.

Treatments	**Density (kg m^{-2})**	**Salinity (‰)**	**N uptake rate**	**P uptake rate**	**SGR (% day^{-1})**
SM	0.50	28-29	n.a.	n.a.	5.70 ± 0.82^{a}
ISP	0.50	28-29	n.a.	n.a.	3.16 ± 0.74^{cb}
ISP1-1	0.50	35-36	0.66 ± 0.03^{a}	0.21 ± 0.02^{a}	3.54 ± 1.35^{b}
ISP2-1	0.50	35-36	0.59 ± 0.03^{b}	0.24 ± 0.01^{ab}	3.24 ± 0.03^{b}
ISP3-1	0.50	35-36	0.55 ± 0.02^{bc}	0.25 ± 0.01^{b}	3.10 ± 0.10^{c}
ISP1-2	0.25	35-36	0.49 ± 0.01^{c}	0.16 ± 0.002^{c}	2.53 ± 0.08^{d}
ISP2-2	0.25	35-36	0.32 ± 0.07^{d}	0.14 ± 0.01^{c}	1.89 ± 0.12^{d}
ISP3-2	0.25	35-36	0.33 ± 0.03^{d}	0.13 ± 0.002^{c}	1.82 ± 0.06^{d}

Note: n.a. indicates no data available as seaweed died at the end of experiment. N uptake rate = Nitrogen uptake rate (mg g^{-1} dry wt day^{-1}); P uptake rate = Phosphorus uptake rate (mg g^{-1} dry wt day^{-1}); SM = seaweed monoculture, ISP = integrated seaweed-prawn culture. *Values followed by different letters (a, b…) of the same column are significantly different at $p < 0.05$.*

Even though *Sargassum* sp. growth was slow, they had a relatively higher capacity to remove nutrients when compared to other seaweed species. For example, Peckol et al. (1994) showed that *Cladophora vagabunda* and *Gracialria tikvahiae* can uptake nitrogen at maximum rate of 0.034 and over 0.054 mg g^{-1} dry wt day^{-1}, respectively. The N uptake rates in the present study are also similar to those

estimated for *Porphyra* species as reported by Carmona et al. (2006). They found that the nitrogen uptake rate varied from 0.4-4.0 mg g^{-1} day^{-1}. However, Subandar et al. (1993) estimated a greater mean N uptake rates of 1.92-3.07 mg g^{-1} dry wt day^{-1} by *Laminaria saccharina* cultured in salmon farm effluents. Thus, it is clear that each seaweed species has different capacity in uptaking nitrogen. However, the mean P uptake rate of *Sargassum* was close to *Porphyra* sp. (Carmona et al. 2006) and to *Gracialria lemaneiformis* (Zhou et al. 2006). These authors found that mean P uptake rates were 0.1-0.32 mg g^{-1} day^{-1} for *Porphyra* sp. and 0.14 mg g^{-1} day^{-1} for *Gracialria lemaneiformis*.

The uptake rate of nutrients is influenced by several biological factors such as, the age of the plant and its past nutritional history or nutrient status of its thalli (Ahn et al. 1998). The nutrient uptake rate of seaweed is also dependant on its stocking density (Jiménez del Río et al. 1994). Neori et al. (1991) reported *Ulva* sp. had high nitrogen uptake and tissue nitrogen content at high stocking densities which is similar in the current study (Table 6.3).

Survival and Growth of prawns

This is the first study of the integration of *Sargassum* into western king prawn, *Penaeus latisulcatus*, culture. The results from three experiments showed that the incorporation of *Sargassum* into prawn culture did not affect the survival rate and growth of prawns in comparison to prawn monoculture (Table 6.4).

However, prawns grew slower when (i) their stocking density was increased as growth rates of aquatic species are inversely related to its stocking biomass (Allan & Maguire 1992b; Martin et al. 1998; Tseng et al. 1998) and (ii) stocking density of seaweed was decreased, except for ISP1-1 where prawn stocking density only increased by 10% and seaweed stocking density was at 0.5 kg m^{-2} (prawn-seaweed ratio = 1:4.72). There was higher nutrient uptake by seaweed in ISP1-1 and therefore, led to the higher prawn growth (Table 6.4).

Table 6.4 Comparison of final SGR of prawns in different nutrient uptake rates with different ratios of prawn-seaweed.

Treatments	Prawn-seaweed ratio	N uptake Rate	P uptake rate	SGR (% day^{-1})	Survival (%)
PM*	-	n.a.	n.a.	0.64 ± 0.21^{b}	55.00 ± 9.57^{a}
ISP*	1:5.21	n.a.	n.a.	0.61 ± 0.15^{b}	60.00 ± 8.71^{a}
ISP1-1	1:4.72	0.66 ± 0.03^{a}	0.21 ± 0.02^{a}	0.69 ± 0.02^{b}	93.33 ± 6.66^{a}
ISP2-1	1:4.31	0.59 ± 0.03^{b}	0.24 ± 0.01^{ab}	0.53 ± 0.06^{a}	93.33 ± 6.66^{a}
ISP3-1	1:3.97	0.55 ± 0.02^{bc}	0.25 ± 0.01^{b}	0.46 ± 0.03^{a}	93.33 ± 6.66^{a}
ISP1-2	1:2.36	0.49 ± 0.01^{c}	0.16 ± 0.002^{c}	0.55 ± 0.01^{a}	83.33 ± 9.62^{a}
ISP2-2	1:2.16	0.32 ± 0.07^{d}	0.14 ± 0.01^{c}	0.51 ± 0.01^{a}	66.30 ± 14.23^{a}
ISP3-2	1:1.98	0.33 ± 0.03^{d}	0.13 ± 0.002^{c}	0.49 ± 0.04^{a}	82.74 ± 10.93^{a}

Note: n.a. indicates no data available as seaweed died and fouled at the end of experiment. N uptake rate = Nitrogen uptake rate (mg g^{-1} dry wt day^{-1}); P uptake rate = Phosphorus uptake rate (mg g^{-1} dry wt day^{-1}); PM = prawn monoculture, ISP = integrated seaweed-prawn culture. *Values followed by different letters (a, b…) of the same column are significantly different at $p < 0.05$.*
** Duration = 42 days*

6.2 Conclusions

1. Integration of seaweed with western king prawns led to the reduction in the concentrations of nitrogen and phosphorus in water.

2. The death of seaweeds can result in an increase in the concentrations of NH_4^+, NO_2^-, NO_3^-, DIN, total nitrogen, PO_4^{3-} and total phosphorus, but still remained relatively lower than in prawn monoculture systems.

3. *Sargassum* cultured together with prawns could effectively remove nutrients without effecting the growth and survival of western king prawns.

4. The integration of seaweed with prawn culture did not alter the nitrogen and phosphorus conversion rates from feed into prawns.

5. The incorporation of *Sargassum* sp. into western king prawn cultures resulted in reducing the nitrogen loss through waste water.

6. The mean nitrogen and phosphorus uptake rates by seaweed are strongly related to the stocking density and the SGR of seaweed.

7. The growth rates of prawns is higher at the prawn:seaweed ratio of 1:4.72.

8. The lower seaweed stocking biomass does not affect the western king prawn growth, but negatively affects the growth, the nutrient conversion rates and nutrient uptake rates of seaweed.

6.3 Recommendations for future research

1. The present study was based on the small scale laboratorial experiments. It is necessary that these results are further validated on a large scale under field conditions.
2. Further study is necessary to examine how low seaweed stocking density can reduce the growth rate, nutrient conversion rates and nutrient uptake rates.
3. Research should be conducted in investigating the underlying biotic and abiotic factors which influence the growth of seaweed and nutrient uptake capacity.
4. Different culture techniques should be tested to reduce the risk of seaweed degradation when cultured with prawns.

REFERENCES

ABARE 2008, *Australian Fisheries Statistics 2007*, Fisheries Research and Development Corporation.

Abeysinghe, DH, Shanableh, A & Rigden, B 1996 'Biofilters for water reuse in aquaculture', in *Water Science and Technology, IWA Publishing* vol. 34, pp. 253-60.

Aboutboul, Y, Arviv, R & van Rijn, J 1995, 'Anaerobic treatment of intensive fish culture effluents: volatile fatty acid mediated denitrification', *Aquaculture,* vol. 133, no. 1, pp. 21-32.

Acosta-Nassar, MV, Morell, JM & Corredor, JE 1994, 'The Nitrogen budget of a Tropical semi-intensive freshwater fish culture pond', *Journal of the World Aquaculture Society,* vol. 25, pp. 261-70.

Ahn, O, Petrell, R & Harrison, P 1998, 'Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm', *Journal of Applied Phycology,* vol. 10, no. 4, pp. 333-40.

Al-Shwafi, N & Rushdi, A, 'Heavy metal concentrations in marine green, brown, and red seaweeds from coastal waters of Yemen, the Gulf of Aden', *Environmental Geology*.

Ali, F, Yamasaki, S & Hirata, H 1994, 'Polyculture of *Penaeus japonicus* larvae and *Ulva sp.* fragments', *Suisanzoshoku (Japan),* vol. 42, no. 3, pp. 453-8.

Allan, GL & Maguire, GB 1992a, 'Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon* Fabricius', *Aquaculture,* vol. 107, no. 1, pp. 33-47.

Allan, GL & Maguire, GB 1992b, 'Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds', *Aquaculture,* vol. 107, no. 1, pp. 49-66.

AOAC 1995, *Officical Methods of Analysis,* 15 edn, Association of Official Analytical Chemists, Washington, DC.

APHA 1998, *Standard methods for the examination of water and wastewater* 20th edn, American Public Health Association, Washington, DC.

Arbiv, R & van Rijn, J 1995, 'Performance of a treatment system for inorganic nitrogen removal in intensive aquaculture systems', *Aquacultural Engineering,* vol. 14, no. 2, pp. 189-203.

Arnold, SJ, Sellars, MJ, Crocos, PJ & Coman, GJ 2006, 'Intensive production of juvenile tiger shrimp *Penaeus monodon*: An evaluation of stocking density and artificial substrates', *Aquaculture,* vol. 261, no. 3, pp. 890-6.

Bartoli, M, Nizzoli, D, Vezzulli, L, Naldi, M, Fanciulli, G, Viaroli, P & Fabiano, M 2005, 'Dissolved oxygen and nutrient budgets in a phytotreatment pond colonised by *Ulva* sp.' *Hydrobiologia,* vol. 550, no. 1, pp. 199-209.

Boaventura, R, Pedro, AM, Coimbra, J & Lencastre, E 1997, 'Trout farm effluents: Characterization and impact on the receiving streams', *Environmental Pollution,* vol. 95, no. 3, pp. 379-87.

Borges, M-T, Silva, P, Moreira, L & Soares, R 2005, 'Integration of consumer-targeted microalgal production with marine fish effluent biofiltration – a strategy for mariculture sustainability', *Journal of Applied Phycology,* vol. 17, no. 3 pp. 187-97.

Boyd, CE & Tucker, CS 1998, *Pond Aquaculture Water Quality Management*, Springer.

Brambilla, F, Lalumera, G, Terova, G, Crosa, G & Saroglia, M 2007, 'Inflow and outflow water quality control in coastal aquaculture systems: a case study', *Aquaculture Research,* vol. 38, no. 15, pp. 1654-63.

Bratvold, D, Burford, M, Jackson, C & Thompson, P 2004, 'Development of methods for the rapid degradation of prawn pond waste'. CSIRO Marine Research, Cleveland, QLD, Australia

Briggs, MRP & Funge-Smith, SJ 1994, 'A nutrient budget of some intensive marine shrimp ponds in Thailand', *Aquaculture and Fisheries Management,* vol. 25, pp. 789-811.

Brown, JJ, Glenn, EP, Fitzsimmons, KM & Smith, SE 1999, 'Halophytes for the treatment of saline aquaculture effluent', *Aquaculture,* vol. 175, no. 3-4, pp. 255-68.

Brzeski, V & Newkirk, G 1997, 'Integrated coastal food production systems -- a review of current literature', *Ocean & Coastal Management,* vol. 34, no. 1, pp. 55-71.

Burford, MA, Costanzo, SD, Dennison, WC, Jackson, CJ, Jones, AB, McKinnon, AD, Preston, NP & Trott, LA 2003, 'A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia', *Marine Pollution Bulletin,* vol. 46, no. 11, pp. 1456-69.

Burridge, TR & Bidwell, J 2002, 'Review of the potential use of brown algal ecotoxicological assays in monitoring effluent discharge and pollution in southern Australia', *Marine Pollution Bulletin,* vol. 45, no. 1-12, pp. 140-7.

Buschmann, AH, Lopez, DA & Medina, A 1996a, 'A review of the environmental effects and alternative production strategies of marine aquaculture in Chile', *Aquacultural Engineering,* vol. 15, no. 6, pp. 397-421.

Buschmann, AH, Mora, OA, Gomez, P, Bottger, M, Buitano, S, Retamales, C, Vergara, PA & Gutierrez, A 1994, '*Gracilaria chilensis* outdoor tank cultivation in Chile: Use of land-based salmon culture effluents', *Aquacultural Engineering,* vol. 13, no. 4, pp. 283-300.

Buschmann, AH, Troell, M, Kautsky, N & Kautsky, L 1996b, 'Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta)', *Hydrobiologia,* vol. 326-327, no. 1, pp. 75-82.

Carmona, R, Kraemer, GP & Yarish, C 2006, 'Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish-algal aquaculture system', *Aquaculture,* vol. 252, no. 1, pp. 54-65.

Chen, J-C, Liu, P-C & Lei, S-C 1990, 'Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents', *Aquaculture,* vol. 89, no. 2, pp. 127-37.

Chen, J-C, Liu, P-C & Lin, Y-T 1989, 'Culture of *Penaeus monodon* in an intensified system in Taiwan', *Aquaculture,* vol. 77, no. 4, pp. 319-28.

Cheng, K-m, Hu, C-q, Liu, Y-n, Zheng, S-x & Qi, X-j 2006, 'Effects of dietary calcium, phosphorus and calcium / phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water', *Aquaculture,* vol. 251, no. 2-4, pp. 472-83.

Chin, KK & Ong, SL 1997, 'Water conservation and pollution control for intensive prawn farms', *Water Science and Technology,* vol. 35, no. 8, pp. 77-81.

Chopin, T, Buschmann, AH, Halling, C, Troell, M, Kautsky, N, Neori, A, Kraemer, GP, Zertuche-Gonzalez, JA, Yarish, C & Neefus, C 2001, 'Integrating seaweeds into marine aquaculture systems: A key toward sustainability', *Journal of Phycology,* vol. 37, no. 6, pp. 975-86.

Chopin, T, Yarish, C, Wilkes, R, Belyea, E, Lu, S & Mathieson, A 1999, 'Developing Porphyra/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry', *Journal of Applied Phycology,* vol. 11, no. 5, pp. 463-72.

Chuntapa, B, Powtongsook, S & Menasveta, P 2001, 'Control of water quality in high density prawn culture by integrating with the microalga, *Spirulina platensis*', *27th Congress on Science & Technology of Thailand*, Technical Information Services (TIS)/KMUTT, Songkla, Thailand.

Chuntapa, B, Powtongsook, S & Menasveta, P 2003, 'Water quality control using *Spirulina platensis* in shrimp culture tanks', *Aquaculture,* vol. 220, no. 1-4, pp. 355-66.

Cohen, I & Neori, A 1991, '*Ulva lactuca* biofilters for marine fishpond effluents 1. Ammonia uptake kinetics and nitrogen content.' *Botanica Marina,* vol. 34, no. 6, pp. 475-82.

Coloso, RM, King, K, Fletcher, JW, Hendrix, MA, Subramanyam, M, Weis, P & Ferraris, RP 2003, 'Phosphorus utilization in rainbow trout (*Oncorhynchus mykiss*) fed practical diets and its consequences on effluent phosphorus levels', *Aquaculture,* vol. 220, no. 1-4, pp. 801-20.

Daniels, HV & Boyd, CE 1989, 'Chemical Budgets for Polyethylene-lined, Brackishwater Ponds', *Journal of the World Aquaculture Society,* vol. 20, no. 2, pp. 53-60.

DeBoer, JA, Guigli, HJ, Israel, TL & D'Elia, CF 1978, 'Nutritional studies two red algae. I. Growth rate as a function of nitrogen source and concentration ', *Journal of Phycology,* vol. 14, no. 3, pp. 261-6.

DeBusk, TA & Ryther, JH 1984, 'Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures', *Botanica Marina,* vol. 27, pp. 357-62.

Diaz-Pulido, G & McCook, LJ 2005, 'Effects of nutrient enhancement on the fecundity of a coral reef macroalga', *Journal of Experimental Marine Biology and Ecology,* vol. 317, no. 1, pp. 13-24.

Dore, I & Frimodt, C 1987, *An Illustrated Guide to Shrimp of the World*, Van Nostrand-Reinhold. New York.

Edwards, P, Pullin, RSV & Gartner, JA 1988, 'Research and education for the development of integrated crop-livestock-fish farming systems in the tropics', in *ICLARM Studies and Reviews*, vol. 16, p. 53.

Ellner, S, Neori, A, Krom, MD, Tsai, K & Easterling, MR 1996, 'Simulation model of recirculating mariculture with seaweed biofilter: development and experimental tests of the model', *Aquaculture,* vol. 143, no. 2, pp. 167-84.

Erler, D, Pollard, P, Duncan, P & Knibb, W 2004, 'Treatment of shrimp farm effluent with omnivorous finfish and artificial substrates', *Aquaculture Research,* vol. 35, no. 9, pp. 816-27.

FAO 2006a, *Fishery and Aquaculture Statistics 2006*, FAO Fisheries Technical Paper No.500.

FAO 2006b, *State of world aquaculture 2006*, FAO Fisheries Technical Paper No.500.

Favero, N & Frigo, MG 2002, 'Biomonitoring of Metal Availability in the Southern Basin of the Lagoon of Venice (Italy) by Means of Macroalgae', *Water, Air, & Soil Pollution,* vol. 140, no. 1, pp. 231-46.

Folke, C & Kautsky, N 1992, 'Aquaculture with its environment: Prospects for sustainability', *Ocean & Coastal Management,* vol. 17, no. 1, pp. 5-24.

Forster, JRM & Beard, TW 1974, 'Experiments to assess the suitability of nine species of prawns for intensive cultivation', *Aquaculture,* vol. 3, no. 4, pp. 355-68.

Foy, RH & Rosell, R 1991, 'Loadings of nitrogen and phosphorus from a Northern Ireland fish farm', *Aquaculture,* vol. 96, no. 1, pp. 17-30.

Funge-Smith, SJ & Briggs, MRP 1998, 'Nutrient budgets in intensive shrimp ponds: implications for sustainability', *Aquaculture,* vol. 164, no. 1-4, pp. 117-33.

Gellenbeck, KW 1984, *The brown alga Sargassum muticum: Biology and mariculture potential (alganic acid, anaerobic digestion)*, University of California.

Goldman, JC, Tenore, RK, Ryther, HJ & Corwin, N 1974, 'Inorganic nitrogen removal in a conbined tertiary treatment-marine aquaculture system. I. Removal efficiencies', *Water Research,* vol. 8, pp. 45-54.

Gordin, H, Krom, MD, Neori, A, Popper, DM, Porter, C & Shpigel, M 1990, 'Intensive integrated seawater fish ponds: fish growth and water quality', in H Rosenthal & S Sarig (eds), *Research in Modern Aquaculture*, vol. Special Publication No. 11, European Aquaculture Society, Bredene, Belgium, pp. 45-64.

Gordin, H, Motzkin, F, Hughes-Games, WL & Porter, CB 1981, 'Seawater mariculture ponds - an integrated system', in H Rosenthal & OH Oren (eds), *Intensive mariculture*, vol. Special Publication No.6, European Aquaculture Society, Bredene, Belgium, pp. 1-14.

Green, BW & Boyd, CE 1995, 'Chemical budgets for organically fertilized fish ponds in the dry tropics', *Journal of the World Aquaculture Society,* vol. 26, pp. 284-96.

Grey, DL, Dall, L & Baker, A 1983, *A Guide to the Australian Penaeid Prawns*, A.B. Caudell Government Printer of the Northern Territory.

Gribble, NA, Wassenberg, TJ & Burridge, C 2007, 'Factors affecting the distribution of commercially exploited *Penaeid* prawns (shrimp) (Decapod:Penaeidae) across the northern Great Barrier Reef, Australia', *Fisheries Research,* vol. 85, no. 1-2, pp. 174-85.

Grommen, R, Van Hauteghem, I, Van Wambeke, M & Verstraete, W 2002, 'An improved nitrifying enrichment to remove ammonium and nitrite from freshwater aquaria systems', *Aquaculture,* vol. 211, no. 1-4, pp. 115-24.

Guanzon, NG, de Castro-Mallare, TR & Lorque, FM 2004, 'Polyculture of milkfish *Chanos chanos* (Forsskal) and the red seaweed *Gracilariopsis bailinae* (Zhang et Xia) in brackish water earthen ponds', *Aquaculture Research,* vol. 35, no. 5, pp. 423-31.

Guimaraens, MAd 1999, *The influence of environmental factors on the seasonal dynamics of Ulva sp. and Sargassum sp. in the Cabo Frio upwelling region of Brazil.* , University of Miami, .

Gutierrez-Wing, MT & Malone, RF 2006, 'Biological filters in aquaculture: Trends and research directions for freshwater and marine applications', *Aquacultural Engineering,* vol. 34, no. 3, pp. 163-71.

Hafting, JT 1999, 'Effect of tissue nitrogen and phosphorus quota on growth of *Porphyra yezoensis* blades in suspension cultures', *Hydrobiologia,* vol. 398-399, no. 0, pp. 305-14.

Hagopian, DS & Riley, JG 1998, 'A closer look at the bacteriology of nitrification', *Aquacultural Engineering,* vol. 18, no. 4, pp. 223-44.

Hanisak, MD & Harlin, MM 1978, 'Uptake of inorganic nitrogen by *Codium fragile* subsp. *tomentosoides* (Cholorophyta) ', *Journal of Phycology,* vol. 14, no. 4, pp. 450-4.

Hanisak, MD & Samuel, MA 1987, 'Growth rates in culture of several species of *Sargassum* from Florida, USA', *Hydrobiologia* vol. 151/152, pp. 399-404

Hansen, GH & Koroleff, F 1999, 'Determination of nutrients', in K Grasshoff, K Kremlong & M Ehrhardt (eds), *Methods of seawater analysis, Completely Revised and Extended Edition*, Third edn, Wiley-VCH, Weinheim, pp. 159-228.

Hanson, RB 1977, 'Pelagic *Sargassum* community metabolism: Carbon and nitrogen', *Journal of Experimental Marine Biology and Ecology,* vol. 29, no. 2, pp. 107-18.

Haque, MM, Narejo, NT, Salam, MA, Rahmatullah, SM & Islam, MA 2003, 'Determination fo optimum stocking density of *Macrobrachium resenbergii* in carp polyculture in earthen pond', *Pakistan Journal of Biological Sciences,* vol. 6, no. 10, pp. 898-901.

Harlin, MM, Thorne-Miller, B & Thursby, GB 1978, 'Ammonium uptake by *Gracilaria* sp. (Florideophycae) and *Ulva lactuca* (Chlorophyceae) in closed system fish culture', *The Interantional Seaweed Symposium*, eds. ARN Jensen & JT Stein, Science Press, Princeton, USA, Santa Barbara, California, pp. 285-192.

Harrison, PJ, Druehl, LD, Lloyd, KE & Thompson, PA 1986, 'Nitrogen uptake kinetics in three year-classes of *Laminaria groenlandica* (Laminariales: Phaeophyta)', *Marine Biology,* vol. 93, no. 1, pp. 29-35.

Hernández, I, Fernández-Engo, M, Pérez-Lloréns, J & Vergara, J 2005, 'Integrated outdoor culture of two estuarine macroalgae as biofilters for dissolved nutrients from *Sparus auratus* waste waters', *Journal of Applied Phycology,* vol. 17, no. 6, pp. 557-67.

Hernández, I, Martínez-Aragón, JF, Tovar, A, Pérez-Lloréns, JL & Vergara, JJ 2002, 'Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium', *Journal of Applied Phycology,* vol. 14, no. 5, pp. 375-84.

Hernández, I, Peralta, G, Pérez-Lloréns, JL, Vergara, JJ & Niell, FX 1997, 'Biomass and dynamic of growth of *Ulva* species in Palmones River estuary', *Journal of Phycology,* vol. 33, no. 5, pp. 764-72.

Hopkins, JS, Sandifer, PA & Browdy, CL 1995, 'A review of water management regimes which abate the environmental impact of shrimp farming', in CL Browdy & JS Hopkins (eds), *Swimming through Troubled Water*, World Aquaculture Society, Baton Rouge, LA, pp. 13-22.

Hurtado-Ponce, AQ, Samonte, GP, Luhan, MR & Guanzon, N 1992, '*Gracilaria* (Rhodophyta) farming in Panay, Western Visayas, Philippines', *Aquaculture,* vol. 105, no. 3-4, pp. 233-40.

Hwang, S-C, Lin, C-S, Chen, IM, Chen, J-M, Liu, L-Y & Dodds, WK 2004, 'Removal of multiple nitrogenous wastes by *Aspergillus niger* in a continuous fixed-slab reactor', *Bioresource Technology,* vol. 93, no. 2, pp. 131-8.

Iwama, G 1991, 'Interactions between Aquaculture and the Environment ', *CRC Critical Reviews in Environmental Control CCECAU,* vol. 21, no. 2, pp. 177-216.

Jackson, C, Preston, N, Burford, M & Thompson, PJ 2003a, 'Managing the development of sustainable shrimp farming in Australia: the role of sedimentation ponds in treatment of farm discharge water', *Aquaculture,* vol. 226, no. 1-4, pp. 23-34.

Jackson, C, Preston, N, Thompson, PJ & Burford, M 2003b, 'Nitrogen budget and effluent nitrogen components at an intensive shrimp farm', *Aquaculture,* vol. 218, no. 1-4, pp. 397-411.

Jiménez del Río, M, Ramazanov, Z & García-Reina, G 1994, 'Optimization of yield and biofiltering efficiencies of *Ulva rigida* C. Ag. cultivated with *Sparus aurata* L. waste waters', *Scientia Marina,* vol. 58, no. 4, pp. 329-35.

Jones, AB 1999, *Environmental Management of Aquaculture Effluent: Development of Biological Indicators and Biological Filters*, The University of Queensland.

Jones, AB, Dennison, WC & Preston, NP 2001a, 'Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study', *Aquaculture,* vol. 193, no. 1-2, pp. 155-78.

Jones, AB, O'Donohue, MJ, Udy, J & Dennison, WC 2001b, 'Assessing ecological impacts of shrimp and sewage effluent: Biological indicators with standard water quality analyses', *Estuarine, Coastal and Shelf Science,* vol. 52, no. 1, pp. 91-109.

Jones, AB, Preston, NP & Dennison, WC 2002, 'The efficiency and condition of oysters and macroalgae used as biological filters of shrimp pond effluent', *Aquaculture Research,* vol. 33, no. 1, pp. 1-19.

Kang, Y, Shin, J, Kim, M & Chung, I 2008, 'A preliminary study of the bioremediation potential of *Codium fragile* applied to seaweed integrated multi-trophic aquaculture (IMTA) during the summer', *Journal of Applied Phycology,* vol. 20, no. 2, pp. 183-90.

Karmer, K & Fong, P 2000, 'A fluctuating salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis* (L.) link', *Journal of Experimental Marine Biology and Ecology,* vol. 254, pp. 53-69.

Karmer, K & Fong, P 2001, 'Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enttermorpha intestinalis*', *Marine Ecology Progress Series,* vol. 218, pp. 87-93.

Kathirvel, M & Selvaraj, V 1987, 'On an experimental seed collection and field culture of king prawn, *Penaues latisulcatus*', *Indian Journal of Fisheries,* vol. 34, no. 4, pp. 365-73.

Kevekordes, K 2001, 'Toxicity tests using developmental stages of *Hormosira banksii* (Phaeophyta) identify ammonium as a damaging component of secondary treated sewage effluent discharged into Bass Strait, Victoria, Australia', *Marine Ecology Progress Series,* vol. 219, pp. 139-48.

Kinne, PN, Samocha, TM, Jones, ER & Browdy, CL 2001, 'Characterization of intensive shrimp pond effluent and preliminary studies on biofiltration', *North American Journal of Aquaculture,* vol. 63, no. 1, pp. 25-33.

Kraemer, GP, Carmona, R, Chopin, T, Neefus, C, Tang, X & Yarish, C 2004, 'Evaluation of the bioremediatory potential of several species of the red alga *Porphyra* using short-term measurements of nitrogen uptake as a rapid bioassay', *Journal of Applied Phycology,* vol. 16, no. 6, pp. 489-97.

Krom, MD, Ellner, S, van Rijn, J & Neori, A 1995, 'Nitrogen and phosphorus cycling and transformations in a prototype 'non-polluting' integrated mariculture system, Eilat, Israel', *Marine ecology progress series,* vol. Vol. 118, pp. 25-36.

Krom, MD & Neori, A 1989, 'A total nutrient budget for an experimental intensive fishpond with circularly moving seawater', *Aquaculture,* vol. 83, no. 3-4, pp. 345-58.

Krom, MD, Porter, C & Gordin, H 1985a, 'Description of the water quality conditions in a semi-intensively cultured marine fish pond in Eilat, Israel', *Aquaculture,* vol. 49, no. 2, pp. 141-57.

Krom, MD, Porter, C & Gordin, H 1985b, 'Nutrient budget of a marine fish pond in Eilat, Israel', *Aquaculture,* vol. 51, no. 1, pp. 65-80.

Lapointe, BE & Tenore, KR 1981, 'Experimental outdoor studies with *Ulva fusciutu* Dclilc. 1. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition', *J. Exp. Mar. Biol. Ecol. ,* vol. 53, pp. 135-52.

Larned, ST 1998, 'Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae', *Marine Biology,* vol. 132, no. 3, pp. 409-21.

Lartigue, J & Sherman, TD 2005, 'Response of *Enteromorpha* sp. (Chlorophyceae) to a nitrate pulse: Nitrate uptake, inorganic nitrogen storage, and nitrate reductase activity', *Marine Ecology Progress Series,* vol. 292, pp. 147-57.

Lartigue, J & Sherman, TD 2006, 'A field study of nitrogen storage and nitrate reductase activity in the estuarine macroalgae *Enteromorpha lingulata* (Chlorophyceae) and *Gelidium pusillum* (Rhodophyceae)', *Estuaries and Coasts,* vol. 29, no. 4, pp. 699-708.

Lee, RE 1980, *Phycology*, Cambridge University Press, New York.

Lemonnier, H & Faninoz, S 2006, 'Effect of water exchange on effluent and sediment characteristics and on partial nitrogen budget in semi-intensive shrimp ponds in New Caledonia', *Aquaculture Research,* vol. 37, no. 9, pp. 938-48.

Lignell, Å & Pedersén, M 1987, 'Nitrogen metabolism in *Gracilaria secundata* Harv', *Hydrobiologia,* vol. 151-152, no. 1, pp. 431-41.

Lin, CK, Ruamthaveesub, P & Wanuchsoontorn, P 1993, 'Integrated culture of the green mussel (*Perna viridis*) in wastewater from an intensive shrimp pond: concept and practice', *World Aquaculture,* vol. 24, pp. 68-73.

Lin, CK & Yi, Y 2003, 'Minimizing environmental impacts of freshwater aquaculture and reuse of pond effluents and mud', *Aquaculture,* vol. 226, no. 1-4, pp. 57-68.

Liu, D, Amy, P & Sun, J 2004, 'Preliminary study on the responses of three marine algae, *Ulva pertusa* (Chlorophyta), *Gelidium amansii* (Rhodophyta) and *Sargassum enerve* (Phaeophyta), to nitrogen source and its availability', *Journal of Ocean University of China (English Edition),* vol. 3, no. 1, pp. 75-9.

Liu, F & Han, W 2004, 'Reuse strategy of wastewater in prawn nursery by microbial remediation', *Aquaculture,* vol. 230, no. 1-4, pp. 281-96.

Liu, S, Jie, Z & Zeng, S 1997, 'The commercial cultivation of *Gracilaria* and its polyculture with prawn in China.' *J.Zhanjiang Ocean University. Zhanjiang Haiyang Daxue Xuebao,* vol. 17, no. 2, pp. 27-30.

Lobban, CS, Harrison, PJ & Duncan, MJ 1985, *The Physiological Ecology of Seaweeds*, Cambridge University Press, Cambridge.

Lombardi, JV, de Almeida Marques, HL, Pereira, RTL, Barreto, OJS & de Paula, EJ 2006, 'Cage polyculture of the Pacific white shrimp *Litopenaeus vannamei* and the Philippines seaweed *Kappaphycus alvarezii*', *Aquaculture,* vol. 258, no. 1-4, pp. 412-5.

Lowther, A 2006, 'Highlight from the FAO database on Aquaculture Statistics', in *FAO Aquaculture Newsletter*, vol. 35, pp. 32-3.

Lu, K, Lin, W & Liu, J 2008, 'The characteristics of nutrient removal and inhibitory effect of Ulva clathrata on Vibrio anguillarum 65', *Journal of Applied Phycology,* vol. 20, no. 6, pp. 1061-8.

Lyngby, JE, Mortensen, S & Ahrensberg, N 1999, 'Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecosystems', *Marine Pollution Bulletin,* vol. 39, no. 1-12, pp. 212-23.

Lyssenko, C & Wheaton, F 2006, 'Impact of rapid impulse operating disturbances on ammonia removal by trickling and submerged-upflow biofilters for intensive recirculating aquaculture', *Aquacultural Engineering,* vol. 35, no. 1, pp. 38-50.

Marinho-Soriano, E 2007, 'Seaweed biofilters: An environmentally friendly solution', *World Aquaculture,* vol. 38, no. 3, pp. 31-3.

Marinho-Soriano, E, Morales, C & Moreira, WSC 2002, 'Cultivation of *Gracilaria* (Rhodophyta) in shrimp pond effluents in Brazil', *Aquaculture Research,* vol. 33, no. 13, pp. 1081-6.

Martin, J-LM, Veran, Y, Guelorget, O & Pham, D 1998, 'Shrimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds', *Aquaculture,* vol. 164, no. 1-4, pp. 135-49.

Martínez-Aragón, JF, Hernández, I, Pérez-Lloréns, JL, Vázquez, R & Vergara, JJ 2002a, 'Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 1. Ammonium', *Journal of Applied Phycology,* vol. 14, no. 5, pp. 375-84.

Martínez-Aragón, JF, Hernández, I, Pérez-Lloréns, JL, Vázquez, R & Vergara, JJ 2002b, 'Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus*

labrax) waste waters 1. Phosphate', *Journal of Applied Phycology,* vol. 14, no. 5, pp. 365-74.

Matos, J, Costa, S, Rodrigues, A, Pereira, R & Sousa Pinto, I 2006, 'Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal', *Aquaculture,* vol. 252, no. 1, pp. 31-42.

Menasveta, P, Panritdam, T, Sihanonth, P, Powtongsook, S, Chuntapa, B & Lee, P 2001, 'Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock', *Aquacultural Engineering,* vol. 25, no. 1, pp. 35-49.

Mendiguchia, C, Moreno, C, Manuel-Vez, MP & Garcia-Vargas, M 2006, 'Preliminary investigation on the enrichment of heavy metals in marine sediments originated from intensive aquaculture effluents', *Aquaculture,* vol. 254, no. 1-4, pp. 317-25.

Menéndez, M, Herrera, J & Comín, FA 2002, 'Effect of nitrogen and phosphorus supply on growth, chlorophyll content and tissu composition of the macroalga *Chaetomorpha linum* (O.F. Miill.) Kütz in a Mediterranean coastal lagoon', *Scientia Marina,* vol. 66, no. 4, pp. 355-64.

Msuya, F, Kyewalyanga, MS & Salum, D 2006, 'The performance of the seaweed *Ulva reticulata* as a biofilter in a low-tech, low-cost, gravity generated water flow regime in Zanzibar, Tanzania', *Aquaculture,* vol. 254, no. 1-4, pp. 284-92.

Msuya, F & Neori, A, 'Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks', *Journal of Applied Phycology*.

Msuya, F & Neori, A 2002, '*Ulva reticulata* and *Gracilaria crassa*: macroalgae that can biofilter effluent from tidal fishponds in Tanzania', *Western Indian Ocean Journal of Marine Science,* vol. 1, pp. 117-216.

Msuya, F & Neori, A 2008, 'Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks', *Journal of Applied Phycology,* vol. 20, no. 6, pp. 1021-31.

Muangkeow, B, Ikejima, K, Powtongsook, S & Yi, Y 2007, 'Effects of white shrimp, *Litopenaeus vannamei* (Boone), and Nile tilapia, *Oreochromis niloticus* L., stocking density on growth, nutrient conversion rate and economic return in integrated closed recirculation system', *Aquaculture,* vol. 269, no. 1-4, pp. 363-76.

Munsiri, P, Boyd, CE & Hajek, BF 1995, 'Physical and chemical characteristics of bottom soil profiles in ponds at Auburn, Alabama, and a proposed system for descrinign pond doil horizons', *Journal of the World Aquaculture Society,* vol. 26, pp. 346-77.

Mwandya, AW, Mtolera, MSP, Pratap, HB & Jiddawi, NS 2001, 'Macroalgae as biofilters of dissolved inorganic nutrients in an integrated mariculture tank system in Zanzibar', *Marine Science Development in Tanzania and Eastern Africa. Proc. 20th Anniversary Conference on Advances in Marine Science in Tanzania*, eds. M Richmond & J Francis, Tanzania, pp. 159-70.

Naldi, M & Wheeler, PA 2002, '^{15}N measurement of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparision of net nutrient disappearance, release of ammonium and nitrate, and ^{15}N accumulation in algal tissue', *Journal of Phycology,* vol. 38, no. 1, pp. 135-44.

Negroni, G 2000, 'Management optimization and sustainable technologies for the treatment and disposal/reuse of fish farm effluent with emphasis on constructed wetlands', *World Aquaculture Society,* vol. 31, no. 3, pp. 16-9.

Nelson, SG, Glenn, EP, Conn, J, Moore, D, Walsh, T & Akutagawa, M 2001, 'Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two-phase polyculture system', *Aquaculture,* vol. 193, no. 3-4, pp. 239-48.

Neori, A 2007, 'Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: an analysis', *Journal of Applied Phycology*.

Neori, A, Chopin, T, Troell, M, Buschmann, AH, Kraemer, GP, Halling, C, Shpigel, M & Yarish, C 2004, 'Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture', *Aquaculture,* vol. 231, no. 1-4, pp. 361-91.

Neori, A, Cohen, I & Gordin, H 1991, '*Ulva lactura* biofilters for marine fish pond effluents. 2. Growth-rate, yield and C:N ratio', *Botan.Marina,* vol. 34, no. 6, pp. 483-9.

Neori, A, Krom, MD, Ellner, SP, Boyd, CE, Popper, D, Rabinovitch, R, Davison, PJ, Dvir, O, Zuber, D, Ucko, M, Angel, D & Gordin, H 1996, 'Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units', *Aquaculture,* vol. 141, no. 3-4, pp. 183-99.

Neori, A, Msuya, FE, Shauli, L, Schuenhoff, A, Kopel, F & Shpigel, M 2003, 'A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture', *Journal of Applied Phycology,* vol. 15, no. 6, pp. 543-53.

Neori, A, Norman, LCR & Shpigel, M 1998, 'The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system', *Aquacultural Engineering,* vol. 17, no. 4, pp. 215-39.

Neori, A, Shpigel, M & Ben-Ezra, D 2000, 'A sustainable integrated system for culture of fish, seaweed and abalone', *Aquaculture,* vol. 186, no. 3-4, pp. 279-91.

Neori, A, Troell, M, Chopin, T, Critchley, A & Buschmann, A 2007, 'The need for a balanced ecosystem approach to Blue Revolution aquaculture', *Environment,* vol. 49, no. 3, pp. 36-43.

NERDI 1994, *Studies on the development of polyculture system in the coastal waters of Korea*, Ministry of Maritime Affairs & Fisheries.

New, MB & Wijkstrom, UN 1990, 'Feed for thought: some observations on aquaculture feed production in Asia', *World Aquaculture,* vol. 21, no. 1, pp. 17-9, 22-3.

Nga, BT, Lürling, M, Peeters, ETHM, Roijackers, R, Scheffer, M & Nghia, TT 2005, 'Chemical and physical effects of crowding on growth and survival of *Penaeus monodon* Fabricius post-larvae', *Aquaculture,* vol. 246, no. 1-4, pp. 455-65.

Ogawa, H 1984, 'Effects of treated municipal wastewater on the early development of sargassaceous plants', *Hydrobiologia,* vol. 116-117, no. 1, pp. 389-92.

Páez-Osuna, F, Gracia, A, Flores-Verdugo, F, Lyle-Fritch, LP, Alonso-Rodríguez, R, Roque, A & Ruiz-Fernández, AC 2003, 'Shrimp aquaculture development and the environment in the Gulf of California ecoregion', *Marine Pollution Bulletin,* vol. 46, no. 7, pp. 806-15.

Páez-Osuna, F, Guerrero-Galván, SR & Ruiz-Fernández, AC 1999, 'Discharge of nutrients from shrimp farming to coastal waters of the Gulf of California', *Marine Pollution Bulletin,* vol. 38, no. 7, pp. 585-92.

Pagand, P, Blancheton, JP, Lemoalle, J & Casellas, C 2000, 'The use of high rate algal ponds for the treatment of marine effluent from a recirculating fish rearing system', *Aquaculture Research,* vol. 31, no. 10, pp. 729-36.

Palacios, GL & Timmons, MB 2001, 'Determining design parameters for recovery of aquaculture wastewater using sand beds', *Aquacultural Engineering,* vol. 24, no. 4, pp. 289-99.

Pan, Q, Chen, X-Y, Li, F, Bi, Y-Z & Zheng, S-X 2005, 'Response of juvenile *Litopenaeus vannamei* to varying levels of calcium phosphate monobasic supplemented to a practical diet', *Aquaculture,* vol. 248, no. 1-4, pp. 97-102.

Parker, HS 1982, 'Effects of simulated current on the growth rate and nitrogen metabolism of *Gracilaria tikvahiae* (Rhodophyta)', *Marine Biology,* vol. 69, no. 2, pp. 137-45.

Peckol, P, DeMeo-Anderson, B, Rivers, J, Valiela, I, Maldonado, M & Yates, J 1994, 'Growth, nutrient uptake capacities and tissue constituents of the

macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loading rates', *Marine Biology,* vol. 121, no. 1, pp. 175-85.

Peckol, P & Rivers, JS 1995, 'Physiological responses of the opportunistic macroalgae *Cladophora vagabunda* (L.) van den Hoek and *Gracilaria tikvahiae* (McLachlan) to environmental disturbances associated with eutrophication', *Journal of Experimental Marine Biology and Ecology,* vol. 190, no. 1, pp. 1-16.

Phang, S-M, Shaharuddin, S, Noraishah, H & Sasekumar, A 1996, 'Studies on *Gracilaria changii* (Gracilariales, Rhodophyta) from Malaysian mangroves', *Hydrobiologia,* vol. 326-327, no. 1, pp. 347-52.

Phillips, MJ, Kwei Lin, C & Beveridge, MCM 1993, 'Shrimp culture and the environment: lessons from the world's most rapidly expanding warmwater aquaculture sector', *Environment and Aquaculture in Developing Countries*, eds. HR Pullin & JL Maclean, International Center for Living Aquatic Resources Management - ICLARM, pp. 171-97.

Phlips, EJ, Willis, M & Verchick, A 1986, 'Aspects of nitrogen fixation in *Sargassum* communities off the coast of Florida', *Journal of Experimental Marine Biology and Ecology,* vol. 102, no. 2-3, pp. 99-119.

Piedrahita, RH 2003, 'Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation', *Aquaculture,* vol. 226, no. 1-4, pp. 35-44.

Porrello, S, Lenzi, M, Tomassetti, P, Persia, E, Finoia, MG & Mercatali, I 2003, 'Reduction of aquaculture wastewater eutrophication by phytotreatment ponds system: II. Nitrogen and phosphorus content in macroalgae and sediment', *Aquaculture,* vol. 219, no. 1-4, pp. 531-44.

Porter, CB, Krom, MD, Robbins, MG, Brickell, L & Davidson, A 1987, 'Ammonia excretion and total N budget for gilthead seabream (*Sparus aurata*) and its effect on water quality conditions', *Aquaculture,* vol. 66, no. 3-4, pp. 287-97.

Prangnell, DI 2007, *Physiological responses of western king prawns, Penaeus latisulcatus, in inland saline water with different potassium concentrations*, Curtin University of Technology.

Prangnell, DI & Fotedar, R 2006a, 'Effect of sudden salinity change on *Penaeus latisulcatus* Kishinouye osmoregulation, ionoregulation and condition in inland saline water and potassium-fortified inland saline water', *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* vol. 145, no. 4, pp. 449-57.

Prangnell, DI & Fotedar, R 2006b, 'The growth and survival of western king prawns, *Penaeus latisulcatus* Kishinouye, in potassium-fortified inland saline water', *Aquaculture,* vol. 259, no. 1-4, pp. 234-42.

Primavera, JH 1985, 'A review of maturation and reproduction in closed thelycum penaeids', *The first international conference on the culture of penaeid prawns/shrimps*, SEAFDEC Aquaculture Department, Iloilo City, Philippines.

Primavera, JH 1993, 'A critical review of shrimp pond culture in the Philippines', *Reviews in Fisheries Science,* vol. 1, pp. 151-210.

Primavera, JH 1997, 'Socio-economic impacts of shrimp culture', *Aquaculture Research,* vol. 28, no. 10, pp. 815-27.

Qian, P-Y, Wu, CY, Wu, M & Xie, YK 1996, 'Integrated cultivation of the red alga *Kappaphycus alvarezii* and the pearl oyster *Pinctada martensi*', *Aquaculture,* vol. 147, no. 1-2, pp. 21-35.

Raveh, A & Avnimelech, Y 1979, 'Total nitrogen analysis in water, soil and plant material with persulphate oxidation', *Water Research,* vol. 13, pp. 911-2.

Ray-Lien Hwang, Tsai, C-C & Lee, T-M 2004, 'Assessment of temperature and nutrient limitation on seasonal dynalics among species of *Sargassum* from a coral reef in southern Taiwan', *Journal of Phycology,* vol. 40, no. 3, pp. 463-73.

Ray, W-M & Chien, Y-H 1992, 'Effects of stocking density and aged sediment on tiger prawn, *Penaeus monodon*, nursery system', *Aquaculture,* vol. 104, no. 3-4, pp. 231-48.

Rees, T, Dobson, B, Bijl, M & Morelissen, B 2007, 'Kinetics of nitrate uptake by New Zealand marine macroalgae and evidence for two nitrate transporters in *Ulva intestinalis* L', *Hydrobiologia,* vol. 586, no. 1, pp. 135-41.

Rodrigueza, MRC & Montaño, MNE 2007, 'Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms', *Journal of Applied Phycology,* vol. 19, no. 6, pp. 755-62.

Rosenberg, G & Ramus, J 1984, 'Uptake of inorganic nitrogen and seaweed surface area: Volume ratios', *Aquatic Botany,* vol. 19, no. 1-2, pp. 65-72.

Ruddle, K 1991, 'Integrated farming systems and future directions for Asian farming systems research and extension', *Asian Farming System Association,* vol. 1, pp. 91-9.

Ryther, JH, Corwin, N, DeBusk, TA & Williams, LD 1981, 'Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979)', *Aquaculture,* vol. 26, no. 1-2, pp. 107-15.

Ryther, JH, Goldman, JC, Gifford, CE, Huguenin, JE, Wing, AS, Clarner, JP, Williams, LD & Lapointe, BE 1975, 'Physical models of integrated waste recycling- marine polyculture systems', *Aquaculture,* vol. 5, no. 2, pp. 163-77.

Sandifer, PA & Smith, IIJ 1975, 'Effects of population density on growth and survival of *Macrobrachium rosenbergii* reared in recirculating water management systems', *6th Annual Workshop World Mariculture Society*, Seattle, WA, pp. 43-53.

Sang, MH 2003, *Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (Penaeus latisulcatus Kishinouye, 1896) reared at different salinities*, Curtin University of Technology.

Sang, MH & Fotedar, R 2004, 'Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities', *Aquaculture,* vol. 234, no. 1-4, pp. 601-14.

Santelices, B & Doty, MS 1989, 'A review of *Gracilaria* farming', *Aquaculture,* vol. 78, no. 2, pp. 95-133.

Schaffelke, B & Klimpp, DW 1998, 'Nutrient-limited growth of the coral reef macroalga *Sargassum baccularia* and experimental growth enhancement by nutrient addition in continuous flow culture', *Marine Ecology Progress Series,* vol. 164, no. 1, pp. 199-211.

Schneider, O, Sereti, V, Eding, EH & Verreth, JAJ 2005, 'Analysis of nutrient flows in integrated intensive aquaculture systems', *Aquacultural Engineering,* vol. 32, no. 3-4, pp. 379-401.

Schuenhoff, A, Mata, L & Santos, R 2006, 'The tetrasporophyte of *Asparagopsis armata* as a novel seaweed biofilter', *Aquaculture,* vol. 252, no. 1, pp. 3-11.

Schuenhoff, A, Shpigel, M, Lupatsch, I, Ashkenazi, A, Msuya, FE & Neori, A 2003, 'A semi-recirculating, integrated system for the culture of fish and seaweed', *Aquaculture,* vol. 221, no. 1-4, pp. 167-81.

Seawright, DE, Stickney, RR & Walker, RB 1998, 'Nutrient dynamics in integrated aquaculture-hydroponics systems', *Aquaculture,* vol. 160, no. 3-4, pp. 215-37.

Seymour, EA & Bergheim, A 1991, 'Towards a reduction of pollution from intensive aquaculture with reference to the farming of salmonids in Norway', *Aquacultural Engineering,* vol. 10, no. 2, pp. 73-88.

Shahidul Islam, M, Jahangir Sarker, M, Yamamoto, T, Abdul Wahab, M & Tanaka, M 2004, 'Water and sediment quality, partial mass budget and effluent N loading in coastal brackishwater shrimp farms in Bangladesh', *Marine Pollution Bulletin,* vol. 48, no. 5-6, pp. 471-85.

Shepherd, CJ & Bromage, NR (eds.) 1988, *Intensive Fish Farming* BSP Professional Books. A Division of Blackwell Scientific Publication Ltd.

Shimoda, T, Fujioka, Y, Srithong, C & Aryuthaka, C 2005, 'Phosphorus budget in shrimp aquaculture pond with mangrove enclosure and aquaculture performance', *Fisheries Science,* vol. 71, no. 6, pp. 1249-55.

Shnel, N, Barak, Y, Ezer, T, Dafni, Z & van Rijn, J 2002, 'Design and performance of a zero-discharge tilapia recirculating system', *Aquacultural Engineering,* vol. 26, no. 3, pp. 191-203.

Shpigel, M, Gasith, A & Kimmel, E 1997, 'A biomechanical filter for treating fish-pond effluents', *Aquaculture,* vol. 152, no. 1-4, pp. 103-17.

Shpigel, M, Lee, J, Soohoo, B, Fridman, R & Gordin, H 1993a, 'The use of effluent water from fish ponds as a food source for the pacific oyster *Crassostrea gigas* Tunberg', *Aquaculture and Fisheries Management,* vol. 24, pp. 529-43.

Shpigel, M, Neori, A & Gordin, H 1991, 'Oyster and clam production in the outflow water of marine fish aquaculture ponds in Israel', *European Aquaculture Society,* vol. Special Publication 14, p. 295.

Shpigel, M, Neori, A, Popper, DM & Gordin, H 1993b, 'A proposed model for "environmentally clean" land-based culture of fish, bivalves and seaweeds', *Aquaculture,* vol. 117, no. 1-2, pp. 115-28.

Siddiqui, SS 2003, 'Wastewater treatment technology in aquaculture', *World Aquaculture Society,* vol. 34, no. 3, pp. 49-52.

Silkin, V & Chubchikova, I 2007, 'Kinetics of uptake of phosphates and nitrates by marine multicellular algae *Gelidium latifolium* (Grev.) Born. et Thur', *Biology Bulletin,* vol. 34, no. 2, pp. 156-62.

Sreesaia, S & Pakpainb, P 2007, 'Nutrient Recycling by *Chlorella vulgaris* from Septage Effluent of the Bangkok City, Thailand', *ScienceAsia* vol. 33, pp. 293-9.

Subandar, A, Petrell, R & Harrison, P 1993, '*Laminaria* culture for reduction of dissolved inorganic nitrogen in salmon farm effluent', *Journal of Applied Phycology,* vol. 5, no. 4, pp. 455-63.

Subasinghe, RP 2006, 'Is aquaculture at its turning point?' in *FAO Aquaculture Newsletter*, vol. 35, pp. 7-9.

Summerfelt, ST, Adler, PR, Glenn, DM & Kretschmann, RN 1999, 'Aquaculture sludge removal and stabilization within created wetlands', *Aquacultural Engineering,* vol. 19, no. 2, pp. 81-92.

Tacon, AGJ & Forster, IP 2003, 'Aquafeeds and the environment: policy implications', *Aquaculture,* vol. 226, no. 1-4, pp. 181-9.

Tanner, JE & Deakin, S 2001, 'Active habitat selection for sand by juvenile western king prawns, *Melicertus latisulcatus* (Kishinouye)', *Journal of Experimental Marine Biology and Ecology,* vol. 261, no. 2, pp. 199-209.

Teichert-Coddington, DR, Martinez, D & Ramirez, E 2000, 'Partial nutrient budgets for semi-intensive shrimp farms in Honduras', *Aquaculture,* vol. 190, no. 1-2, pp. 139-54.

Teichert-Coddington, DR, Rouse, DB, Potts, A & Boyd, CE 1999, 'Treatment of harvest discharge from intensive shrimp ponds by settling', *Aquacultural Engineering,* vol. 19, no. 3, pp. 147-61.

Thakur, DP & Lin, CK 2003, 'Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems', *Aquacultural Engineering,* vol. 27, no. 3, pp. 159-76.

Tian, X, Li, D, Dong, S, Yan, X, Qi, Z, Liu, G & Lu, J 2001, 'An experimental study on closed-polyculture of penaeid shrimp with tilapia and constricted tagelus', *Aquaculture,* vol. 202, no. 1-2, pp. 57-71.

Tidwell, JH & Allan, GL 2001, *Fish as food: aquaculture's contribution. Ecological and economic impacts and contributions of fish farming and capture fisheries*, European Molecular Biology Organization.

Tilley, DR, Badrinarayanan, H, Rosati, R & Son, J 2002, 'Constructed wetlands as recirculation filters in large-scale shrimp aquaculture', *Aquacultural Engineering,* vol. 26, no. 2, pp. 81-109.

Timmons, MB, Ebeling, JM, Summerfelt, ST & Vinci, BJ 2002, *Recirculating aquaculture systems,* 2 edn, Northeasten Regional Aquaculture Center.

Torres, AI, Gil, MN & Esteves, JL 2004, 'Nutrient uptake rates by the alien alga *Undaria pinnatifida* (Phaeophyta) (Nuevo Gulf, Patagonia, Argentina) when exposed to diluted sewage effluent', *Hydrobiologia,* vol. 520, no. 1, pp. 1-6.

Tovar, A, Moreno, C, Manuel-Vez, MP & Garcia-Vargas, M 2000a, 'Environmental impacts of intensive aquaculture in marine waters', *Water Research,* vol. 34, no. 1, pp. 334-42.

Tovar, A, Moreno, C, Manuel-Vez, MP & Garcia-Vargas, M 2000b, 'Environmental Implications of Intensive Marine Aquaculture in Earthen Ponds', *Marine Pollution Bulletin,* vol. 40, no. 11, pp. 981-8.

Troell, M, Halling, C, Neori, A, Chopin, T, Buschmann, AH, Kautsky, N & Yarish, C 2003, 'Integrated mariculture: asking the right questions', *Aquaculture,* vol. 226, no. 1-4, pp. 69-90.

Troell, M, Halling, C, Nilsson, A, Buschmann, AH, Kautsky, N & Kautsky, L 1997, 'Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales,

Rhodophyta) and salmon cages for reduced environmental impact and increased economic output', *Aquaculture,* vol. 156, no. 1-2, pp. 45-61.

Troell, M, Neori, A, Chopin, T & Buschmann, A 2005, 'Biological wastewater treatment in aquaculture-More than just bacteria', *World Aquaculture Society,* vol. 36, no. 1, pp. 27-9.

Troell, M & Norberg, J 1998, 'Modelling output and retention of suspended solids in an integrated salmon- mussel culture', *Ecological Modelling,* vol. 110, pp. 65-77.

Troell, M, Ronnback, P, Halling, C, Kautsky, N & Buschmann, A 1999, 'Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture', *Journal of Applied Phycology,* vol. 11, pp. 89-97.

Tseng, CK, Yoshida, T & Chiang, YM 1985, 'East Asiatic species of *Sargassum subgenus* Bactrophycus J.Agardh (Sargassaceae, Fucales), with keys to the sections and species', in IA Abbott & JN Norris (eds), *Taxonomy of Economic Seaweeds with reference to some Pacific and Caribbean species*, vol. 1, California Sea Grant Program, pp. 1-14.

Tseng, K, Su, H & Su, M 1998, 'Culture of *Penaeus monodon* in a recirculating system', *Aquacultural Engineering,* vol. 17, no. 2, pp. 138-47.

Ugarte, R & Santelices, B 1992, 'Experimental tank cultivation of *Gracilaria chilensis* in central Chile', *Aquaculture,* vol. 101, no. 1-2, pp. 7-16.

van Rijn, J 1996, 'The potential for integrated biological treatment systems in recirculating fish culture--A review', *Aquaculture,* vol. 139, no. 3-4, pp. 181-201.

van Rijn, J, Fonarev, N & Berkowitz, B 1995, 'Anaerobic treatment of intensive fish culture effluents: digestion of fish feed and release of volatile fatty acids', *Aquaculture,* vol. 133, no. 1, pp. 9-20.

Vandermeulen, H & Gordin, H 1990, 'Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: mass culture and treatment of effluent', *Journal of Applied Phycology,* vol. 2, no. 4, pp. 363-74.

Viera, MP, Gomez Pinchetti, JL, Courtois de Vicose, G, Bilbao, A, Suarez, S, Haroun, RJ & Izquierdo, MS 2005, 'Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve', *Aquaculture,* vol. 248, no. 1-4, pp. 75-82.

Waite, T & Mitchell, R 1972, 'The effect of nutrient fertilization on the benthic alga *Ulva lactuca* ', *Botanica Marina* vol. 15, no. 3, pp. 151-6.

Wang, J-K 1990, 'Managing shrimp pond water to reduce discharge problems', *Aquacultural Engineering,* vol. 9, no. 1, pp. 61-73.

Wang, W-N, Wang, A-L, Chen, L, Liu, Y & Sun, R-Y 2002, 'Effects of pH on survival, phosphorus concentration, adenylate energy charge and Na+-K+ ATPase activities of *Penaeus chinensis* Osbeck juveniles', *Aquatic Toxicology,* vol. 60, no. 1-2, pp. 75-83.

Westermeier, R, Gómez, I & Rivera, P 1993, 'Suspended farming of *Gracilaria chilensis* (Rhodophyta, Gigartinales) at Cariquilda River, Maullín, Chile', *Aquaculture,* vol. 113, no. 3, pp. 215-29.

Wickins, JF 1976, 'The tolerance of warm-water prawns to recirculated water', *Aquaculture,* vol. 9, pp. 19-37.

Wu, R 1995, 'The environmental impact of marine fish culture: toward a sustainable future', *Mar Pollut Bull,* vol. 31, pp. 159-66.

Zhou, Y, Yang, H, Hu, H, Liu, Y, Mao, Y, Zhou, H, Xu, X & Zhang, F 2006, 'Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China', *Aquaculture,* vol. 252, no. 2-4, pp. 264-76.

Ziemann, DA, Walsh, WA, Saphore, EG & Fulton-Bennet, K 1992, 'A survey of water quality characteristics of effluent from Hawaiian aquaculture facilities', *Journal of the World Aquaculture Society,* vol. 23, pp. 180-91.

APPENDICES

Appendix 1

Analytical methods

1. Preparation of molybdovanadate reagent for analysis of phosphorus in tissue samples: 60 g $(NH_4)_6Mo_7)O_{24}\times 4H_2O$ (ammonium molybdate) was dissolved in 900 ml hot deionised water, cool, and dilute to 1000 ml. 1.5 g ammonium metavanadate was dissolved in 690 ml hot deionised water. 300 ml HNO_3 was then added into ammonium metavanadate solution, cool, and dilute to 1000 ml. Gradually add molybdate solution to vanadate solution with stirring.

2. Preparation of the standard curve for analysis of phosphorus in tissue samples: 0.2397 g of pure and dried KH_2PO_4 was dissolved to obtain stock solution of 0.5 mg P_2O_5 ml^{-1}. The stock solution was then diluted to obtain of 0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 and 0.35 mg P_2O_5 10 ml^{-1}. 5ml of molybdovanadate reagent was then added to each standard solution. Using these solutions of standard curve was then constructed at a wave length of 400nm.

3. Indophenol blue method:

An intensely blue compound, indophenol, is formed by reaction of ammonia, hydrochlorite, and phenal catalyzed by sodium nitroprusside. 25 ml filtered water sample was added with thorough mixing after each addition as follow: 1 ml phenol solution[1], 1 ml sodium nitroprusside solution[2], and 2.5 ml oxidizing solution[3]. Let samples color develop at room temperature (22-27^{0}C) in subdued light for at least 1h and measured absorbance at 640nm. Blank and standard solutions[4] were prepared to have standard curve to compute concentration ammonium of water sample by comparing water sample absorbance with the standard.

Note:

[1] Phenol solution: mix 11.1 ml liquefied phenol (≥89%) with 95% v/v ethyl alcohol to a final volume of 100 ml.

[2] Sodium nitroprusside solution, 0.5%: 0.5g sodium nitroprusside was dissolved in 100 ml deionised water.

[3] Oxidising solution: mixed alkaline citrate solution with 5% sodium hypochloride (4:1 v/v). Alkaline citrate solution was prepared by diluting 200 g trisodium citrate and 10 g sodium hydroxide in 1000 ml of deionised water.

[4] Using NH_4Cl to prepare standard solution: 0.3819 g of pure and dried NH_4Cl was dissolved to obtain stock solution of 100 mg l^{-1}. The stock solution was then diluted to obtain of 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}.

4. Colorimetric method:

2 ml color reagent was added into 50 ml filtered water sample and standard solution and then left them to develop color and measured absorbance at 543nm. Blank and standard solutions were prepared to have standard curve to compute concentration nitrite of water sample by comparing water sample absorbance with the standard. The color reagent and standard solution were prepared as follow:

- 10g sulfanilamide was dissolved in 800 ml of distilled water and 100ml 85% phosphoric acid. After dissolving sulfanilamide completely, 1g N-(1-naphythyl)-ethylenediamine dihydrochloride was added and diluted to 1000 ml with distilled water.
- Standard solution: 0.4925 g of pure and dried $NaNO_2$ was dissolved to obtain stock solution of 0.5 mg l^{-1}. The stock solution was then diluted to obtain of 0.0, 0.02, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg l^{-1}.

5. Ascorbic acid method:

50 ml water sample was added 0.05 ml (1 drop) phenolphthalein indicator. If a red color develops add 5N H_2SO_4 solution dropwise to just discharge the color. 8 ml combined reagent was added into samples and mixed thoroughly. After at least 10 minutes but no more than 30 minutes, measure absorbance of each sample at 880 nm, using reagent bank as the reference solution. Blank and standard solutions were prepared to have standard curve to compute concentration phosphate of water sample by comparing water sample absorbance with the standard. The combined reagent and standard phosphate solution were prepared as follow:

- Combined reagent: Mix reagents in the following proportions for 100 ml of the combined reagent and follow in the order: 50 ml 5N H_2SO_4, 5 ml 0.001M potassium antimonyl tartrate, 15 ml 0.003M ammonium molybdate solution, and 30 ml 0.01M ascorbic acid solution. If turbidity forms in the combined

- Standard solution: 0.2195 g of pure and dried KH_2PO_4 was dissolved to obtain stock solution of 0.5 mg l^{-1}. The stock solution was then diluted to obtain of 0.0, 0.02, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg l^{-1}.

Appendix 2

Table I: Water quality parameters (mg l^{-1}) of each treatment over the experimental period (mean ± S.E.) (Chapter 4).

Treatment	**Temperature (^{0}C)**	**Salinity (‰)**	**pH**	**DO (mg l^{-1})**
PM	25.08 ± 1.26	28.96 ± 0.03	7.91 ± 0.01	5.81 ± 0.29
SM	23.81 ± 0.10	30.19 ± 0.09	8.17 ± 0.01	6.16 ± 0.03
ISP	23.60 ± 0.06	29.06 ± 0.04	7.99 ± 0.01	5.96 ± 0.02

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed-prawn culture.

Table II: Water quality parameters (mg l^{-1}) each treatment over the experimental period (mean ± S.E.) (Chapter 5).

Treatment	**Temperature (^{0}C)**	**Salinity (‰)**	**pH**	**DO (mg l^{-1})**
ISP1-1	21.60 ± 1.28	35.14 ± 0.13	7.43 ± 0.04	5.81 ± 0.29
ISP2-1	22.31 ± 0.17	36.19 ± 0.79	8.02 ± 0.03	6.16 ± 0.03
ISP3-1	22.04 ± 0.78	35.76 ± 1.04	7.99 ± 0.01	5.96 ± 0.02
ISP1-2	22.48 ± 0.34	36.06 ± 0.79	8.07 ± 0.09	6.02 ± 0.04
ISP2-2	21.98 ± 1.02	35.22 ± 1.11	7.89 ± 0.44	5.99 ± 0.07
ISP3-2	22.08 ± 0.56	36.01 ± 0.74	7.54 ± 0.67	5.92 ± 0.05

Note: ISP = integrated seaweed- prawn culture.

Appendix 3

Table I: The concentration of nitrogen and phosphorus in feed for prawns (%) (mean ± S.E.).

Nutrient	Chapter 4	Chapter 5
Nitrogen	8.12 ± 0.22	5.34 ± 0.32
Phosphorous	1.29 ± 0.135	1.78 ± 0.25

Table II: Total N and P biomass gained of prawns and seaweed in treatments in Chapter 4.

Species	Treatment	mg N gained in culture (g)	mg P gained in culture (g)
Prawn	PM	102.95 ± 33.40	4.07 ± 1.67
	ISP	96.79 ± 40.99	13.37 ± 6.14
Seaweed	SM	-106.95 ± 6.92	-67.69 ± 5.32
	ISP	-111.42 ± 44.60	-103.71 ± 15.61

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed-prawn culture.

Table III: Total N and P biomass gained of prawns and seaweed in treatments in Chapter 5.

Species	Treatment	mg N gained in culture (g)	mg P gained in culture (g)
Prawn	ISP1-1	137.96 ± 13.90	27.17 ± 5.60
	ISP2-1	136.36 ± 9.90	28.17 ± 2.45
	ISP3-1	131.35 ± 6.33	39.52 ± 3.33
	ISP1-2	149.01 ± 6.10	26.99 ± 3.24
	ISP2-2	135.07 ± 13.34	23.50 ± 6.20
	ISP3-2	153.36 ± 18.72	23.72 ± 4.98
Seaweed	ISP1-1	138.96 ± 16.69	69.33 ± 6.72
	ISP2-1	121.09 ± 21.62	103.77 ± 17.00
	ISP3-1	129.09 ± 5.55	103.58 ± 10.90
	ISP1-2	91.18 ± 13.74	71.49 ± 14.15
	ISP2-2	97.27 ± 17.34	63.48 ± 11.76
	ISP3-2	104.25 ± 25.58	63.67 ± 15.97

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed-prawn culture.

Appendix 4

Table I: Intensity of light for all experiments (mean ± S.E.).

Chapter	Experiment	Treatment	Intensity (Lux)
Chapter 4		PM	115.57 ± 13.03
		SM	111.05 ± 10.25
		ISP	114.75 ± 11.47
Chapter 5	Experiment 1	ISP1-1	182.40 ± 13.83
		ISP2-1	145.32 ± 17.84
		ISP3-1	181.19 ± 12.95
	Experiment 2	ISP1-2	177.61 ± 13.01
		ISP2-2	189.57 ± 14.56
		ISP3-2	141.73 ± 16.05

Note: PM = prawn monoculture, SM = seaweed monoculture, ISP = integrated seaweed-prawn culture.

Appendix 5

Conference article

Tropentag 2008
University of Hohenheim, October 7-9, 2008
Conference on International Research on Food Security, Natural Resource Management and Rural Development

Removal of inorganic nitrogen by integrating seaweed *Sargassum sp.* into western king prawn (*Penaeus latisulcatus,* Kishinouye 1896*)* culture

Huong Mai, Ravi Fotedar and Jane Fewtrell

Curtin University of Technology, Muresk Institute, Technology Park (Brodie Hall Building) 1 Turner Ave Bentley, 6102 Perth, Western Australia. E-mail: mhuongria1@yahoo.com

Abstract

Effluent water from intensive prawn culture ponds typically has high concentrations of dissolved nutrients such as nitrogen. An experiment was conducted for 28 days to investigate the nitrogen flow where seaweed (*Sargassum sp.)* was integrated into western king prawn (*Penaeus latisulcatus)* culture. Three treatments were used, each consisting of four, 0.1m^3 plastic tanks. Treatment 1 and 2 were the monocultures of western king prawns (5.48 ± 0.29 g) and seaweed (young seaweed). Treatment 3 was an integrated culture of prawns and seaweed. Five prawns were stocked in each tank of treatment 1 and 3. About 137 ± 0.36 g of biomass seaweed was stocked in the treatment 2 and 3**.** Prawns in prawn monoculture and integrated culture were fed twice a day at a rate of 2.5% of total body weight. The concentration of dissolved inorganic nitrogen (DIN) discharged from the prawn monoculture increased from 0.126 to 10.98 mg/L during the experiment. The concentration of total ammonium nitrogen (TAN), nitrite-nitrogen (NO_2^-) and nitrate-nitrogen (NO_3^-) in the integrated culture was significantly lower at the termination of the experiment than the prawn monoculture ($p \leq 0.05$). The concentration of TAN, NO_2^-, NO_3^- and DIN in the integrated culture remained within non-toxic limits for the duration of the experiment. Integrating *Sargassum sp*. with prawns did not alter the specific growth rate (SGR) and survival rate of the prawns ($p > 0.05$). The mean biomass of seaweed in the integrated culture increased at the rate of 3.16 ± 0.74% g per day after 7 days of the experiment, which was significantly lower ($p \leq 0.05$) than the growth rate of the seaweed in the monoculture (5.70 ± 0.82 % g per day). The results suggest that integrating seaweed into prawn culture can benefit prawn farming by assisting in the maintenance of optimum water quality and thereby, reduce environmental impacts on surrounding areas.

Key words: Integrated aquaculture, nitrogen, western king prawn, *Sargassum sp*

1. Introduction

Prawn farming has developed steadily over the last decades in response to increasing world market demand. The western king prawn (*Penaeus latisulcatus,* Kishinouye 1896*)* is considered as one of the candidate species for culture and has been widely cultured in several Asian countries (Kathirvel and Selvaraj, 1987). To increase prawn productivity, the management practices have been intensified by

using high quality and quantity of feed (Brzeski and Newkirk, 1997, Shepherd and Bromage, 1988, Seymour and Bergheim, 1991) which accounts for more than 95% of the nutrient input (Krom and Neori, 1989). However, less than one third of nutrients are assimilated into the shrimp biomass (Briggs and Funge-Smith, 1994) and the remainder is lost to the system (Wu, 1995, Piedrahita, 2003). In addition, aquatic species excrete to the water 70-80% of their ingested protein, the majority of which (80%) are composed of dissolved nitrogen in ammonium forms (Porter et al., 1987).

The discharged wastewater from intensive prawn culture may cause environmental concerns. The effluents, which consist of excess feeds and excretory products, can promote eutrophication and result in harmful algal blooms and anoxia conditions (Wu, 1995). In order to mitigate the environmental impacts due to effluent discharge and maintain sustainable prawn farming, various methods have been proposed to address the issue of nutrients discharged from intensive prawn aquaculture (Neori et al., 2004). One possible approach is integrating prawns and macroalgae where macroalgae is expected to absorb nutrients.

Macroalgae species such as *Ulva, Porphyra* and *Gracilaria* have been proven to effectively reduce the nutrient load in effluents and assist in maintaining water quality at acceptable levels (Neori et al., 2004). However, there is limited literature available on integrating *Sargassum sp.* with king prawn farming. *Sargassum* species are common macroalgae occurring worldwide and inhabits in subtidal areas in both warm and temperate water, such as in the Indo-west Pacific region and Australia (Tseng et al., 1985). Furthermore, *Sargassum* species have potential to act as a biofilter because of its capacity of nitrogen metabolism in the ocean environment (Hanson, 1977, Phlips et al., 1986). The aim of this study was to evaluate the efficacy of *Sargassum sp.* in assimilating nitrogen when integrated with western king prawn culture.

2. Materials and Methods

2.1 Materials and experimental design

Western king prawns (size: 5.48 ± 0.29 g) were collected from the mouth of Swan River in Bicton, Western Australia (32^0 40”S 115^0 13”E). Prawns were acclimated to the laboratory conditions for 14 days before commencing the experiment. *Sargassum sp.* was collected from the Cottesloe coast in Western Australia (31^0 57’ S 115^0 05”E). Seaweed was rinsed with ocean water and epiphytes were removed.

The system used in this trial consisted of twelve, 100L (0.1 m^3) plastic tanks. Four replicates of three treatment group were set up in a completely randomized design. Treatment groups 1 (PM) and 3 (IPS) were monocultures of western king prawn and seaweed, respectively. Treatment 2 (SM) was a co-culture of prawns and seaweed. Prawns and seaweed were stocked at densities of 18 animals/m^2 (27 g per tank) and 0.5 kg/m^2 (140 g per tank), respectively. Prawns were fed 2.5% of the total tank prawn biomass twice a day. Mortalities in each tank were removed and weighed and any sign of cannibalism was noted. The trial was conducted over a period of 28 days.

2.2 Analytical procedures

Prawns were weighed at the commencement of the experiment and were re-weighed once a week to obtain the data required to determine specific growth rates (SGR %) and weight gain (WG g) by using formulas:

$$SGR = 100\ (\ln W_t - \ln W_0)/t \text{ and } WG = W_t - W_0$$

where: W_0 = initial weight; W_t = weight at time t since the beginning.

The survival rate (S_{tn}) of the prawns in each tank was also calculated using the formulas:

$$S_{tn} = N_{tn} \times 100/N_i$$

where: N_{tn}: number of prawn surviving at the time n; N_i: number of prawn at the beginning of the trial.

The concentrations of total ammonia nitrogen (TAN: NH_3^- & NH_4^+), nitrite nitrogen (NO_2^-) and nitrate nitrogen (NO_3^-) in all tanks were measured weekly. TAN and NO_2^- were analysed using standard methods for water and waste water analysis (APHA, 1998). NO_3^- was analysed by using a DR/890 Colorimeter. Nitrogen removal (NR %) in the integrated systems was estimated according to the following equation:

$$NR = 100 \times (C_{cnl} - C_p)/C_{cnl}$$

where C_{cnl} = nutrient concentration in the prawn monoculture treatment (mg/L); C_p = nutrient concentration in the integrated culture treatment (mg/L).

2.3 Statistical analysis

SPSS (versions 15) and Microsoft Excel were used for data analysis. LSD post hoc tests in One way of Analysis of Variance (ANOVA) were used to determine any significant differences ($p \leq 0.05$) among treatment means. Regression analysis was used to assess relationships between SGR of prawn and nutrients in water.

3. Results and discussion

3.1 Water quality parameters

The concentration of nitrogen metabolites gradually increased over the 21-day experimental period in all treatments, except for NO_3^- in seaweed monoculture which remained undetectable after 14 days of the experiment (Figure 1). There was a significant increase in the concentration of nitrogen metabolites after 21 days of the experiment, with DIN at 11 mg/l in prawn monoculture, 4.27 mg/l in the integrated culture and 1.77 mg/l in seaweed monoculture. The observed decay of seaweed would have contributed to this increase in nitrogen loading (Jones, 1999). In this study, the thallus of *Sargassum* began to deteriorate and disintegrate after 7 days and 100% mortality was recorded by the end of the experiment. Similarly, DIN was greater than 14 mg/l when red seaweed (*Gracilaria)*, was cultivated in *P. monodon* effluents, died (Marinho-Soriano et al., 2002).

In this study, the concentration of TAN in all treatments remained below 1.0 mg/l until day 21 of experiment and then significantly increased to 2.92 mg/l in prawn

monoculture, 2.34 mg/l in integrated culture and 1.66 mg/l in seaweed monoculture. The concentration of NO_2^- in prawn monoculture and integrated culture increased to nearly 0.7 mg/l by day 21 and remained at this level until the conclusion of the experiment. TAN and NO_2^- levels remained within the known acceptable concentration for successful prawn culture (3.0 mg/l and 1.0 mg/l, respectively) in both prawn monoculture and integrated culture treatments (Timmons et al., 2002).

In all treatments, NO_2^- was generally the form of DIN at the lowest concentration. In addition, NO_2^- concentration did not significantly differ over the experimental period, while the concentration of TAN, NO_3^- and DIN after 28 days of the experiment was significantly higher than at the previous days of the experiment (Figure 1). This suggests that NO_2^- could be accumulating in the tanks due to incomplete nitrification with the kinetic reaction being controlled by ammonia oxidation over the experimental period (Timmons et al. 2002).

Overall, integrating prawn culture with seaweed resulted in lower concentration of TAN, and NO_3^- than in prawn monoculture. With *Sargassum sp.* absent, TAN concentration ranged from 0.03-2.91 mg/l, while NO_3^- concentrations reached 7.4 mg/l by day 28. However, integrating *Sargassum* with western king prawns the concentration of nitrogen metabolites was significantly lower than in prawn monoculture, with only 2.34 mg/l of TAN and 1.25 mg/l of NO_3^-. The concentration of NO_2^- in the integrated culture remained lower than in prawn monoculture until day 21 of the experiment, but at the end of the experiment NO_2^- reached 0.67 mg/l in both prawn monoculture and integrated culture. Generally, these results suggest that *Sargassum* improved water quality when integrated with prawn culture.

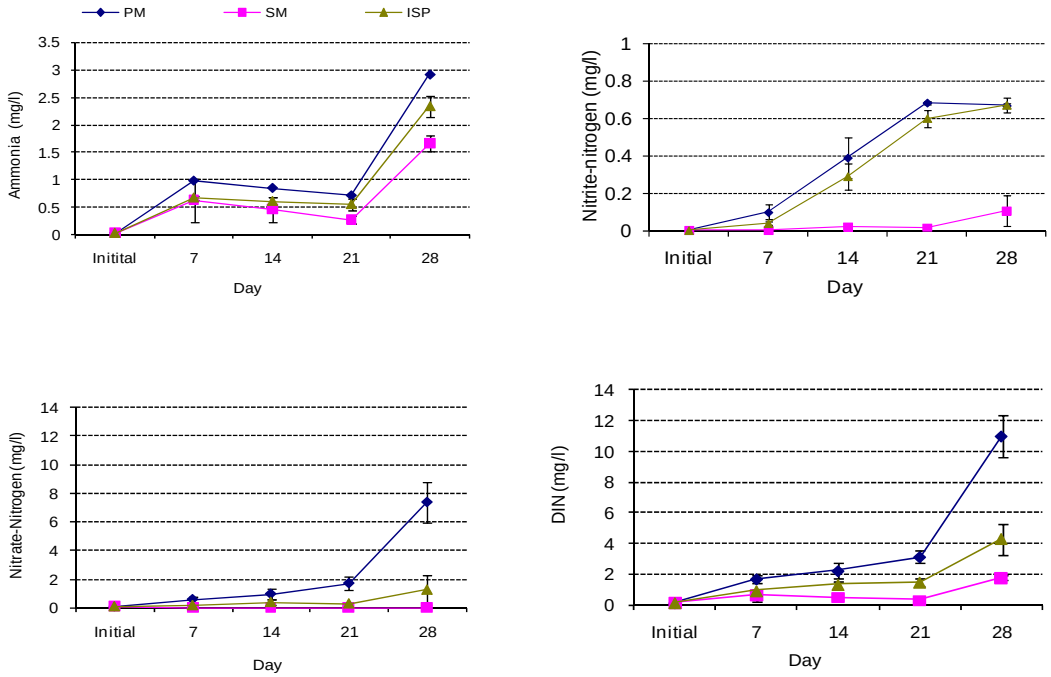


Figure 1: Concentrations of TAN, NO_2^-, NO_3^- and DIN in different systems over 28-day experiment. *(PM- prawn monoculture, SM - seaweed monoculture, ISP- integrated seaweed & prawn)*

3.2 Nitrogen removal

The removal rates of nitrogen metabolites from water when *Sargassum sp.* was present in prawn culture were not significantly different over the period of the experiment, except for NO_2^- which showed a significant decrease (Table 1). The removal rate of NO_3^- generally increased with increasing NO_3^- concentration, while the removal rate of TAN decreased with increasing TAN concentration (Figure 1, Table 1). The presence of *Sargassum sp.* resulted in more efficient at removal of NO_3^- than TAN, with removal rates of 69.28% and 28.25%, respectively. Similarly, previous research has shown that seaweeds, such as green seaweed *Codium fragile* (Hanisak and Harlin, 1978), brown seaweed *Laminaria groenlandica* (Harrison et al., 1986) and red seaweed *Porphyra yezoensis* (Hafting, 1999) removed NO_3^- more efficiently than TAN. However, other research has shown *Ulva pertusa*, *Gelidum amansii* and *Sargassum enerve* to be more useful in removal of TAN than NO_3^- from water (Liu et al., 2004). In this study, *Sargassum sp.* was able to remove a maximum of 52.57% of DIN. This is higher than the values reported for red seaweed *Gracilaria longissima* where such seaweed removed only 17% of DIN when integrated with fish (*Sparus auratus)* culture (Hernández et al., 2005). This indicates that *Sargassum sp.* has a potential to act as a nitrogen sink when integrated with western king prawn culture.

Table 1: Removal rate of nitrogen metabolites over the experimental period

Variable	D7	D14	D21	D28	Mean
TAN (%)	29.22±7.49[a]	29.35±11.28[a]	24.23±15.77[a]	20.75±8.31[a]	28.25±1.28
NO_2^- (%)	55.58±6.78[a]	24.10±10.54[b]	12.87±6.43[c]	nd	30.85±12.78
NO_3^- (%)	68.09±7.32[a]	61.79±11.03[b]	72.19±16.54[a]	75.04±22.25[a]	69.28±2.87
DIN (%)	44.65±6.66[a]	37.89±8.45[a]	49.37±14.41[a]	52.57±3.73[a]	46.12±3.19

Values in any one row not followed by the same superscript letters are significantly different at $p < 0.05$; nd = *not detectable*

3.3 Survival and growth performance of prawns and seaweed

Table 2: Specific growth rate (SGR), weight gain (WG) and survival rate of prawns and seaweed biomass in different treatments over the experimental period

Variable	Prawn monoculture	Seaweed monoculture	Integrated prawn & seaweed
Prawns			
SGR (% g day^{-1})	0.39 ± 0.12[a]	-	0.32 ± 0.08[a]
Weight gain (g)	3.27 ± 0.92[a]	-	2.47 ± 0.69[a]
Survival (%)	85.00 ± 9.57[a]	-	80.00 ± 0.00[a]
Seaweed			
SGR (% g day^{-1})*		5.70 ± 0.82[a]	3.16 ± 0.74[b]

Values in any one row not followed by the same superscript letters are significantly different at $p < 0.05$

** Biomass of live seaweed after 7 days of the experiment*

Integrating *Sargassum sp.* with prawn culture did not alter the SGR or weight gain of prawns (Table 2). Similarly, Lombardi et al. (2006) reported no signifcant differences in weight gain between monoculture and integrated culture when

seaweed (*Kappaphycus alvarezii*) was integrated into Pacific white prawn (*Litopenaeus vanamei*) culture. Compared with studies on *P. monodon* (Chen et al., 1989, Thakur and Lin, 2003), the growth rate of western king prawns in both the monoculture and integrated culture of this study was higher, possibly as a result of lower stocking densities. In the present study, the stocking density of western king prawn was 18 prawns per m^2 (5 prawn per tank), while *P. monodon* were stocked at approximately 70 postlarvae per m^2 (PL_{25-27}) by Chen et al. (1989) and 20-25 juveniles per m^2 by Thakur and Lin (2003). Mean prawn survival rate was not significantly affected by the presence of seaweed, with 85% survival in prawn monoculture and 80% survival in integrated prawn and seaweed culture.

No correlation between the SGR of prawns and TAN or DIN was found in either prawn monoculture or integrated culture, suggesting that prawn growth rate was not affected by any measured water quality parameter. To investigate if a relationship between prawn growth rate and nitrogen concentration exists an extended study period is suggested for future studies.

When seaweed was integrated with prawn culture, the mean biomass of seaweed increased at the rate of 3.16% g per day after 7 days of the experiment, while the growth rate of seaweed in the monoculture system was significantly greater with 5.70% g per day (Table 2). Similarly, Guimaraens (1999) found that *Sargassum* growth rates decreased in nitrogen enriched conditions. Liu et al. (2004) reported that *Sargassum enerve* had a high capacity to assimilate nitrogen, but the increase in fresh weight gain was slow at high nitrogen concentration condition. Different species of seaweed, for example *Ulva* and *Gracilaria*, have also shown that high nitrogen levels can result in an inhibition in growth rate (Waite and Mitchell, 1972, Parker, 1982, Lignell and Pedersén, 1987, Marinho-Soriano et al., 2002).

4. Conclusions

Integrating *Sargassum sp.* into western king prawn culture can improve the water quality when compared to prawn monoculture systems. Although the results of this study showed that this integration had no significant effect on prawn growth, the addition of *Sargassum* did assist in the maintenance of optimum water quality and could thereby reduce the environmental impacts of the effluent on surrounding areas.

Acknowledgements

This work was supported by AusAID scholarship program, Australia. The authors thank their colleagues in the Department of Applied Bioscience for their assistance in conducting the experiment and analysing parameters.

Reference

APHA (1998) *Standard methods for the examination of water and wastewater* American Public Health Association, Washington, DC.

BRIGGS, M. R. P. & FUNGE-SMITH, S. J. (1994) A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquacult. fish.Manag.,* 25**,** 789-811.

BRZESKI, V. & NEWKIRK, G. (1997) Integrated coastal food production systems -- a review of current literature. *Ocean & Coastal Management,* 34**,** 55-71.

CHEN, J.-C., LIU, P.-C. & LIN, Y.-T. (1989) Culture of Penaeus monodon in an intensified system in Taiwan. *Aquaculture,* 77**,** 319-328.

GUIMARAENS, M. A. D. (1999) The influence of environmental factors on the seasonal dynamics of Ulva spp. and Sargassum sp. in the Cabo Frio upwelling region of Brazil. . . Florida, The United States, University of Miami, .

HAFTING, J. T. (1999) Effect of tissue nitrogen and phosphorus quota on growth of Porphyra yezoensis blades in suspension cultures. *Hydrobiologia,* 398-399**,** 305-314.

HANISAK, M. D. & HARLIN, M. M. (1978) Uptake of inorganic nitrogen by *Codium fragile* subsp. *tomentosoides* (Cholorophyta) *Journal of Phycology,* 14**,** 450-454.

HANSON, R. B. (1977) Pelagic Sargassum community metabolism: Carbon and nitrogen. *Journal of Experimental Marine Biology and Ecology,* 29**,** 107-118.

HARRISON, P. J., DRUEHL, L. D., LLOYD, K. E. & THOMPSON, P. A. (1986) Nitrogen uptake kinetics in three year-classes of Laminaria groenlandica (Laminariales: Phaeophyta). *Marine Biology,* 93**,** 29-35.

HERNÁNDEZ, I., FERNÁNDEZ-ENGO, M., PÉREZ-LLORÉNS, J. & VERGARA, J. (2005) Integrated outdoor culture of two estuarine macroalgae as biofilters for dissolved nutrients from Sparus auratus waste waters. *Journal of Applied Phycology,* 17**,** 557-567.

JONES, A. B. (1999) Environmental Management of Aquaculture Effluent: Development of Biological Indicators and Biological Filters. *Department of Botany.* Queensland, The University of Queensland.

KATHIRVEL, M. & SELVARAJ, V. (1987) On an experimental seed collection and field culture of king prawn, *Penaues latisulcatus*. *Indian Journal of Fisheries,* 34**,** 365-373.

KROM, M. D. & NEORI, A. (1989) A total nutrient budget for an experimental intensive fishpond with circularly moving seawater. *Aquaculture,* 83**,** 345-358.

LIGNELL, Å. & PEDERSÉN, M. (1987) Nitrogen metabolism in Gracilaria secundata Harv. *Hydrobiologia,* 151-152**,** 431-441.

LIU, D., AMY, P. & SUN, J. (2004) Preliminary study on the responses of three marine algae, Ulva pertusa (Chlorophyta), Gelidium amansii (Rhodophyta) and Sargassum enerve (Phaeophyta), to nitrogen source and its availability. *Journal of Ocean University of China (English Edition),* 3**,** 75-79.

LOMBARDI, J. V., DE ALMEIDA MARQUES, H. L., PEREIRA, R. T. L., BARRETO, O. J. S. & DE PAULA, E. J. (2006) Cage polyculture of the Pacific white shrimp Litopenaeus vannamei and the Philippines seaweed Kappaphycus alvarezii. *Aquaculture,* 258**,** 412-415.

MARINHO-SORIANO, E., MORALES, C. & MOREIRA, W. S. C. (2002) Cultivation of Gracilaria (Rhodophyta) in shrimp pond effluents in Brazil. *Aquaculture Research,* 33**,** 1081-1086.

NEORI, A., CHOPIN, T., TROELL, M., BUSCHMANN, A. H., KRAEMER, G. P., HALLING, C., SHPIGEL, M. & YARISH, C. (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture,* 231**,** 361-391.

PARKER, H. S. (1982) Effects of simulated current on the growth rate and nitrogen metabolism of Gracilaria tikvahiae (Rhodophyta). *Marine Biology,* 69**,** 137-145.

PHLIPS, E. J., WILLIS, M. & VERCHICK, A. (1986) Aspects of nitrogen fixation in Sargassum communities off the coast of Florida. *Journal of Experimental Marine Biology and Ecology,* 102**,** 99-119.

PIEDRAHITA, R. H. (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture,* 226**,** 35-44.

PORTER, C. B., KROM, M. D., ROBBINS, M. G., BRICKEL, L. & DAVIDSON, A. (1987) Ammonia excretion and total N budget for Gilthead Seabream (Sparus aurata) and its effect of water quality conditions. *Aquaculture,* 66**,** 287-297.

SEYMOUR, E. A. & BERGHEIM, A. (1991) Towards a reduction of pollution from intensive aquaculture with reference to the farming of salmonids in Norway. *Aquacultural Engineering,* 10**,** 73-88.

SHEPHERD, C. J. & BROMAGE, N. R. (Eds.) (1988) *Intensive Fish Farming* BSP Professional Books. A Division of Blackwell Scientific Publication Ltd.

THAKUR, D. P. & LIN, C. K. (2003) Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Engineering,* 27**,** 159-176.

TIMMONS, M. B., EBELING, J. M., SUMMERFELT, S. T. & VINCI, B. J. (2002) *Recirculating aquaculture systems*, Northeasten Regional Aquaculture Center.

TSENG, C. K., YOSHIDA, T. & CHIANG, Y. M. (1985) East Asiatic species of Sargassum subgenus Bactrophycus J.Agardh (Sargassaceae, Fucales), with keys to the sections and species. IN ABBOTT, I. A. & NORRIS, J. N. (Eds.) *Taxonomy of Economic Seaweeds with reference to some Pacific and Caribbean species.* California Sea Grant Program.

WAITE, T. & MITCHELL, R. (1972) The effect of nutrient fertilization on the benthic alga *Ulva lactuca Botanica Marina* 15**,** 151-156.

WU, R. (1995) The environmental impact of marine fish culture: toward a sustainable future. *Mar Pollut Bull,* 31**,** 159-166.