

Polyculture crevette Litopenaeus stylirostris (Stimpson, 1974) et poisson Siganus lineatus (Valenciennes, 1835) : faisabilité technique et effets sur le fonctionnement écologique des bassins d'élevage de crevettes

Cong Trung Luong

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(Spécialité Aquaculture)

Présentée par **Luong Cong Trung**

en vue d'obtenir de grade de Docteur de l'Université de la Nouvelle-Calédonie

Polyculture crevette *Litopenaeus stylirostris* (Stimpson, 1974) et poisson *Siganus lineatus* (Valenciennes, 1835) : Faisabilité technique et effets sur le fonctionnement écologique des bassins d'élevage de crevettes

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LIST OF ABBREVIATIONS

DIN: Dissolved inorganic nitrogen

DIP: Dissolved inorganic phosphorus

DO: Dissolved oxygen

DON: Dissolved organic nitrogen

DWG: Daily weight gain

Eh: Redox potential

FAO: Food and Agriculture Organization of the United Nations

FCR: Food conversion ratio

GPP: Gross primary productivity

GNP: Gross natural production

MPB: Microphytobenthos

NPP: Net primary productivity

PN: Particulate nitrogen

POC: Particulate organic carbon

POM: Particulate organic matter

R: Respiration

SdR: Sedimentation rate

SGR: Specific growth rate

SOM: Sediment organic matter

SPC: Secretariat of the Pacific community

SR: Survival rate

SRP: Soluble reactive phosphorus

TAN: Total ammonia nitrogen

TDN: Total dissolved nitrogen

TSS: Total suspended solids

CHAPTER 1

GENERAL INTRODUCTION

1.1 World aquaculture

World aquaculture production of food-fish (fishes, crustaceans, mollusks, amphibians, reptiles (except crocodiles), sea cucumber, sea urchin, etc.) for human consumption reached 62.7 million tonnes in 2011 (Fig. 1.1), up by 4.7% from 59.9 million tonnes in 2010 and 81.2% from 34.6 million tonnes in 2001 (FAO 2012, 2013a). Aquaculture continues to be the fastest-growing, impressive and important production sector for high-protein food. In the period 1980 – 2011, world food fish production of aquaculture has increased by over 13 times (from 4.7 to 62.7 million tonnes), at an average annual growth rate of 8.8% (FAO 2012, 2013a).

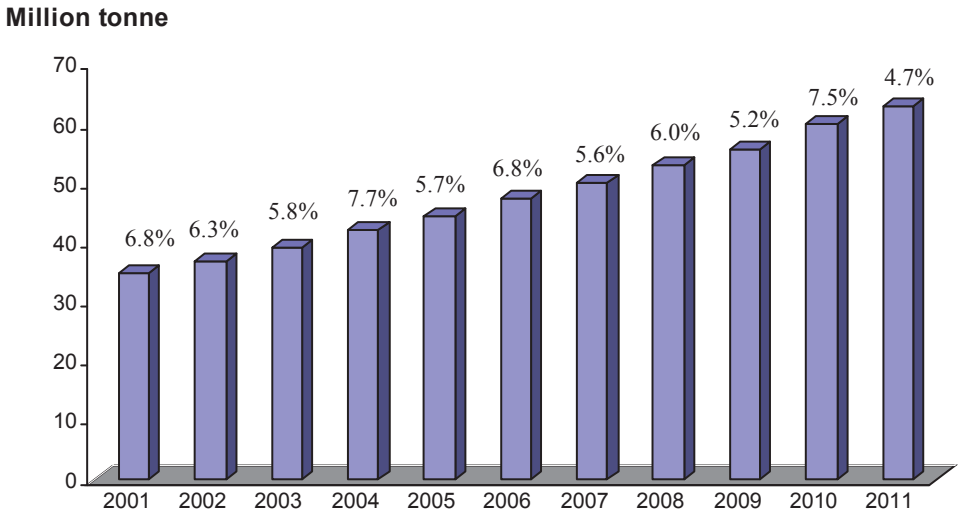


Figure 1.1: World yearly food-fish production of aquaculture and percentage of growth from 2001 to 2011. Data source: FAO, 2013a.

Since the mid-1990s, aquaculture has been the dynamic promoting growth in total fish production as global capture production has stagnated. Its contributions to world total fish production climbed steadily from 20.9% in 1995 to 32.4% in 2005 and 40.3% in 2010, and to world food fish production for human consumption was 47% in 2010 compared with only 9% in 1980 (FAO, 2012). It is further estimated by 2020 more than 50% of global food fish consumption will derive from aquaculture due to static global capture fishery production and a growing population (FAO, 2010). As population is rapidly increasing in the world, from 6.6 billion in 2006 to 7 billion in 2011, fish food requirements have risen dramatically. While overall global capture fisheries production continues to remain stable at about 90 million tonnes, aquaculture production has steadily increased from 47.3 million tonnes in 2006 to

62.7 million tonnes in 2011 that contributes to increase food fish supply per capita from 17.4 to 18.8 kg in the same period (FAO, 2012).

Aquaculture is currently practiced in 190 countries and territories worldwide, with about 600 aquatic species are bred in captivity for production in a variety of culture systems and facilities of varying input intensities and technological degrees, using freshwater, brackish water and marine water (FAO, 2012). The world top aquaculture producers of food-fish are China, India, Viet Nam, Indonesia, Bangladesh, Thailand, Norway, Egypt, Myanmar and Philippine (FAO, 2010). The Asia continues to dominate the aquaculture sector, accounting for 88.5% of world aquaculture production by volume in 2011, while America, Europe, Africa, and Oceania account for 4.7%, 4.3%, 2.2%, and 0.3%, respectively (FAO, 2013a). Freshwater fishes dominate global aquaculture production (56.4%), followed by mollusks (23.6%), crustaceans (9.6%), diadromous fishes (6.0%), marine fishes (3.1%) and other aquatic animals (1.4%).

Aquaculture expansion has already raised many concerns on environmental and social impacts. The environmental effects include the destruction of coastal mangrove to converse to culture ponds, salinization of groundwater and land, pollution of receiving waters from pond effluents, biodiversity issues from the collection of wild seed and broodstock; introduction and transfer of exotic species, spread diseases and misuse of chemicals (Lewis et al., 2003; Pillay, 2004; Primavera, 2006; FAO, 2011). The socioeconomic impacts consist of privatization of public lands and waterways and social conflicts between aquaculturists and other aquatic resource users, loss of fisheries livelihoods, food insecurity, and urban migration (Lewis et al., 2003; Primavera, 2006).

Aquaculture production is vulnerable to adverse impacts of disease and environmental conditions. Disease outbreaks in several last years have affected farmed Atlantic salmon in Chile, oysters in Europe, and marine shrimp farming in several countries in Asia, South America and Africa, resulting in partial or sometimes total loss of production (FAO, 2012).

1.2 World Penaeid shrimp culture

Penaeid shrimp farming is one of the most economically successful and fastest-growing sections of the aquaculture industry. World farmed shrimp production has grown

continuously from under 10.000 tonnes in the early 1970s to over 1 million tonnes by the late 1990s (Tacon, 2002) and near 3.8 million tonnes in 2010 (FAO, 2010). From 2000 to 2010, farmed shrimp production has grown at an annual average of 13%, and exceeded captured shrimp production in recent years. Meanwhile, the total catch of shrimp from capture fisheries has grown at a low rate of 0.6% per year over the same period (Figure 1.2) (FAO, 2008, 2010). Shrimp farming is currently practised in over 50 countries worldwide (Tacon, 2002), and almost restricted to developing countries; specially concentrated in the Asia region, mainly China, Thailand, Viet Nam, Indonesia, India, Bangladesh, Philippine and Malaysia (Tacon, 2002; Valderrama and Anderson, 2011). The Latin America region also produces significant farmed shrimp, mainly Ecuador, Mexico, Brazil, Colombia, Honduras, Nicaragua (Tacon, 2002; Valderrama and Anderson, 2011). Remaining regions produce small amounts, including Oceania (mainly Australia and New Caledonia), Africa (mainly Madagascar and South Africa), North America and lastly Europe (mainly Spain and Italy) (Tacon, 2002).

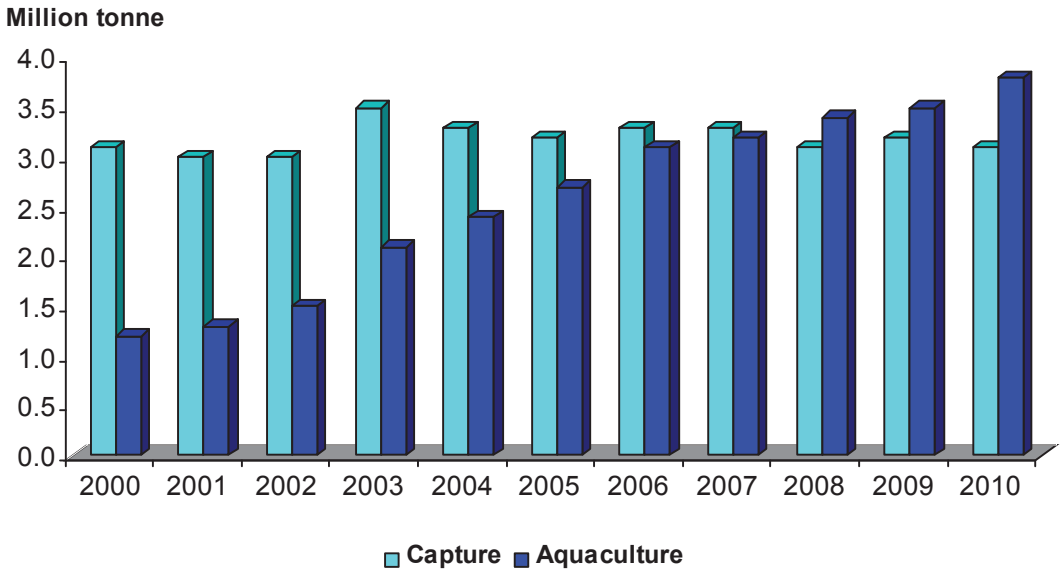


Figure 1.2: Yearly World shrimp capture and aquaculture production, 2000 - 2010. Data source: FAO (2008, 2010) (freshwater prawn *Macrobrachium rosenbergii* is not included).

The farming systems currently employed can be roughly divided into four basic categories: extensive, semi-intensive, intensive and super-intensive farming systems (Tacon, 2002; Lewis et al., 2003). Extensive farming systems usually employ large earthen ponds (> 5–100 ha) with low input of shrimp stocking (< 5 shrimp.m^{-2}), water exchange, fertilizer and/or complementary food and low output of shrimp yields. Semi-intensive farming systems usually use small to moderate-sized earthen ponds (> 1–20 ha), with medium water exchange,

intermediate shrimp stocking densities (5 – 25 shrimp.m^{-2}), partial or continuous aeration, fertilization and/or supplementary feeding and produce moderate shrimp yields. Intensive farming systems usually practice in small earthen ponds (0.1–2 ha), with high water exchange and high shrimp stocking densities (>25 shrimp.m^{-2}), provide continuous aeration, formulated high protein pellet feed, and produce high shrimp yields (Tacon, 2002; Lewis et al., 2003). Super-intensive farming systems use small ponds (0.1 ha) with continuous aeration, low water exchange and very high densities (> 100 shrimp.m^{-2}). In this case, the reared species is *Litopeneaus vannamei*. As a consequence, extensive farming systems have usually inconsiderably impacted on the environment; in contrast intensive farming systems have raised environmental problems due to high enriched nutrient and organic matter effluent discharged from shrimp farms into receiving water (Briggs and Funge-Smith, 1994; Phillips, 1995; Funge-Smith and Briggs, 1998; Páez-Osuna, 2001a; Thomas et al., 2010).

The development of shrimp farming industries in many countries has been accompanied by sporadic growth, local collapses of the industry, and sometimes abandonment of shrimp ponds (Phillips, 1995). Such problems, widely attributed to disease outbreaks associated to environmental deterioration, have raised major questions about the sustainability of shrimp farming. Thus, although shrimp aquaculture has contributed to rural employment and economic development in the Asia-Pacific, concerns have grown over the sustainability of the industry (Phillips, 1995). The rapid increase in world cultured shrimp production and its equally rapid decline in some countries like Ecuador, China and Indonesia have left environmental, social and financial problems in its wake. Extensive farms have an enormous requirement for land and the development of intensive culture practice increases nutrient exports and eutrophication of coastal environment (Funge-Smith and Briggs, 1998). Typically, the pattern of production from a shrimp farm is that of an initial 'honeymoon period,' characterized by success and good production followed by gradual decrease in yields over successive crops. Depending upon a wide range of factors, decreased yields are manifested as reduced growth, higher food conversion ratio (FCR), and disease outbreaks that require emergency harvesting. The worst case is that of complete mortality of stock and this is being encountered more frequently with the increasing incidence of extremely pathogenic viral diseases (Funge-Smith and Briggs, 1998).

Almost from the beginning disease was recognized as a biological threat to the shrimp culture industry, and some diseases caused serious shrimp and economic losses (Lightner and

Redman, 1998**)**. Lightner (2003) reported at least four virus caused pandemics have adversely affected the global penaeid shrimp farming industry since 1980. These viruses in the approximate order of their discovery are Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), and White Spot Syndrome Virus (WSSV). The White Spot Syndrome Virus (WSSV) has emerged globally as one of the most prevalent, widespread and lethal for shrimp populations (Sánchez-Paz, 2010**,** Tendencia et al., 2011). It was first detected in Taiwan in 1992, and then it spread to Japan and almost all Asian countries. The first diagnosed case of WSSV in the Americas occurred in 1995 in a South Texas shrimp farm. The virus caused massive mortalities in some farms in Ecuador in 1999, while the most recent outbreak in an area with WSSV-free status occurred in Brazil in 2005 (Sánchez-Paz, 2010). WSSV still continues to plague the shrimp industry, despite the bulk of information available. There is no treatment for WSSV and prevention is the best way to avoid outbreaks (Tendencia et al., 2011). Taura Syndrome Virus (TSV) was first detected in 1992 in samples of *Penaeus vannamei* collected from shrimp farms located near the mouth of the Taura River. The rapid spread of TSV throughout the Americas, together with the resulting economic loss suffered by *P. vannamei* farmers, makes this one of the most important and detrimental pathogens affecting the shrimp industry in the Western hemisphere (Bonami et al., 1997). Frozen commodity shrimp have been implicated as the route by which WSSV was moved from Asia to the Americas, while TSV was moved in the opposite direction with infected live broodstock from Central America (Lightner, 2003; Sánchez-Paz, 2010).

Shrimp bacterial disease caused by bacteria (Vibrio) is also a significant problem among the countries where marine shrimp is the main aquaculture product (Ruangpan, 1998; Moriaty, 1999; Lightner, 2011). Luminous bacteria *Vibrio harveyi* is claimed to be the causative agent associated with shrimp mortality. In grow-out ponds, luminous disease frequently causes mortality with 2-3 month old stock of *Penaeus monodon*, and has also been reported to cause economic losses to the shrimp industry in the Philippines, Viet Nam, India, and Indonesia, and seems to be common problem among the Asian countries where shrimp farming is the main aquaculture activity (Ruangpan, 1998).

An unknown disease of cultured shrimp commonly known as Early Mortality Syndrome (EMS) or more technically known as Acute Hepatopancreatic Necrosis Syndrome (AHPNS) appears to have been infecting the shrimp aquaculture sector in Asia. This disease is considered idiopathic, i.e. it is not known whether the cause is infectious or toxic. Some of

the earlier hypotheses pointed to a range of agents and other causes such as cypermethrin (an insecticide), other pesticides, pollution, something in the feed, parasites, harmful algae, probiotics and inbreeding (FAO, 2013b). The first occurrence of this disease reported was in southern China and Hainan Island in 2010 and subsequently in Viet Nam and Malaysia in 2011. In terms of impacts on production, Viet Nam reported on the affected hectare of shrimp farms in the Mekong Delta was about 39,000 ha in 2011. In Malaysia, it was estimated production losses at USD 0.1 billion in 2011. It was also estimated that losses to the Asia shrimp culture sector could be USD 1 billion. The shortage of shrimp supply subsequently had an impact on shrimp prices (FAO, 2013b).

1.3 World Siganidae culture

The fish family Siganidae, popularly known as rabbitfishes, is widely distributed in the Indo-Pacific region, from the east coast of Africa to Polynesia, southern Japan to northern Australia (Lam, 1974; Duray, 1998), and in the Red Sea and Mediterranean Sea (Popper and Gundermann, 1975). The family Siganidae consists of a single genus, *Siganus*, which has been subdivided into sub-genera *Siganus* and *Lo*, and 28 species (Randall and Kulbicki, 2005; Borsa et al., 2007), some of which are abundant in the Indo-Malaysian area, but scarce in French Polynesia (Duray, 1998). Some species are commercially-important contributing to the total fishery production where they appear (Duray, 1998; Soliman et al., 2008).

As being excellent food fishes, rabbitfishes (Siganidae) traditionally contribute a major part to commercial fisheries production in several Pacific countries, such as Philippines, Guam, and Palau (Lam, 1974; Duray, 1998; Soliman et al., 2008) and are considered high potential candidates for mariculture long years ago (Lam, 1974). Rabbitfishes possess most of the desirable characteristics for aquaculture, such as high tolerance to different environmental factors, mainly temperature and salinity, rough handling and crowding (Lam, 1974), palatability and high demand and market prices for both export and local consumption. Many studies have been attempted in order to learn how to culture rabbitfishes, and several species have been tried in many countries for the ultimate purposes of commercial culture, for example *Siganus canaliculatus, S. fuscescens, S. guttatus, S. punctatus, S. spinus,* and *S. virgatus* in the Philippines (Von Wersternhagen and Rosenthal, 1976), *S. canaliculatus* in the East coast of Africa (Bwathondi, 1982), *S. canaliculatus* and *S. rivulatus* in the Red sea and Mediterranean region (Ben-Tuvia et al., 1973; Lichatowich et al., 1984; Stephanou and

Georgiou, 2000; Yousif et al., 2005; El-Dakar et al., 2010), *S. canaliculatus* in India (Jaikumar, 2012), *S. fuscescens* in Taiwan (Nelson et al., 1992), and finally *S. canaliculatus*, *S. lineatus,* and *S. randalli* in the Pacific Islands (Brown et al., 1994; SPC, 2008). Rabbitfishes commonly are grown in monoculture in brackish water ponds, and cages/pens, some of them are also commercially co-cultured with other fish species, such as with milkfish in the Philippines (Von Wersternhagen and Rosenthal, 1975). Farming of rabbitfish is profitable. However, it has its economic limitations (Ben-Tuvia et al., 1973). In general rabbitfishes grow slowly but mature early (Duray, 1998). Rabbitfish ponds yield 1051 kg/ha (Anon, 1976). S. *guttatus* cultured in cages produced a benefit cost ratio of 2.03. At a stocking rate of 50 fish/m^3 and using commercial feeds supplemented with natural food, they gained 108.1 g of highest mean weight and 9.8 cm length increment (Duray, 1998).

Although rabbitfishes constitute a major part of fisheries production, and some species are traditionally farmed in many countries, commercial farming has not yet been well developed in any of these countries. There is lack of information on the economics and sociocultural aspects of rabbitfish farming (Duray, 1998).

1.4 Environmental impacts

Aquaculture provides an annually increasing proportion of total fisheries production up to now from over 40% in 2010 (FAO, 2012). Half of the total aquaculture yield comes from land-based ponds and water-based pens, cages, longlines and stakes in brackish water and marine habitats (Primavera, 2006). Aquaculture practices rely upon the use of natural resources such as land and water that are parts of overall environment shared by other living beings (FAO, 2011). Besides providing employment, income and foreign exchange, aquaculture has been overshadowed by negative environmental effects (Primavera, 2006). The most common negative environmental impacts from aquaculture practices include alteration or destruction of natural habitats; discharge of aquaculture effluent leading to degrade water quality; misuse of chemicals; bycatch during collection of wild seed and broodstock; introduction and transmission of aquatic animal diseases; and the negative impact of escaped farmed fish on populations, communities and genetic diversity (Wu, 1995; Pillay, 2004; Primavera, 2006; FAO, 2011). Among the most important pollutant effects of aquaculture are the output of dissolved nutrients, suspended solids and organic matters

(Tovar et al., 2000). The expansion of intensive aquaculture has raised environmental concerns due to increasing nutrient accumulation in culture system and lack of effective waste treatment (Phillip et al., 1993; Briggs and Funge-Smith, 1994; Jackson et al., 2003).

The environmental impact of marine fish-farming depends very much on species, culture method, stocking density, feed type, and hydrography of the site and husbandry practices (Wu, 1995). In intensive shrimp aquaculture systems, a large proportion of the pellet feed is not assimilated by shrimp (Primevera, 1994); and approximately 10% of the feed is dissolved and 15% remains uneaten. The remaining 75% is ingested, but 50% is excreted as metabolic wastes, producing large amounts of gaseous, dissolved and particulate wastes (Lin et al., 1993). Shrimp (Penaeid) could only convert around 9 – 27% of total nitrogen input (intake water and feed) (Briggs and Funge-Smith, 1994; Funge-Smith and Briggs, 1998; Jackson et al., 2003; Lemonnier and Faninoz, 2006; Le and Fotedar, 2010), 5 – 13% of total phosphorus input (Briggs and Funge-Smith, 1994; Le and Fotedar, 2010), and 6 – 11% of carbon input to harvested biomass. A large proportion of total nitrogen input, 35 – 66% (Briggs and Funge-Smith, 1994; Jackson et al., 2003; Lemonnier and Faninoz, 2006; Le and Fotedar, 2010), and total phosphorus input, 46 – 65% (Le and Fotedar, 2010) would be discharged into the surrounding environment from shrimp farming.

Wu (1995) estimated 52 – 95% of nitrogen input into a marine fish culture system as feed may be lost into the environment through feed wastage, fish excretion, faeces production and respiration. Tovar et al. (2000) calculated the amounts of 9105 kg TSS (total suspended solids), 843 kg POM (particulate organic matter), 235 kg BOD (biochemical oxygen demand), 36 kg $N–NH_4^+$, 5 kg $N–NO_2^-$, 7 kg $N–NO_3^-$, and 3 kg $P–PO_4^{3-}$, dissolved in the seawater, that would to be discharged to the environment for each tonne of fish cultured.

The effluent discharged from intensive farming may lead to deterioration in receiving waters if the assimilative capacity of the environment is exceeded (Primavera, 2006). The effluent contains elevated concentrations of dissolved nutrients, suspended solids and organic matter (Ziemann et at., 1992; Tovar et al., 2000). The dissolved nutrients and organic matter would stimulate rapid growth of bacteria, phytoplankton and zooplankton (Lin et al., 1993). Furthermore, the composition of phytoplankton communities may be shifted by nutrients added to the water column from aquaculture farm wastes (Primavera, 2006; Thomas et al., 2010). The untreated wastes may promote hyper-nutrification and eutrophication, low dissolved oxygen, low pH, organic enrichment and turbidity as well as sedimentation in the

receiving waters (Burford et al., 2001, Thomas et al., 2010). In addition, organic matter settled on the bottom of the receiving waters may lead to the development of anoxic and reducing conditions in the sediment and the production of toxic gases (e.g. ammonia, methane and hydrogen sulphide) (Wu, 1995).

As a consequence, the polluted surrounding environment couples with the degraded water quality in culture systems have serious influences on aquaculture activities, cause farmed animal disease outbreak, and lead to reducing farming production and economic loss for farmers.

1.5 Aquaculture pond ecology

In tropical shrimp ponds, the increasing of feed input, concomitantly with the stocking shrimp biomass, induces an eutrophication of the ecosystem (Burford et al., 2003; Lemonnier et al., 2010; Lucas et al., 2010). Although difficult to maintain, its stability is required to guarantee the success of the culture. Two compartments can be taking into account to survey and analyse pond ecosystem, the water column and the sediment. A wide range of organic matter (loading from food input, organic fertilizer, excretion, organism metabolites, etc.) occurs in pond water (Boyd, 2002). In the oxygen rich water column organic matter can undergo chemical oxidation processes and relatively fast aerobic microbial degradation processes (Joyni et al., 2011), yielding dissolved nutrients to the water that support phytoplankton growth (Boyd, 1998; Lazur, 2007) (Fig. 1.3). Sedimentation of organic matter in ponds steadily increases during the culture cycle, mainly because the daily feed portion increases in concordance to the growing animal biomass (Torres-Beristain, 2005). Organic matter accumulates at the sediment-water interface, and microbial activity is very intensive in this surface layer (Massuda and Boyd, 1994; Boyd, 2002). The extend of decomposition of organic matter at the sediment-water interface is very high compared to that occurring in the water column (Hargreaves, 1998). Mineralization of organic matter and the consequent regeneration of nutrients at the sediment–water interface is important as a source of nutrients for microphytobenthos growth (Fig. 1.3) and recycle to the water column (Hargreaves, 1998; Buford and Longmore, 2001; Boyd, 2002).

Diffusion is one major mechanism controlling the fluxes of dissolved materials across the sediment water interface (Avnimelech et al., 1999; Joyni et al., 2011) (Fig. 1.3). The

movement of nutrients across the sediment-water interface may have a profound impact on the availability of nutrients to support primary production (Avnimelech et al., 1999).

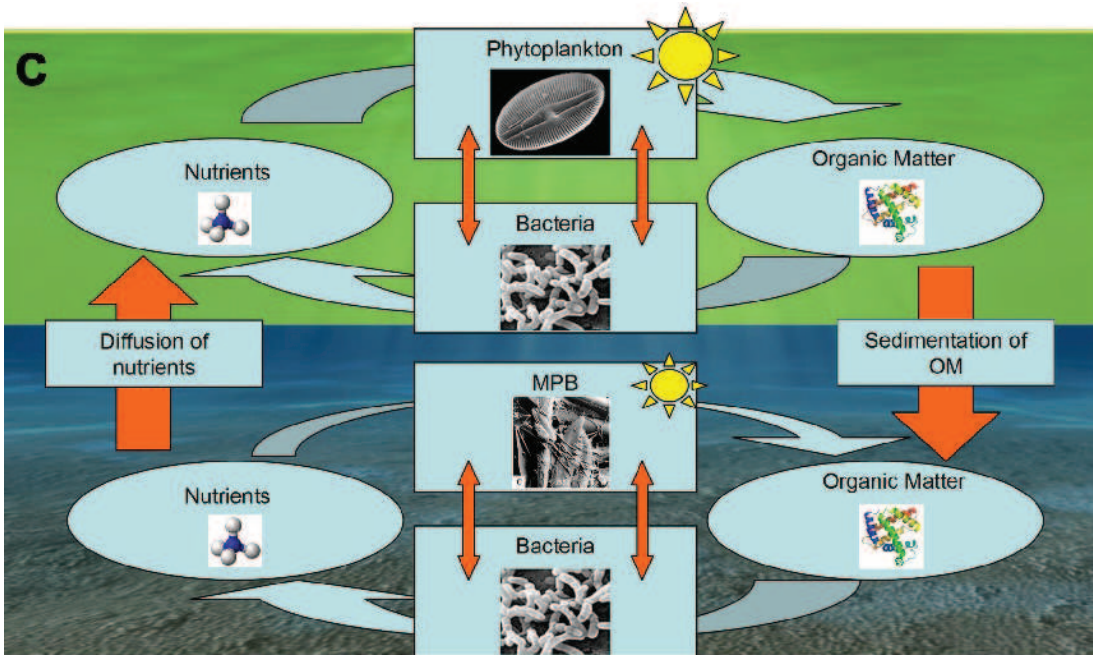


Figure 1.3: Scheme of exchange between sediment and water column in shallow ecosystem (Hochard et al., 2010 & 2012).

Materials settling from the water column to the sediment can be derived from two sources, including particulate organic matters, specific gravities are greater than water, and particles resuspended from the sediment bottom (Avnimelech et al., 1999). Settled particles in aquaculture ponds may be continuously resuspended by fish movement or foraging near the pond bottom (Avnimelech et al., 1999; Jiménez-Montealegre et al., 2002**)**. Sediment particles may be also periodically resuspended following exposure to a critical shear force from wind-induced water movements (Avnimelech et al., 1999). The cycling of organic matter in the pond is influenced by sedimentation and resuspension processes (Torres-Beristain, 2005).

Fish species, size, density, and foraging behavior (benthic feeding) are critical factors affecting sediment disturbance. Increased fish size and increased nutrient input, as time progressed, are positively correlated to increased sedimentation rate (Avnimelech et al., 1999). According to Jiménez-Montealegre et al. (2002), total solids sedimentation and resuspension rates are highly correlated to fish weight and biomass, chlorophyll a, total suspended solids, total feed input and Secchi disk visibility.

The process of bioturbation which involves both the dispersal of sediment particles and transport of interstitial pore water by benthic organisms is of global importance. Through

various activities (mainly feeding, burrowing, locomotion and ventilation), benthic fauna modifies the physical, chemical and biological properties of the sediment (Aller, 1982). Joyni et al. (2011) reported finfish, such as tilapia and pearlspot *Etroplus suratensis*, may be a natural control of bottom soil characteristics may improve shrimp production and add a valuable product to shrimp production in a shrimp polyculture system.

Principal sources of ammonia include fish excretion and flux derived from the mineralization of organic matter in the sediment and in the water column and molecular diffusion from reduced sediment (Fig. 1.3), and principal sinks for ammonia include phytoplankton uptake and nitrification (Hargreaves, 1998). Interactions between pond sediment and water are important regulators of nitrogen (N) biogeochemistry. Sediment represents a source of ammonia and a sink for nitrite and nitrate (Hargreaves, 1998).

The productivity/respiration (p/r) ratio of the habitats is an important control on the rates, direction (uptake, efflux) and composition dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), N_2 of N fluxes across the sediment water interface, with an efflux below p/r = 1.5 and an uptake above p/r = 1.5 (Eyre et al., 2010). Net heterotrophic communities tend to release N and phosphorus (P) to the water column, and N_2 to the atmosphere, with the rate of release determined by factors such as organic matter quantity and quality, temperature, and biological and physical advective flow (Cowan and Boynton, 1996; Kristensen and Hansen, 1999). In contrast, net autotrophic communities tend to remove DIN, dissolved inorganic phosphorus (DIP) (Rizzo et al., 1992; Sundback et al., 2000) and DON from the water column (Linares, 2005; Vonket et al., 2008) but can be also a source of DON (Ferguson et al., 2003) and enhance N_2 efflux (An and Joye, 2001). Heterotrophically dominated sediments have the potential to degrade water quality, whereas photoautotrophy in the sediments ameliorate this impact (Torres-Beristain, 2005). Microphytobenthos (MPB) can ameliorate water quality by stabilizing sediments and altering sediment-water nutrient fluxes. Sediments dominated by MPB have lower rates of ammonium, phosphate, and nitrate + nitrite release to the water column, and in many cases become sinks for nutrients rather than sources (Torres-Beristain, 2005).

For ecological purposes, photosynthesis and respiration may be thought of as reversible reactions. When photosynthesis is progressing faster than respiration during daylight, oxygen will accumulate and carbon dioxide will decline. At night, photosynthesis stops but respiration must continue during day and night, leading oxygen declines and carbon dioxide

increases (Boyd, 1998). Dissolved oxygen is one of the critical factors affecting processes and conditions at the sediment-water interface. Oxygen is consumed in ponds during aerobic bacteria break down of organic matter. A large accumulation of organic matter in pond soil increases oxygen demand and favors anaerobic conditions. Bacteria consume large amounts of oxygen and sediments become anoxic below the surface (Avnimelech and Ritvo, 2003). As anaerobic processes taking place, bacteria release reduced metabolites such as nitrite, ferrous iron, hydrogen sulfide, and various organic compounds (Boyd, 1995). Anaerobic conditions may affect aquaculture production both due to the unfavorable conditions at the pond bottom, or affect it through the diffusion of the reduced compounds from the sediment upward to the water column (Avnimelech and Ritvo, 2003).

1.6 Shrimp polyculture potential and practice

Reducing negative environmental impacts from aquaculture activities and maintain good environmental quality in ponds are the key issues for ensuring long-term sustainability of the industry (Troell et al., 2003). Different methods have been tried to minimize the effects of nutrient loading from intensive farming on the environment. The feasible methods have been widely applied are polyculture and integrated aquaculture (Casalduero, 2001). Polyculture refers to two or more species mixed in the same pond or aquaculture unit without partitioning (Martínez-Porchas et al., 2010). Numerous fish species used in aquaculture cover a wide range of feeding niches, including carnivorous, benthivorous, omnivorous, zooplanktivorous, phytoplanktivorous, and herbivorous niches (Bosma and Verdegem, 2011). Fish species in an ideal polyculture pond occupy different niches and possess feeding habits which are different from and complementary to each other, therefore are able to utilize food available in the pond more efficiently than single species (Yuan et al., 2010). The benefits of polyculture as already determined include the mitigation of ecological impacts and amelioration of yield and environmental quality (Chien and Liao, 1995; Martínez-Porchas et al., 2010; Yuan et al., 2010; Bosma and Verdegem, 2011).

Penaeid shrimp spend most of their life in contact with the bottom sediment (Dall et al., 1990) and have wide-range of food habits in natural systems. They have been described as omnivores, scavengers, detritus feeders, carnivores, and predators (Dall et al., 1990; Rothlisberg, 1998). The widely diverse feeding behaviors offer possibility to culture shrimp

in polyculture as either the main species or a secondary species (Jackson and Ozbay, 2008; Yuan et al., 2010).

Polyculture of shrimp with other species such as shrimp (Penaeid) (Martínez-Córdova and Pena-Messina, 2005), milkfish (*Chanos chanos*), mullet (*Mugil cephalus, Liza tade, L. parsia*) (Biswas et al., 2012), tilapia (*Oreochromis niloticus*, *Oreochromis* sp., hybrid tilapia) (Wang et al., 1998; García-Pérez et al., 2000; Uddin et al., 2008; Cruz et al., 2008; Yuan et al., 2010), mollusks (Martínez-Córdova and Matínez-Porchas, 2006), sandfish (*Holothuria scabra*) (Purcell et al., 2006; Bell et al., 2007), seaweed (*Kappaphycus alverazii*) (Lombardi et al., 2006), tilapia and mollusks (Tian et al., 2001) have been practised with the purpose of increasing overall production and controlling water quality.

Tilapia has been shown to feed on excess organic matter, improving water quality and thus increasing shrimp production (Wang et al., 1998). Juan et al. (2010) found that polyculture shrimp with red tilapia at suitable stocking densities and sizes can improve productivity, nutrient utilization, and environmental friendliness. García-Pérez et al. (2000) reported that prawn-tilapia polyculture increased economic returns by 21% when cultured in earthen ponds. Polyculture shrimp-tilapia has shown an important role to control luminous bacterial disease caused by *Vibrio harveyi*, possibly due to fish feeding on organic wastes and conversion to faeces; selective fish foraging to increase the dominance of beneficial phytoplankton; bioturbation of pond sediments; and release in the water column of antimicrobials, fungi, or competing bacteria from the skin and gut mucus of tilapia (Cruz et al., 2008). Antibacterial activity against luminous bacteria and gram-negative bacteria was found in mucus of some fish species of grouper, tilapia, milkfish, seabass and rabbitfish (Nagashima, 2001; Tendencia, 2006 a, b & c). Thus, the presence of these species inhibits growth of luminous bacteria and positively affects shrimp survival (Tendencia, 2006b). Of these species, rabbifish being herbivorous could be an excellent species for polyculture with shrimp (Tendencia, 2006a). However, it seemed that there is no practical polyculture shrimp with rabbitfish that has been conducted in laboratorial scale or in semi-intensive and intensive culture scales. Further studies are needed to determine the efficiency of shrimp and rabbitfish polyculture on technical and environmental aspects.

1.7 State of the aquaculture in New Caledonia

In the Pacific Islands, aquaculture is a relatively new development, which has been initiated

since 30 years ago in most areas. Shrimp (*Penaeus* spp.) farming has been a focus of commercial development in several islands with varying degrees of success (Adams et al., 2001). Tilapia (*Oreochromis niloticus*) aquaculture has entered the subsistence economy in some areas, such as Fiji Islands, Guam, Vanuatu, Samoa, American Samoa, Cook Islands and Northern Mariana Islands (Adams et al., 2001; Ponia, 2010). Seaweed (*Kappaphycus* spp.*)* is considered a future commercial export prospect by the region. By the 1980s, French Polynesia and New Caledonia were successful after investment of significant resources into technology, financing and marketing, in creating industries for two commodities, black pearl and blue shrimp. And now, large-scale commercial enterprises were established and these territories were regarded as world leaders in their fields (Ponia, 2010).

In New Caledonia, a French oversea territory in Pacific region, aquaculture has been developing since 1980s, mainly on shrimp farming. Farmed shrimp production achieved 2108 metric tonnes in volume (FAO, 2010) and 1.5 billion XPF (about US$ 17.25 million) in value in 2008 (SPC, 2010). Besides, about 45 metric tonnes of oysters (*Crassostrea gigas*) were produced for the local market in 1999 (Adams et al., 2001). New species have been domesticated and raised in captivity conditions in recent years, including sea cucumber, lobsters, crabs, giant clams, seaweed, pacific oysters and rabbitfish (*Siganus lineatus*) (SPC, 2010).

1.7.1 Shrimp culture development

Profitable aquaculture of penaeid shrimp has been established in New Caledonia for over 30 years (SPC, 2010) with only one shrimp species farmed, blue shrimp *Litopenaeus stylirostris* Stimpson (Mexican strain), and has developed significantly up until now. During the period 1999 to 2009, production fluctuated between 1680 and 2400 metric tonnes per year (Fig. 1.4) However, in the 2009/2010 production year, there was a sharp decline in production as a result of poor survival on most farms (AquaSol, 2011). Shrimp farming is almost practised in flow through semi-intensive ponds, stocking densities at 15 to 30 shrimp.m^{-2}; produce a yield of 4.2 – 5 tonne.ha^{-1}.y^{-1} (Galinié, 1989; Mermoud et al., 1998). Nowadays, there is 18 shrimp farms, with total 670 ha in area, locating along the west coast of the mainland, most of them in the southern Province, two processing plants, and two feed mills in New Caledonia, and about 1000 workers involving in the shrimp industry (SPC, 2010). Production of farmed shrimp is an important agricultural crop and a major exported product of New Caledonia.

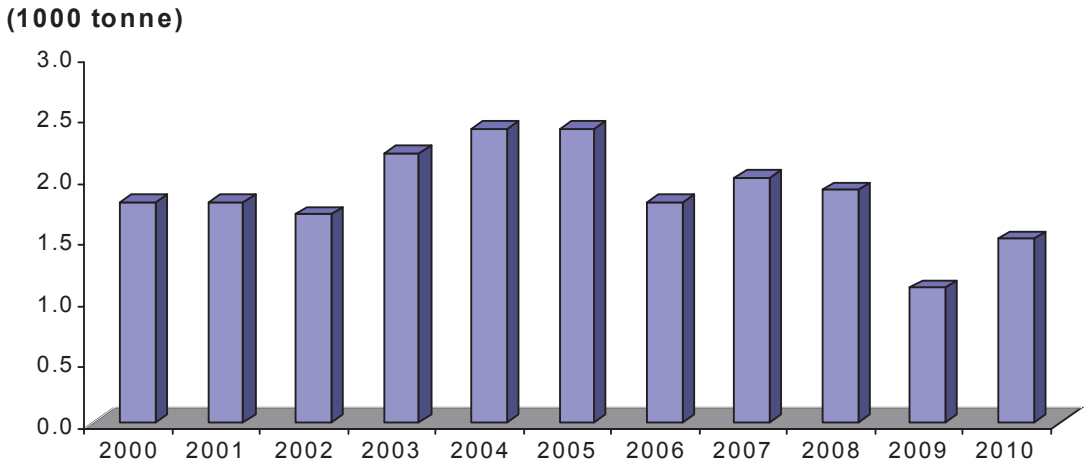


Figure 1.4: Yearly farmed shrimp production in volume in period 2000 – 2010, data: AquaSol (2011).

In historical development, from the 1980s to 1992, shrimp production increased progressively (538 tonnes in 1990 to 734 tonnes in 1992), resulted from improved techniques and expanded productive surface area. However, in 1993, the production unexpectedly decreased to 621 tonnes, due to abnormal mortalities associated with the pathogen named as "syndrome 93" or "winter syndrome". The vibriosis disease caused by *Vibrio penaeicida*, appeared during the period of drought and winter season (mid-May to mid-August) (Mermoud et al., 1998; Goarant et al., 1999). The mortalities were likely related to a variation in the rearing system, to unusual climate and to presence and virulence of pathogens, as well as the physiological condition of shrimp (Mermoud et al., 1998; Chim et al., 2008). The second pathogen observed since 1997, named as "summer syndrome", has affected shrimp farms in the hot season and associated another species of vibrio, *V. nigripulchritudo* (Goarant et al., 2006a). The highly variable environment could favor the expression and intensification of these diseases (Lemonnier et al., 2006). These two pathogens reduce the profitability and threaten the sustainability of the shrimp industry and are therefore its great concern.

1.7.2 Interest of polyculture development

To solve the problem of two vibriosis pathogens, especially "winter syndrome", the producers in New Caledonia have stocked an annual production cycle in order to avoid the cool season when mortality rates are particularly high. Approximately three-quarters of farm production are, therefore, reared from December to June/July (SPC, 2010). However, the solution is no longer suitably applied today due to free-used shrimp farms in a long time of year (August to November), which possibly leads to reduce expected aquaculture production

and to surfeit situation of human resource and facilities involving in the shrimp industry. Moreover, the summer syndrome is at that time, geographically restricted within one bay (the bay of Saint-Vincent). If it affected all shrimp farms in New Caledonia, the profitability of the industry would be seriously threatened (Goarant et al., 2006b).

New Caledonia has a number of native fish and invertebrates that could potentially be good targets for aquaculture such as sea cucumber, oyster, giant clam, crab, seaweed and fish (snapper, rabbitfish, grouper, etc). Nowadays, New Caledonia is looking to diversify its aquaculture production. Firstly, they are researching suitable species for stocking in the earthen ponds in the cool season to maintain the production around year. The rotation of crops between shrimp and other species could make full-used the earthen ponds to increase the aquaculture production, and also avoid shrimp high mortality occurrence from disease. The other way for sustainable development that they are trying is polyculture shrimp with other aquatic species, which could help to solve the problems facing the sustainable aquaculture by improving the rearing environment, minimizing shrimp pathogen, and increasing aquaculture production and benefit.

The study on polyculture shrimp with other species such as sea cucumber has been conducted in New Caledonia (Purcell et al., 2006; Bell et al., 2007). Purcell et al. (2006) reported that co-culture juveniles of sea cucumber (*Holothuria scabra*) and blue shrimp (*Litopenaeus stylirostris*) in earthen ponds appeared feasible, without adverse impact on shrimp production, presenting a cost-effective method for growing sandfish to larger sizes for restocking. Inversely, Bell et al. (2007) reported that grow-out of *L. stylirostris* with *H. scabra* in ponds is not viable. In co-culture *L. stylirostris* with *H. scabra*, high stocking density of sandfish juvenile had no significant effects on growth and survival of shrimp, but shrimp had a significant negative effect on survival and/or growth of sandfish in sand-bottom tanks, and furthermore all sandfish were dead or moribund after a month of grow-out trial in ponds (Bell et al., 2007).

Continuously, to find suitable species for polyculture with blue shrimp, which could be used in practical development, we conducted this study on feasibility of polyculture blue shrimp with goldlined rabbitfish and we obtained significant results that are described in following chapters.

1.8 Thesis general objectives

The shrimp industry in New Caledonia has not been highly profitable but has persisted mainly because of the lucrative, niche market for *L. stylirostris* in Japan and subsidies to farmers from the New Caledonian government (AquaSol, 2011). The decline in blue shrimp production in recent years was alarming to both the shrimp farming industry and the government. The government agency sought to ascertain possible reasons for the decline in shrimp production and desired to work with the producer association, and the shrimp farmers to improve the structure of the industry and to facilitate adoption of more efficient production methods (AquaSol, 2011). One of the efficient production methods to improve the shrimp industry and increase shrimp production is probably polyculture shrimp with suitable species, which could support shrimp growth and survival by stabilizing the rearing environment of shrimp ponds and preventing shrimp disease occurrence. Of aquatic species that have been domesticated and raised in captivity in New Caledonia, goldlined rabbitfish *Siganus lineatus* is considered a high potential candidate for polyculture with blue shrimp in earthen pond due to it is herbivorous, adapts well in captivity and would have the capacity to inhibit the growth of Vibrio. Goldlined rabbitfish showed a high growth rate in cage culture trials, reaching marketable size under one year (SPC, 2008). However, there are still some questions given when raise this species in a closed system as an earthen pond alone or with blue shrimp for polyculture. The questions include 1) how goldlined rabbitfish *Siganus lineatus* grow in a closed system, 2) how the environment vary in a *S. lineatus* culture system, 3) how *S. lineatus* affect blue shrimp *Litopenaeus stylirostris* growth performance when stocking for polyculture, and 4) how polyculture *L. stylirostris* and *S. lineatus* impact on production, environment and ecological functioning of a culture system. To find the answers, we carry out the present study. The main objectives of the study are as follow:

- Estimate the adaptive capacity of goldlined rabbitfish *S. lineatus* cultured in a closed system,

- Estimate the effects of stocking density on growth performance of *S. lineatus* and environment in a closed system,

- Estimate the technical feasibility of *L. stylirostris* and *S. lineatus* polyculture,

- Estimate the effects of *L. stylirostris* and *S. lineatus* polyculture on animal growth performances, environment and ecological functioning in a culture system,

- Estimate the effects of *L. stylirostris* and *S. lineatus* monoculture and shrimp-fish polyculture with high stocking biomass on production, environment and ecological functioning in a culture system.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Environmental conditions in New Caledonia

New Caledonia, in the southwest Pacific Ocean (Photo 2.1), is located in the southeast trade-wind belt, and its climate is oceanic tropical. The shrimp farms are located on the leeward side of the island from about 22° to 21° south latitude between Noumea (22° 18' S) and Voh (20° 58' S) (AquaSol, 2011).



Photo 2.1: The map of New Caledonia, source: www.infoplease.com.

Climate of the shrimp farming area varies during the year with a cool season (May to November) and a warm season (December to April). Average annual rainfall varies widely from place to place with a range of 1,000 to 2,000 $mm.y^{-1}$. Rainfall is typically higher in the warmer season, and it may exhibit large year to year variation (AquaSol, 2011). The shrimp farming is subjected to two clearly differentiated seasons with average water temperatures of 29 °C and 19 °C, respectively, between April and September and between October and May (Lemaire et al., 2002). In general, the northern location is slightly warmer and has more rainfall than the southern zone. Shrimp farms are supplied with water that is quite clear and near oceanic salinity (28 – 36) (slightly lower in the rainy season and a little greater in the dry season) (AquaSol, 2011). The potential for culture of *L. stylirostris* is favored by the slightly higher water temperature in ponds in the northern area. Díaz et al. (2004) reported that the optimum temperature for culture species is around 28 °C. So, water temperature is assumed to impose a major limitation on growth of shrimp in New Caledonia. It is also likely that the lowest water temperatures cause considerable stress to cultured shrimp (AquaSol, 2011), and sudden water temperature drop has an impact on the development of the shrimp disease in

New Caledonia (Mermoud et al., 1998; Wabete et al., 2008). The yearly air temperature for Saint-Vincent varies in a range from 9.5 to 36.0 ^{o}C (Fig. 2.1), the average of max and min temperatures are 28.7 and 18.0 ^{o}C, respectively. The highest temperature is in February and the lowest one is in July (Saint-Vincent Station, 2013).

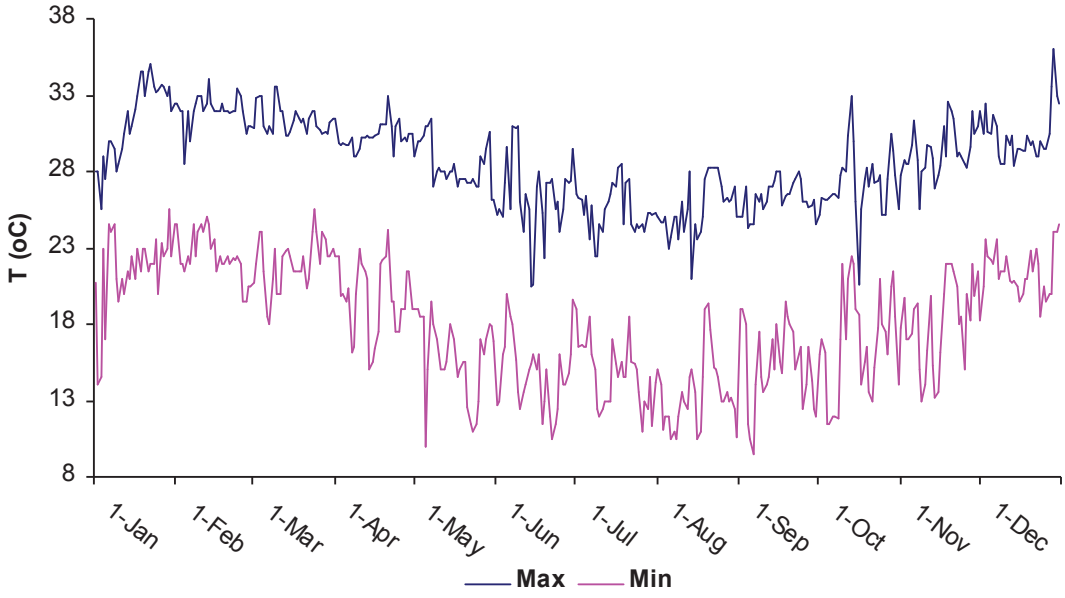


Figure 2.1: The variation of air temperature around year at Ouenghi, Saint-Vincent, source: Saint-Vincent Research Station.

A survey on shrimp farms recorded that the pH values ranged from 6.4 to 8.4, and thus most pond soils in New Caledonia do not require agricultural limestone treatment. Water supplies for shrimp ponds in New Caledonia are clear with low concentrations of suspended mineral particles (AquaSol, 2011).

Because of the low population density in rural areas, pollution is probably not a major problem. The main, country-level pollution problem is thought to be mining, particularly nickel mining. However, there is opportunity for pollution of the coastal waters near shrimp farms and hatcheries by suspended solids originating from erosion and by agricultural chemicals (AquaSol, 2011).

Shrimp farming in New Caledonia typically uses a flow-through system with water exchange rates as a tool to maintain optimum hydrological and biological parameters for the crop (Thomas et al., 2010). The effluent discharged from shrimp farm contains hydrobiological characteristics significantly higher than that of the receiving environment. Net loads of nitrogen export from the semi-intensive and the intensive farms amount to 0.68 and 1.36

$kg.ha^{-1}.d^{-1}$, respectively. Besides, the release of nutrients causes an increased eutrophication of the ecosystem, lead to a shift in composition of phytoplankton from offshore towards the coast (Thomas et al., 2010).

2.2 The research aquaculture station of Saint-Vincent

2.2.1 General description

Ifremer (French Research Institute for Exploration of the Sea) was established in New Caledonia since 1973. The Institute provides a primarily scientific and technical support to the livestock sector of the blue shrimp (*Litopenaeus stylirostris*). This support is applied research in rearing environment, and pathology and ecophysiology of shrimp. For this work, the research contributes to the sustainability of the shrimp industry in the territory. It also aims to diversify into matters relating to the lagoon environment, the valuation of resources and marine biodiversity, as part of a project "Lagoons of New Caledonia".

The Ifremer in New Caledonia consists of two components, Noumea site and the station of Saint-Vincent (Photo 2.2). The Noumea site is located on the campus of the Institute of Research for Development (IRD). Research resources and experimental facilities are located in the station of Saint-Vincent that is about 90 km north of Noumea, in the Boulouparis District. Formerly Aquaculture Station of Saint-Vincent, its facilities were in service since 1970. The station has contributed to the historical development of shrimp farming in New Caledonia, and handing majority of research in aquaculture.



Photo 2.2: Overview of Saint-Vincent Aquaculture Research Station, © J. Patrois, Ifremer 2011.

The station includes the departments of zootechny (pond and hatchery), ecophysiology, environment, pathology (pathogens, infections, epidemiology, clinical monitoring biosafety) and logistic. The ecophysiology focuses on some aspects of the physiology of shrimp in interaction with their breeding environment. Physiological studies focus on the breathing, management of different reactive oxygen species (oxidative stress), osmoregulation, nutrition and growth. These functions are studied at different stages of livestock from the larval stages (nauplius, zoea, mysis and post-larvae) through sub-adult (20 – 30 g) to the spawner (40 –70 g). The environmental researches are mainly focused on shrimp pond ecology and the impact on close environment (mangrove and lagoon). The zootechny support concentrates on reproduction broodstock at the station, the provision of biological materials and technical assistances to private hatcheries. The pathology department researches focus on the study of vibrio, and two bacterial species (*V. penaeicida* and *V. nigripulchritudo*) responsible for diseases affecting shrimp farming in New Caledonia. The clinical monitoring ensures the transfer of clinical monitors Aquaculture Technology Centre (CTA) and the implementation of biosecurity measures. The logistic supports the research teams in terms of infrastructure, information technology, fleet and orders from external providers (http://wwz.ifremer.fr).

2.2.2 Experimental facilities

2.2.2.1 Experimental closed system

The experimental closed system included 12 – 700 L outdoor circular fiberglass tanks (1.0 m^2, 70 cm in height) that set up at the experimental zone in the Saint-Vincent station (Photo 2.2). Sediment taken from salt-marsh near the station was mixed and spread evenly in all the tanks up to 10 cm (per tank). Each tank was equipped with a central standpipe for water outlet and a spherical air stone with a diameter of 4 cm suspended 5 cm above the bottom center. The tanks were shaded near 50% area above tank surfaces by green sheets to limit direct sunlight (Photo 2.3).



Photo 2.3: The experimental closed tanks set up at the experimental zone.

2.2.2.2 Experimental mesocosm system

The experimental mesocosm system includes 16 – 1600L outdoor circular mesocosm fiberglass tanks (1.7 m^2, 109.5 cm in height) that set up at the experimental zone in the Saint-Vincent station (Photo 2.2). Sediment taken from an earthen shrimp pond located at the station was mixed and spread evenly in all the tanks up to 20 cm (per tank). This sediment was clay-like in texture and its organic content was 1.2%. Each tank was equipped with a central standpipe for water outlet and a spherical air stone with a diameter of 4 cm suspended 10 cm above the bottom center (Photo 2.4).



Photo 2.4: The mesocosm system set up at the experimental zone, © J. Patrois, Ifremer 2011.

2.2.3 Characteristics of the water source

Water used for experiments of the study was pumped in Saint-Vincent lagoon and settled in a sedimentation pool (Photo 2.2). The table 2.1 shows the characteristics of the input water recorded during previous experimental trials.

Table 2.1: Characteristics of the water source measured during an experiment pumped in the lagoon and supplied to the experimental tanks, source: Ifremer.

Salinity	33.4-34.2
Dissolved oxygen (DO) ($mg.L^{-1}$)	6.3
pH	8.1
Turbidity (NTU)	3.5
Fluorescence ($\mu g.L^{-1}$)	3.99
Chlorophyll a ($\mu g.L^{-1}$)	0.63
Total ammonia nitrogen (TAN) ($\mu mol.L^{-1}$)	1.57
(NO_2+NO_3)-N ($\mu mol.L^{-1}$)	1.13
N-Urea ($\mu mol.L^{-1}$)	0.84
N-Organic ($\mu mol.L^{-1}$)	8.99
Soluble reactive phosphorus (SRP) ($\mu mol.L^{-1}$)	0.08

2.3 Characteristics of Penaeid and blue shrimp *Litopenaeus stylirostris* (Stimpson, 1974)

2.3.1 Biology and ecology

Penaeid shrimps are found throughout the tropical and subtropical regions, where they are one of the most valuable fishery resources, particularly in areas where conditions are favorable (mangroves, lagoons, wide shallow shelf areas, etc.) (Gacia, 1988; Dall et al., 1990; Motoh, 1995; Bonddad-Reantaso et al., 2005). Blue shrimp *Litopenaeus stylirostris* originates on the Pacific coast of Latin America and has a natural range extending from Peru to Mexico. During the late 1970s and early 1980s, the species was introduced to Hawaii and the eastern Atlantic coast of the Americas, from South Carolina and Texas to Central America and Brazil (Bonddad-Reantaso et al., 2005). The shrimp inhabits lagoons, estuaries and bays, places with a varying hydrography through the year (Díaz, et al., 2004). This species recently was commercially cultivated in United States, Central and South America, particularly in Ecuador and Mexico, and now essentially in New Caledonia and Brunei (Hernandez-Llamas et al., 1995; Lemaire et al., 2002).

Penaeid shrimps generally have an annual life cycle, with postlarval and juvenile stages of

their life cycle occur in estuarine waters of less than sea water salinity and settle in shallow areas rich in detritus, such as seagrass beds, mangrove swamps or floating sargasso whilst the adolescent, sub-adult, adult, egg and larval stages occur offshore in oceanic waters (Fig 2.2) (Gacia, 1988; Motoh, 1995; Montgomery, 2010). *L. stylirostris* has a typical penaeid life cycle in which the postlarval stages develop in coastal areas (Bondad-Reantaso et al., 2005).

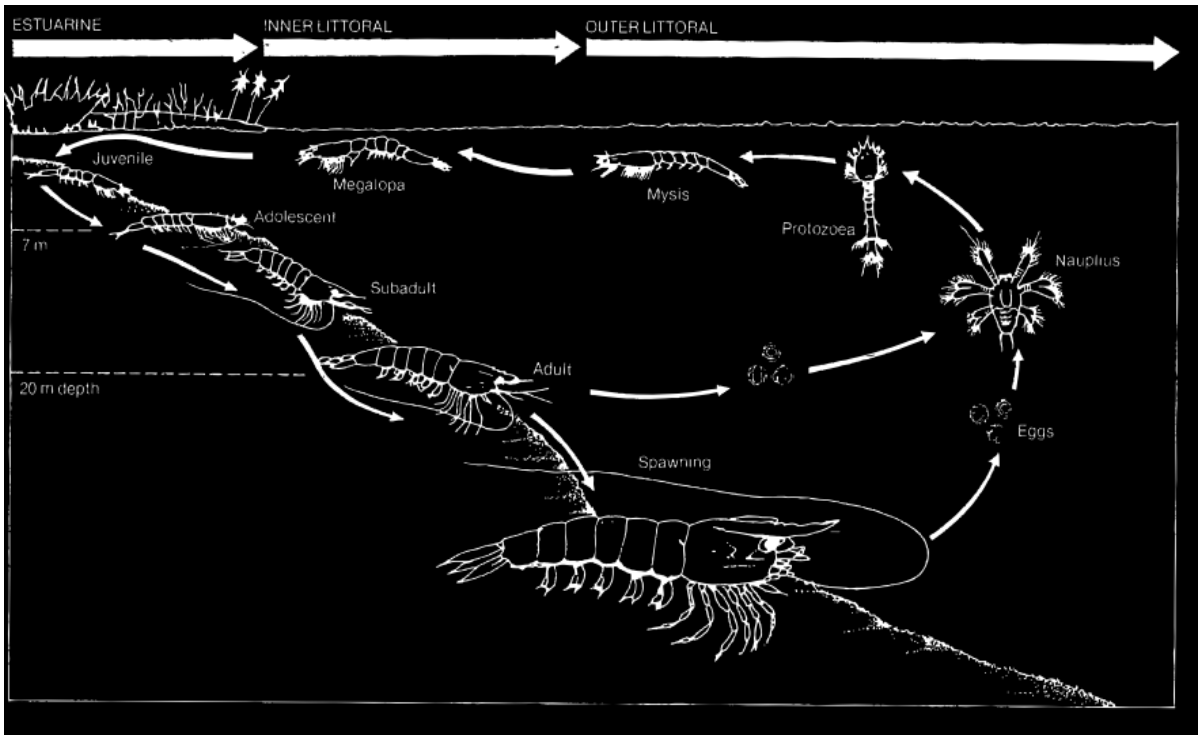


Figure 2.2: Diagrammatic representation of the life history of Penaeid shrimp, source: Motoh (1985).

Juvenile and adult shrimps eat a wide variety of microinvertebrates (gastropods, bivalves, crustaceans and polychaetes) and plant material (Dall et al., 1990; Motoh, 1995; Rothlisberg, 1998; Montgomery, 2010). They generally eat what is available, but in the wild they do not eat carrion (Rothlisberg, 1998). In aquaculture shrimp are fed artificial diets, and most digestion is rapid and complete in about six hours (Montgomery, 2010). Shrimp undergo an ontogenetic shift in diet in the different larval stages even within the progressing postlarvae stage (Rothlisberg, 1998). For instance, protozoea are generally herbivorous while mysis and postlarvae become increasingly carnivorous. When juveniles are small they eat mangrove detritus, microinvertebrates and some plant material, epiphytes on seagrasses, and even seagrass seeds; as they grow they eat larger invertebrates and less plant material. The diet also changes seasonally, depending on prey availability (Rothlisberg, 1998). *L. stylirostris* is omnivorous, preying on small marine invertebrates (e.g. worms, small crustaceans, etc.). When raised at high stocking densities, they can exhibit interspecific aggressive behavior;

however, in the wild, the expansive habitat should preclude this behavior (Bondad-Reantaso et al., 2005). Martínez-Córdova and Pena-Messina (2005) found that *L. stylirostris* had restricted sources of feed in the ponds, and is probably more carnivorous than *L. vannamei*.

Penaeid shrimps have a discontinuous growth pattern at individual levels, but the obvious lack of synchronism of moulting at the population level and the relatively high number of moults per year lead to the consideration that continuous growth models of the von Bertalanffy type are appropriate (Gacia, 1988). Growth is very fast and the maximum size, which varies from 15 – 16 cm total length in some smaller penaeid species (*Metapenaeus* spp., *Xiphopenaeus* spp.) to 30 cm in the giant tiger prawn, *Penaeus monodon*, is reached in about 2 years (Motoh, 1985; Gacia, 1988). For the first 6 to 9 months penaeids grow very rapidly and then reach a plateau, and the rapid growth period is exploited in aquaculture production (Rothlisberg, 1998). The growth of *L. stylirostris* is best between 23 – 30 ^{o}C, with optimal growth occurring at 30 ^{o}C for small (1 g) and 27 ^{o}C for larger (12 – 18 g) shrimp (Bondad-Reantaso et al., 2005). In fertilizered ponds, *L. stylirostris* fed pellet feed obtained growth range from 0.06 to 0.33 g.day^{-1}, average 0.22 g.day^{-1} (Hernandez-Llamas et al., 1995) and when cultured in tanks gained higher growth, range 0.17 – 0.35 g.day^{-1}, average 0.26 g.day^{-1} (Kumaraguru vasagam et al., 2009).

L. stylirostris is not so tolerant of low salinities, but tolerate a wide range of temperatures. The species also tolerate temperatures down to 15 ^{o}C and up to 33 ^{o}C without problems, but at reduced growth rates (Bondad-Reantaso et al., 2005). Wabete et al. (2008) reported that the low edge of the thermopreferendum for *L. stylirostris* is 20 – 22 ^{o}C, and shrimp is thermally stressed and mortality of up to 70% after 2 days at 20 – 22 ^{o}C. *L. stylirostris* can tolerate colder temperatures than *L. vannamei*, *P. monodon* and *Fenneropenaeus indicus*, but requires higher oxygen levels (Bondad-Reantaso et al., 2005). The optimum temperature for *L. stylirostris* is reported to be about 28 ^{o}C (Díaz et al., 2004; Bondad-Reantaso et al., 2005). Díaz et al. (2004) emphasized that thermal stress of *L. stylirostris* would increase as temperatures decreased or increased with respect to the optimum temperature of 28 ^{o}C.

2.3.2 Animal behaviour and rearing possibilities

The behavior of penaeids can be divided into activities related to the physiological functions, such as feeding, moulting, or reproduction and activities that are responses to such environmental factors: light, tide, or temperature (Dall et al., 1990). Penaeids live in the

sediment and burrow in the substratum during the day, and at night emerge from it for such activities as feeding, moulting and reproducing (Dall et al., 1990; Montgomery, 2010). Penaeids spend most of their life in contact with the bottom sediment, and generally favor fine sediments and most species prefer substrata with high mud content, probably because they are easier to burrow in (Dall et al., 1990; Montgomery, 2010). In culture ponds, shrimp normally live on or near the bottom, are exposed to conditions on the pond bottom. Exposure to toxic materials endanger the well being of the cultured shrimp, leading to reduced feeding, slower growth, mortality and possibly higher sensitivity to disease (Avnimelech and Ritvo, 2003).

The mariculture of shrimp may provide one of the best opportunities for polyculture and integrated systems due to shrimp require higher water quality standards than many other cultured species and, thus, would benefit from a more stable ecosystem. The ability of shrimp to use a broad spectrum of the food web would allow them to be co-cultured with a number of other species. Feed and fertilization management can be directed to support the food web to produce food items that shrimp prefer, rather than relying on the direct consumption of pellet feed (Jackson and Ozbay, 2008).



Photo 2.5: Blue shrimp *Litopenaeus stylirostris*, unique Penaeid species commercially cultured in New Caledonia, source: wwz.ifremer.fr.

2.3.3 Environmental characteristics of the rearing structure

Temperature and salinity of the water are among the most important environmental factors

that affect the life of the penaeid shrimp. The adaptative capacity of the penaeids are specific and determined by a number of factors that have caused shrimp species to be distributed differently in the marine-estuarine gradient (Díaz et al, 2004**)**. Key water quality parameters for shrimp culture are dissolved oxygen, pH, salinity, nitrate, ammonia, nitrite, biochemical oxygen demand, and hydrogen sulfide. Lazur (2007) recommended the water quality criteria for shrimp culture, as follows temperature 26 – 30 ^{o}C, salinity 15 – 32, dissolved oxygen > 4 mg.L^{-1}, pH 7.0 – 8.5, ammonia (NH_3) < 0.15 mg.L^{-1}, nitrite (NO_2) < 4.5 mg.L^{-1}, hydrogen sulfide (H_2S) < 0.1 mg.L^{-1}. Shrimp are stressed when dissolved oxygen (DO) falls below 2.0 mg.L^{-1}.

The recommended ranges for salinity and temperature for growing *L. stylirostris* are 25 – 38 and 20 – 30 °C, respectively (Spanopoulos-Hernández et al., 2005). To optimize the culture of *L. stylirostris* in controlled conditions, however, Díaz et al. (2004) proposed that it should be cultivated in salinity of 25 and temperatures of 28 ^{o}C because these are considered the optimal conditions, and this environment also is free of stress, and for this reason the growth of shrimp is increased. *L. stylirostris* subjected to diurnal cycles of 1.5 mgO_2.L^{-1} to saturation showed lowered survival and growth, and their molting cycle was modified (Aquacop et al., 1988). Mugnier and Soyez (2005) observed no mortality of *L. stylirostris* occurred in tanks maintained under normoxia at both 28 and 22 ^{o}C, and mortality started 24 min after oxygen level reached 0.6 mgO_2.L^{-1} at 28 ^{o}C and after 135 min at 22 ^{o}C when oxygen level reached 0.45 mgO2.L^{-1}, 50% of the shrimps died after 100 min at 28 ^{o}C and 0.6 mgO_2.L^{-1}, and after 153 min at 22 ^{o}C and 0.45 mgO_2.L^{-1}. Re and Díaz (2011) reported that growth, oxygen consumption, ammonium excretion, scope for growth and apparent heat increment of *L. stylirostris* were significantly affected when exposed at different oxygen concentrations (2 – 6 mgO_2.L^{-1}) at 28 ^{o}C, and, thus, recommend maintaining *L. stylirostris* at 6 mgO_2.L^{-1}. Mugnier and Soyez (2005) reported that no mortality of *L. stylirostris* occurred within a range of DO concentrations from 1 – 5 mgO_2.L^{-1} (28 ^{o}C), but shrimp hypo-OC was significantly negatively affected as DO concentrations below 3 mgO_2.L^{-1}. *L. stylirostris* showed a short-term stress response when exposed to pond sediments where TAN in pore water was around 8.51 mg.L^{-1} (607.9 µM) (Mugnier et al., 2006). Lemonnier et al. (2006) reported an early eutrophication of water was observed concomitantly with the beginning of the mortality outbreaks in *L. stylirostris* ponds, indicating this could play a role by inducing directly or indirectly a stress for shrimp and/or a growth and/or virulence factors of the pathogen.

The farming of *L. stylirostris* in New Caledonia is subjected to two clearly differentiated seasons with average water temperatures of 29 and 19 ^{o}C, respectively, between April and September and between October and May. Within these average temperatures, nyctemeral fluctuations can reach 7 ^{o}C (Lemaire et al., 2002). The seasons of abrupt water temperature changes correspond to shrimp mass mortalities known as 'syndrome 93' (Mermoud et al., 1998). Apart from the decreased yield originating from the high mortality related to 'syndrome 93', the cold season is also characterized by the production of animals of significantly lower quality (Lemaire et al., 2002).

2.4 Characteristics of Siganidae and goldlined rabbitfish *Siganus lineatus* (Valenciennes, 1835)

2.4.1 Biology and ecology

Generally, rabbitfishes (Siganidae) are gregarious, often occur in large or small school on and around grass or reef flats (Lam, 1974), in reef front, mangroves, estuaries and estuarine lakes (Borsa et al., 2007), and in shallow water over sandy and rocky substrates (Popper and Gundermann, 1975).

The juveniles and adults are primarily herbivorous, they browse on a variety of benthic algae, mangrove roots and sea grass (Lam, 1974; Von Westernhagen, 1974; Gundermann et al., 1983; Duray, 1998). Feeding takes place most actively in the dim light of morning and evening for *S. luridis* and *S. rivulatus* (Popper and Gundermann, 1975), or during the day and in the evening for *S. vermiculatus* (Gundermann et al., 1983). Chitravadivelu and Sivapalan (1984) reported that goldlined rabbitfish *S. lineatus* is purely a herbivore and prefers to feed during night. Von Westernhagen (1973) found that four species of rabbitfishes, *Siganus concatenatu, S. oramin, S. striokzta* and *S. virgata* were held in rearing tanks and fed with benthic algae, ate only 67 species of 101 algae species and 4 vascular plants offered. However, rabbitfishes are also potentially omnivorous. Some observations indicated that siganids are not obligatory herbivores (Lam, 1974). *S. argentus* was found feeding waste meat scraps. *S. lineatus* is often found with large quantity of sponge in its stomach and intestine. It is also reported that in stomach contents of rabbitfish, animal matters is sometimes found mixed with algae. In captivity, *S. canaliculatus* showed to feed on all kinds of food offered, such as aquatic plants, chicken food pellets, cooked rice, dried shrimps and

even fish scraps (Lam, 1974). *S. rivulatus* kept in aquaria readily feed on a great variety of food stuff such as chopped fish or mollusks, fish meal, and pellets (Ben-Tuvia et al., 1973). Besides pellet feed, *S. canaliculatus* and *S. guttatus* feed voraciously on live halved mussels as introduced into the tanks (Von Westernhagen and Rosenthal, 1975).

Rabbitfishes reach their maximum size differently of each species (Darsono, 1993). *S. canaliculatus* in the Philippines grows up to 22 cm in length, and in Indian coasts grows to about 25 cm (Von Westernhagen and Rosenthal, 1976). *S. guttatus* has been reported to grow to more than 50 cm in length (Von Westernhagen and Rosenthal, 1976); it can attain a weight of more than 1 kg and grows fastest in floating cages (Juario et al., 1985). *S. spinus* is as a rather small species, maximum 22 cm. *S. virgatus* does not grow very large, reported length to 19 cm. *S. punctatus* is one of the larger members of the rabbitfish family, and attains a length of over 30 cm. *S. fuscescens* is rarely larger than 22 cm (Von Westernhagen and Rosenthal, 1976), while *S. vermiculatus* is one of the largest of the rabbitfishes, females can reach 45 cm and weigh 2.3 kg (Gundermann et al., 1983). *S. concatenuta, S. oramin, S. striolata* and *S. virgata* can reach an approximate size of 35 – 40 cm as fully grown (Von Westernhagen, 1974). Goldlined rabbitfish *S. lineatus* has a larger size than some species of Siganidae. Its common size is 25 – 30 cm and up to 43 cm in maximum size (Fishbase, 2005).

Growth rates of Siganidae are not similar. *S. canaliculatus* in Tanzania can reach a marketable size of 20 cm fork length in 6 months (Bwathondi, 1982), and grows to about 120 g in about 9 – 11 months in Singapore (Lam, 1974). *S. rivulatus* weighs about 150 g at the end of its first year in the Mediterranean coast (Ben-Tuvia et al., 1973), while it grows from 3 to 150 g in 105 days in extensively managed sea cage system in Red Sea (Lichatowich et al., 1984). *S. lineatus* in cage culture trials showed that it reaches commercial size 300 g in less than one year (SPC, 2008).



Photo 2.6: Goldlined rabbitfish *Siganus lineatus*, a herbivorous fish considered as an important candidate for co-culture with shrimp.

Rabbitfishes have high tolerance of a fairly wide range of changes in salinity and temperature (Lam, 1974; Duray, 1998). *S. canaliculatus* can suffer direct transfer to at least one-third sea water and can be adapted gradually to live in low salinity, down to 5, and can be maintained in open tanks where the temperature of the water may vary from 25 to 34 ^{o}C. (Lam, 1974). Popper and Gundermann (1975) stated that juvenile *S. rivulatus* and *S. luridis* are able to survive in salinity up to 50. Mortality was 28% for *S. guttatus* after 2 days in freshwater, whereas *S. spinus* had total mortality in 3 days in salinity at 2 (Duray, 1998). Bwathondi (1982) observed that *S. canaliculatus* grew well at salinities ranging from 23 to 35.8 in the creek area. This wide range of salinity tolerance is of advantages to the culture of this species in estuaries and creeks where salinity regularly fluctuates. Rabbitfishes are generally capable of adapting to reduced oxygen concentration (Ben-Tuvia et al., 1973), the lower limit is 2 mg.L^{-1} for *S. canaliculatus* (Lam, 1974), 0.7 mg.L^{-1} for *S. guttatus*, 1.0 mg.L^{-1} for *S. argenteus*, and 2.0 mg.L^{-1} for *S. vermiculatus* (Duray, 1998). The variation in tolerance to low oxygen is related to the differences in metabolic rate among species. *S. rivulatus* stops feeding at 14 and 36 ^{o}C, and its maximum growth rate is at 27 ^{o}C (Saoud et al., 2008b).

2.4.2 Animal behaviour and rearing possibilities

Rabbitfishes are divided into two groups of species on the basis of behavioral characteristics, habitat, and coloration (Gundermann et al., 1983). One group includes species that live in pairs, are site-tenacious, brightly colored and associated strictly with coral reefs. These coral-dwelling species are fragile, sensitive to physico-chemical changes and usually show interspecific aggressive behavior; a typical example is *S.corallinus* (Gundermann et al., 1983; Duray, 1998; Borsa et al., 2007). The other group includes species which school at some stage in life, move over substantial distances, and are gray or drab. They are strong and apparently resistant to considerable variations in salinity and temperature. Typical examples are *S. canaliculatus*, *S. luridis* and *S rivulatus* (Gundermann et al., 1983; Borsa et al., 2007). These schooling species are important food fishes and currently the subject of a number of mariculture studies (Duray, 1998).

Ben-Tuvia et al. (1973) observed that rabbitfishes are tolerant of rough handling and overcrowding, which allow to stock somewhat high density in captivity for culture. Lam (1974) characterized rabbitfishes are gregarious, swimming and feeding in schools whose size seems to vary not only among species but also among schools of the same species. Fry often occur in great abundance during certain seasons, so they may be collected easily at that time (Lam, 1974). Rabbitfishes are wary and seem easily frightened and to be attracted by diffuse light. They may be immobilized at night as being shone a flashline directly upon them. Besides, Rabbitfishes can tolerate any type of pond soil, as long as vegetation is present, and seem to adapt well to captivity (Lam, 1974). These behaviors along with biological and ecological characteristics refer high suitability and feasibility of rabbitfish culture in coastal ponds, tanks, enclosures, and floating cages.

2.4.3. Environmental characteristics of the rearing structure

As possessing suitable characteristics, rabbitfishes can be reared to marketable size in suitably designed holding structures such as ponds, tanks or enclosures in natural area and in floating cages (Lam, 1974). The ideal pond for rabbitfish is one that directly draws brackish water from a river. A slight rain or even a shower can kill the fish if pond water is shallow and not refilled with fresh brackish water (Duray, 1998).

The cages should be placed in well-protected areas where the water is exposing to plentiful

air circulation and has a depth of at least 3 m at the lowest tide. It should be the free-pollution area, and the water temperature should be 28 – 32 °C, and salinity of 15 – 27 (Duray, 1998). Yousif et al. (2005) cultured *S. canaliculatus* in sea net cages at water temperature ranged 29 – 33 ^{o}C (max. 35 ^{0}C), salinity ranged 50 – 53 (max. 55), dissolved oxygen 4.7 – 6.0 mg.L^{-1}, and pH 8.0 – 8.6. Bwathondi (1982) reared *S. canaliculatus* in wooden cages located in a coastal creek with dissolved oxygen ranged 3.7 – 6.4 mg.L^{-1}, and salinity ranged 30.0 – 33.5. Popper and Gundermann (1975) found that *S. rivulatus* grows in a salinity of 30 has a greater weight gain than those kept in 20 and 50. Saoud et al. (2008b) reported *S. rivulatus* is a eurythermal fish whose optimal temperature for growth is 27 ^{o}C, and stops feeding at 14 and 36 ^{o}C.

CHAPTER 3

FEASIBILITY OF GOLDLINED RABBITFISH S*iganus lineatus* CULTURE IN EARTHEN POND: A MESOCOSM APPROACH

3.1 Introduction

Siganidae (Rabbitfishes) is a family of 28 marine herbivorous species, inhabiting the Indo-West Pacific (Lam, 1974; Duray, 1998; Borsa et al., 2007). Four species also occur in the Red sea, two of which (*Siganus luridus* and *S. rivulatus*) have migrated through the Suez Canal and large populations established in the eastern Mediterranean (Lam, 1974; Popper and Gundermann, 1975; Bariche, 2005). Rabbitfishes traditionally contribute an important part to commercial fisheries production in some countries, and have recently attracted the attention of commercial mariculture in many areas of their distribution (Lam, 1974; Duray, 1998; Bariche, 2005). Many studies have been conducted on biological and ecological aspects of rabbitfishes for mariculture (Ben-Tuvia et al., 1973; Lam, 1974; Popper and Gundermann, 1975; Von Westernhagen and Rosenthal, 1976; Gundermann et al., 1983; Wassef and Addul Hady, 1997; Duray, 1998; Bariche, 2005; Jaikumar, 2012). The authors characterized rabbitfishes as suitable candidates for mariculture for a numbers of reasons, including their desirability as excellent food fish and their high demand and market. In addition, rabbitfishes are primarily herbivores but may turn to other diets readily. Thus, in captivity they have shown to feed on a wide variety of foods offered, and grow rapidly on a variety of natural foods or artificial food pellets (Ben-Tuvia et al., 1973; Lam, 1974). Some species are gregarious and thus may be able to tolerate crowded conditions. They also have tolerance of changes in salinity and temperature (Ben-Tuvia et al., 1973; Lam, 1974; Durray, 1998).

Many attempts have been done in order to learn more about culturing rabbitfishes. For stocking in culture systems (coastal ponds, enclosures, tanks or floating cages), rabbitfish fry can be collected with large numbers from coastal waters during recruitment seasons. Besides, many studies have been successful in spawning and rearing larvae to juvenile of some species, such as *Siganus canaliculatus* and *S. rivulatus* (Lam, 1974), *S. vermiculatus* (Popper and Gundermann, 1976; Popper et al., 1976), *S. lineatus* (Bryan and Madraisau, 1977; SPC, 2008), *S. guttatus* (Juario et al, 1985; Hara et al., 1986; Duray and Juario, 1988; Duray, 1998). The successes of juvenile reproducing help to limit relying natural seed for stocking and promote further development of rabbitfish mariculture.

Several studies have been carried out on the food and nutrient requirements of rabbitfishes in captivity. Rabbitfishes in nature are browsing herbivores feeding in large to small schools on variety of benthic algae and occasionally on seagrass. However, they grow faster on pellet diets than on ordinary seaweed (Bwathondi, 1982). Based on the omnivorous potential of

rabbitfishes, Parazo (1990) tried to look for economical formulated feed for commercial scale and showed that a diet with 35% protein and 3832 kcal/kg energy was found to be the best for rabbitfish fry. Ghanawi et al. (2011) found that the dietary lipid requirement for optimal growth in juvenile marbled spinefoot *Siganus rivulatus* is 9.8%. Furthermore, soybean oil may be a suitable dietary lipid source for *S. canaliculatus*, and can replace up to 67% or 45% of total dietary fish oil without negatively compromising growth performance or nutritional quality of fish, respectively (Xu et al., 2011).

Many species (*Siganus canaliculatus*, *S. fuscescens*, *S. guttatus*, *S. punctatus*, *S. spinus*, and *S. virgatus*) have already been farmed in coastal ponds in the Philippines either in monoculture or co-culture with milkfish (*Chanos chanos*) (Lam, 1974; Von Westernhagen and Rosenthal, 1976; Duray, 1998). Nowadays, rabbitfish mariculture has been widely expanded in many countries, under diversity of suitable designed structures. For example, *Siganus randalli* in Guam and *S. fuscescens* in Taiwan have been cultured in coastal ponds (Nelson et al., 1992); *S. rivulatus* in the Red sea and Mediterranean region (Ben-Tuvia et al., 1973; Lichatowich et al., 1984; Stephanou and Georgiou, 2000; El-Dakar et al., 2010); *S. canaliculatus* in UAE (Yousif et al., 2005), East coast Africa (Bwathondi, 1982), and India (Jaikumar et al., 2011; Jaikumar, 2012); *S. randall* in Guam (Brown et al., 1994), and *S. lineatus* in New Caledonia (SPC, 2008) have been cultured in sea cages. Yet rabbitfish aquaculture has not advanced on a commercial scale, possibly due to the fishes grow slowly but mature early and are difficult to handle (Von Westernhagen and Rosenthal, 1976; Duray, 1998). Furthermore, there was lack of economical formulated feeds (Parazo, 1990), and many aspects of rabbitfish performance in different grow-out facilities remained unsolved (Yousif et al., 2005). Almost studies on rabbitfish grow-out were conducted in the cages or ponds/tanks with water flow through and mainly focused on production performance. The environmental variations in culture systems as well as the mutual effects between the environment and rabbitfish production have not been well reported. More researches are needed to carry out to evaluate the effects of rabbitfish culture on the environment and *vice versa* the effects of environment on rabbitfish growth and survival.

Several species (*Siganus argenteus*, *S. canaliculatus*, *S. doliatus*, *S. lineatus*, *S. punctatus*, *S. randalli*, and *S. woodlandii*) (Lam, 1974; Thollot et al., 1999; Randall and Kulbicki, 2005; Thibeaud, unpublished data) are found in New Caledonia, of which *S. lineatus*, reffered to as goldlined rabbitfish or 'Picot Rayé', is the biggest and favourite species. It reaches 14 - 18 $US (1300 - 1800 XPF) per kg in local market and its current production (fishing only) is

around 50 tonnes per year (SPC, 2008). Research on this species started in 2003 in collaboration with the *Laboratoire d'Etudes des Resources Vivantes et de l'Environnement Marin* (LERVEM) at the University of New Caledonia. In 2004, a pilot scale hatchery was installed to demonstrate technical feasibility. During the initial phases of the research, broodstock was routinely secured and a good understanding of their maturation was developed. A complete goldlined rabbitfish life cycle was achieved repeatedly during the initial trials and specific requirements for live phytoplankton and zooplankton during larval rearing were developed. Cage trials proved that *S. lineatus* reaches commercial size (300 g) in less than a year (SPC, 2008). As having high demand and market price, goldlined rabbitfish attracts increasing attention from the government and aquaculture enterprises for commercial scale development. In the context of the sustainability of shrimp industry was threatened by bacterial pathogens. New Caledonian authorities are attempting to diversify aquaculture species, and goldlined rabbitfish emerges as an important candidate for beneficial and sustainable development. So, we conducted the study: "feasibility of goldlined rabbitfish *Siganus lineatus* (Valenciennes, 1835) culture in earthen pond: a mesocosm approach". However, some questions remain as rabbitfish cultured in such closed system, including 1) how goldlined rabbitfish grows in a closed system at different stocking densities, and 2) how goldlined rabbitfish culture affect the environment in a closed system. To answer theses question, the objectives of the study include:

- Estimate the adaptive capacity of goldlined rabbitfish *S. lineatus* under culture conditions of a closed system,
- Estimate the effects of different stocking densities on *S. lineatus* growth performance in a closed system,
- Estimate the effects of *S. lineatus* culture on the environmental quality in a closed system.

The results of this study would be useful for determining whether *S. lineatus* is a good candidate for commercial culture and/or particularly being a suitable species for co-culture with blue shrimp *Litopenaeus stylirostris* in earthen ponds.

3.2 Materials and methods

3.2.1 Preliminary trial

The trial was conducted in the closed system as described in the paragraph 2.2.2.1 of chapter

2. The experimental tanks were filled with fresh seawater up to 50 cm one week before stocking. Neither aeration nor water exchange was applied.

S. lineatus juveniles (4.8 ± 1.0 g, 6.5 ± 0.5 cm in total length, TL), hatchery-reproduced, were randomly stocked at three different densities to form three treatments, including low density (LD) (8 fish.m^{-2}, also 8 fish.$tank^{-1}$); medium density (MD) (16 fish.m^{-2}) and high density (HD) (24 fish.m^{-2}). All treatments were randomly distributed among tanks with four replicates per treatment. Fish in all tanks were fed with pellet feed (35-40% protein, SICA Manufacturer, New Caledonia), twice daily at 8:00 am and 16:00 pm, with a feeding rate of 10% of fish biomass per day.

Water temperature and dissolved oxygen (DO) concentrations were recorded twice daily (07:30 am and 15:00 pm) at mid depth of each tank using an OxyGuard meter (Handy Polaris, Birkerod, Denmark). Salinity was measured daily (08:00 am) using refractometer (Cond 3210, Welheim, Germany). Turbidity, fluorescence and pH were measured twice a week using turbidimeter (TN-100, Eutech Instruments, Singapore), Aquafluor (Turner Designs, Sunnyvale, CA. USA), and pH meter (pH 197i, Welheim, Germany), respectively. A day before stocking, water samples (1 L for each tank) were collected (08:00-08:15 am) and filtered through pre-combusted (450 ^{o}C, 4 hrs) GF/C Whatman fiberglass filters (47mm), then analysed for total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Koroleff, 1976) and soluble reactive phosphorus (SRP) (Murphy and Riley, 1962).

The trial was lasted three days after stocking because of fish mortality occurrence in the early morning of the fourth day in the HD treatment.

3.2.2 Experimental design

This experiment was conducted in the same closed system. The tanks were filled with fresh seawater one week before stocking up to 50 cm (500 L in volume). Aeration was continuously supplied into the tanks via 4 cm diameter spherical air-stones hanging 5 cm above bottom centers, one air-stone per tank. No water exchange was applied during the experiment.

S. lineatus juveniles (5.7 ± 1.2 g, 6.8 ± 0.5 cm TL), hatchery-reproduced, were randomly stocked at three different densities to form three treatments, including low density (LD) (7

fish.m-2, also 7 fish.tank^{-1}); medium density (MD) (14 fish.m^{-2}) and high density (HD) (21 fish.m^{-2}). All treatments were randomly distributed among tanks with four replicates per treatment. Fish were fed with commercial pellet feed (35 – 40% protein, SICA Manufacturer, New Caledonia), twice daily at 8:00 am and 16:00 pm, with a feeding rate of 3–5% of fish biomass per day during the experiment. Every two weeks, all fish in a replicate per treatment were captured and individually weighted, and the daily amount of feed was adjusted accordingly. To avoid overfeeding and underfeeding, the pellet feed was put in feeding trays (30 cm diameter) set on tank bottoms to observe feeding activities and feed amount consumptions. The experiment lasted for 8 weeks (May-July, 2012) from stocking to harvesting.

3.2.3 Sampling and analyzing

At stocking, 30 fish were randomly sampled, individually weighed and measured. At harvesting, all fish in each tank were counted, individually weighed and measured. The weight was scaled to the nearest 0.1 g using an electronic balance, and the total length (TL) was measured to the nearest 0.1 cm using a technical ruler.

The average weight of fish and the coefficient of variation (CV = SD/mean) for weight of individual fish within each tank were compared among treatments. The length and weight measurements were used to calculate Fulton's condition index: $K = 100 * W/Lth^3$, where W is the weight (g), and Lth is the total length (cm).

Fish growth performance was evaluated in terms of survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), and yield.

SR (%) = harvesting number/stocking number*100

DWG (g.day^{-1}) = Weight gain (g)/time (days)

SGR (%.day^{-1}) = (Ln Wf – Ln Wi)/time (days)*100

Yield (g.m^{-2}) = harvesting biomass (g)/area of culture tank (m^2)

where Wi: initial mean weight (g), Wf: final mean weight (g)

Food conversion ratio (FCR) was calculated as followed:

FCR = total feed fed (dry weight, g)/total weight gain (fresh weight, g)

Water temperature and dissolved oxygen (DO) concentrations were recorded twice daily (07:30 am and 15:00 pm) at mid depth of each tank using an OxyGuard meter (Handy

Polaris, Birkerod, Denmark). Salinity was measured daily (08:00 am) using refractometer (Cond 3210, Welheim, Germany). Turbidity, fluorescence and pH were measured twice a week using turbidimeter (TN-100, Eutech Instruments, Singapore), Aquafluor (Turner Designs, Sunnyvale, CA. USA), and pH meter (pH 197i, Welheim, Germany), respectively. On the day before fish stocking and one a week thereafter, water samples (1 L for each tank) were collected in all tanks (08:00-08:15 am) and filtered through pre-combusted (450 ^{o}C, 4 hrs) GF/C Whatman fiberglass filters (47mm). Water parameters were analysed, including total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Koroleff, 1976) and soluble reactive phosphorus (SRP) (Murphy and Riley, 1962). To estimate chlorophyll a (Chl *a*), water sample of 25 mL was filtered onto GF/F Whatman fiberglass filter (25 mm), and the filter was immediately frozen until analysing. The filter was analysed using fluorometer (TD 700) with methanol extraction of the filter before and after adding with HCl 1% following Holm-Hansen et al. (1965). The ratio of phaeopigment to total chlorophyll pigments was calculated as (Phaeo)/(Phaeo + Chl *a*) and expressed in %.

Sediments in all tanks were sampled on the day before fish stocking and one every three weeks thereafter from 1 cm deep core by using 50 ml cut-off syringes. Sediment samples were collected at three different points within each tank and combined together to provide one sample per tank for measuring pH, organic matter content (loss on ignition), and nutrient concentrations in pore water. pH was directly measured by pushing the glass electrode (pH 197i, Welheim, Germany) into freshly collected sediment in the sample vials. Then, the samples were centrifuged at 2000 rpm for 20 minutes. The supernatant parts (pore water) were used to analyze total ammonia nitrogen (TAN) and soluble reactive phosphorus (SRP) following the methods described by Koroleff (1976) and Murphy and Riley (1962), respectively. The sediment parts were dried at 60 °C to constant weight (one week), and then analysed for loss on ignition in a muffle furnace at 350 °C for 8 hours (Nelson and Sommers, 1996). Sediment chlorophyll *a* concentration was analysed from three different samples (1 cm core layer) per tank following the method from Holm-Hansen et al. (1965), and the values were calculated for the average for each tank.

3.2.4 Statistical analysis

All data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (HOV, Brown Forsythe test), and statistically analyzed using one-way ANOVA

with IBM SPSS software version 16.0; with possible differences among data being tested by Duncan's multiple range tests. Percent data were arcsine-transformed before statistical analyses, but non-transformed data are presented in tables. Statistical comparisons of experimental data among treatments were performed for overall mean values and for each time of analyses. Non-parametric test (Kruskal-Wallis test, H test) and Tamhane's T2 (Post-hoc, one-way ANOVA) were used when data were not normally distributed or the variances were heterogeneous.

3.3 Results

3.3.1 Preliminary trial

Mean temperature, pH, salinity, turbidity and fluorescence were similar among treatments in the first three days of the trial (Table 3.1). Total ammonia nitrogen and soluble reactive phosphorus were 0 $\mu mol.L^{-1}$ for all treatments. Dissolved oxygen (DO) concentrations were significantly different ($P<0.05$) among treatments (Table 3.1).

Rabbitfish mortality occurred in the early morning in all replicates of the HD treatment at the fourth day of culture. The survival rate was significantly lower ($P<0.05$) in the HD treatment than those in the LD and the MD treatments (Table 3.1).

Table 3.1: Water parameters and survival rate of rabbitfish stocked at different densities; T: temperature, DO: Dissolved oxygen. Values are means ± SD. Values in parentheses are min – max.

	Treatment		
	Low density (8 fish.m^{-2})	Medium density (16 fish.m^{-2})	High density (24 fish.m^{-2})
T (07:30) (^{0}C)	20.6 ± 0.1	20.6 ± 0.3	20.6 ± 0.2
T (15:00) (^{0}C)	24.4 ± 0.4	24.5 ± 0.8	24.6 ± 0.5
DO (07:30) ($mg.L^{-1}$)	5.8 ± 0.3^a (4.7 – 7.0)	5.0 ± 0.2^b (2.9 – 6.9)	4.6 ± 0.1^c (2.0 – 7.0)
DO (15:00) ($mg.L^{-1}$)	6.2 ± 0.3^a (5.4 – 7.0)	5.4 ± 0.3^b (3.8 – 7.1)	4.8 ± 0.4^c (2.6 – 7.0)
Salinity	35.8 ± 0.2	35.8 ± 0.1	35.8 ± 0.2
pH	8.1 ± 0.1	8.0 ± 0.0	8.0 ± 0.1
Turbidity (NTU)	3.9 ± 1.4^a	7.5 ± 3.5^a	6.4 ± 2.4^a
Fluorescence ($\mu g.L^{-1}$)	5.4 ± 0.7^a	5.7 ± 0.4^a	6.3 ± 2.0^a
Survival rate (%)	100^a	100^a	92.3 ± 3.2^b

Mean values in a same row with different superscript letters are significantly different ($P<0.05$).

3.3.2 Experimental results

3.3.2.1 Rabbitfish growth performance

Rabbifish final mean weight, survival rate (SR), daily weight gain (DWG), and specific growth rate (SGR) were not significantly different (P>0.05) among treatments (Table 3.2). Rabbitfish yield was significantly greater (P<0.05) in the MD and the HD treatments than that in the LD treatment, but it was not significantly different (P>0.05) between the MD and the HD treatments (Table 3.2). Food conversion ration (FCR) was not significantly different (P>0.05) between the MD and the LD treatments. In the HD treatment, FCR was not calculated because of negative weight gain in a replicate where high mortality occurred.

Table 3.2: Growth performance of rabbitfish cultured at different stocking densities. Values are means ± SD.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
Stocking			
Biomass (g.m^{-2})	39.9	79.9	119.8
Initial mean weight (g.fish^{-1})	5.7 ± 1.2	5.7 ± 1.2	5.7 ± 1.2
Initial mean length (cm.fish^{-1})	6.8 ± 0.5	6.8 ± 0.5	6.8 ± 0.5
CV (%) *	7.4	7.4	7.4
K **	1.80	1.80	1.80
Harvesting			
Final mean weight (g.fish^{-1})	10.9 ± 2.0^{a}	11.9 ± 2.7^{a}	10.3 ± 3.4^{a}
DWG (g.d^{-1})	0.09 ± 0.01^{a}	0.11 ± 0.01^{a}	0.08 ± 0.06^{a}
SGRw (%.d^{-1})	1.16 ± 0.11^{a}	1.31 ± 0.09^{a}	0.98 ± 0.67^{a}
SR (%)	100 ± 0.0^{a}	100 ± 0.0^{a}	92.1 ± 13.7^{a}
Yield (g.m^{-2})	76.5 ± 4.8^{a}	166.8 ± 8.3^{b}	205.7 ± 89.5^{b}
FCR***	2.72 ± 0.43^{a}	2.44 ± 0.09^{a}	
Final mean length (cm.fish^{-1})	8.5 ± 0.6^{a}	8.8 ± 0.7^{a}	8.4 ± 0.7^{a}
DLG (cm.d^{-1})	0.03 ± 0.0^{a}	0.03 ± 0.0^{a}	0.03 ± 0.0^{a}
SGR_L (%.d^{-1})	0.39 ± 0.05^{a}	0.45 ± 0.01^{a}	0.37 ± 0.03^{a}
CV (%) *	17.9 ± 7.3 ^{a}	22.3 ± 4.5 ^{a}	21.7 ± 1.5 ^{a}
K **	1.81 ± 0.06 ^{a}	1.77 ± 0.05 ^{a}	1.70 ± 0.17 ^{a}

Mean values in a same row with different superscript letters are significantly different (P<0.05).
(*): Coefficient of variations (CV) was significantly higher (P<0.05) at harvesting than at stocking in all treatments.
(**): There was no significant difference (P>0.05) in Fulton's composition index between stocking and harvesting (P<0.05) in all treatments.
(***): FCR was not calculated for the high density treatment.

The growth in rabbitfish length was similar among all treatments (Table 3.2). The final Fulton's condition indices (K) were similar in all treatments, and there was no significant

difference (P>0.05) between the initial and the final K. The coefficient of variations (CV) in weight were not significantly different (P>0.05) among treatments at harvesting, yet they significantly higher (P<0.05) than the initial values for all treatments (Table 3.2).

3.3.2.2 Water parameters

Mean values of temperature, DO, salinity and pH were similar in all treatments throughout the experiment (Table 3.3). The temperature varied in ranges that seemed to be lower recommended suitable temperatures for rabbitfish growth while DO, salinity and pH remained in suitable ranges for rabbitfish growth during the experiment.

Table 3.3: Water parameters in the experimental treatments of rabbitfish culture at different stocking densities. Values in parentheses are min – max. Values are means ± SD.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
T (07:30) (^{0}C)	19.7 ± 0.1 (16.6 – 24.2)	19.7 ± 0.1 (16.7 – 24.3)	19.7 ± 0.1 (16.5 – 24.3)
T (15:00) (^{0}C)	22.8 ± 0.2 (20.2 – 27.7)	23.0 ± 0.2 (20.3 – 27.8)	22.9 ± 0.2 (20.4 – 27.7)
DO (07:30) (mg.L^{-1})	6.5 ± 0.1 (5.1 – 8.9)	6.4 ± 0.1 (4.5 – 9.0)	6.3 ± 0.1 (4.1 – 9.1)
DO (15:00) (mg.L^{-1})	8.0 ± 0.2 (6.2 – 10.1)	8.1 ± 0.2 (6.2 – 10.9)	8.2 ± 0.2 (6.0 – 11.6)
Salinity	33.2 ± 0.4	33.1 ± 0.1	33.1 ± 0.2
pH	8.1 ± 0.0	8.0 ± 0.1	8.0 ± 0.1
Turbidity (NTU)	6.4 ± 3.5^a	7.1 ± 1.6^{ab}	12.6 ± 3.0^b
Fluorescence (µg.L^{-1})	23.5 ± 9.7^a	18.6 ± 6.0^a	44.1 ± 24.0^a
Chl *a* (µg.L^{-1})	9.8 ± 5.1^a	7.4 ± 0.6^a	27.5 ± 11.8^b
Phaeopigment (%)	38.3 ± 1.1^a	43.7 ± 2.6^b	36.0 ± 3.0^a
TAN (µmol.L^{-1})	4.6 ± 1.0^a	18.0 ± 5.3^a	52.4 ± 10.6^b
SRP (µmol.L^{-1})	1.8 ± 0.6^a	4.8 ± 1.6^{ab}	7.8 ± 2.6^b

Mean values in a same row with different superscript letters are significantly different (P<0.05).

Mean turbidity was not significantly different (P>0.05) between the MD treatment with the others, but it was significantly higher (P<0.05) in the HD treatment than in the LD treatment (Table 3.3). Turbidity strongly increased in the day 7 after stocking in the HD treatment, and remained at high values towards the end of the experiment while it raised within small ranges in the MD and the LD treatments (Fig. 3.1).

Mean fluorescence was not significantly different (P>0.05) among treatments (Table 3.3). Chl *a* concentration was significantly greater (P<0.05) in the HD treatment than those in the

other treatments (Table 3.3). However, phaeopigment ratio was significantly higher (P<0.05) in the MD treatment than those in the HD and the LD treatments. Chl *a* gradually increased in the LD and the MD treatments during the experiment whilst it fluctuated within a small range from stocking to the day 42, then dramatically increased towards the end of the experiment in the HD treatment (Fig. 3.2).

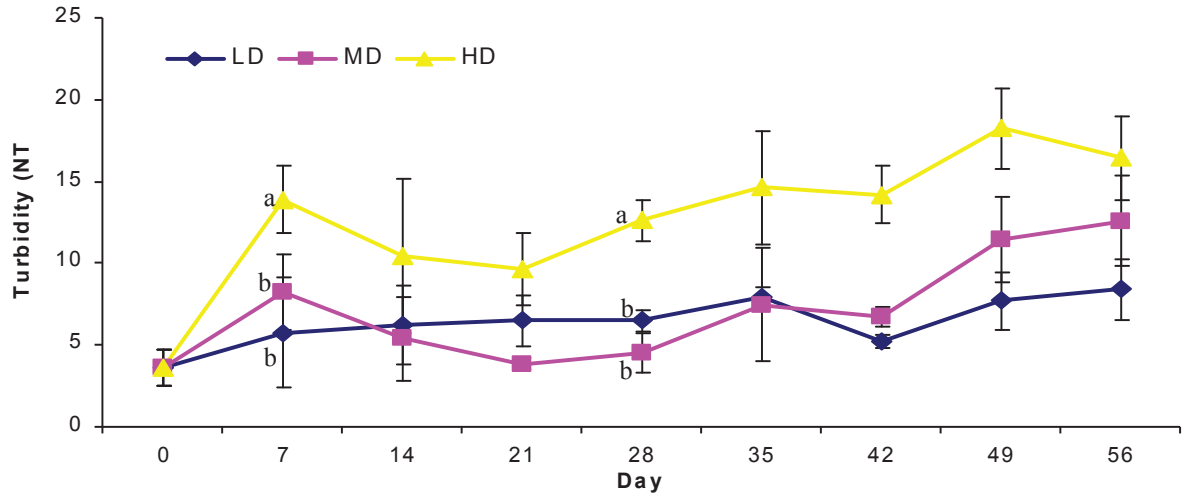


Figure 3.1: Temporal variations of turbidity in the experimental treatments of rabbitfish culture at different stocking densities. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

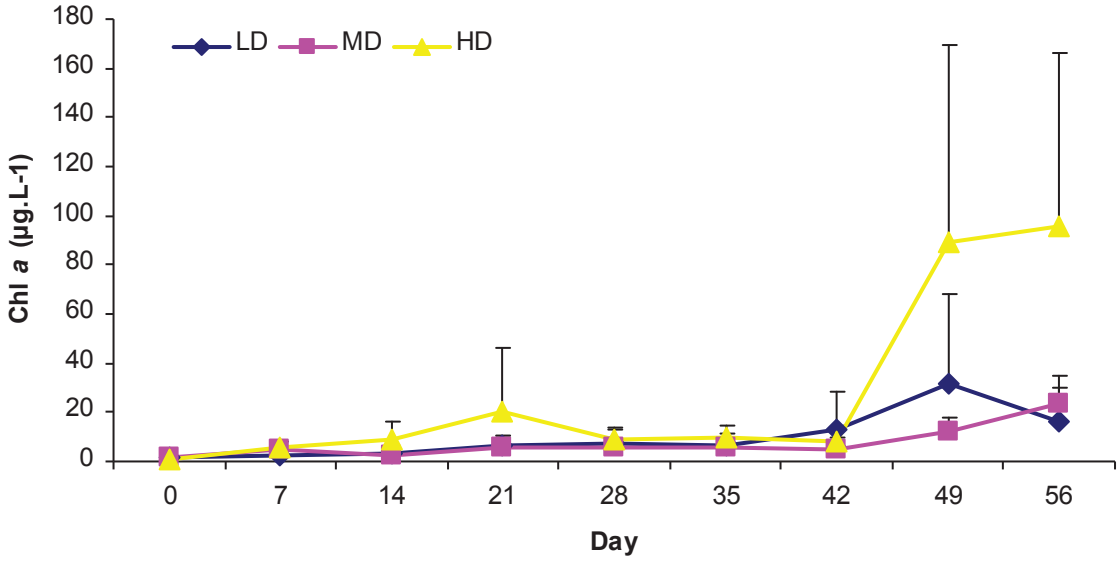


Figure 3.2: Temporal variations of water chlorophyll a in the experimental treatments of rabbitfish culture at different stocking densities. Bars present standard deviations.

Mean value of total ammonia nitrogen (TAN) was significantly greater (P<0.05) in the HD treatment than those in the other treatments (Table 3.3). Just 7 days after stocking, TAN in the HD treatment was significantly higher (P<0.05) than that in the LD treatment, and this significant difference was kept to the end of the experiment (Fig. 3.3). TAN dramatically

increased at the day 21 in the HD treatment and remained at high values towards the end of the experiment (Fig 3.3) whereas it varies within small range in the LD treatment, and fluctuated with two its highest values at the day 21 and at the end in the MD treatment.

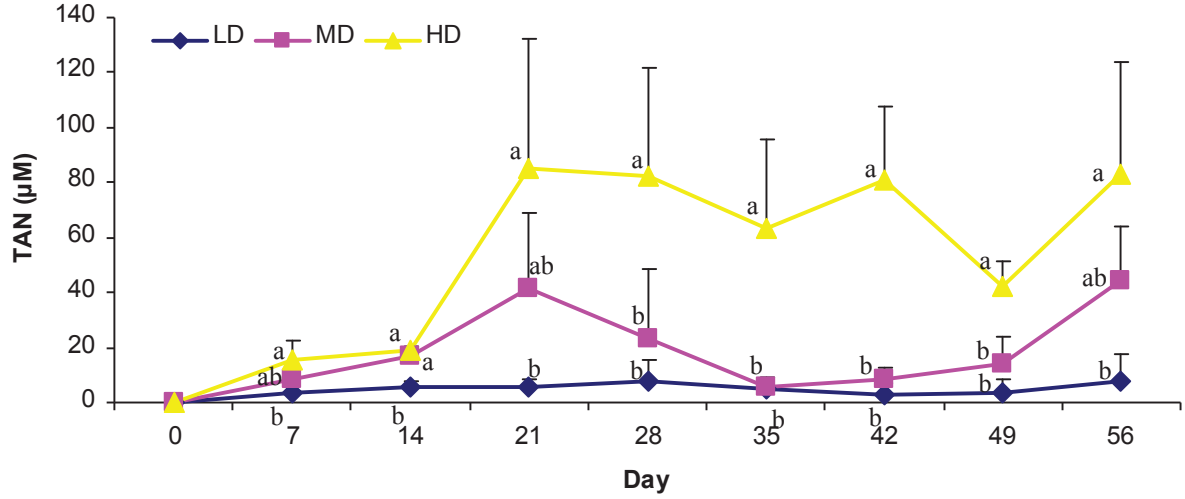


Figure 3.3: Temporal variations of total ammonia nitrogen (TAN) in the experimental treatments of rabbitfish culture at different stocking densities. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

Mean value of soluble reactive phosphorus (SRP) was significantly higher (P<0.05) in the HD treatment than that in the LD treatment, but there was no significant difference (P>0.05) in SRP between the HD and the MD treatments, as well as between the MD and the LD treatments (Table 3.3). SRP temporal variations had a same trend in all treatments, which continuously increased from stocking to harvesting (Fig 3.4). However, the values were always highest in the HD treatment, followed by those in the MD treatment, and lowest in the LD treatment throughout the experiment.

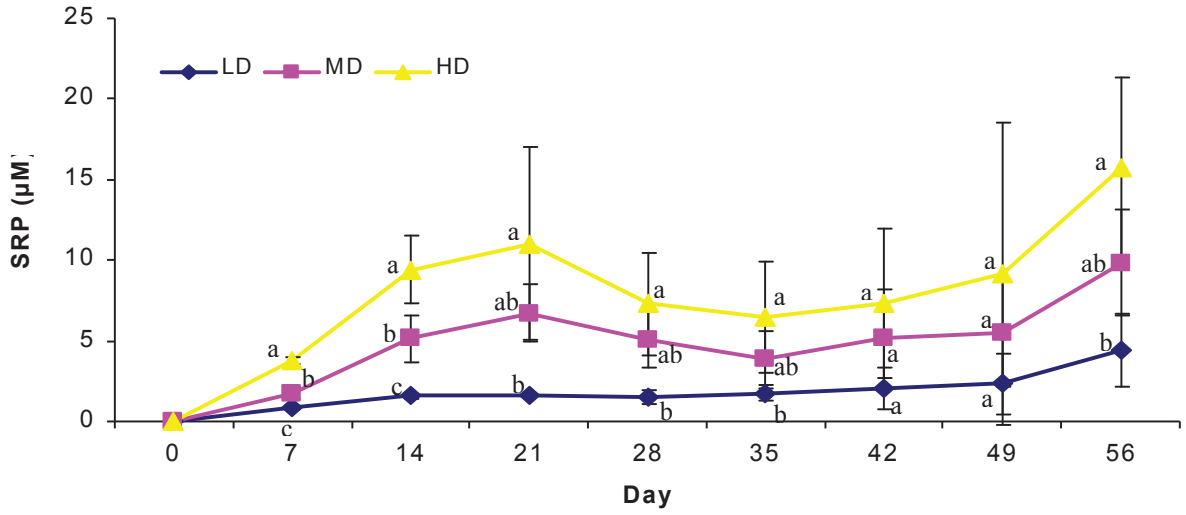


Figure 3.4: Temporal variations of soluble reactive phosphorus (SRP) in the experimental treatments of rabbitfish culture at different stocking densities. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

3.3.2.3 Sediment parameters

Sediment pH was similar among treatments, and relatively stable throughout the experiment (Table 3.4). Sediment Chl *a* concentration was significantly greater (P<0.05) in the HD treatment than those in the MD and the LD treatments. From stocking to the day 42, Chl *a* highly increased in the HD treatment and moderately raised in the MD treatment, then it was stable in the HD treatment and slightly decreased in the MD treatment to the end of the experiment (Fig. 3.5). Meanwhile, Chl *a* gradually increased in the LD treatment throughout the experiment. There were similar in phaeopigment ratio and loss on ignition among treatments (Table 3.4). Mean value of pore water TAN was significantly greater (P<0.05) in the HD treatment than that in the LD treatments. There were no significant differences (P>0.05) in mean pore water SRP among treatments (Table 3.4).

Table 3.4: Sediment parameters in the experimental treatments of rabbitfish culture at different stocking densities. Values are means ± SD.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
pH	7.4 ± 0.1	7.3 ± 0.1	7.2 ± 0.2
Chlorophyll a (mg.m^{-2})	107.5 ± 21.9^{a}	137.6 ± 32.9^{a}	221.6 ± 59.2^{b}
Phaeopigment (%)	27.4 ± 2.1^{a}	29.2 ± 3.2^{a}	30.3 ± 0.5^{a}
Loss on ignition (%)	1.8 ± 0.1^{a}	1.9 ± 0.2^{a}	1.8 ± 0.2^{a}
Pore water TAN (µmol.L^{-1})	188.4 ± 51.0^{a}	313.8 ± 116.2^{ab}	386.0 ± 52.7^{b}
Pore water SRP (µmol.L^{-1})	6.7 ± 2.6^{a}	6.9 ± 1.3^{a}	7.2 ± 1.7^{a}

Mean values in a same row with different superscript letters are significantly different (P<0.05).

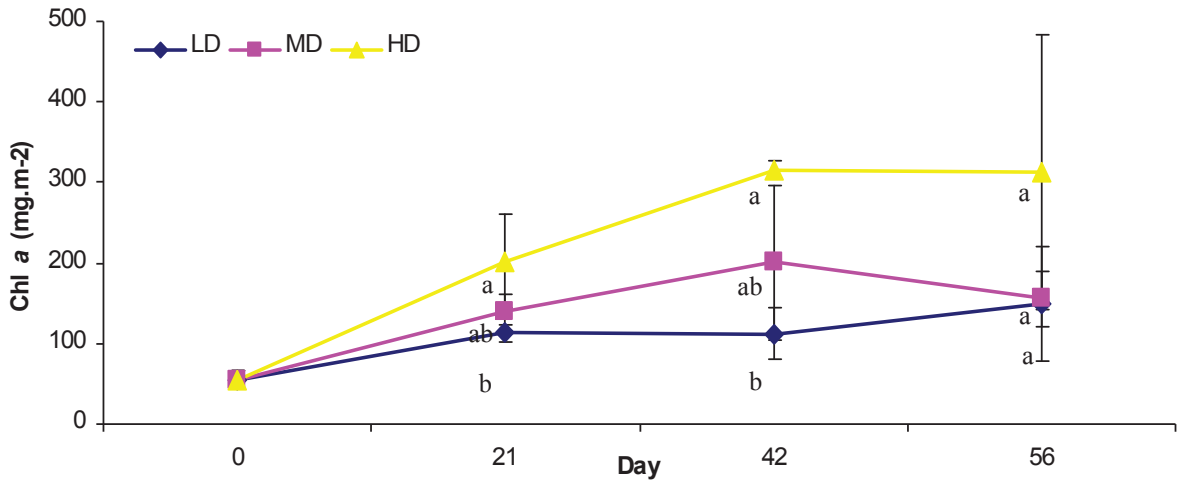


Figure 3.5: Temporal variations of sediment Chl *a* in the experimental treatments of rabbitfish culture at different stocking densities. Bars present standard deviations. Values in the same day with different letters are significantly different ($P<0.05$).

3.4 Discussion

3.4.1 Preliminary trial

Decrease of DO concentrations after stocking was mainly resulted from rabbitfish consumption. As biomass increased following the density, oxygen consumption was highest in the HD treatment, followed by the MD treatment. In the culture tanks, oxygen was produced by algae photosynthesis and diffusing from the air. However, at the beginning when algae have not well grown, showed by low fluorescence (Table 3.1), the amount of DO produced was insufficient to compensate the depletion by rabbitfish and other organisms' consumption. DO concentration depleted more rapidly in the HD treatment than the other treatments, and was the main cause of rabbitfish mortality as it reduced down to the lethal level to rabbitfish. The DO concentration at which cultured rabbitfish mortality occurred was not known. However, the DO was from 2.0 to 2.7 $mg.L^{-1}$ in the morning of the third day in all replicates of the HD treatment, and there was no mortality at such concentrations was observed. So it was probably attributed that the DO concentration that caused rabbitfish mortality was likely below 2.0 $mg.L^{-1}$. The low limit of DO was recorded for some rabbitfishes, such as 2 $mg.L^{-1}$ for *S. canaliculatus* (Lam, 1974), 0.7 $mg.L^{-1}$ for *S. guttatus*, 1.0 $mg.L^{-1}$ for *S. argenteus*, and 2.0 $mg.L^{-1}$ for *S. vermiculatus* (Duray, 1998). The present result was consistent with these reports; however, further research on the lethal level of DO to goldlined rabbitfish *S. lineatus* should be conducted.

3.4.2 Environmental variability in the experiment

The significant differences in most environmental parameters between the HD and the LD treatments, and similarly in some environmental variables between the HD and the MD treatments (Table 3.3 & 3.4) indicated the effects of rabbitfish stocking density on environmental variation in the culture tanks. These effects were possibly derived from the food amount feeding daily and rabbitfish activities. As increased stocking density, fish biomass raised, concomitantly higher amount of food was offered daily. Food input and rabbitfish metabolic products were remarkable sources of nutrient wastage into the tanks and hence the causes of the variations of water and sediment parameters. Boyd and Tucker (1998) stated that most of the feed were eaten directly by fish, but usually only 10 – 30% of phosphorus (P) and 20 – 40% of nitrogen (N) applied in feed were retained by cultured animals. The remainder of the N and P entered pond ecosystems in faeces or other metabolic products. Wu (1995) reported that depending on the species and culture techniques, up to 85% of P and 52 – 95% of N input into a marine fish (samonid fish, groupers, sea breams, seabass, snappers and yellow tails) culture system as feed might be lost into the environment through feed wastage, fish excretion, faeces production and respiration. Results from various studies also showed that some of 21% of N and 53% of P of feed input into the culture system accumulated in the bottom sediments (Wu, 1995). Organic metabolites and uneaten feed are decomposed by microorganisms, and N and P are mineralized. N in sediment organic matter may be mineralized to ammonia and recycled to the pond water. P released by decomposition of organic matter in pond bottoms is rapidly adsorbed by sediment and little of it enters the water (Boyd et al., 2002). As the experiment was carried out in the closed tanks, all released wastages and nutrients were retained and accumulated in the water columns and sediments over the course of the experiment. This led to the increases and the variations of the environmental parameters in the culture tanks, especially in the HD treatment. The high increases of TAN and SRP occurred in a short time after stocking in the HD treatment (Fig. 3.3 & 3.4) were probably derived from large quantity of wastage, rabbitfish excretion, nutrients loading from large amount of feed used in comparison with the lower quantities in the MD and the LD treatments.

In the HD treatment, high concentrations of TAN and SRP might bring about well development of phytoplankton and microphytobenthos (MPB), as expressed by the high Chl *a* concentrations in both water column and sediment (Table 3.2 & 3.3). In pond aquaculture,

N and P are the two most important nutrients because they are often present in short supply and limit phytoplankton growth (Boyd, 1998). Ammonia is the preferred nitrogen substrate for phytoplankton (Hargreaves, 1988), and P can be rapidly absorbed by phytoplankton from water (Boyd, 1998). So the variations of water and sediment Chl *a* were probably affected by the increases of TAN and SRP throughout the experiment, particularly obviously observed in the HD treatment (Fig. 3.2 & 3.5). Sediment Chl *a* rapidly increased in the MD and the HD treatments possibly resulted from high concentration of N and P and shallow water column in the culture tanks that strongly supported for MPB growth.

The algae growth, rabbitfish activities and particulate loading from the pellet feed were possibly the factors that caused the increases in turbidity (Ritvo et al., 1997; Boyd, 1998). As the tank was shallow, rabbitfish activities for feeding, normally on the bottom, easily created suspension of particulate matter from sediment into the water column. High turbidity and its rapid increase on day 7 in the HD treatment reflected the effects of higher rabbitfish density, daily food input and algae density in comparison with the LD and the MD treatments.

The nutrient concentrations likely increased following the stocking density, and thus got the highest values and wide ranges of variations in the HD treatment (Table 3.3, Fig. 3.3 & 3.4). However, these values still lied in acceptable ranges for ammonia, NH^{+}_{4} 0.2 - 2 $mg.L^{-1}$ (14.3 – 143.0 μM), NH_3< 0.1 $mg.L^{-1}$ (7.1 μM), and phosphorus, 0.005 – 0.2 $mg.L^{-1}$ (0.2 – 6.5 μM) in pond aquaculture water (Boyd, 1998). Notably, the present experiment was conducted in a closed system without water exchange, including small (500 L in water volume) and shallow (50 cm water column) tanks. So the environmental parameters were easily fluctuated under variations of ambient factors (temperature, light, etc.), and especially nutrients released by feed loading and metabolic products would be accumulated within the tanks that probably led to degradation of water quality and effects on rabbitfish growth and survival.

3.4.3 Effects of density and environment on rabbitfish growth performance

There was no significant difference in rabbitfish survival and growth performance among all treatments, indicating that stocking densities at tested levels had no negative effect on rabbitfish survival and growth. Similar results were recorded by other authors. Saoud et al. (2008a) cultured *Siganus rivulatus* juvenile (6.5 g) at stocking density of 10, 20, 30, and 40 fish per recirculating aquarium (52 L) and found no significant difference in survival and growth among treatments. Yousif et al. (2005) stocked *S. canaliculatus* (3.4 g) in floating

cage nets at 8 and 12 fish.m^{-3} and recorded no differences in the growth performance and survival of the two groups. Stocking density may or may not cause adverse effects on fish survival and growth, depending on the species of fish being reared and their development stages. Jorgensen et al. (1993) found Arctic charr (*Salvelinus alpinus*) grew faster at high stocking densities than at low density due to differences in food intake between groups, and low levels of food intake for the groups of fish stocked at low density. Inversely, El-Sayed (2002) reported a negative correlation between stocking density with fish survival and growth rate of tilapia (*Oreochromis niloticus* L.) fry. According to the author, cannibalism could be a main cause of tilapia fry mortality and "social stress" led to impaired fish growth as increasing fish densities. Since rabbitfish are schooling fish (Lam, 1974) and have tolerance of overcrowding (Ben-Tuvia et al., 1973), little competitive behaviour is expected among individuals reared at high densities.

Compared to the results gained by other researchers for different species of rabbitfish reared in recirculating tanks or floating cages (Parazo, 1990; Brown, 1994; Yousif et al., 2005; Saoud et al., 2008a; Ghanawi et al., 2010b), the growth rate of rabbitfish gained in this study was lower (SGR 0.98 – 1.31 %.d^{-1}). This could be attributed to the differences in species genetics, culture facilities, food and environmental conditions, but the most important factor was likely the water temperature. Most species obtained higher growth rates were reared under suitable temperatures for their growth. For example, *Siganus randalli* got specific growth rate (SGR) 1.9 – 2.0 %.d^{-1} at 26 – 33 ^{o}C (Brown, 1994), *S. canaliculatus* attained SGR 1.9 %.d^{-1}, at 29 – 33 ^{o}C (Yousif et al., 2005), *S. guttatus* obtained SGR 3.3 – 3.9 %.d^{-1}, at 27.2 – 29.0 ^{o}C (Parazo, 1990), and *S. rivulatus* got SGR 2.6 – 3.2 %.d^{-1}, at 27.3 ^{o}C (Ghanawi et al., 2010b). Saoud et al. (2008b) reported that rabbitfish stopped feeding at 14 and 36 ^{o}C, and their maximum growth rate was at 27 ^{o}C. Duray (1998) suggested the water temperature for rabbitfish rearing was 28 – 32 ^{o}C, and according to Boyd (1998) warm-water species grow best at temperature ranged 25 – 32 ^{o}C. There was no report on optimum temperature for optimal growth of goldlined rabbitfish *S. lineatus*, but the water temperature in culture tanks (average 19.5 – 23.0 ^{o}C) seemed to be lower than species requirement for good growth during the experiment. The experiment took place in the cool season; the air temperature at the area of Saint-Vincent fluctuated in range of 10.5 – 20.0 ^{o}C in the morning and 20.5 – 31.0 ^{o}C in the afternoon (data from the Saint-Vincent station). Such air temperature largely affected leading to the decrease of water temperature in the culture tanks over the experiment, which possibly had adverse effect on cultured rabbitfish growth. As

outdoor, small and shallow tanks, the water temperature easily varied following the air temperature, and was impossible to control. So, low water temperature could be the main reason of low growth of reared rabbitfish. However, this result also showed that *S. lineatus* could resist low temperature of the cold season without mortality occurrence. For shrimp farming in New Caledonia, sudden water temperature change in the cold season was responsible for shrimp mass mortalities (Mermoud et al., 1998). Our results showed that *S. lineatus* can be stocked into the ponds in the cold season for commercial production, replacing for shrimp production with high risk of mortality.

Although ammonia might not cause rabbitfish mortality, it could affect the growth. The overall mean value of TAN was 54.2 µM (± 10.6) in the HD treatment, at a temperature range of 19.7 – 22.9 ^{o}C and pH 8.0, from calculation, average NH_3 concentration could reach to 2.46 µM (~ 0.03 mg.L^{-1}). At this value, ammonia could be considered as an important factor that has negative effect on fish growth. The suitable NH_3 concentration for pond aquaculture water was below 0.1 mg.L^{-1} (Boyd, 1998), but low concentrations of ammonia could damage gills, reduce growth and cause mortality (Lazur, 2007).

Rabbitfish survival gained 100% in the MD and the LD treatments, and mortality occurred in one of four replicates of the HD treatment. There was no apparent reason for the mortality, but this phenomenon happened near the end of the culture when phytoplankton was blooming in the tank. The water colour became too dark and Chl *a* concentration reached its highest value (179.2 µL^{-1}) (Fig. 3.6). At that time, cultured rabbitfish also stopped feeding completely that led to poor body weight for the harvested remainders. Cultured rabbitfish could be died by low dissolved oxygen (DO) when concentration depressed below 2 mg.L^{-1} as previously described. However, DO concentrations were maintained in suitable ranges (Table 2.2) during the experiment by opening aeration. The toxic gas, such as NH_3, was lower than lethal level for fish at that time (TAN 0.5 – 1.33 mg.L^{-1}, and NH_3 (calculated from TAN) 0.02 – 0.09 mg.L^{-1}), which was probably not a reason of rabbitfish mortality. Nagle and Paul (1998) recorded a massive die-off of juveniles *Siganus argenteus* and *S. spinus* that simultaneously occurred with a bloom of cyanobacteria at Tumon Bay, Guam. Bubble-filled brown masses were floating throughout the bay and washing onto nearby beaches. At the same time, juvenile siganids were dying and washing onto beach areas. This event was attributed to floating material of cyanobacteria. So, factor(s) associated with phytoplankton bloom could be supposed causing cultured rabbitfish mortality. However, further researches

need to be conducted to find the effect of environmental eutrophication on rabbitfish mortality that would be one of the most importances for practical rabbitfish rearing.

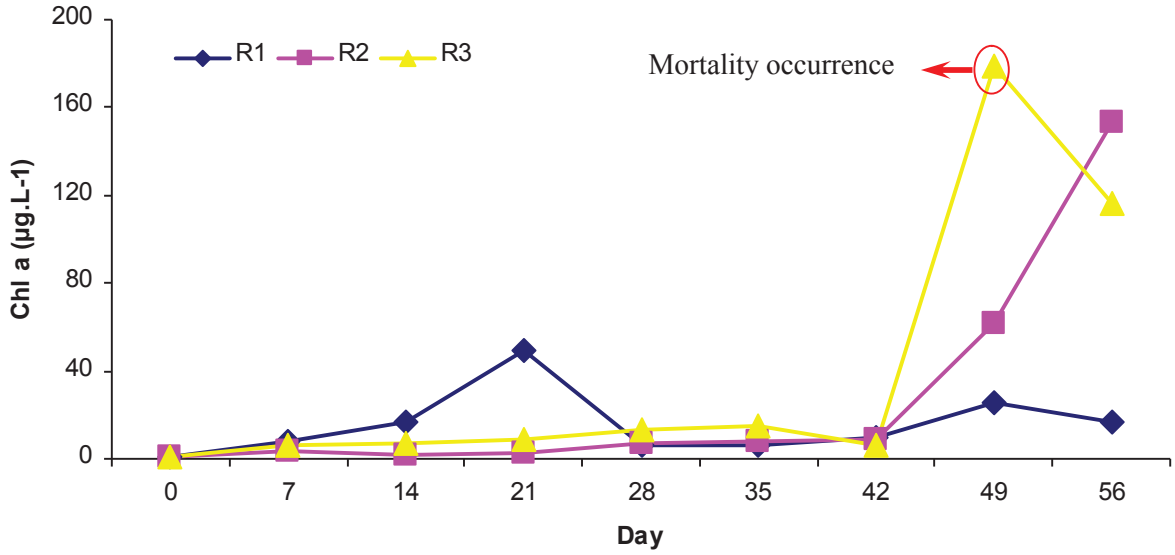


Figure 3.6: Temporal variations of Chl *a* in replicates of the HD treatment and the Chl *a* concentration, around which mortality occurred in the replicate 3; R1, R2, R3: replicate 1, 2 and 3.

The coefficient of variation (CV) in weight was high (17.7 – 21.7%) among the harvested rabbitfish. An increase in CV was an indication of large size distribution within a group of fish (Jobling and Baardvik, 1994). In aquaculture, it is desirable to have homogeneous animal size since that would facilitate feeding, harvesting, marketing and processing (Saoud et al., 2008a). In a population in which the CV increases with time, hierarchy effects might be responsible for the suppression in growth of certain individuals (Purdom, 1974). In this experiment, juveniles at stocking were hand-sorted, and thus CV among individuals was low, 7.4%. The size distribution of harvested rabbitfish remarkably increased in all treatments (Table 3.2). High CV values were also found in other cultured rabbitfish (Saoud et al., 2008a; Ghanawi et al., 2010b). Such results indicated that growth depensation was not because of social constraint by large rabbitfish on smaller rabbitfish. A possible reason for the growth depensation observed was genetic variability among individuals, causing an unequal growth potential. As rabbitfish aquaculture develops and cultured populations become more domesticated, size depensation can be expected to decrease (Saoud et al., 2008a).

The weight–length relationships, such as the Fulton's condition index (K), are relative measures of well-being among the same fish species in nature (Anderson and Neumann, 1996) or evaluating the physiological health of fish. Ghanawi et al. (2010a) found condition index of *S. canaliculatus* was better at harvest than at stocking. They attributed to an

availability of more protein and lipid in used diets was higher than in diets for fish in nature. In this experiment, there were no differences in K of rabbitfish between stocking and harvesting in all treatments as well as among treatments. The same pellet feed used for feeding was attributed to such similar K values before and after experiment as well as among treatments.

The rabbitfish yield was significantly lower in the LD treatment than in the HD and the MD treatments. This was because of remarkably lower stocking density, and thus lower number of harvested rabbitfish. However, there was no significant difference in the yield between the HD and the MD treatments. This was due to mortality and poor growth of rabbitfish occurred in a replicate of the HD treatment. Food conversion ratios (FCR) were not significantly different between the MD and the LD treatments indicated food intake and digestibility of cultured rabbitfish were probably not affected by density. However, these effects, if any, could not be obviously seen because low temperatures most likely affected feeding intensity, digestibility and growth of rabbitfish in all tanks.

An increase in number of fish per culture unit is desirable since high stocking densities generally reduce production costs per unit of fish (Huguenin, 1997). However, as biomass per tank increases, so does the quantity of feed offered, resulting in potential eutrophication and oxygen concentration depletion. The results of this study showed that stocking density had no directly negative effect on growth and survival of *Siganus lineatus*, but high stocking density (21 fish.m^{-2}) might cause high environmental variability that led to adverse effects on survival and growth. In a closed system, the environment can be quickly deteriorated by waste accumulating from uneaten feed and metabolic products, oxygen depletion and toxic gases increase. At low density (7 fish.m^{-2}), the environment was well maintained, but low yield was produced. Stocking density at 14 fish.m^{-2} seemed to be more suitable for rabbitfish rearing in a closed system, produced a relative high yield without widely environmental variations. However, further researches need to be carried out for long period of culture with different stocking densities at various size groups of rabbitfish to determine optimal stocking density and size to optimize high production *versus* low environmental changes in a closed system. Besides, experiments on tolerance to various water parameters should be investigated separately from density experiments, and then possible total biomass per unit volume calculation could be based on rabbitfish requirements and the metabolic parameters.

3.4.4 Links between environment and reared species: implication for the polyculture

Rabbitfishes are euryhaline and eurythemic species, tolerate wide environmental variations, (Lam, 1974; Saoud et al., 2008a, 2008b; Ghanawi et al., 2010a). They can adapt at low salinity, down to 5 (Lam, 1974) and survive at high salinity, up to 50 (Popper and Gundermann, 1975) or even 55 (Yousif et al., 2005). Rabbitfishes also tolerate a wide range of temperature, but stop feeding at 14 and 36 ^{o}C, and grow best at 27 ^{o}C (Saoud et al., 2008b). The wide range of salinity and temperature tolerance is of advantages to the culture of rabbitfishes in brackish water ponds, raceways, recirculating tanks, and cages/pens in estuaries and creeks in tropical, subtropical and even some temperate regions. In practice, rabbitfish have been commercially cultured in many countries and territories in the world, monoculture or in conjunct with other species in culture systems.

There has been a remarkable increase in rabbitfish rearing research in recent years; however, most studies have focused on production (Brown et al., 1994; Duray, 1998; Saoud et al., 2008a; El-Dakar et al., 2010; Jaikumar et al., 2011; Jaikumar, 2012). Few studies have been carried out on the environmental factors that affect rabbitfish survival and growth. Saoud et al. (2008b) studied effects of temperature on survival and growth of *S. rivulatus* and reported that this species is eurythermal and optimal temperature for growth is ca. 27 ^{o}C. Ghanawi et al. (2010a) studied effect of continuous water movement on growth and body composition of *S. rivulatus* and suggested that juveniles should not be reared in self-cleaning circular tanks with constantly water moving (13.3 cm/sec).

This study has reported the effects of rabbitfish culture at different densities on environmental variations and the growth performance of rabbifish under environmental conditions of a closed system. The results showed that *S. lineatus* has capacity to grow in such closed system; it could suffer to some extent of environmental variations. However, it could get poor growth at low range of temperature, ca 20 ^{o}C, and die at low water dissolved oxygen (< 2 mg.L^{-1}). Furthermore, hypereutrophication could induce problems, such as reducing growth and possible mortality of cultured rabbitfish. At low stocking density, the environmental quality was well maintained, and there was no mutual negative effect between cultured rabbitfish and the environment. At high stocking density, the environmental parameters quickly changed, including an increase in nutrient concentrations, phytoplankton density (as Chl *a*), turbidity, and a decrease in dissolved oxygen that possibly cause adverse effects on fish growth and survival. These variations can be partially managed by exchanging

water, supplying aeration and properly reducing feeding levels (Lazur, 2007).

The results also showed that *S. lineatus* could be commercially cultured in brackish earthen pond in monoculture form or co-culture with various warm-water species. Besides herbivorous feeding habit and high tolerance to environmental variations, *S. lineatus* showed a little competition among individuals in high density and could grow in low water temperature, ca. 20 ^{o}C. So this species should be considered as a suitable candidate for co-culture with other species, fish or shrimp, which are different in feeding habit and the same requirement for environment. Especially, it could be stocked in the cool season when shrimp farming encounter problems from abrupt water temperature changes.

3.5 Highlights and limits of this experiment

3.5.1 Highlights

- The results of this study show that goldlined rabbitfish *Siganus lineatus* can well adapt and grow in a closed culture system, even at low temperature ca. 20 ^{o}C.

- *S. lineatus* has little competitive behavior among individuals when stocked at size and density of 5.7 g, 7 – 21 fish.m^{-2}. The density has no effect on growth performance of *S. lineatus*, increase stocking density from 7 to 14 fish.m^{-2} can elevate harvested yield.

- The environmental quality can be adversely affected as increasing stocking density (7 – 21 fish.m^{-2}) in a closed culture system, leading to environmental deterioration by eutrophication, high concentrations of nutrients and phytoplankton bloom.

- *S. lineatus* mortality occurs when water dissolved oxygen deplete down to below 2 mg.L^{-1}. The factors associated with hypereutrophication (phytoklanton bloom, high variation of environmental parameters) can cause fish mortality and reduce growth.

3.5.2 Limits

- The culture is practised in a short period, so it is impossible to estimate the growth of rabbitfish and the environmental quality when rabbitfish size and biomass increase up to higher than harvested values.

- The water temperature is low during the culture period, *S. lineatus* reduce activity, including feeding, and thus get low growth at all densities. In this case, it is impossible to assess if density affect rabbitfish growth performance and competition among individuals.

CHAPTER 4

FEASIBILITY OF BLUE SHRIMP *Litopenaeus stylirostris* AND GOLDLINED RABBITFISH S*iganus lineatus* POLYCULTURE IN EARTHEN POND: A MESOCOSM STUDY

4.1 Introduction

Shrimp farming has been a major aquaculture activity worldwide and has rapidly expanded over the past three decades. Production of farmed shrimp increased from ~ 88,000 tonnes in 1981 to 3,788,000 tonnes in 2010 (FAO, 2010). Attracted by the demand from North America, Europe and Japan, together with high profitability and the generation of foreign exchange, shrimp culture has globally expanded and large-scale shrimp farming has arisen (Primavera, 1997; Páez-Osuna, 2001b). These expansions have occurred in the tropical and subtropical coastal lowlands and, as with other aquaculture practices, can compete for wet-lands along with waste disposal from different economic activities (industrial, agriculture, tourism, traditional fisheries) and rural development (Páez-Osuna, 2001b).

Growth of shrimp culture has also raised controversy in both the shrimp producing and shrimp importing countries. Public opinion is being impacted by increasing concerns over environmental and social impacts of shrimp culture development, food safety issues and, more generally, the long-term sustainability of shrimp farming practices (Lewis et al., 2003). Although it has brought significant benefits to some areas, shrimp farming has also been associated with environmental degradation. These include habitat conversion; expropriation, conversion and privatization of mangroves and lands from other valuable uses; nutrients and organic matter in effluent; chemicals used in soil, water, and disease treatment; salinization; and the introduction of non-native species or genetically distinct varieties (Phillips, 1995; Primavera, 1997; Páez-Osuna, 2001b; Lewis et al., 2003). These impacts have led to reductions in production, disease outbreaks and implementation of regulations on aquaculture operations and the use of the coastal zone (Páez-Osuna, 2001b). The nutrients and organic matter discharged from shrimp farms may potentially result in hyper-nutrification, eutrophication with phytoplankton blooms, low dissolved oxygen as well as sedimentation and changes in productivity and community structure of benthic organisms in receiving waters (Phillips et al., 1993, Phillips, 1995; Páez-Osuna, 2001 a & b; Lewis et al., 2003, Thomas et al., 2010). In addition, the discharge of untreated pond effluent represents an economic loss of costly nutrient, thus reducing farm profitability (Burford et al., 2001). To reduce and mitigate negative impacts and improve sustainability of shrimp farming, one of feasible and efficient methods that can be applied is integrated and polyculture system of shrimp with other aquatic species (Primavera, 2006; Jackson and Ozbay, 2008; Martínez-Porchas et al., 2010).

Shrimp polyculture has been shown to be an ecologically and economically sound method to increase the sustainability of shrimp industry (Akiyama and Angawati, 1999; Tian et al., 2001; Martínez-Córdova and Martínez-Porchas, 2006; Cruz et al., 2008), and an effective choice for solving and/or minimizing some of the current problems associated with shrimp culture (i.e. environmental pollution, disease and decreasing profitability) (Martínez-Porchas et al., 2010).

Shrimp are benthic animals spending most of their life in contact with the bottom (Dall et al., 1990) and have wide-ranging food habits in natural systems. The ability of shrimp to utilize a broad spectrum of the food web would allow them to be cultured with a number of other species (Jackson and Ozbay, 2008; Yuan et al., 2010). Besides, the mariculture of shrimp may provide one of the best opportunities for polyculture and integrated systems. Shrimp require higher water quality standards than many other cultured species and, thus, would benefit from a more stable ecosystem (Jackson and Ozbay, 2008).

In recent years, research on shrimp polyculture has increased, showing in some cases, its successfulness and sustainability, both in freshwater and marine systems (García-Pérez et al., 2000; Martínez-Pochas et al., 2010; Yuan et al., 2010). Many species from various trophic levels, for instance seaweed (*Kappaphycus alvarezii, Ulva clathrata*) (Lombardi et al., 2006; Cruz-Suárez et al., 2010), mollusks (*Sinonovacula constricta, Crassostrea gigas*, *Chione fluctifraga*) (Tian et al., 2001; Martínez-Córdova and Martínez-Porchas, 2006) and fish (*Oreochromis* spp., *Mugil cephalus*, *Liza tade*, *L. parsia* and *Chanos chanos*) (Eldani and Primavera, 1981; Wang et al., 1998; García-Pérez et al., 2000; Yuan et al., 2010; Biswas et al., 2012) have been co-cultured with penaeid shrimps with the purpose of increasing overall production and improving culture water quality. Polyculture improved shrimp growth and quality (Akiyama and Angawati, 1999; Cruz-Suárez et al., 2010), raised production and economic benefits (García-Pérez et al., 2000; Tian et al., 2001; Yuan et al., 2010) and reduced environmental impacts (Tian et al., 2001; Yuan et al., 2010).

Some studies on the polyculture of penaeid shrimps with omnivorous fish such as tilapias showed an improvement in water quality as fish feed on organic wastes (Akiyama and Angawati, 1999; Cruz et al., 2008) and an increase nutrient retention in fish biomass (Wang et al., 1998; Yuan et al., 2010). Furthermore, some fish species such as *Epinephelus coioides*, *Lates calcarifer*, *Lujanus argentimaculatus*, *Oreochromis niloticus*, *Siganus guttatus* and *Tilapia hornorum* have the capacity to inhibit the growth of luminous bacteria *Vibrio harveyi*

in shrimp rearing water and thus positively affect shrimp survival (Tendencia et al., 2006 a, b & c; Cruz et al., 2008). Siganids, herbivorous fish, are excellent candidates for polyculture with shrimp and considered having high potential to decrease the disease impact, prevent environmental deterioration and increase overall production (Tendencia et al., 2006a). However, to date no study has been conducted on polyculture of shrimp with rabbitfish in practical semi-intensive and intensive shrimp culture systems. So researches need to be done on co-culture shrimp with rabbitfish to find technical feasibility and proper solution to improve shrimp monoculture.

Shrimp aquaculture in New Caledonia has initiated at an experimental stage in 1970s, actually reached the industrial stage in 1980s with the first exports and progressively grown up to now. Farmed shrimp production is about 2000 tonnes per year (SPC, 2010). It is the second largest export industry after nikel. With its XPF 1.5 billion (~ 16 million $US) turnover in 2008, and 500-strong workforce, it is a significant source of employment and income for the territory's rural population (SPC, 2010).

Shrimp farming in New Caledonia has been affected by two seasonal mortalities. The first occurred during the drought and cold season, named as "syndrome 93", was associated with high biomass and sudden water temperature drop in shrimp ponds, and caused by *Vibrio penaeicida* (Mermoud et al., 1998; Goarant et al., 1999). The second appeared during the warm grow-out season, named as "summer syndrome", caused by *V. nigripulchritudo* (Goarant et al., 2006; Lemonnier et al., 2006). The highest densities and shortest drying duration between crops in the farms and an early eutrophication of water were considered as the factors that related to the disease (Lemonnier et al., 2006). In both these bacterial diseases, mortalities were related to septicemic vibriosis. These two pathogens seriously affected farmed shrimp yield and thus reduced the profitability of the industry and were therefore of great concerns.

To solve these problems, producers opted for an annual production cycle in order to avoid the cold season when mortality rates considered be particularly high. However, it is no longer workable today due to decreases of shrimp production and exported market prices (SPC, 2010). New Caledonia is now looking for suitable solutions that can improve shrimp farming sustainability as well as aquaculture production. Diversification farmed species and culture approaches have been prioritized to sustain aquaculture production. These include domesticating of some valuable species (sea cucumber, oyster, lobster and rabbitfish) to raise

in captivities (cages, ponds) (Adam et al., 2002; SPC, 2008) and co-culture blue shrimp with other species in ponds. Polyculture blue shrimp with sea cucumber have been studied in New Caledonia (Purcell et al., 2006; Bell et al., 2007). However, the results have not shown prospects for further development because shrimp had a significant negative effect on survival and/or growth of sea cucumber (Bell et al., 2007). As reported, rabbitfishes are considered suitable candidates for co-culture with shrimp, so a polyculture of blue shrimp with rabbitfish should be attempted to determine the technical feasibility and thus introduce an appropriate approach for sustaining shrimp farming.

The results found in Chapter 3 showed goldlined rabbitfish *Siganus lineatus* can well adapt and grow in a closed culture system where dissolved oxygen is above 2 $mg.L^{-1}$ and eutrophication is limited. This species is less competitive among individuals in high stocking density and is able to grow at low temperature during the cold season. At stocking density from 7 to 14 $fish.m^{-2}$ (5.7 ± 1.2g), the environment of rabbitfish culture would be maintained at adequate condition during the time of culture. It is probably assumed that *S. lineatus* could grow well in earthen ponds where environmental conditions are maintained better than those of the experimental closed tanks through proper management approach. Besides, natural food source for rabbitfish (periphyton, macroalgae, etc.) is easily promoted in earthen ponds by, for example, fertilizing and/or supplying necessary substrates. In this case, pellet feed using will be reduced, and thus benefit increases. Another important consideration is that *S. lineatus* can be an excellent species for co-culture with blue shrimp *Litopenaeus stylirostris* in earthen ponds because at least it dose not devour shrimp nor strongly compete with shrimp for food. In addition, rabbitfish could consume uneaten pellet feed in shrimp ponds, so prevent further degradation of the environment and increase total production (Tendencia et al., 2006a). However, questions still remain when practise co-culture *L. stylirostris* with *S. lineatus*, including 1) how *L. stylirostris* and *S. lineatus* grow as cultured together, 2) how polyculture of *L. stylirostris* and *S. lineatus* affect the environmental quality and ecological functioning in the culture system in comparison with shrimp monoculture.

The process of bioturbation by benthic organisms is one major mechanism controlling the fluxes of particulate matter and dissolved materials across the sediment-water interface (Aller, 1982; Avnimelech et al., 1999; Joyni et al., 2011). Fish species, size, density, and foraging behavior are critical factors affecting sediment disturbance. Settled particles in aquaculture ponds may be continuously resuspended by fish movement or foraging near the

pond bottom (Avnimelech et al., 1999; Jiménez-Montealegre et al., 2002**)**. Resuspension would increase the exchange rate of materials from sediment to water and thus favor the aerobic decomposition of organic matter occur in the water column generating less toxic products than those of anaerobic decomposition in bottom sediment (Jiménez-Montealegre et al., 2002; Torres-Beristain, 2005; Joyni et al., 2011). A large accumulation of organic matter in pond sediment increases oxygen demand and favors anaerobic conditions (Aller, 1982). Photosynthesis produces oxygen during daylight (Boyd, 1998; Avnimelech and Ritvo, 2003), whereas respiration continues during day and night (Boyd, 1998) and oxygen is consumed during aerobic bacteria break down of organic matter (Torres-Beristain, 2005), leading oxygen declines and carbon dioxide increases in pond ecosystem. Heterotrophically dominated sediments tend to release nitrogen (N) and phosphorus (P) to the water column and have the potential to degrade water quality (Cowan and Boynton, 1996; Kristensen and Hansen, 1999; Torres-Beristain, 2005). In contrast, net autotrophic communities tend to remove dissolved inorganic N (DIN) and P (DIP) (Rizzo et al., 1992; Sundback et al., 2000), and dissolved organic N (DON) from the water column (Linares, 2005; Vonket et al., 2008), but can also be a source of DON (Ferguson et al., 2003). Finfish, such as tilapia and pearlspot *Etroplus suratensis*, may be a natural control of bottom sediment characteristics may improve shrimp production and add a valuable product to shrimp production when co-stocking with shrimp (Joyni et al., 2011).

The stable isotope analysis technique provides a useful tool for investigating the food web in terrestrial and aquatic ecosystems. The stable carbon isotope ratio ($^{13}\delta C$) have been used to elucidate sources and pathways of organic matter for consumers and the stable nitrogen isotope ratio ($^{15}\delta N$) has been used to elucidate the trophic position of organism in the food webs (Yokoyama et al., 2002). The stable isotope signature of a consumer reflects the isotopic signatures of material assimilated and provides an integration of feeding over time (Peterson and Fry, 1987). Stable isotope analysis has proved particularly effective in the study of aquatic food webs where there are often obvious differences in the isotope signatures of the major primary sources (Boon and Bunn, 1994). In aquaculture, stable isotope analysis is increasingly used to determine the relative contributions of natural biota and supplementary food to the nutrition of cultured animals (Anderson et al., 1987; Lilyestrom et al., 1987; Lochmann and Philipps, 1996; Burford et al., 2004), to identify the role of the natural biota in processing dietary nitrogen (N) and determine the effect of dietary N on ecosystems (Burford et al., 2002) and also to evaluate waste assimilation in polyculture systems (Yokoyama et al.,

2002). Anderson et al. (1987) estimated the contribution of pond biota and added feed to growth carbon of *Penaeus vannamei* (1.5 g) sequestered in bottomless cages (20 shrimp.m^{-2}) in contact with the pond sediment by using feeds with different $^{13}\delta C$ ratios. The authors reported that the added feed supplied between 23 and 47% of growth carbon, and between 53 and 77% of the shrimp growth was due to grazing on pond biota. Burford et al. (2004) determined the sources of dietary nitrogen (N) and carbon (C) for shrimp during the rearing phase in extensive rice-shrimp ponds using stable isotope analysis. They reported that biota from beam trawls and benthic organic matter were the most likely sources of nutrition for shrimp growth. Yokoyama et al. (2002) quantified the contribution by co-cultured animals to waste assimilation in a shrimp polyculture system by using $^{13}\delta C$ and $^{15}\delta N$ to examine the food web structures of the macrobenthos. The authors found that shrimp feed was the main food source for benthic animals in the culture pond and for green mussels in the treatment pond, but sediments in the reservoir pond and particulate organic matter and/or sediments in the treatment pond supplied carbon for most macrobenthic animals.

To answer the given questions, an experiment on polyculture of blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* was conducted in a mesocosm system with the objectives were as follows:

- Estimate the effects of polyculture *L. stylirostris* with *S. lineatus* on cultured animal's growth performance,

- Estimate the effects of polyculture *L. stylirostris* with *S. lineatus* on the environmental quality and ecological functioning in a mesocosm system in comparison with shrimp monoculture,

- Estimate if polyculture of *L. stylirostris* and *S. lineatus* is possible.

In this experiment, stable isotope analysis was used to identify the potential nutrient sources from natural biota and supplied pellet feed for shrimp and rabbitfish nutrition, and determine the relative contributions of natural biota and pellet feed to shrimp and rabbitfish growth. The results would be useful for determination stocking ratio between co-cultured animals and proper feeding management. The stable isotope ($^{13}\delta C$ and $^{15}\delta N$) values of the pellet feed, particulate organic matter (POM) and sediment organic matter (SOM) as food sources; of *L. stylirostris* and *S. lineatus* as consumers were analysed over the experiment.

4.2 Materials and methods

4.2.1 Experimental design

The experiment was conducted for a period of 12 weeks, from shrimp stocking (August – November, 2012), in 12 outdoor circular mesocosm fibreglass tanks as described in the paragraph 2.2.2.2 of Chapter 2. The tanks were filled with fresh seawater one week before shrimp stocking. A daily water exchange of around 10% was applied by regulating individual valve in each tank and water height was maintained at 75 cm (1275 L in volume) above the sediment surface during the experiment. The aeration was continuously supplied to the tanks.

Blue shrimp *L. stylirostris* juveniles (2.9 ± 1.1 g) were randomly selected and stocked to the experimental tanks at a density of 15 shrimp.m^{-2} (26 shrimp.tank^{-1}). One month later, goldlined rabbitfish *S. lineatus* were added to the shrimp tanks to form polyculture treatments. Rabbitfish (25.5 ± 2.9 g, 11.2 ± 0.4 cm TL), hatchery-reproduced, were stocked to the shrimp tanks at either a low density (LDRB) of 1.2 fish.m^{-2} (2 fish.tank^{-1}) or a high density (HDRB) of 2.4 fish.m^{-2} (4 fish.tank^{-1}). Four additional tanks with shrimp monoculture were used as a control treatment. All treatments were randomly distributed among tanks with four replicates per treatment. Shrimp in all tanks were fed similarly with commercial pellet feed (35 – 40 % protein, SICA Manufacturer, New Caledonia), twice daily at 08:00 am and 16:00 pm, with a feeding rate of 3 – 5 % of shrimp biomass per day during the experiment. Feed quantity was adjusted by using feeding trays (30 cm diameter) placed in the control treatment tanks at seven day intervals. Feed was distributed over the entire bottom of the tank and on the feeding tray (20 % of total amount per time). Feed consumption in the tray was closely observed to determine and adjust the feed ration (Salame, 1993). The same amount of feed as for control tanks was applied to all other tanks. Rabbitfish were not given any supplementary feed after being added to the experimental tanks.

4.2.2 Shrimp and rabbitfish sampling and analyses

At stocking, 30 shrimps were randomly sampled and weighed individually to the nearest 0.1 g. All fish at stocking and harvesting as well as all shrimp at harvesting in each tank were counted and weighed individually to the nearest 0.1 g and fish body total length (TL) were measured to the nearest 0.1 cm with a technical ruler.

Shrimp and fish growth performance were evaluated at harvesting in terms of survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), and yield.

SR (%) = harvesting number/stocking number*100

DWG ($g.day^{-1}$) = weight gain (g)/time (days)

SGR ($\%.day^{-1}$) = (Ln Wf – Ln Wi)/time (days)*100

Yield ($g.m^{-2}$) = harvesting biomass (g)/area of culture tank (m^2)

where Wi: initial mean weight (g), Wf: final mean weight (g)

Shrimp food conversion ratio (FCRs) was calculated as followed:

FCRs = total feed used (dry weight, g)/total shrimp weight gain (fresh weight, g)

Overall food conversion ratio (FCRsf) was calculated as followed:

FCRsf = total feed used (dry weight, g)/total shrimp and fish weight gain (fresh weight, g)

4.2.3 Water sampling and analyses

Water temperature and dissolved oxygen (DO) concentrations were recorded twice daily (07:30 am and 15:00 pm) at mid depth of each tank using an OxyGuard meter (Handy Polaris, Birkerod, Denmark). Salinity, turbidity, fluorescence and pH were measured three times a week (08:00 am) using refractometer (Cond 3210, Welheim, Germany); turbidimeter (TN-100, Eutech Instruments, Singapore), Aquafluor (Turner Designs, Sunnyvale, CA. USA), and pH meter (pH 197i, Welheim, Germany), respectively.

On the day before rabbitfish stocking and once a week thereafter, water samples (2 L) were collected in all tanks (08:00 am) at mid depth and filtered through pre-combusted (450 ^oC, 4 hrs) GF/C Whatman fiberglass filters (47mm). Filtered water were analysed for total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Koroleff, 1976), soluble reactive phosphorus (SRP) (Murphy and Riley, 1962) by every week. Nitrite and nitrate nitrogen (NO_2^-+NO_3^-)-N and total dissolved nitrogen (TDN) were estimated every two weeks following the methods described by Wood et al. (1967) and Raimbault et al. (1999), respectively. Dissolved organic nitrogen (DON) was expressed as the difference between total dissolved nitrogen and total dissolved inorganic nitrogen [(NH_4^+-NH_3)-N + (NO_2^-+NO_3^-)-N].

To estimate chlorophyll *a*, a water sample of 25 mL was filtered through GF/F Whatman fiberglass filter (25 mm) and the filter was immediately frozen until analysing. The filter was

analysed using fluorometer (TD 700) with methanol extraction of the filter before and after adding HCl 1% following Holm-Hansen et al. (1965). The ratio of phaeopigment to total chlorophyll pigments was calculated as (Phaeo)/(Phaeo + Chl *a*) and expressed in %.

A water sample of 50 mL was filtered through pre-combusted (450 ^{o}C, 4 hrs) GF/F Whatman fiberglass filter (25 mm) to analyse for particulate nitrogen (PN) and particulate organic carbon (POC), which were determined on a Nitrogen–Carbon Analyzer **(**ThermoFinnigar with microbalance**)** after decarbonation with H_2SO_4 (Hedges and Stern, 1984).

Total suspended solids (TSS, mg.L^{-1}) was estimated by filtering 250 mL sample water collected at mid depth of each tank through pre-dried (60 ^{o}C, 24 hrs) and pre-weighed (Wi) GF/C Whatman fiberglass filter (47 mm). The filter was dried to constant weight (60 ^{o}C, 24 hrs), then weighed again (Wf), and TSS was calculated as follows:

$$TSS = (Wf – Wi)/V*1000$$

where Wf and Wi: the final weight and initial weight of the filter, V: volume of water sample (mL), 1000: index converted from L to mL.

4.2.4 Sediment sampling and analyses

Sediments in all tanks were sampled on the day prior to the addition of rabbitfish and every two weeks thereafter from 1 cm deep cores by using 50 ml cut-off syringes (2.3 cm diameter). Sediment samples were collected at three different points within each tank and combined to provide one sample per tank for the analysis organic matter content, pH, redox potential, and nutrient concentrations in pore water. The redox potential (Eh) was estimated with a specific electrode (Consort P901, electrochemical analyzer, Beverly, MA, USA) and using the method described by Hussenot and Martin (1995). pH was directly measured by pushing the glass electrode (pH 197i, Welheim, Germany) into freshly collected sediment in the sample vials. After that sediment samples were centrifuged at 2000 rpm for 20 minutes. The supernatant parts (pore water) were used to analyse TAN and SRP following the methods as described above for water. The sediment samples were dried at 60 °C for one week and then analysed for loss on ignition in a muffle furnace at 350 °C for 8 hrs (Nelson and Sommers, 1996). Sediment chlorophyll a (Chl *a*) concentration was analysed from three different samples (1cm core layer) per tank. Frozen sediment samples were freeze-dried (lyophilisated) for 24 hrs, 14 ml of methanol were added to the samples for 20 minutes to

extract the pigments. After homogenization, 100 µl of the supernatant were removed and added to 7 ml of methanol. The extracts were analysed before (Fo) and after acidification (Fa) with 50 µl of 1% HCl using a TD-700 fluorometer. The values were calculated taking into account the dilution and Fo/Fa. The concentration of Chl *a* was expressed by $\mu g.m^{-2}$.

4.2.5 Ecological functioning

To estimate and compare ecological processes with the control, the HDRB treatment was chosen as a representation for polyculture treatments. Sedimentation, metabolism and nutrient fluxes were estimated on the day prior to rabbitfish stocking and every two weeks thereafter.

4.2.5.1 Sedimentation processes

Sedimentation rates (SdR) were determined by using sets of sediment traps. A set of sediment trap included a rectangular plastic tray, 6 cm in height, which was covered by a plastic sheet with rows of holes (~ 1 cm diameter for each) to catch settled particulate matter into the tray, and a heavy smooth lead bar to stabilize the tray. The area of the trap (total hole areas) was 0.01425 m^2. The trap was placed on the bottom of each tank at opposite side with water inlet and retrieved after ~ 48 hrs. All settled particulate matter in a trap was carefully transferred to a bottle. Total solid weight of trapped particulate matter was determined gravimetrically following drying at 60 ^oC to constant weight and sedimentation rate (SdR) was calculated and expressed as $g.m^{-2}.h^{-1}$.

$$SdR = W/(S*T)$$

where W: weight of dry trapped particulate matter (g), S: surface area of sediment trap (m^2), T: setting time (h)

Loss on ignition of deposit was determined in a muffle furnace at 350 °C for 8 hrs following Nelson and Sommers (1996).

4.2.5.2 Metabolism

Primary productivity (PP) and respiration (R) were determined by using the light and dark bottle method (Strickland and Parsons, 1972) under natural light. Two pairs of light and dark bottles were incubated in the water column at 20 cm under water surface and 20 cm above the sediment surface, respectively. Two chambers (light and dark) were placed on bases set up on

the bottom sediment. Dissolved oxygen (DO) concentrations were recorded in the bottles and in the chambers every hour, between 10:30 am and 13:30 pm, using an Oxygenmeter probe (Fibox 3 LCD – trace, Present, Germany).

Net primary productivity (NPP) and R were assessed as the rates of oxygen variations in the incubated bottles and in the chambers. NPP, R and gross primary productivity (GPP) were calculated for the water column and sediment using followed formulas.

$$NPPw = (Slsb + Slbb)/2*H*1000$$

$$Rw = (Sdsb + Sdbb)/2*H*1000$$

$$NPPs = (Slc - Slbb)*(V/S)*1000$$

$$Rs = (Sdc - Sdbb)*(V/S)*1000$$

$$GPP = NPP + R$$

where NPPw: net primary productivity of the water column ($\mu mol.m^{-2}.h^{-1}$); Slsb: oxygen slope in light surface bottle ($\mu mol.L^{-1}.h^{-1}$), Slbb: oxygen slope in light bottom bottle ($\mu mol.L^{-1}.h^{-1}$); Rw: respiration of water column ($\mu mol.m^{-2}.h^{-1}$), Sdsb: oxygen slope in dark surface bottle ($\mu mol.L^{-1}.h^{-1}$), Sdbb: oxygen slope in dark bottom bottle ($\mu mol.L^{-1}.h^{-1}$); H: the height of the water column (m). NPPs: net primary productivity of sediment ($\mu mol.m^{-2}.h^{-1}$), Slc: oxygen slope in the light chamber ($\mu mol.L^{-1}.h^{-1}$); Rs: respiration of sediment ($\mu mol.\ m^{-2}.h^{-1}$); Sdc: oxygen slope in the dark chamber ($\mu mol.\ L^{-1}.h^{-1}$); V: volume of the benthic chamber (m^3), and S: surface area of the benthic chamber (m^2); GPP: gross primary productivity ($\mu mol.m^{-2}.h^{-1}$).

Oxygen self produced budget from oxygen metabolism in whole tanks was estimated as differences between total GPP and total R during day long.

$$OB = (\sum GPP*12) - (\sum R*24)$$

where OB: oxygen self produced budget ($mgO_2.m^{-2}.d^{-1}$); GPP: gross primary productivity in whole tank ($mg\ O_2.m^{-2}.h^{-1}$); R: respiration in whole tank ($mg\ O_2.m^{-2}.h^{-1}$); 12: the duration (hour) of photoperiod during the experiment and 24: the duration (hour) of respiration.

Shrimp oxygen demand was calculated based on shrimp predicted biomass ($g.m^{-2}$) in the culture tank and respiration rate ($mg\ O_2.g^{-1}.d^{-1}$) for *L. stylirostris* (Wabete et al., 2008) during the experiment.

Gross natural production in the term of organic product produced by photosynthesis, expressed by $gC.m^{-2}.d^{-1}$, was converted from total gross primary productivity, as follow:

$$GNP = (\sum GPP*12)/(32*1000)$$

where GNP: gross natural production ($gC.m^{-2}.d^{-1}$), $\sum GPP$: total gross primary productivity ($mgO_2.m^{-2}.d^{-1}$) in whole tank in day, 12: carbon atomic density weight and 32: oxygen molecular weight, 1000: index converted from g to mg.

The quantity of carbon supplied daily to the tank through feeding was considered to account for 42.5% of dry weight of the feed pellet (unpublished data).

4.2.5.3 Nutrient fluxes

Nutrient fluxes were determined by using the light and dark bottles and chambers as in metabolism determination. The bottles and chambers were incubated into the tanks like what done for metabolism measurement.

Nutrient fluxes on sediment – water interface were determined from temporal variations of nutrient concentrations (TAN, SRP, TDN, NO_2+NO_3-N and DON) in the chambers and corresponding bottles during the time of incubation. Thirty minutes after installing, water samples were withdrawn through a sealed sampling tube at the top of each chamber, by using syringes and then filtered on pre-dried GF/C (47 mm) Whatman fiberglass filters. The filtered samples were analysed for initial nutrient concentrations in chambers. At the same time, water samples were collected in the water columns for analysing initial nutrient concentrations of the water column.

Similarly, final nutrient concentrations were analysed at the end of incubation for water withdrawn from chambers and water in incubated bottles. TAN, SRP, TDN, NO_2+NO_3-N concentrations were analysed following the methods as described above for water. DON concentration was as difference between TDN with corresponding TAN and NO_2+NO_3-N. The time of incubation was recorded for each measurement to calculate nutrient slopes.

Nutrient fluxes on sediment-water interface were calculated following the formula:

$$NE = [(FC - IC) - (FB - I)]/T*(V/S)*1000$$

where NE: nutrient fluxes (TAN, SRP, NO_2+NO_3-N and DON) ($\mu mol.m^{-2}.h^{-1}$); FC: final nutrient concentrations in benthic chamber (light or dark) ($\mu mol.L^{-1}$); IC: initial nutrient concentrations in benthic chamber (light or dark) ($\mu mol.L^{-1}$); FB: final concentrations in

bottom bottle (light or dark) (µmol.L^{-1}); I: initial nutrient concentrations in the water column (µmol.L^{-1}); T: time of incubation (hr); V: Volume of benthic chamber (m^3) and S: surface area of benthic chamber (m^2).

4.2.6 Stable isotope analysis

Pellet feed samples were taken at the beginning of the experiment, after one month, and close to the end of the experiment. SOM and POM were collected on the day prior to shrimp stocking, on the day before rabbitfish stocking, and at the end of the experiment. POM samples were obtained by filtering water (0.5 – 1.5 L), collected at mid depth, on pre-combusted (350 ^oC, 4 hrs) Whatman GF/F fiberglass filters (47mm). The filters were dried in an oven at 60 ^oC for 24 hrs and then stored in dark condition until analysis. SOM samples were collected from 1cm deep core using 50 ml cut-off syringes at three different points within each tank and combined together to provide one sample per tank. The samples were frozen until analysis. At stocking, three shrimps and three rabbitfish were randomly collected and immediately frozen, and at harvesting one shrimp and one rabbifish per tank were randomly sampled and frozen until analysis.

The surfaces of dried filters were scraped to collect POM materials for stable isotope analysis. Sediment samples were dried at 60 ^oC for 24 hrs and ground to fine powder using mortar and pestle. POM and SOM samples were divided into two subsamples. One subsample, for carbon isotope analysis, was acidified with 1% HCl solution to remove carbonates, as carbonates present higher $\delta^{13}C$ than organic carbon (De Niro and Epstein, 1978), and oven-dried at 60 ^oC for 24 hrs (Jadine et al., 2003; Lorrain et al., 2003; Jacob et al., 2005). The other one, for nitrogen isotope analysis, was not acidified.

Shrimp and rabbitfish samples were washed with distilled water and then filleted to get muscle tissues. The tissue samples were freeze-dried and ground into fine powder using mortar and pestle. Triplicates (1mg of powder for each) per sample were analyzed and the values were calculated for the average for each sample.

The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio in the sample were analyzed by continuous flow isotope ratio mass spectrometry. The spectrometer (Europa Scientific ANCA-NT 20-20 stable-isotope analyser with ANCA_NT solid/liquid preparation module; Europa Scientific, Crewe, U.K.) was operated in dual isotope mode. The analytical precision was 0.2‰ for both N and C,

estimated from standard analysed along with the samples. Internal standards were 1mg leucine calibrated against 'Europa flour' and IAEA standard N_1 and N_2 (Scrimgeour and Robinson, 2003). Isotope ratios were expressed as parts per thousand or per mil (‰) differences from the given standards, and were calculated according to the formula:

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] * 1000$$

where X is ^{13}C or ^{15}N, R is the corresponding ratio, $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and δ is the measure of heavy to light isotope in the sample. The international standard references are Vienna Pee Dee Belemnite (vPDB) for carbon and atmospheric N_2 for nitrogen.

The fractionation factor was calculated following the equation defined by Hobson and Clark (1992).

$$\Delta dt = \delta t - \delta d$$

where δt and δd are the isotopic signature of the consumer tissue and of the diet or food source.

The relative contribution of natural production and the pellet feed to animal growth carbon was determined using the equation defined by Anderson et al. (1987).

$$Wp/Wf = (\delta f - \delta g)/(\delta g - \delta p)$$

where Wp is the weight gained from pond production, Wf is the weight gained from pellet feed; δp, δf and δg are the $\delta^{13}C$ values for the pond biota, feed and growth, respectively. The δg was calculated from the equation:

$$\delta g = (Wt\delta t - Wi\delta i)/Wg$$

where δt, δi are the $\delta^{13}C$ values of the harvested and initial tissues of animal. Wt, Wi and Wg are mean harvested weight, initial weight and weight gained.

The proportion of a particular food type contributing to animal growth nitrogen was calculated using the "mixing model" following Tiunov (2007).

$$X = (\delta t - \delta_B)/(\delta_A - \delta_B)$$

where δt, δ_A and δ_B are the $\delta^{15}N$ isotope signature of the animal tissue and resource A and B, respectively. X is the proportion of resource A in the diet.

4.2.7 Statistical analysis

All data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (HOV, Brown Forsythe test), and statistically analysed using one-way ANOVA with IBM SPSS software version 16.0; with possible differences among data being tested by Duncan's multiple range tests. Percent data were arcsine-transformed before statistical analyses, but non-transformed data are presented in tables. Statistical comparisons of experimental data among treatments were performed for overall mean values and for each time of analyses. Non-parametric test (Kruskal-Wallis test, H test) and Tamhane's T2 (Post-hoc, one-way ANOVA) were used when data were not normally distributed or the variances were heterogeneous.

The data of sedimentation, TSS, metabolism between the HDRB treatment and the control were statistically compared by using a paired Student's t-test in MS-Excel.

4.3 Results

4.3.1 Shrimp and rabbitfish growth performance

Shrimp final mean weight, survival rate (SR), daily weight gain (DWG), specific growth rate (SGR) and yield were not significantly different ($P>0.05$) among treatments. The variability in shrimp SR and yield among replicates of each treatment reduced from the control to the HDRB treatment (Table 4.1).

Rabbitfish SR was 100% in polyculture treatments. Rabbitfish final mean weight, DWG, and SGR were similar between the LDRB and the HDRB treatments (Table 4.1). The rabbitfish yield was significantly higher ($P<0.05$) in the HDRB than in the LDRB treatment. Similarly, the total combined shrimp and rabbitfish yield was significantly higher ($P<0.05$) in the HDRB treatment than in the LDRB treatment and the total productions in polyculture treatments were significantly greater ($P<0.05$) than shrimp production alone in the control treatment (Table 4.1).

Shrimp food conversion ratio was not significant different ($P>0.05$) among treatments. However, the overall FCR was significantly lower ($P<0.05$) in the HDRB treatment than that in the control treatment (Table 4.1).

Table 4.1: Blue shrimp and goldlined rabbitfish growth performances for polyculture of shrimp with rabbitfish at different densities and shrimp monoculture (control). Values are means ± SD.

	Treatment		
	Low density rabbitfish	High density rabbitfish	Control
Shrimp			
Final mean weight ($g.shrimp^{-1}$)	13.4 ± 0.9^{a}	13.9 ± 1.5^{a}	14.0 ± 0.7^{a}
DWG ($g.d^{-1}$)	0.13 ± 0.01^{a}	0.13 ± 0.02^{a}	0.13 ± 0.01^{a}
SGR ($\%.d^{-1}$)	1.83 ± 0.08^{a}	1.88 ± 0.12^{a}	1.89 ± 0.06^{a}
SR (%)	71.2 ± 13.1^{a}	80.8 ± 7.0^{a}	66.3 ± 20.7^{a}
Shrimp yield ($g.m^{-2}.84d^{-1}$)	145.4 ± 24.7^{a}	170.3 ± 27.0^{a}	143.2 ± 48.6^{a}
FCRs	3.7 ± 0.7^{a}	2.9 ± 0.5^{a}	3.8 ± 1.6^{a}
Fish			
Final mean weight ($g.fish^{-1}$)	57.5 ± 11.4^{a}	53.1 ± 6.5^{a}	
DWG ($g.d^{-1}$)	0.58 ± 0.22^{a}	0.50 ± 0.09^{a}	
SGR ($\%.d^{-1}$)	1.44 ± 0.41^{a}	1.33 ± 0.15^{a}	
Fish yield ($g.m^{-2.}.55d^{-1}$)	67.7 ± 13.5^{a}	125.0 ± 15.4^{b}	
Combined			
Total yield ($g.m^{-2}$)	213.1 ± 34.3^{a}	295.3 ± 24.4^{b}	$143.2 \pm 4\ 8.6^{c}$
Overall FCRsf	2.6 ± 0.5^{ab}	2.0 ± 0.3^{b}	3.8 ± 1.6^{a}

Mean values in a same row with different superscript letters are significantly different ($P<0.05$).

4.3.2 Environmental variations

4.3.2.1 Water parameters

Mean values and temporal variation trends of temperature, dissolved oxygen (DO), salinity, and pH were similar in all treatments over the experiment (Table 4.2). Salinity and pH were in suitable ranges for shrimp and rabbitfish growth during the experiment. In general, DO fluctuated within suitable ranges for shrimp and rabbitfish growth (Fig. 4.1), while temperature varies in the ranges that seemed to be lower than the required temperatures for optimal growth of both shrimp and rabbitfish (Fig 4.2).

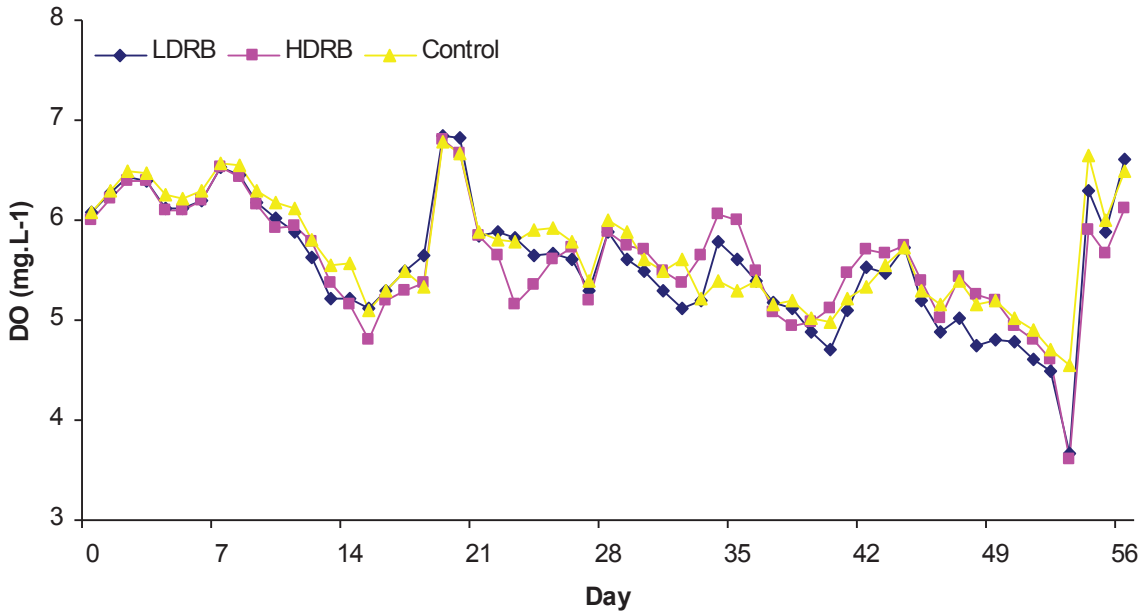


Figure 4.1: Temporal variations of DO concentration in the morning in the experimental treatments during the experiment.

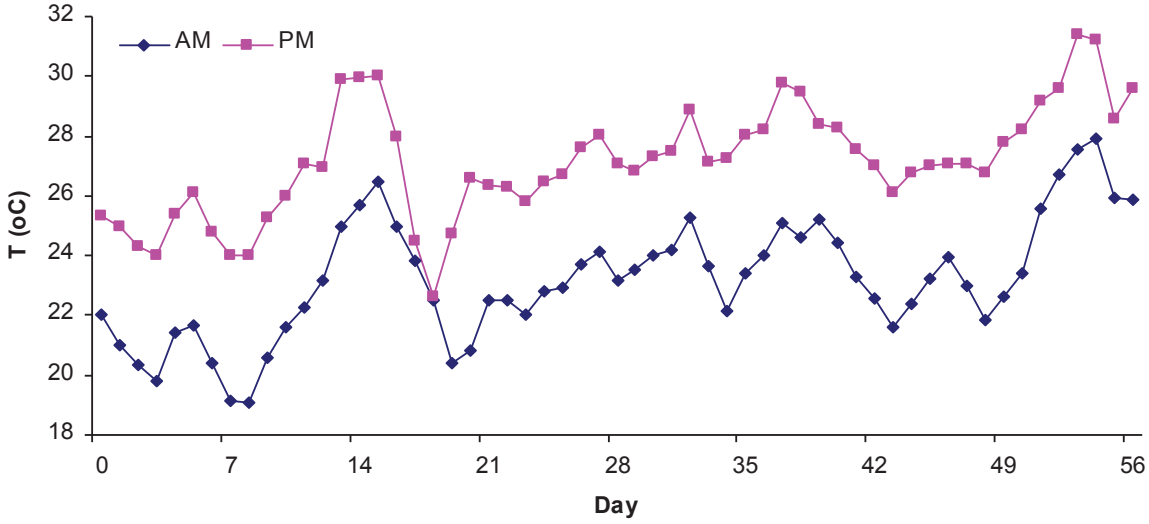


Figure 4.2: Temporal variations of average temperature in the morning (AM) and in the afternoon (PM) in all experimental tanks during the experiment (n = 12 for each value).

Mean turbidity and Chl *a* were not significantly different (P>0.05) among treatments (Table 4.2). The trend of temporal variation of turbidity was similar across all treatments during the experiment (Fig. 4.3). Chl *a* temporal variations showed slightly different trends among treatments (Fig. 4.4). Large standard deviation of Chl *a* values in the control at the end of the experiment indicated a high variability among replicates for this treatment at that time. The phaeopigment ratio was similar in all treatments (Table 4.2).

Except for SRP, mean nutrient concentrations (TDN, TAN, NOx-N, and DON) were similar for all treatments (Table 4.2). Different trends in TAN temporal variations were observed among treatments. TAN gradually increased during the experiment in the LDRB treatment (Fig 4.5). In the control, TAN fluctuated within smaller range than those in the HDRB treatment throughout the experiment. Mean SRP concentration was significantly higher ($P<0.05$) in the LDRB treatment than those in the HDRB treatment and the control. SRP temporal variation showed a strong increase in the LDRB in the last four weeks whilst the other treatments had a same trend with a slow increase at the end of the experiment (Fig. 4.6).

Table 4.2: Water parameters in polyculture and in the control treatments throughout the experimental period. Values in parentheses are min – max. Values are means ± SD.

	Treatment		
	Low density rabbitfish	High density rabbitfish	Control
T (07:30) (^{o}C)	23.1 ± 0.1 (18.9 - 28.0)	23.0 ± 0.2 (18.0 - 28.1)	23.0 ± 0.3 (18.5 - 28.2)
T (15:00) (^{o}C)	27.3 ± 0.1 (22.5 - 31.4)	27.3 ± 0.4 (22.1 - 32.8)	27.4 ± 0.3 (22.4 - 31.7)
DO (07:30) (mg.L^{-1})	5.6 ± 0.2 (2.4 - 7.6)	5.6 ± 0.1 (2.9 - 7.4)	5.8 ± 0.3 (3.1 - 7.6)
DO (15:00) (mg.L^{-1})	10.3 ± 0.1 (6.3 - 15.0)	10.6 ± 0.2 (7.1 - 16.1)	10.4 ± 0.7 (6.4 - 15.3)
pH (n = 24)	8.1 ± 0.0	8.1 ± 0.0	8.2 ± 0.0
Salinity (n = 24)	36.4 ± 0.1	36.5 ± 0.1	36.5 ± 0.1
Fluorescence (n = 24)	36.8 ± 2.2^{a}	41.4 ± 10.8^{a}	45.7 ± 24.5^{a}
Turbidity (NTU) (n = 24)	7.1 ± 1.7^{a}	8.7 ± 2.3^{a}	6.6 ± 1.3^{a}
Chlorophyll *a* (µg.L^{-1}) (n = 9)	18.3 ± 4.3^{a}	20.7 ± 4.5^{a}	28.5 ± 15.3^{a}
Phaeopigment (%) (n = 9)	35.0 ± 2.8^{a}	32.8 ± 5.0^{a}	30.2 ± 2.6^{a}
TDN (µM) (n = 5)	26.08 ± 4.27^{a}	26.41 ± 5.07^{a}	21.45 ± 2.1^{a}
TAN (µM) (n = 9)	1.84 ± 1.35^{a}	1.83 ± 1.05^{a}	0.96 ± 0.70^{a}
(NO_2+NO_3)-N (µM) (n = 5)	0.19 ± 0.06^{a}	0.25 ± 0.08^{a}	0.20 ± 0.04^{a}
DON (µM) (n = 5)	23.65 ± 1.94^{a}	23.06 ± 3.40^{a}	20.55 ± 2.15^{a}
SRP (µM) (n = 9)	0.26 ± 0.04^{a}	0.17 ± 0.03^{b}	0.16 ± 0.07^{b}

Mean values in a same row with different superscript letters are significantly different ($P<0.05$).

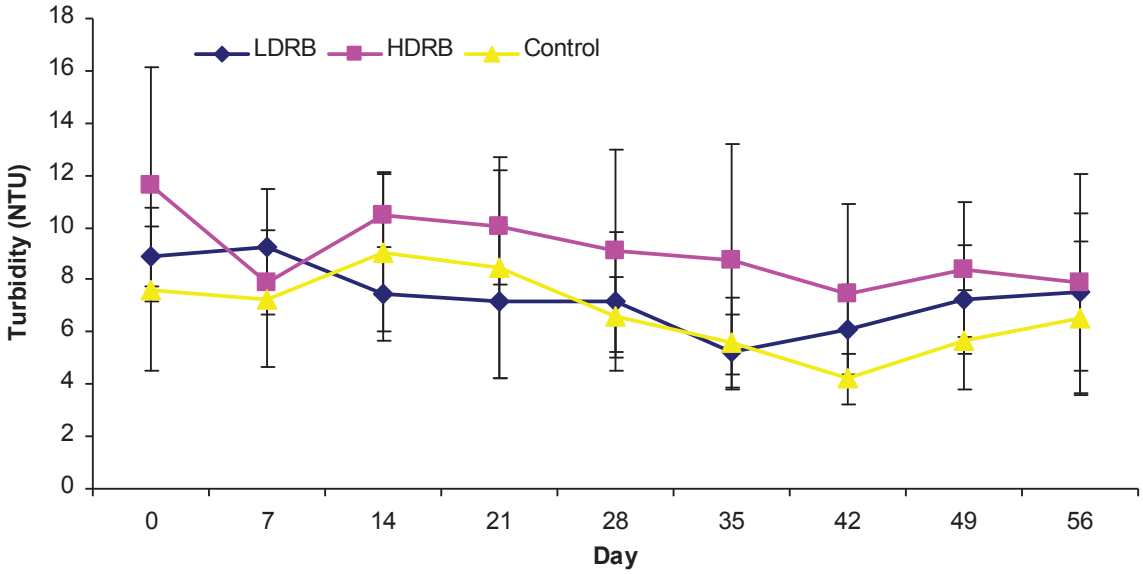


Figure 4.3: Temporal variations of turbidity in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Day 0: the day before rabbitfish stocking to the experimental tanks. Bars present standard deviations.

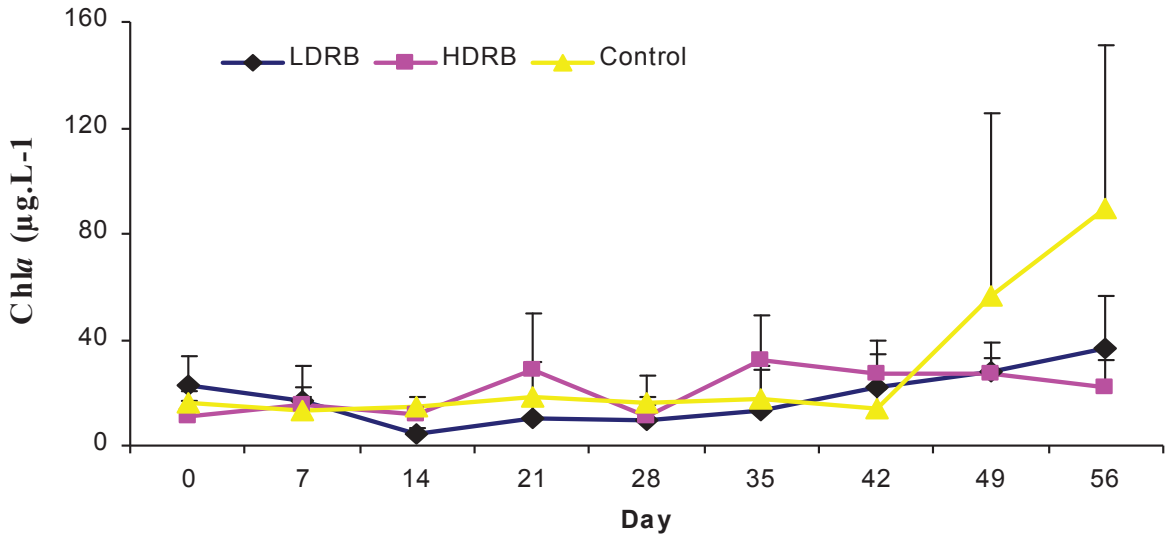


Figure 4.4: Temporal variations of water chlorophyll a in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Day 0: the day before stocking rabbitfish to the shrimp tanks. Bars present standard deviations.

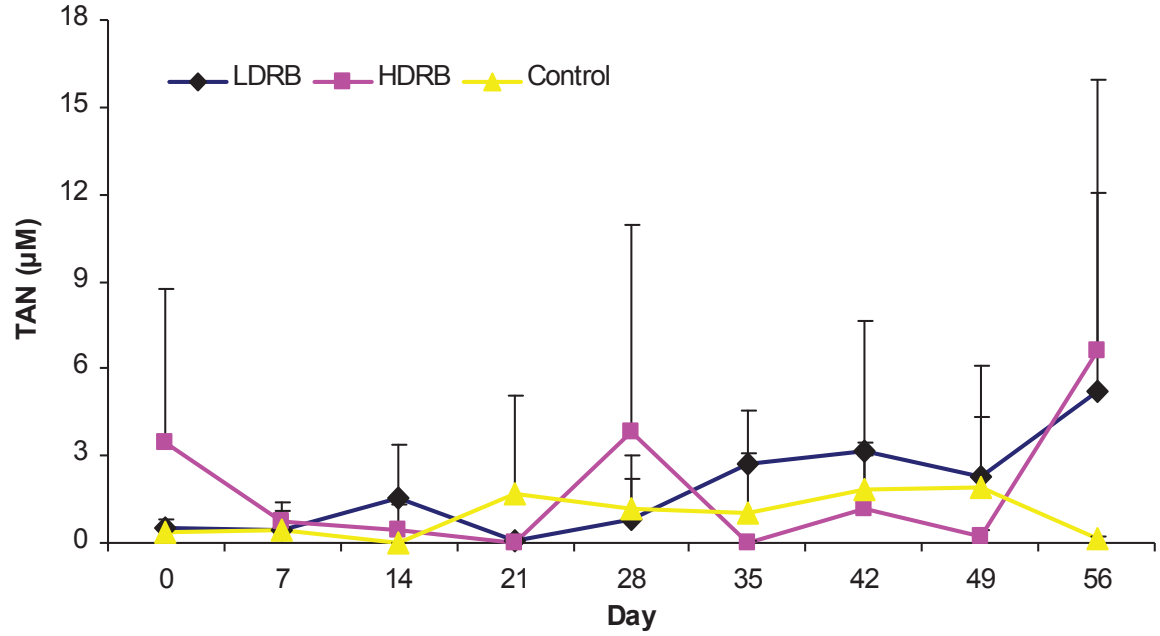


Figure 4.5: Temporal variations of total ammonia nitrogen (TAN) in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Bars present standard deviations.

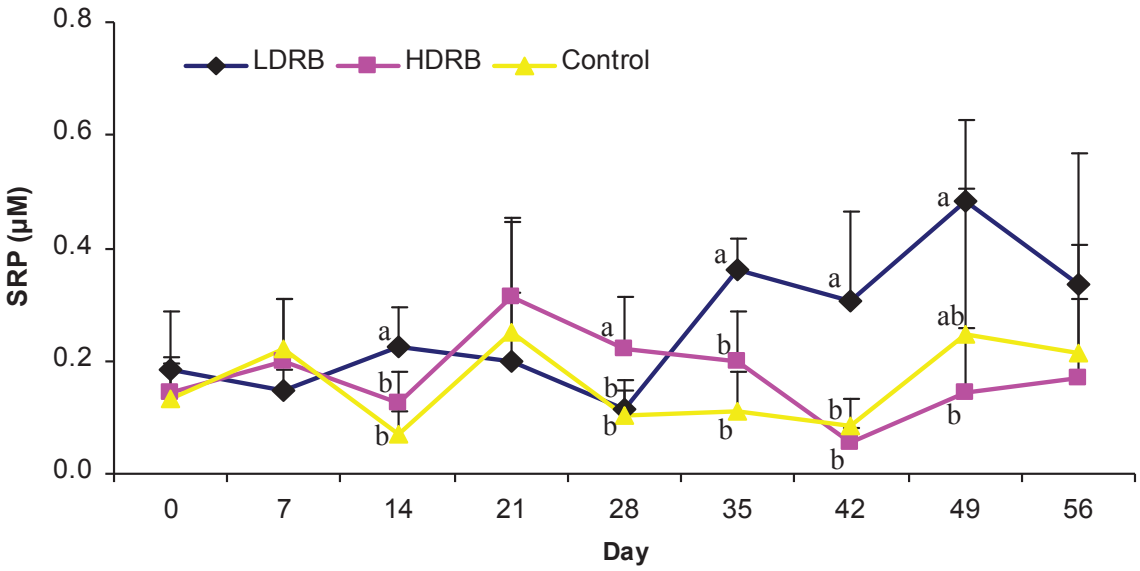


Figure 4.6: Temporal variations of soluble reactive phosphorus (SRP) in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Bars present standard deviations. Values in the same day with different letters are significantly different ($P<0.05$).

4.3.2.2 Sediment parameters

No significant difference ($P>0.05$) was observed in all sediment parameters among polyculture treatments and the control (Table 4.3). Trends in redox potential were similar across all treatments. However, there was high variability among replicates of each treatment.

Polyculture treatments and the control had the same trend in sediment Chl *a* variation throughout the experiment that fluctuated in the first four weeks and gradually increased during the last four weeks (Fig. 4.7).

Table 4.3: Sediment parameters in polyculture and in the control treatments throughout the experimental period. Values are means ± SD.

	Treatment		
	Low density rabbitfish	High density rabbitfish	Control
pH	6.9 ± 0.0	6.8 ± 0.1	6.9 ± 0.1
Redox potential (mV)	-37.6 ± 11.4[a]	-27.1 ± 23.8[a]	-34.0 ± 11.9[a]
Loss on ignition (%)	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
Chlorophyll *a* ($mg.m^{-2}$)	166.3 ± 20.7[a]	180.0 ± 41.8[a]	144.7 ± 15.8[a]
Phaeopigment (%)	26.6 ± 1.9[a]	25.8 ± 3.6[a]	25.6 ± 3.7[a]
Pore water TAN (µM)	277.6 ± 96.5[a]	283.3 ± 206.0[a]	261.3 ± 40.6[a]
Pore water SRP (µM)	2.41 ± 0.77[a]	1.40 ± 0.38[a]	2.32 ± 1.14[a]

Mean values in a same row with same superscript letters are not significantly different (P>0.05).

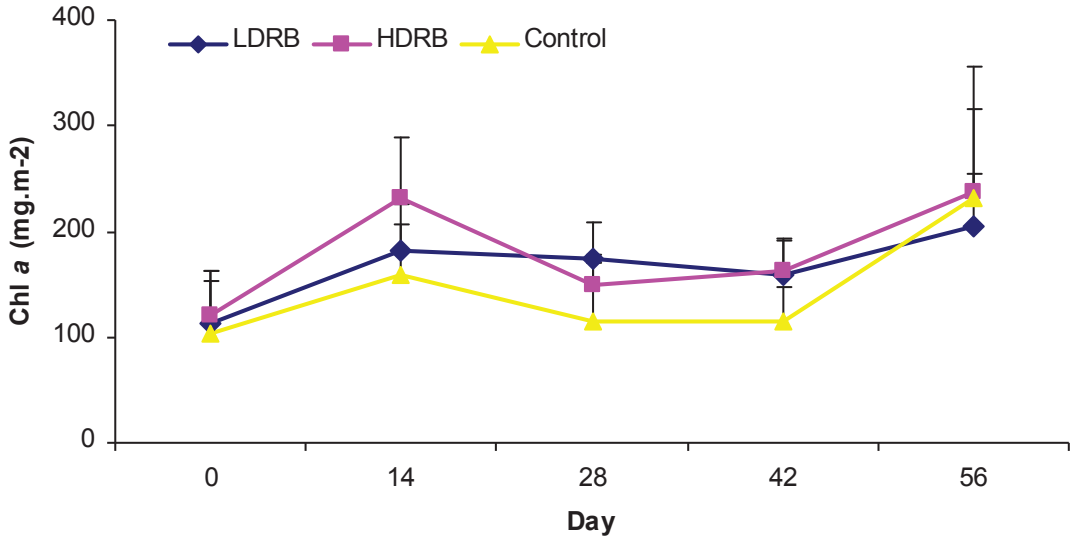


Figure 4.7: Temporal variations of sediment chlorophyll a in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Bars present standard deviations.

Both polyculture treatments had the same trend in pore water TAN temporal variations, which showed a fluctuation within small range in the first six weeks and a strong increase at the end of the experiment. Meanwhile, it gradually raised throughout the experiment in the control (Fig. 4.8). The HDRB treatment and the control had the same trend of pore water SRP temporal variation that fluctuated within small range during the experiment, while SRP strongly increased in the last four weeks of the experiment in the LDRB treatment (Fig. 4.9).

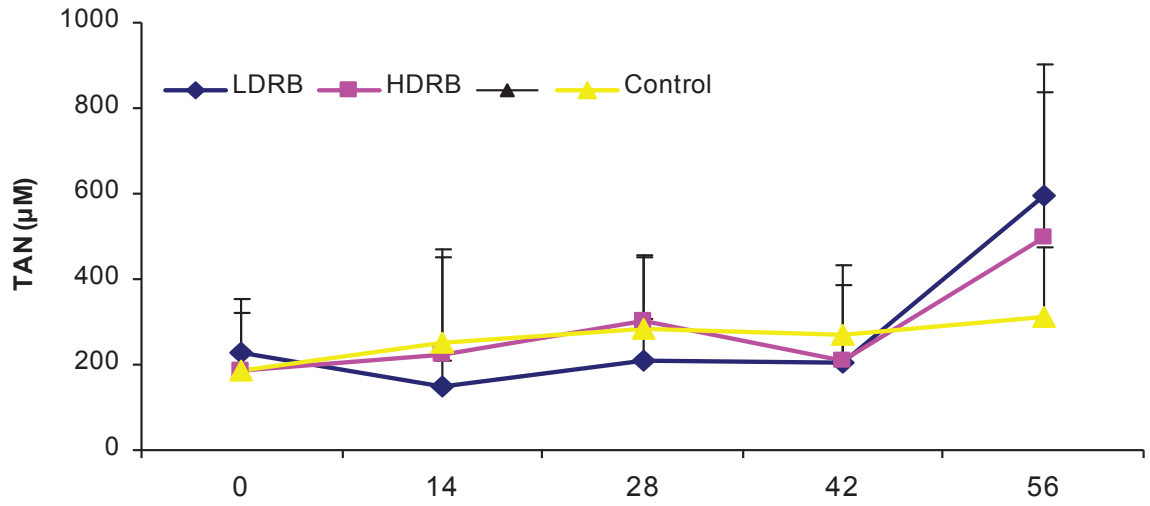


Figure 4.8: Temporal variations of pore water total ammonia nitrogen (TAN) in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Bars present standard deviations.

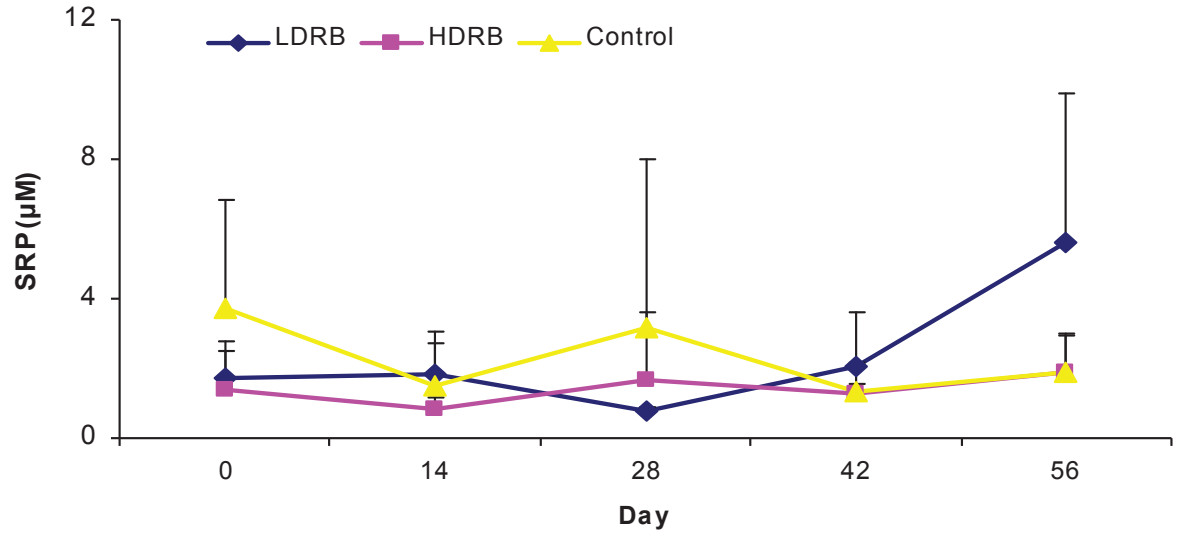


Figure 4.9: Temporal variations of pore water soluble reactive phosphorus (SRP) in low density rabbitfish (LDRB) and high density rabbitfish (HDRB) treatments and the control throughout the experimental period. Bars present standard deviations.

4.3.3 Ecological functioning of the ecosystem

4.3.3.1 Sedimentation processes

There was no significant difference ($P>0.05$) in mean sedimentation rate between the HDRB treatment and the control throughout the experiment, despite much higher value was in the HDRB treatment (Table 4.4). The deposit loss on ignition was significantly higher ($P<0.05$)

in the control than that in the HDRB treatment (Table 4.4). Mean TSS, particulate nitrogen and particulate organic carbon concentrations were similar between the HDRB treatment and the control (Table 4.4). Similarly, the particulate C:N ratio was not different between these treatments.

Table 4.4: Sedimentation process, total suspended solid (TSS), particulate nitrogen and particulate organic carbon in high density rabbitfish treatment and shrimp monoculture. Values are means ± SD.

	Treatment	
	HDRB	Control
Sedimentation rate ($g.m^{-2}.h^{-1}$)	37.8 ± 16.9[a]	14.07 ± 5.2[a]
Deposit loss on ignition (%)	4.33 ± 0.02[a]	5.83 ± 0.9[b]
TSS ($mg.L^{-1}$)	26.7 ± 2.8[a]	28.4 ± 2.3[a]
Particulate nitrogen (µM)	55.3 ± 2.7[a]	64.0 ± 8.6[a]
% N in particulate	3.9 ± 0.2[a]	4.5 ± 0.6[a]
Particulate organic carbon (µM)	432.4 ± 18.4[a]	489.5 ± 39.4[a]
% C in particulate	26.0 ± 1.1[a]	29.4 ± 2.4[a]
C:N	6.7 :1	6.6 :1

Mean values in a same row with same superscript letters are not significantly different (P>0.0).

Sedimentation process and TSS slowly raised through almost the course of the experiment, and dramatically increased at the end of the experiment (Fig. 4.10 & 4.11). There was a large variability of sedimentation between replicates of the HDRB treatment at the end of the experiment.

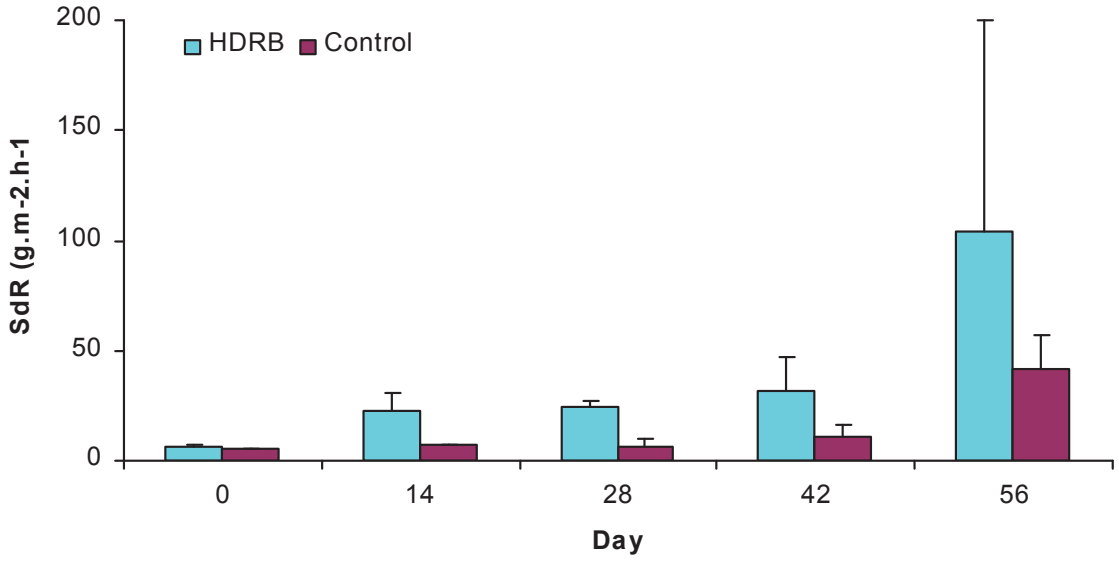


Figure 4.10: Sedimentation rates in high density rabbitfish (HDRB) treatment and shrimp monoculture (control) during the experiment. Bars present standard deviations.

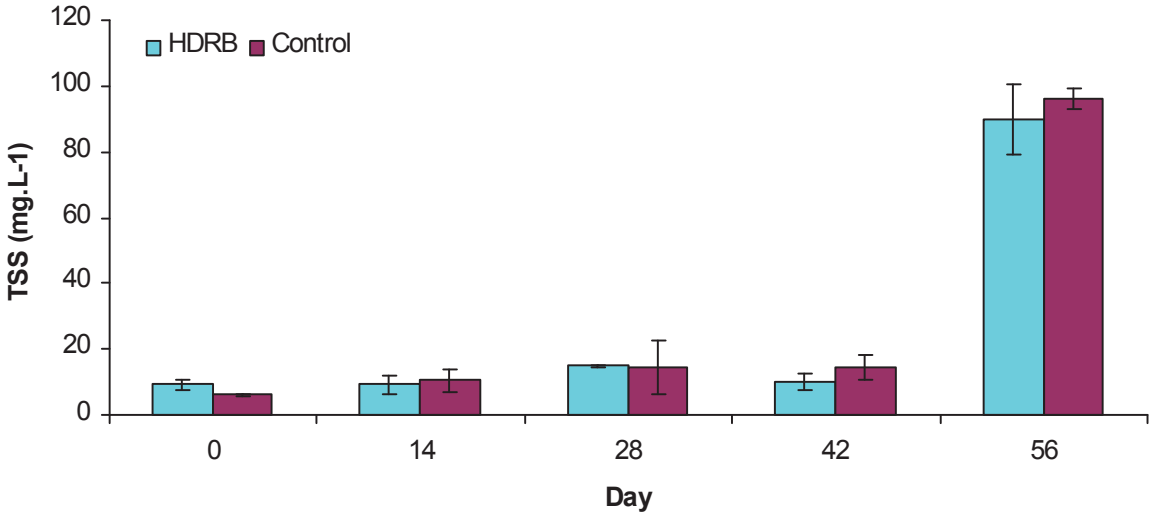


Figure 4.11: Total suspended solids in high density rabbitfish treatment (HDRB) and shrimp monoculture (control) during the experiment. Bars present standard deviations.

4.3.3.2 Nutrient fluxes

Nitrite/nitrate – nitrogen (NOx – N) was the smallest in nitrogen fluxes and without typical temporal pattern in the HDRB treatment as well as in the control (Fig. 4.12). In the HDRB treatment, except on the end day, light NOx – N fluxes were effluxes with values ranging from 1.2 to 15.4 $\mu mol.m^{-2}.h^{-1}$. Meanwhile, dark NOx – N fluxes included effluxes and uptakes appearing alternately over the experiment (Fig. 4.12), its values ranged from -24.7 to 12.0 $\mu mol.m^{-2}.h^{-1}$. So, net NOx – N fluxes consisted of effluxes alternating with uptakes in the HDRB treatment, with values ranged from -31.8 to 27.3 $\mu mol.m^{-2}.h^{-1}$. In the control, dark NOx – N, except for the day 0, and light NOx – N fluxes were uptakes throughout the experiment. Dark NOx – N uptakes ranged from -3.7 to -17.8 $\mu mol.m^{-2}.h^{-1}$, and light NOx – N uptakes were from -0.5 to -49.6 $\mu mol.m^{-2}.h^{-1}$ (Fig. 4.12). So net NOx – N fluxes were uptakes over the experiment, from -6.0 to -67.4 $\mu mol.m^{-2}.h^{-1}$.

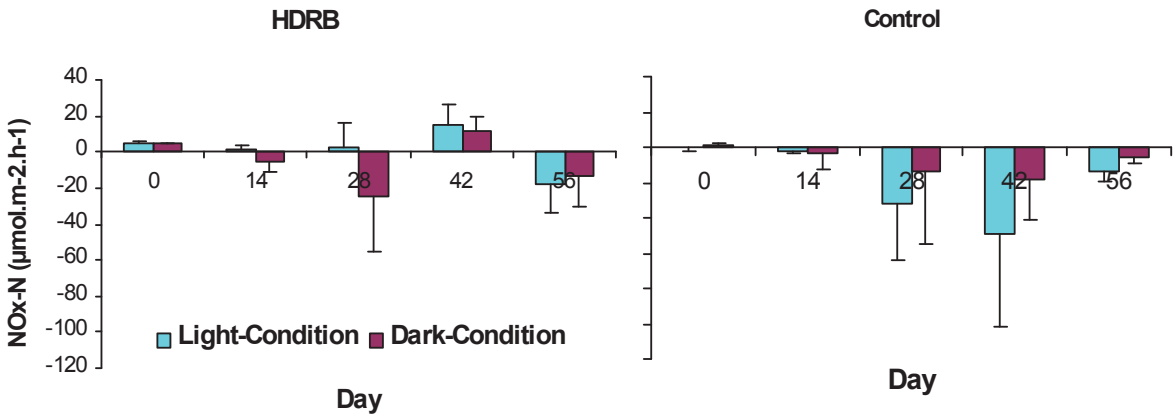


Figure 4.12: $(NO_2 + NO_3)$-N fluxes in water-sediment interface of the high density rabbitfish treatment (HDRB) and the control in light and dark conditions over the experiment. Positive fluxes represent efflux from the sediment, while negative fluxes represent uptake by the sediment. Bars present standard deviations.

TAN fluxes also had no typical temporal pattern in both treatments (Fig. 4.13). In the HDRB treatment, very high values but large variabilities of dark and light TAN fluxes were observed on the end day. Dark TAN fluxes ranged form -41.3 to 171.6 µmol.m^{-2}.h^{-1}, included effluxes alternating with uptakes. Light TAN fluxes, except for the day 14, were effluxes over the experiment (Fig. 4.13). Almost net TAN fluxes were effluxes, ranging from 9.2 to 264.2 µmol.m^{-2}.h^{-1}, except for the day 42. In the control, light TAN fluxes, except on the end day, were effluxes with the highest value was 68.8 µmol.m^{-2}.h^{-1} on the day 42 (Fig. 4.13). Dark TAN fluxes were uptakes in three first times followed by effluxes in two last times of measurements, its values ranged from -39.4 to 50.7 µmol.m^{-2}.h^{-1}. Likewise, net TAN fluxes had the same trend with dark TAN fluxes, ranging from -38.6 to 119.6 µmol.m^{-2}.h^{-1}.

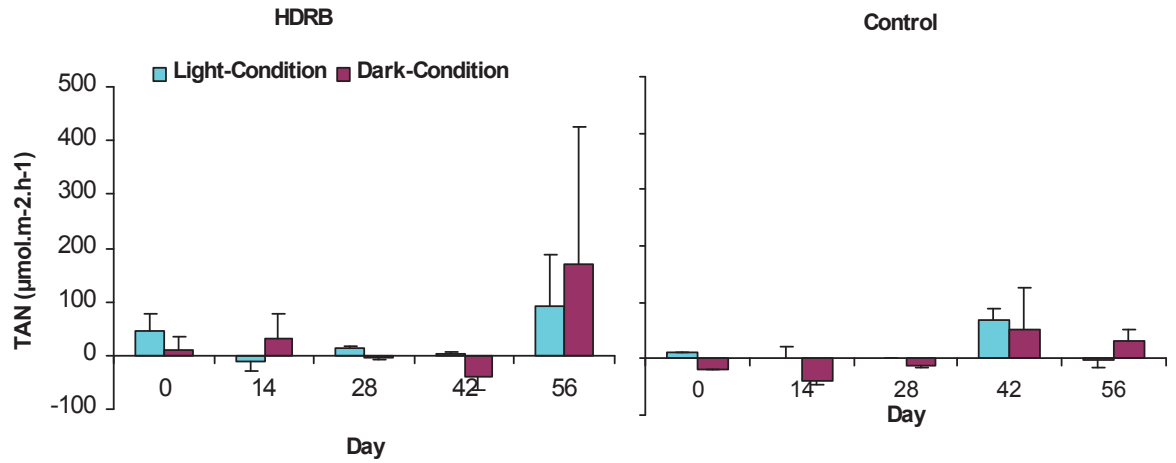


Figure 4.13: Total ammonia nitrogen (TAN) fluxes in water-sediment interface of the high density rabbitfish treatment (HDRB) and the control in light and dark conditions over the experiment. Positive fluxes represent efflux from the sediment, while negative fluxes represent uptake by the sediment. Bars present standard deviations.

DON dominated the nitrogen fluxes and showed no typical temporal pattern in both treatments (Fig. 4.14). In the HDRB treatment, dark DON fluxes ranged from -1022 to 426 µmol.m^{-2}.h^{-1}, and the lowest value of uptake was on the end day. In the control, dark DON fluxes were from -217 to 603 µmol.m^{-2}.h^{-1}, and the highest value of efflux was on the end day. The HDRB treatment had light DON effluxes, only one exception on day 14, ranged from 52 to 642 µmol.m^{-2}.h^{-1}. In the control, except small effluxes on day 28 and 56, light DON fluxes were uptakes with the lowest value on the day 42 (Fig 4.14). Both treatments had large ranges of net DON fluxes, from - 970 to 1068 µmol.m^{-2}.h^{-1}, and from - 363 to 671 µmol.m^{-2}.h^{-1} in the HDRB treatment and the control, respectively.

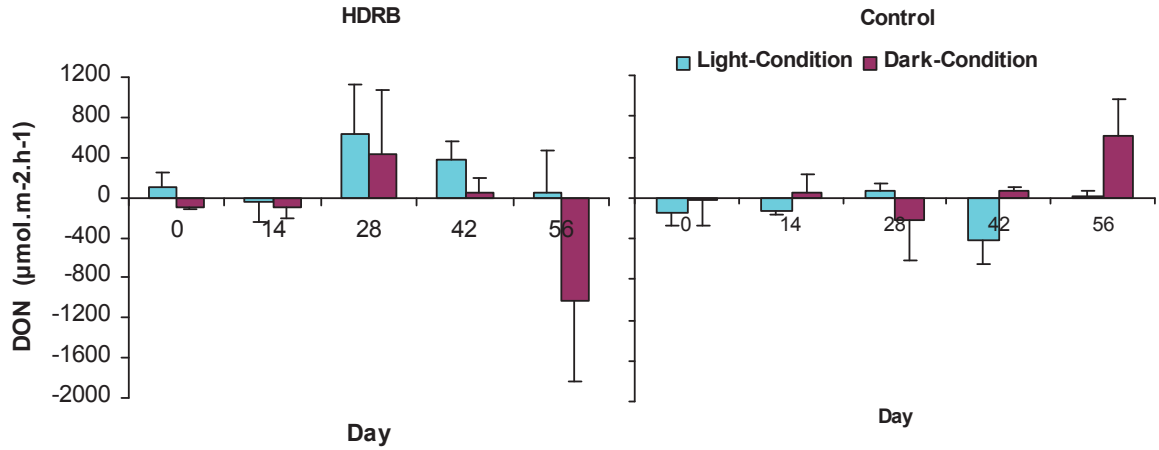


Figure 4.14: Dissolved organic nitrogen (DON) fluxes in water-sediment interface of the high density rabbitfish treatment (HDRB) and the control in light and dark conditions over the experiment. Positive fluxes represent efflux from the sediment, while negative fluxes represent uptake by the sediment. Bars present standard deviations.

Like nitrogen fluxes, there was no typical temporal pattern in dark and light SRP fluxes in both treatments (Fig. 4.15). Except on the day 28, light SRP fluxes were uptakes within a small range of rate, from -0.2 to -6.2 µmol.m^{-2}.h^{-1} in the HDRB treatment. A high uptake but large variability of dark SRP fluxes was observed on the end day, -28.15 µmol.m^{-2}.h^{-1} (Fig. 4.15). The remainders of dark SRP fluxes showed both effluxes and uptakes with small rates, from -0.1 to 2.9 µmol.m^{-2}.h^{-1}. The uptake dominated in net SRP fluxes than effluxes over the experiment in the HDRB treatment. The values ranged from -31.3 to 3.0 µmol.m^{-2}.h^{-1}. Similarly, small rates of uptakes and effluxes of light and dark SRP fluxes were observed throughout the experiment in the control (Fig. 4.15). Dark, light and net SRP fluxes ranged from -6.4 to 9.8, -2.8 to 2.3, and -6.4 to 12.1 µmol.m^{-2}.h^{-1}, respectively.

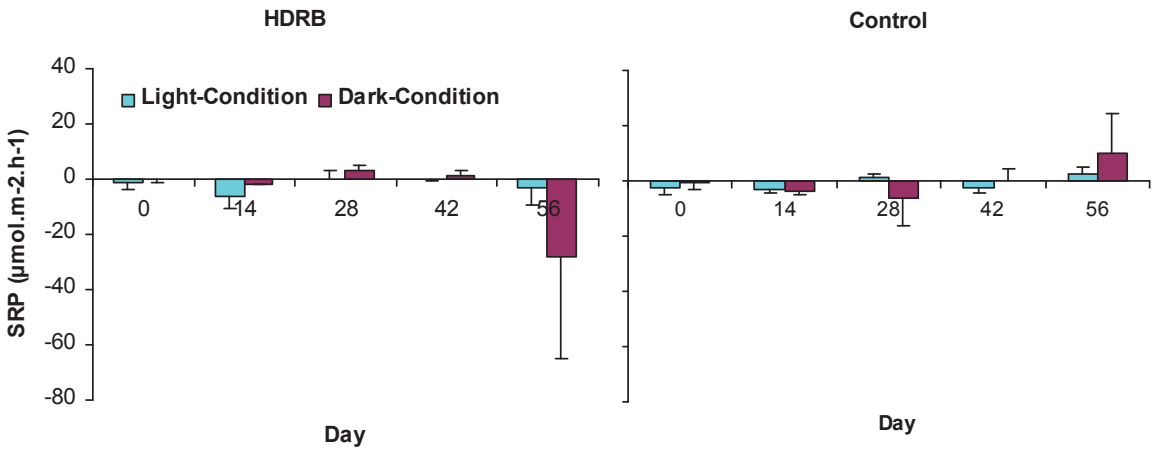


Figure 4.15: Soluble reactive phosphorus (SRP) fluxes in water-sediment interface of high density rabbitfish treatment (HDRB) and the control in light and dark conditions over the experiment. Positive fluxes represent efflux from the sediment, while negative fluxes represent uptake by the sediment. Bars present standard deviations.

4.3.3.3 Metabolism

There was no significant difference ($P>0.05$) in gross primary productivity (GPP) of the water column, sediment and entire ecosystem between the HDRB treatment and the control over the experiment. However, the trend of GPP temporal variation was different between two treatments (Fig. 4.16). GPP was significantly different ($P<0.05$) between the water column and sediment within each treatment. GPP dominated in water column and had opposite trend of temporal variation with sediment in both treatments (Fig. 4.16).

In the HDRB polyculture, GPP temporal variation had the same trend between the surface and bottom water, which increased in the first four weeks and reached the maximum values of 481 and 386 mg $O_2.m^{-2}.h^{-1}$, respectively, then rapidly decreased in the last four weeks (Fig. 4.16). In contrast, GPP in sediment was lowest in the fourth week and reached a maximum of 309 mg $O_2.m^{-2}.h^{-1}$ at the sixth week (Fig. 4.16).

In the control, GPP of surface water rapidly rose in the first two weeks, then highly decreased in next four weeks and dramatically increased and reached the maximum value, 941 mg $O_2.m^{-2}.h^{-1}$, at the end of the experiment, (Fig. 4.16). In bottom water, GPP moderately increased and attained the maximum of 390 mg $O_2.m^{-2}.h^{-1}$ at the second week, then gradually decreased to the end of the experiment. Meanwhile in sediment, GPP gained the maximum of

231 mg $O_2.m^{-2}.h^{-1}$ at the beginning, maintained until the sixth week and then decreased toward the end of the experiment.

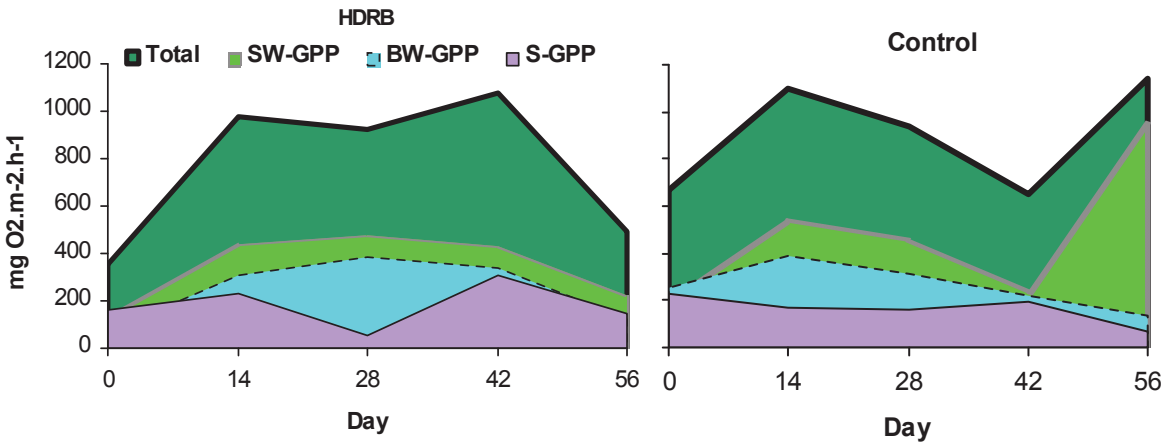


Figure 4.16: Temporal variations of Gross Primary Productivity (GPP) in high density rabbitfish (HDRB) treatment and shrimp monoculture (control); S-GPP: GPP in sediment, BW-GPP: GPP in bottom water, SW-GPP: GPP in surface water, Total: GPP in entire ecosystem.

There was no significant difference ($P>0.05$) in respiration of the water column, sediment and entire ecosystem between the HDRB polyculture and the control. Within each treatment, respiration was significantly higher ($P<0.05$) in the water column than in sediment (Fig. 4.17). The trend of entire respiration temporal variation was different between the HDRB treatment and the control, but sediment respiration temporal variation had same trend in both treatments (Fig. 4.17).

In the HDRB treatment, the water respiration moderately decreased in the first two weeks, followed by a rapid increase to the maximum of 175 mg $O_2.m^{-2}.h^{-1}$ in the sixth week, and then slightly reduced to the end of the experiment. In the control, the water respiration gradually raised in the first six weeks and then dramatically increased and reached the maximum of 353 mg $O_2.m^{-2}.h^{-1}$ at the end of the experiment (Fig. 4.17). Sediment respiration progressively increased throughout the experiment and reached the maximum of ~ 85 mg $O_2.m^{-2}.h^{-1}$ at the end of the experiment in both treatments.

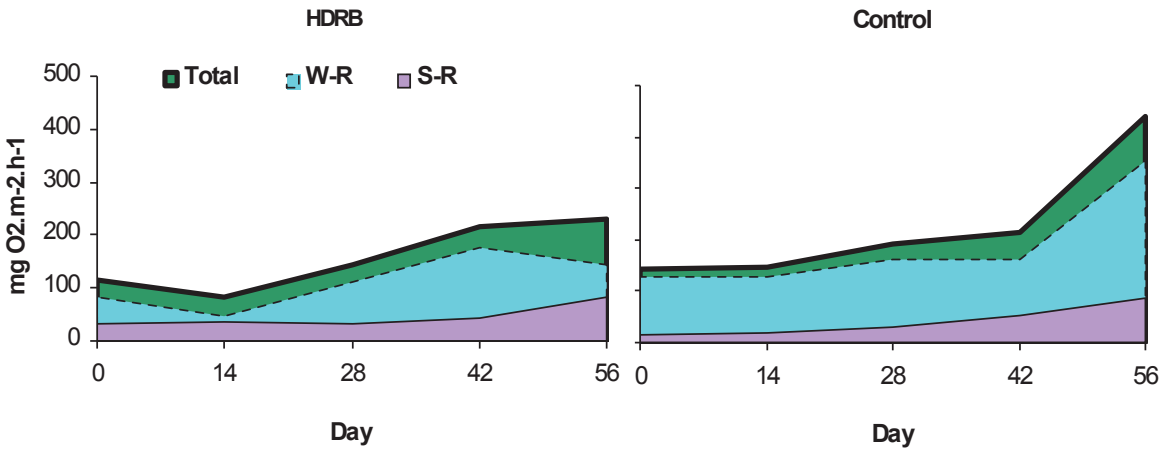


Figure 4.17: Temporal variations of Respiration (R) in high density rabbitfish treatment (HDRB) and shrimp monoculture (control); S-R: Sediment Respiration, W-R: Water column Respiration, total: respiration in entire ecosystem.

There was no significant difference (P>0.05) in the P/R ratio between the HDRB treatment and the control. The P/R ratio in entire ecosystem had the same trend in both treatments. It rapidly increased from beginning and attained the highest value at the second week, then progressively decreased to the end of the experiment (Fig. 4.18).

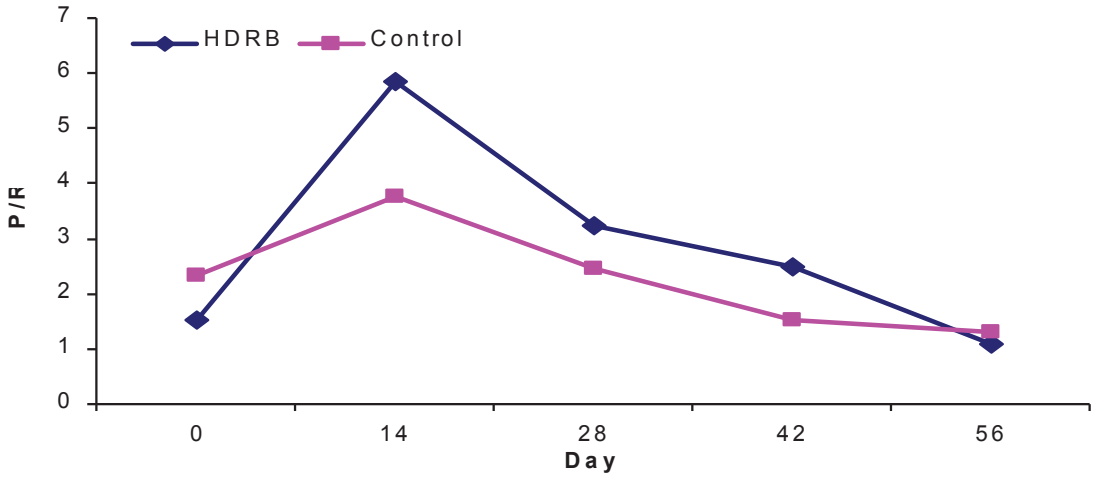


Figure 4.18: Temporal variations of Primary Productivity and Respiration ratio (P/R) in the high density rabbitfish treatment (HDRB) and the control over the experiment.

4.3.4 Stable isotope signatures

The same pellet feed was used for feeding to the culture system during the whole experiment, so the pellet feed $\delta^{13}C$ and $\delta^{15}N$ values were similar among analyses, and the average values were -20.89 ± 0.17 ‰ for $\delta^{13}C$, and 12.31 ± 0.34 ‰ for $\delta^{15}N$. At the beginning, the $\delta^{13}C$ values of particulate organic matter (POM) and sedimentary organic matter (SOM) were

significantly higher ($P<0.05$) than the pellet feed $\delta^{13}C$ value. There was also significant difference ($P<0.05$) in $\delta^{13}C$ value between POM and SOM.

Throughout the experiment, the POM $\delta^{13}C$ values decreased in all treatments. However, the final POM $\delta^{13}C$ values were not significantly lower ($P>0.05$) than the initial value, but became similar with the pellet feed $\delta^{13}C$ value ($P>0.05$) for all treatments. The SOM $\delta^{13}C$ values slightly reduced over the course of the experiment (Table 4.5); however, they were still significantly different ($P<0.05$) from the pellet feed $\delta^{13}C$ value in all treatments.

The $\delta^{15}N$ values for both POM and SOM were significantly lower ($P<0.05$) than the pellet feed $\delta^{15}N$ value at the beginning, and the $\delta^{15}N$ was also significantly different ($P>0.05$) between POM and SOM. Over the course of the experiment, the POM $\delta^{15}N$ value significantly increased in all treatments (Table 4.5). Similarly, the final SOM $\delta^{15}N$ values were higher than the initial $\delta^{15}N$ value in all treatments (Table 4.5). However, except in the LDRB treatment, the final SOM $\delta^{15}N$ values did not significantly ($P>0.05$) differ from the initial $\delta^{15}N$ value in other treatments (Table 4.5). Although the POM and SOM $\delta^{15}N$ values highly increased over the experiment in all treatments, they still were significantly lower ($P<0.05$) than the pellet feed $\delta^{15}N$ value at the end of the experiment.

Table 4.5: Stable isotope ($\delta^{13}C$, $\delta^{15}N$) values in particulate organic matter (POM) and sedimentary organic matter (SOM) at the beginning and at the end of the experiment.Values are means ± SD, expressed in ‰.

	Initial	Final		
		Low density rabbitfish	High density rabbitfish	Control
POM $\delta^{13}C$	-19.35 ± 1.30^{a}	-20.68 ± 1.66^{a}	-19.91 ± 2.25^{a}	-19.61 ± 0.51^{a}
$\delta^{15}N$	5.67 ± 1.45^{a}	9.89 ± 1.84^{b}	9.69 ± 0.56^{b}	10.64 ±0.37^{b}
SOM $\delta^{13}C$	-17.70 ± 0.57^{a}	-17.98 ± 0.45^{a}	-18.14 ± 0.30^{a}	-18.28 ± 0.32^{a}
$\delta^{15}N$	6.99 ± 1.39^{a}	9.89 ± 0.02^{b}	7.09 ± 1.23^{a}	7.67 ± 0.00^{ab}

Mean values in a same row with same superscript letters are not significantly different ($P>0.05$).

At stocking, the shrimp $\delta^{13}C$ value was significant higher ($P<0.05$) than the initial $\delta^{13}C$ values of the pellet feed, POM and SOM. Over the experiment, the shrimp $\delta^{13}C$ values significantly decreased in all treatments (Table 4.6), which were not significantly different ($P>0.05$) from the final SOM $\delta^{13}C$ values, but still significantly higher ($P<0.05$) than the pellet feed $\delta^{13}C$ isotope value.

The rabbitfish $\delta^{13}C$ value was higher than the initial $\delta^{13}C$ values of the pellet feed ($P<0.05$), and POM ($P>0.05$), but lower than the initial $\delta^{13}C$ values of SOM ($P<0.05$) and shrimp

(P<0.05) at stocking. Unlike with shrimp $\delta^{13}C$, the rabbitfish $\delta^{13}C$ values significantly increased over the course of the experiment in all treatments (Table 4.6). Particularly, the rabbitfish $\delta^{13}C$ values were not significantly different (P>0.05) with the shrimp and SOM $\delta^{13}C$ values at the end of the experiment.

At stocking, the shrimp $\delta^{15}N$ value was significantly lower (P<0.05) than the pellet feed $\delta^{15}N$ value, but significantly higher (P<0.05) than the initial POM and SOM $\delta^{15}N$ values. Throughout the experiment, the shrimp $\delta^{15}N$ values significantly increased in all treatments (Table 4.6), except for the HDRB treatment, it did not significantly differ (P>0.05) from the pellet feed $\delta^{15}N$ value. The shrimp $\delta^{15}N$ values were enriched by 1.23 – 2.06 ‰ relative to POM, and by 2.06 – 4.44 ‰ relative to SOM at harvesting.

The rabbifish $\delta^{15}N$ value was significantly higher (P<0.05) than the $\delta^{15}N$ values of the pellet feed, POM and SOM at stocking. The rabbifish $\delta^{15}N$ value did not change over the experiment (Table 4.5). At harvesting, the $\delta^{15}N$ fractionation factors of rabbitfish to the pellet feed, POM and SOM were 0.95 – 1.17 ‰, 3.58 – 3.59 ‰, and 3.59 – 6.18 ‰, respectively.

Table 4.6: Stable isotope ($\delta^{13}C$, $\delta^{15}N$) values in shrimp and rabbitfish at stocking and harvesting of the experiment.Values are means ± SD, expressed in ‰.

	Stocking	Harvesting		
		Low density rabbitfish	High density rabbitfish	Control
Shrimp $\delta^{13}C$	-13.37 ± 0.09[a]	-17.81 ± 0.78[b]	-18.01 ± 0.15[b]	-17.86 ± 0.27[b]
$\delta^{15}N$	10.11 ± 0.11[a]	11.95 ± 0.18[b]	11.53 ± 0.41[b]	11.87 ± 0.45[b]
Rabbitfish $\delta^{13}C$	-18.60 ± 0.18[a]	-17.79 ± 0.51[b]	-18.06 ± 0.21[b]	
$\delta^{15}N$	13.49 ± 0.35[a]	13.49 ± 0.26[a]	13.26 ± 0.25[a]	

Mean values in a same row with same superscript letters are not significantly different (P>0.05).

4.4 Discussion

4.4.1 Shrimp and rabbitfish growth performances

Shrimp growth performance, including final mean weight, daily weight gain (DWG) and specific growth rate (SGR) were not significantly different (P>0.05) between polyculture treatments and the control, indicating that the presence of rabbitfish had no negative effect on shrimp growth. One problem in polyculture shrimp with free-swimming fishes is that the competition for food could potentially negatively affect shrimp growth (Wang et al., 1998;

García-Pérez et al., 2000; Yuan et al., 2010) because fish are faster swimmers than shrimp and can quickly monopolize the food, and thus can suppress shrimp growth. Rabbitfish are herbivorous and gregarious fishes (Lam, 1974), and exhibit little competitive behaviour (Saoud et al., 2008a) even when reared at high densities as described in Chapter 3. In captivity, rabbitfish become opportunistic omnivores and can feed on a great variety of foods such as aquatic plants, cooked rice, chopped fish or mollusks, fish meal, and pellets (Ben-Tuvia et al., 1973; Lam, 1974). Tendencia et al. (2006a) supposed that rabbitfish consume uneaten feed, and thus prevent further deterioration of the environment in shrimp culture system. In our study, it was expected that *S. lineatus* would eat uneaten pellet feed offered to shrimp for their growth and that interspecies competition for food would be so low that it would have no negative effect on shrimp growth and survival.

Shrimp growth in this study was similar with the result recorded for blue shrimp reared in earthen ponds (0.14 – 0.16 $g.d^{-1}$; Lemonnier and Faninoz, 2006), but lower than those of blue shrimp cultured in circulating tanks (0.17 – 0.33 $g.d^{-1}$; Kumaraguru vasagam et al., 2009). Quite low temperatures (Table 4.2 & Fig. 4.2) could be one of the possible reasons for the "low" growth of shrimp in our results. The temperature range for growing *L. stylirostris* is 20 – 30 °C (Bondad-Reantaso et al., 2005; Spanopoulous-Hernández et al., 2005), and the optimum temperature is reported to be about 28 ^{o}C (Díaz et al., 2004; Bondad-Reantaso et al., 2005). Díaz et al. (2004) emphasized that thermal stress of *L. stylirostris* would increase as temperatures decreased or increased with respect to the optimum temperature of 28 ^{o}C. Wabete et al. (2008) reported that the low limit of the thermo-preferendum for *L. stylirostris* is 20-22 ^{o}C, and also showed that at 20 to 22 ^{o}C shrimp were thermally stressed. In our experiment, water temperature fluctuated over the lower half of the temperature range for *L. stylirostris* growth. Morning temperatures were even below the lower limit of this range (Fig 4.2). This low water temperature might have negatively affected shrimp growth, survival and food conversion ratio (Wyban et al., 1995).

Adding rabbitfish in our experiment decreased the variability of the shrimp survival observed between replicates. This suggested that rabbitfish activity caused a possible increase in the stability of environmental conditions in the culture tanks subsequently reducing shrimp mortality in some tanks. However, this hypothesis was not well supported yet and should be tested in further experiments. Shrimp mortality was observed, particularly at the end of the experiment when the eutrophication level of the ecosystem was highest as already observed

in production ponds (Lemonnier et al., 2006). The water quality parameters were similar among treatments and varied within ranges (Table 4.2 and Fig. 4.3, 4.4 & 4.5) that are unlikely to cause shrimp mortality. TAN concentrations were well below safe levels of ammonia recommended for rearing penaeid shrimp, 4.26 $mg.L^{-1}$ (304.3 μM) TAN and 0.08 $mg.L^{-1}$ (5.7 μM) NH_3-N (Chen et al., 1990); or for grow-out ponds, NH_3-N < 0.15 $mg.L^{-1}$ (10.7 μM) (Lazur, 2007). The (NO_2+NO_3)-N concentrations were also well under safe levels of nitrite for rearing penaeid shrimp, 10.6 $mg.L^{-1}$ (757.1 μM) (Chen et al., 1990); or for grow-out pond, 4.5 $mg.L^{-1}$ (321.4 μM) (Lazur, 2007). pH in sediments and in the water columns varied within small and suitable ranges (6.5 – 8.0) for animal health and growth in all treatments (Lemonnier et al., 2004). The effect of hypoxic conditions on TAN accumulation may have a negative impact on shrimp growth (Joyni et al., 2011) and lead to animal mortality. However, mean TAN in pore water in this study were lower than stressful values recorded in a shrimp pond in New Caledonia (607.9 μM, Mugnier et al., 2006) and were below safe level of TAN defined for rearing penaeid shrimp, 304.3 μM TAN (Chen et al., 1990). During the experiment, DO concentrations fluctuated within suitable ranges (Table 4.2 & Fig 4.1) for shrimp and rabbitfish requirement, and also could provide for biochemical processes in all tanks (Boyd, 1998; Lazur, 2007).

Rabbitfish growth in this study (DWG: 0.50 – 0.58 $g.d^{-1}$, SGR: 1.33 – 1.44 $\%.d^{-1}$) was remarkably improved compared with the results of rabbitfish monoculture described in Chapter 3 (DWG: 0.08 – 0.11 $g.d^{-1}$, SGR: 0.98 – 1.16 $\%.d^{-1}$). The main reason was likely due to higher water temperature, average 22.5 – 26.4 ^{o}C, in this study than those in the previous experiment, average 19.7 – 23.0 ^{o}C. Rabbitfishes are eurythermal species and tolerate large variation of temperature (Lam, 1974). The suitable range of water temperature for rabbitfish rearing is 27 – 32 ^{o}C (Duray, 1998; Saoud et al., 2008b), and optimal temperature is at 27 ^{o}C, but they stop feeding at 14 and 36 ^{o}C (Saoud et al., 2008b). Our results showed that goldlined rabbitfish could well adapt low temperature (ca. 20 ^{o}C, Fig. 4.2 & Table 4.2) and its growth could rapidly improve as increasing temperature within the suitable range.

Rabbitfish gained SR of 100% and had high growth rate, and growth performance was similar at all rabbitfish stocking densities. This growth of rabbitfish indicated that food supplied in tanks was sufficient for rabbitfish and the tank environment might be able to support even more rabbitfish biomass. The polyculture of blue shrimp *L. stylirostris* with goldlined rabbitffish *S. lineatus* resulted in a significant increase of total production

compared with shrimp monoculture. The total production significantly raised by 46.8% and 106.2% in the LDRB and the HDRB treatments, respectively, compared with shrimp production without more additional production cost, indicating polyculture these species could be a highly economic possibility. Furthermore, rabbitfish polyculture decreased significantly overall FCR by 31.6% and 47.4% in the LDRB and the HDRB treatments, respectively, compared with the control. Lower overall FCR in rabbitfish polyculture contributed to increasing productive benefit and preventing further degradation of the environment caused by uneaten feed.

4.4.2 Environmental characteristics and ecological functioning

Most nutrient concentrations were low and varied within small ranges during the experiment. This indicated that water quality remained stable over the course of the experiment in all treatments. DON accounted for 87.3 – 95.8 % of TDN in all treatments. In growing ponds, DON derives from formulated feed leaching, gill excretion and faeces leaching, and is one of the major sources of nitrogen in pond water (Burford and William, 2001). The low TAN concentrations were likely due to absorption by phytoplankton (Boyd, 1998; Lazur, 2007). This would also explain the decrease of TAN in the control coinciding with a rapid increase of Chl *a* at the end of the experiment.

High concentration of pore water TAN might be resulted from increasing accumulated organic matter decomposition and mineralization on the sediment bottom. In intensive shrimp ponds, the accumulated sediment was the sink for 58 – 70 % of the organic matter in the system (Funge-Smith and Briggs, 1998), and 24 – 31 % of nitrogen input was retained in the bottom sediments (Briggs and Funge-Smith, 1994; Funge-Smith and Briggs, 1998). Nitrogen in sediment organic matter may be mineralized to ammonia and recycled to the pond water (Boyd et al., 2002). As increased organic source from senescent phytoplankton, animal faeces and uneaten feed accumulated to the sediment, the pore water TAN concentration increased throughout the experiment (Fig. 4.8). The sediment was also the sink for 84% of phosphorus input (Briggs and Funge-Smith, 1994). Phosphorus released by decomposition of organic matter in pond bottoms is rapidly absorbed by sediment and little of it enters the water (Boyd et al., 2002). This process possibly led partially to the higher concentrations of pore water SRP than those in the water columns in all treatments.

The fluxes of NOx-N, TAN, DON and SRP had no typical temporal pattern irrespective of the treatment or light and dark conditions, indicating that there was possibly a variety of factors that influence the nutrient fluxes across sediment-water interface in the culture ecosystem. Resuspension was an important mechanism for materials and nutrients transfer across the sediment-water interface in aquaculture ponds (Funge-Smith and Briggs, 1998; Avnimelech et al., 1999), and diffusion was a major mechanism controlling the fluxes of dissolved materials across the sediment-water interface (Avnimelech et al., 1999).

Eyre et al. (2010) found productivity/respiration (P/R) ratio is an important control on the rates, direction (uptake, efflux) and composition dissolved inorganic N (DIN), dissolved organic N (DON) and N_2 of nitrogen (N) fluxes across the sediment-water interface, with an efflux below P/R = 1.5 and an uptake above P/R = 1.5. In our study, net NOx-N fluxes were weakly correlated with sediment P/R ratio in the HDRB treatment ($R^2 = 0.55$, $P>0.05$) and in the control ($R^2= 0.35$, $P>0.05$). In the control, almost NOx-N fluxes were uptakes and the large net uptake of NOx-N was driven by a combination of the highest dark uptake and light uptake on the day 42 when P/R ratio was 1.8. In the HDRB, net large uptake NOx-N was a combination of a dark uptake and a light uptake of NOx-N occurred on the end day when P/R ratio was 1.5. The daily net mean NOx-N fluxes were uptakes in both treatments throughout the experiment (Table 4.7), indicating sediment represents a sink for nitrite and nitrate (Hargreaves, 1998). The net mean rates of NOx-N uptake in this study were much smaller than those reported in an earthen marine fishpond, -5100 – -8500 $\mu mol.m^{-2}.d^{-1}$ (Blackburn et al., 1988).

There was no correlation between net TAN fluxes and sediment P/R ratios in both treatments ($R^2 = 0.13$, $P>0.05$). However, in the control, the net uptake of TAN occurred as P/R ratio was above 3 and the net efflux of TAN fluxes when P/R ratio was below 2. The large net efflux of TAN was driven by a combination of the largest dark efflux and light efflux of TAN occurring on the day 42 when sediment P/R was 1.8 that was opposite the NOx-N flux. In the HDRB treatment, almost light and net TAN fluxes were effluxes, and the large net efflux of TAN was a combination of the largest dark efflux and light efflux of TAN on the end day when P/R ratio was 1.5, which was reverse the NOx-N flux. In both treatments, the daily net mean TAN fluxes were effluxes throughout the experiment (Table 4.7), indicating sediment represents a source of ammonia (Hargreaves, 1998), and heterotrophic sediment dominated close to the end of the experiment (Cowan and Boynton, 1996; Kristensen and Hansen,

1999). The net mean rate of TAN effluxes in both treatments were very smaller than those of ammonia (NH_4^+) flux in an earthen marine fish pond, 15800 – 23500 µmol.m^{-2}.d^{-1} (Blackburn et al., 1988).

The only and large net efflux of DON occurred on the end day as sediment P/R ratio below 1 in the control. Similarly, in the HDRB treatment, the largest net efflux of DON was on the day 28 when P/R ratio was as low as 1, indicating that large DON released to the water when sediment were net heterotrophic (Eyre et al., 2010**)**. The daily net mean of DON flux indicated sediment was a source of DON in the HDRB treatment, while, inversely, the sediment was a sink of DON in the control when the daily net mean DON flux was uptake throughout the experiment (Table 4.7).

The dark effluxes and light effluxes of SRP occurred simultaneously when sediments were net heterotrophic in both treatments (Cowan and Boynton, 1996; Kristensen and Hansen, 1999). The large net effluxes of SRP were a combination of the largest dark effluxes and light effluxes of SRP on the end day in the control as sediment P/R ratio below 1, and on the day 28 in the HDRB treatment when P/R ratio equal 1. However, the daily net mean uptake of SRP flux (Table 4.7) showed sediments were sinks of SRP in both treatments.

In general, the total net N fluxes (NOx-N + TAN + DON) through sediment-water interfaces (Table 4.7) were in very small rates in both treatments compared with quantity of daily nitrogen input from pellet feed supplied to the tank ecosystem, average 23366 µmol.m^{-2}.d^{-1}, during the experiment. These results suggested that water quality was negligibly affected by biochemical processes occurred on sediment and subsequently generated dissolved nutrients releasing into the water. The results also showed that rabbitfish adding to the shrimp tanks did not clearly change nutrient fluxes compared with shrimp monoculture. However, additional researches should be conducted on this area with different stocking ratios and sizes between shrimp and rabbitfish.

Table 4.7: The daily mean net nutrient fluxes, expressed by µmol.m^{-2}.d^{-1} (n =5), for two treatments throughout the experiment. Positive values represent efflux from the sediment, while negative values represent uptake by the sediment.

	HDRB	Control
NOx-N	-51.6	-331.3
TAN	750.8	229.1
DON	980.0	-351.2
SRP	-87.2	-10.1

The equal daily food input, water exchange rate and similar Chl *a* concentration might lead to the similarity of mean value and temporal variation of turbidity for all treatments. Except the first week after shrimp stocking, turbidity highly increased in all treatments (not shown) probably resulted from shrimp strong activities for feeding on the sediment bottoms because they were not fed during catching and maintaining for two days. Turbidity tended slightly decrease over the experiment in all treatments (Fig. 4.3), although the daily food portion, shrimp and rabbitfish sizes and biomasses increased. In the culture systems with soil substrate, turbidity increased with shrimp/fish length and feeding because the elevated movement or feeding activity of the larger shrimp/fish increased the suspension of soil particles (Ritvo et al., 1997). Our results showed that sediment disturbance caused by shrimp and/or rabbifish activity did not affect turbidity in culture tanks.

There were significant positive correlations between sedimentation rate (SdR) and total suspended solid (TSS) in the HDRB polyculture ($R^2 = 0.95$, $P = 0.005$) and the control ($R^2 = 0.99$, $p = 0.001$). The increases of SdR and TSS were probably derived from increasing shrimp and rabbitfish sizes and biomasses, and increased nutrient input, which caused increasing suspension and resuspension of particulate matter from the bottom to the water column over the course of the experiment (Avnimelech et al., 1999). Shrimp/fish size, density and foraging behavior were key factors affecting sediment disturbance. Both shrimp/fish weight increase and the increased feed input led to higher suspended solids in the water column. Materials depositing from the water column to the sediment could be originated from two sources, i.e. suspended particulate matter and particles resuspended from the pond bottom (Avnimelech et al., 1999). In the HDRB polyculture, rabbitfish movement and feeding activity might generate more additional suspension and resuspension, consequently SdR was higher than in the control. In addition, this increase might be derived from an increased portion of inorganic solids in sedimented materials, and thus led to a lower percent of deposit loss on ignition in the HDRB polyculture compared with the control (Table 4.4).

C:N ratio is a useful tool to assess the nature of organic matter. In both treatments, C:N ratio of suspended organic particulate was in a range for fresh living phytoplankton (Fernandes et al., 2009). Mean Chl *a* concentrations were not different among polyculture treatments and the control, that might be due to equal nutrient inputs provided into the tanks from daily pellet feed supplied and water intake. Furthermore, rabbitfish, in juvenile and adult stages, was unlikely feeding on phytoplankton (Lam, 1974).

As conditions (nutrients, turbidity, water level, light, etc.) for microphytobenthos (MPB) development were similar among treatments, sediment Chl *a* had similar concentration (Table 4.3) and the same trend of temporal variation throughout the experiment (Fig. 4.6). The increases of sediment Chl *a* seemed to following increasing concentrations of TAN and SRP in pore water of sediment (Fig 4.6, 4.7 & 4.8), however there was no correlation between sediment Chl *a* with pore water TAN ($R^2 = 0.006$) and between Chl *a* with pore water SRP ($R^2 = 0.002$). The high concentrations of Chl *a* (Table 4.3) indicated sediments dominated by MPB in all treatments, which would limit rates of TAN release to the water column, and would be sinks of NOx-N and SRP as described above (Beristain, 2005). This therefore brought about the low concentrations of TAN, SRP, and NOx-N in the water column and possibly did not negatively affect water quality as seen in this study. In addition, the presence of MPB may also reduce sediment resuspension through the formation of mats on the surface, and binding of sediment particles with extracellular exudates (MacIntyre et al., 1996).

The changes of GPP in the water column and in sediment followed opposite trends in the HDRB polyculture and the control. As phytoplankton biomass increases, light availability at the bottom was reduced, resulting in limited photosynthesis of MPB. This process might also be enhanced by resuspended particulate matter caused by rabbitfish and/ or shrimp activities. Furthermore, in well-mixed or turbulent environment, resuspension of MPB attached to sediment particles also contributes significantly to primary productivity in water columns (MacIntyre et al., 1996). At the beginning, the water column and sediment were autotrophic in both treatments, the increases of GPP and P/R of entire ecosystems provided by strong phytosynthesis processes of phytoplankton and MPB. From middle to the end of the experiment, the water column and sediment became heterotrophic, total GPP of water and sediment tended to reduce, while total respiration in entire tank increased throughout the experiment. As increasing organic matter loads accumulated in the tanks, bacteria decomposition processes increased continuously, leading to increasing oxygen demands throughout the experiment in the water columns as well as in sediments in both treatments (Fig. 4.17). Suplee and Cotner (1996) found an increase in the sediment oxygen demand throughout the shrimp growing season, and Ellis (1992) reported that sediment oxygen demand consisted of more than 50% of the total shrimp pond oxygen demand at the end of the growing season. Our results agreed with these findings.

The decrease of total GPP coupled with the increase of total respiration in entire ecosystem leading to reducing P/R ratio (Fig. 4.18) and differential oxygen amount between daily GPP and respiration tended to decrease close to the end of the experiment (Fig. 4.19). The excess oxygen produced through photosynthesis was essential to supply the reared animal and other organisms with oxygen. In the HDRB treatment, oxygen budget generated from daily GPP and respiration was lower than estimated shrimp oxygen demand (not including rabbitfish oxygen demand) at the end day. Whilst in the control, the oxygen budget could fulfil shrimp oxygen demand over the course of the experiment (Fig. 4.19). However, the tanks were continuously supplied aeration and further sources from air diffusion and water inflow (10% volume per day) provided oxygen to the tanks that maintained DO concentration in suitable ranges for cultured animal demand during the experiment (Table 4.2). To develop polyculture in semi-intensive earthen ponds, our results suggest that extra oxygen will be needed such as aeration to satisfy the animal's growth requirements.

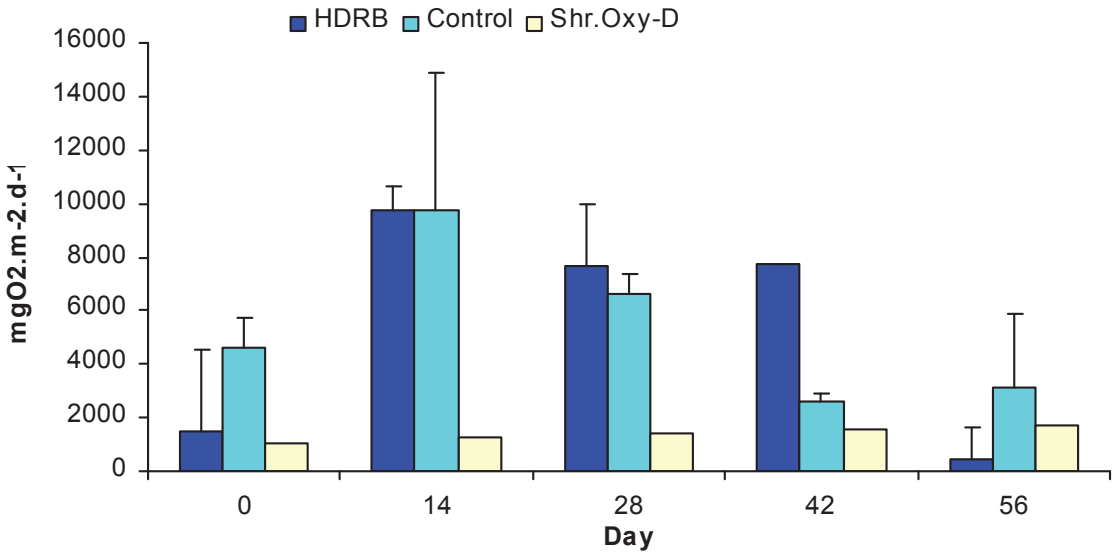


Figure 4.19: Oxygen budget in high density rabbitfish (HDRB) polyculture and shrimp monoculture (control) in comparison with shrimp oxygen demand (Shr.Oxy-D) throughout the experiment.

GNP, in terms of organic matter, produced by algae in the tanks was higher than organic carbon sources from food ration provided in both treatments (Fig. 4.20). Most of produced GNP might transfer to the bottom through sedimentation and to the surrounding environment through water exchange. This organic matter source can be considered as lost for the culture. This primary organic source could be consumed and transferred through the food web and ultimately be converted to animal biomass and thus contributed to increase production. In

crustacean-fish polyculture, the use of artificial substrates, has showed good results because the contact surface is increased, promoting biotic communities such as phytoplankton, zooplankton and periphyton, which contribute to the nutrition of the shrimp and the co-cultured species (Martínez-Porchas et al., 2010). In prawn-tilapia polyculture ponds, Uddin et al. (2007) placed bamboo sticks as substrates for periphyton growth, which resulted in a more favourable environment and provided an extra source of food for both species. Blue shrimp can utilize a wide variety of food, such as detritus, macroalgae, exuviae, and prey and formulated feed in semi-intensive ponds (Martínez-Córdova and Pena-Messina, 2005). Rabbitfish are herbivores and primarily feed on benthic algae and filamentous algae (Lam, 1974). Our results suggest that an important area for further research is the use of artificial substrates to promote periphyton development, which could provide an extra food source for both blue shrimp and goldlined rabbitfish and reduce pellet feed use and nutrient loss to the environment.

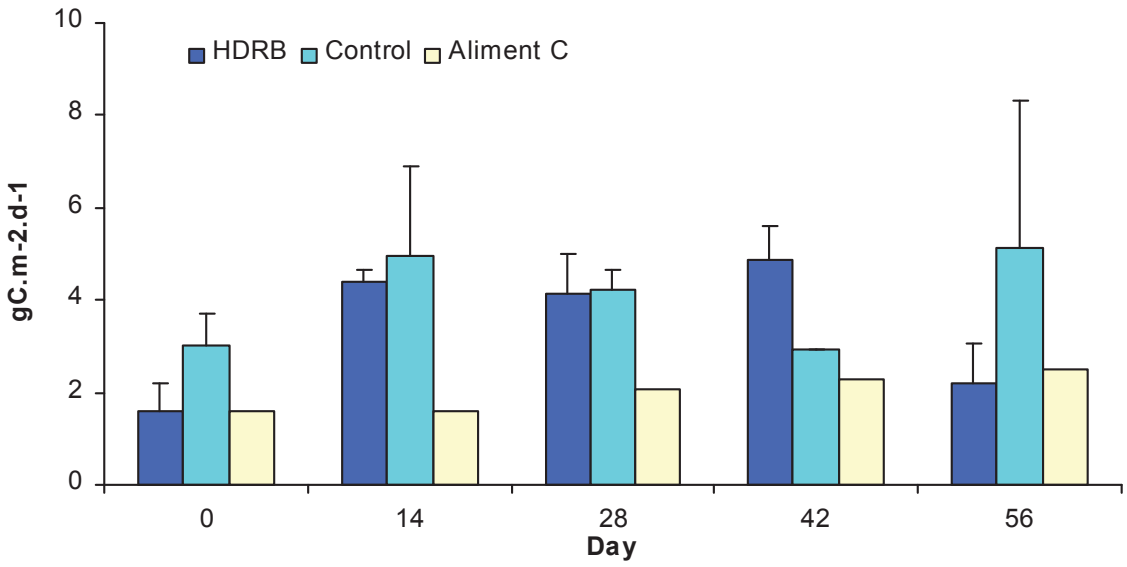


Figure 4.20: Gross Natural Production (GNP) in high density rabbitfish (HDRB) polyculture and shrimp monoculture (control) compared with carbon daily supplied from the pellet feed (Aliment C).

4.4.3 Food sources for shrimp and rabbitfish

The high increase of POM $\delta^{15}N$ values throughout the experiment and the similarity of $\delta^{13}C$ values between POM and the pellet feed at the end of the experiment suggested that POM isotope variations were likely affected by the pellet feed isotope signatures. The POM to feed $\delta^{13}C$ fractionation reduced from 1.54 ‰ at the beginning to 0.21 – 1.28 ‰ (0.82 ‰), and the $\Delta\delta^{15}N$ changed from -6.64 ‰ to -1.67 – -2.63 ‰ (-2.24 ‰) by the end of the experiment in

all treatments. The POM $\delta^{15}N$ largely increased from the beginning to the middle and then remained stable to the end of the experiment (Fig. 4.21). While the variation of the POM $\delta^{13}C$ was more complicated, as it increased from beginning to the middle and then reduced and became similar with the feed $\delta^{13}C$ value at the end of the experiment in all treatments (Fig. 4.21). This trend suggested that at the early the pellet feed did not largely affect the $\delta^{13}C$ signature of POM. It was possible that the low amount of organic carbon loading from the pellet feed during that time was rapidly decomposed and mineralized into inorganic carbon in the water column. In addition, phytoplankton possibly absorbed and fixed more air-diffused CO_2 through photosynthesis processes, which was most likely different in $\delta^{13}C$ ratio from the pellet feed ($\delta^{13}C$ air-CO_2: -7.4 ‰, Peterson and Fry, 1987) and that possibly led to increases in the POM $\delta^{13}C$ ratios. Nevertheless, by the time of the second sampling for POM and SOM isotope analysis, phytoplankton and microphytobenthos had not shown strong development in the system as expressed by relative low Chl *a* concentrations measured at the day 0 (Fig. 4.4 & 4.7). So, the reason of the increases remains unclear and it is necessary to implement more investigations. By using traced $\delta^{15}N$-nitrogen-enriched formulated feed fed to shrimp in outdoor culture tanks, Burford et al. (2002) found the particulate fraction in the water column accounted for 4 – 5 % of the $\delta^{15}N$-nitrogen fed to shrimp after one day and increased to 16 % by day 15. Our results showed that after 30 days, POM $\delta^{15}N$ increased by 4.00 – 4.26 ‰ compared with the initial value, and this changes was probably affected by the pellet feed. The effect of dietary nitrogen on POM $\delta^{15}N$ value was faster than that of dietary carbon, perhaps because dietary nitrogen was more accessible to the phytoplankton community.

The $\Delta\delta^{13}C$ SOM-feed lightly reduced from 3.19 ‰ at the beginning to 2.61 – 2.91 ‰ (2.76 ‰) at the end of the experiment. The $\Delta\delta^{15}N$ SOM-feed changed from -5.32 ‰ at the beginning to -2.42 – -5.22 ‰ (-4.09 ‰) at the end of the experiment. Additionally, the variations of SOM $\delta^{13}C$ and $\delta^{15}N$ signatures had different trends among treatments from the beginning to the end of the experiment (Fig. 4.21). These results suggested a low effect of the pellet feed on the SOM $\delta^{13}C$ and $\delta^{15}N$ signatures. Organic matter wastes from uneaten feed, senescent plankton, and animal excretion settled on the sediments probably were rapidly decomposed by active microorganism communities, mineralized and partly resuspended into the water column. This was supported by the low sediment loss on ignition in all treatments throughout the experiment (Table 4.3). Besides, the settled organic matter might be partly used by organisms, including shrimp and/or rabbitfish, in the culture system. These processes

led to reduce organic matter amount accumulated on sediments, and thus limited the effect of the pellet feed on the SOM stable isotope signatures.

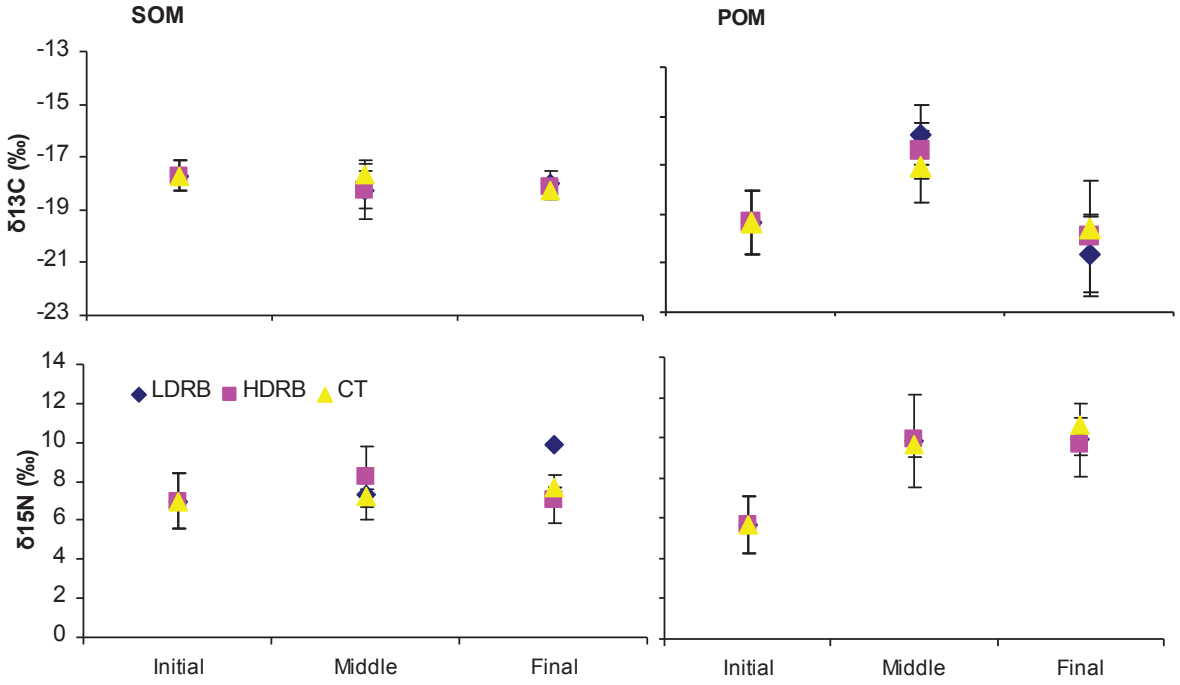


Figure 4.21: The $\delta^{13}C$ and $\delta^{15}N$ values in sediment organic matter (SOM) and particulate organic matter (POM) at beginning, middle and end of the experiment in low density rabbitfish (LDRB) and high density rabbitfish polyculture (HDRB) and the control (CT).

At harvest, the $\Delta\delta^{13}C$ shrimp-feed were from 2.9 to 3.1 ‰ (3.0 ‰), which higher than the $\Delta\delta^{13}C$ shrimp-SOM, 0.12 – 0.42 ‰ (0.24 ‰) and the $\Delta\delta^{13}C$ shrimp-POM, 1.8 – 2.8 ‰ (2.17 ‰). Animal tissue carbon tend to be $\delta^{13}C$ enriched roughly 1‰ (0.6 – 2.7 ‰) relative to the diet (DeNiro and Epstein, 1978). These results suggested that the pellet feed was not a major source of carbon for shrimp, and SOM was an important carbon source for shrimp. This was consistent with previous studies that reported that natural biota, including SOM, was a major carbon source for shrimp growth (Anderson et al., 1987; Nunes et al., 1997; Burford et al., 2004). To calculate the relative contribution of potential food sources to shrimp carbon growth, SOM was chosen as a major carbon source beside the pellet feed as a secondary one. The results showed that the pellet feed contributed 28.8 – 39.9% to the shrimp carbon growth (Table 4.8), and the remainder attributed for natural biota, 60.1–71.2%. This was similar with the results of other studies, which showed that pond natural biota contributed 53 – 77% of carbon requirements to *L. vannamei* growth (Anderson et al., 1987; Nunes et al., 1997).

Table 4.8: Range of percent feed carbon contribution to the shrimp weight gained in the experimental treatments. The values were calculated following the equation suggested by Anderson et al. (1987); δt and δg: the $\delta^{13}C$ values of harvested tissue and tissue gained, Wg: weight gained. Wf: weight gained from feed, Wp: weight gained from natural biota (SOM).

	δt (‰)	Wg (g)	δg (‰)	Wp/Wf	% feed contribution
LDRB	-17.81	10.5	-19.04	1.74	36.4
HDRB	-18.61	11.0	-19.23	1.51	39.9
Control	-17.86	11.1	-19.03	2.48	28.8

The stable isotope signature of a consumer reflects the isotopic signatures of dietary material assimilated and provides an integration of feeding over time (Peterson and Fry, 1997; Jardin et al., 2003). Consumer nitrogen tends to be $\delta^{15}N$ enriched on average 3.4 ‰ (-0.5 – 9.2 ‰) relative to diet (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). At harvest, the $\Delta\delta^{15}N$ shrimp-feed was from -0.78 to -0.36 ‰ (-0.53 ‰), the $\Delta\delta^{15}N$ shrimp-POM ranged 1.23 – 2.06 ‰ (1.71 ‰), and the $\Delta\delta^{15}N$ shrimp-SOM was 2.06 – 4.44 ‰ (3.56 ‰). These data suggested that the pellet feed was an important nitrogen source and POM represented as another major nitrogen source for shrimp nutrition. The mixing models have been employed to determine the relative contribution of the potential food sources, the pellet feed and POM, to the shrimp growth. The result showed that the pellet feed contributed 70 – 85% of nitrogen to the shrimp growth (Table 4.9), and hence the contribution of natural biota was 15 – 30%. This result was similar with a previous study, which reported that 31% of nitrogen requirements for *L. vannamei* (3.5 g), reared in zero-water exchange 1200 L outdoor mesocosms (50 shrimp.m^{-2}), were derived from pond ecosystem dynamics (Epp et al., 2002).

Table 4.9: Range of percent feed nitrogen contribution to the shrimp weight gained in the experimental treatment. The values were calculated following mixing model equation (Tiunov, 2007); δ_A: the pellet feed $\delta^{15}N$ value, δ_B: the POM $\delta^{15}N$ values.

	δt (‰)	δ_A (‰)	δ_B (‰)	$(\delta t - \delta_B)/(\delta_A - \delta_B)$	% feed contribution
LDRB	11.95	12.31	9.89	0.85	85
HDRB	11.53	12.31	9.69	0.70	70
Control	11.87	12.31	10.64	0.73	73

The similarity of $\delta^{13}C$ values of shrimp and rabbitfish in polyculture treatments at harvest suggested that shrimp and rabbitfish possibly had the same source of carbon for their growth. The $\Delta\delta^{13}C$ rabbitfish-SOM was from 0.07 to 0.19 ‰ (0.13 ‰) and the $\Delta\delta^{13}C$ rabbitfish-POM, 1.85 – 2.89 ‰ (2.37 ‰), which lied in the range of relationship of $\delta^{13}C$ between consumer and its diet, 0.6 – 2.7 ‰ (DeNiro and Epstein, 1978). Meanwhile, the $\Delta\delta^{13}C$ rabbitfish-feed, 2.83 – 3.10 ‰ (2.96 ‰), were out of the usual range. These results suggested

that SOM was an important carbon source and POM was possibly a secondary source for rabbitfish growth in the culture system.

The stable isotope ^{15}N is excreted in lower amounts than the abundant ^{14}N, so that organisms become enriched in $\delta^{15}N$ compared with their food (Peterson and Fry, 1987). The $\Delta\delta^{15}N$ rabbitfish-feed did not change over the study, and $\delta^{15}N$ rabbitfish were enriched by 0.95 – 1.17 ‰ (1.06 ‰) relative to the pellet feed at harvest. Meanwhile, the $\Delta\delta^{15}N$ rabbitfish-POM was 3.59 ‰, and the $\Delta\delta^{15}N$ rabbitfish-SOM ranged 3.59 – 6.18 ‰ (4.88 ‰). These results suggested that the pellet feed was a major nitrogen source and POM and SOM were possibly other sources for rabbitfish growth.

It was impossible to calculate the relative contribution of potential food source to the rabbitfish growth due to the high $\delta^{13}C$ and $\delta^{15}N$ fractionation presented by rabbitfish.

Rabbitfish are herbivorous in the wild (Lam, 1974), but they become opportunistic omnivorous in captivity and could feed on a great variety of food offered, such as aquatic plants, benthic algae, cooked rice, chicken food pellet, chopped fish and mollusks, fish meal, and pellets (Von Westernhagen, 1974; Ben-Tuvia et al., 1973; Lam, 1974; Von Westernhagen and Rosenthal, 1975). Preferable feeding habit of rabbitfish is still grazing on benthic algae and macrophytes when the sources are available. In this study, it was incidentally seen that rabbitfish grazing on periphyton growing on the tank walls during the culture. Furthermore, filamentous algae were found to be more abundant in the control than in the polyculture treatments, being detected through cleaning the tank walls every week. It was probable that periphyton and filamentous algae found in the tanks could be potential food sources for rabbitfish, and thus be other carbon and nitrogen sources for rabbitfish growth. The lack of data of periphyton and filamentous algae $\delta^{13}C$ and $\delta^{15}N$ values would not allow concluding and calculating possible contribution of these sources to rabbitfish growth. More researches need to be conducted to confirm this hypothesis.

4.5 Highights and limits of this experiment

4.5.1 Highlights

- The results of this study show that the presence of goldlined rabbitfish *S. lineatus* at tested sizes and densities did not affect growth and survival of blue shrimp *L. stylirostris* in

polyculture system, but *S. lineatus* adding significantly increased total production of the culture system. Furthermore, *S. lineatus* adding reduced FCR of *L. stylirostris* culture, and thus increased the benefit.

- The addition of *S. lineatus* to the *L. stylirostris* culture system did not largely change the pond ecology and had no or little effect on the environmental quality.

- Nutrient fluxes (NOx-N, TAN, DON and SRP) across the sediment-water interface were in small rates in both polyculture and *L. stylirostris* monoculture, so sediments did not highly impact on the availability of nutrients in the water column. Sediments have shown as sinks of NOx-N and SRP and sources of TAN in both culture systems.

- Particulate exchange between water column and sediment increased throughout the experiment, as expressed by increasing sedimentation rate, due to cultured animal's bioturbation.

- In the first half of the culture period, the ecosystem was autotrophic expressed by increasing total GPP and P/R ratio. Whereas in the second one, the ecosystem became heterotrophic as increasing organic matter loads from increased food input and organism metabolites, expressed by reduction of total GPP, and increases of respiration and released nutrients from decomposition.

- *L. stylirostris* and *S. lineatus* had the same sources of carbon and nitrogen for their nutrition. The pellet feed was not an important source of carbon for both species, and the natural biota (SOM and POM) was the main source carbon for both species. Meanwhile, the pellet feed was the major source of nitrogen, and natural biota (SOM and POM) was a second one for both species.

- In polyculture system, the oxygen produced by photosynthesis was lower than estimated shrimp oxygen demand at the end of the culture period, suggesting a need of extra oxygen source to fulfill cultured animals requirements.

- The entire ecosystem could produce remarkable source of carbon organic matter in comparison with those of daily external source from supplied pellet feed, which would be a nutrient source for both cultured species.

4.5.2 Limits

- At harvest, shrimp and rabbitfish have not reached marketable sizes yet. In the case of the culture would be continue to get larger sizes and higher biomass of shrimp and rabbitfish, what would happen for the cultured animals, the environment and ecological functioning in the culture system. To understand these matters, further study investigating with large size and high biomass stocking of shrimp and rabbitfish should be implemented.

- Large sizes and high biomasses stocking and different stocking ratios would also allow to investigate the food source and competitive behavior between *L. stylirostris* and *S. lineatus* in polyculture system.

CHAPTER 5

EFFECTS OF BLUE SHRIMP AND GOLDLINED RABBITFISH MONOCULTURE AND SHRIMP - RABBITFISH POLYCULTURE ON PRODUCTION AND ENVIRONMENTAL CONDITIONS

5.1 Introduction

Shrimp polyculture represents a feasible method to solving and/or minimizing some problems that shrimp industry has faced in the past years, such as environmental pollution, disease, and reducing prices (Martínez-Porchas et al., 2010). Polyculture of shrimp-fish can improve shrimp growth performance due to water quality maintained stable (Akiyama and Angawati, 1999; Cruz-Suárez et al., 2010), increase productivity and profitability (García-Pérez et al., 2000; Tian et al., 2001; Yuan et al., 2010), improve nutrient utilization (Yuan et al., 2010), and minimize the pollution of coastal waters caused by pond effluents (Tian et al., 2001) (see also Chapter 4).

However, in polyculture systems only a proper combination of ecologically different species at adequate densities will efficiently use the available resources, maximize the synergistic between cultured species and species-environment relationships and minimize the antagonistic ones (Milstein, 1992). Polyculture system increases production per unit area when compatibility and optimum stocking combinations are considered. Yuan et al. (2010) reported that red tilapia (*Oreochromis* spp.) at 13.7 g stocked into intensive white shrimp (*Litopenaeus vannamei*) monoculture (60 PL.m^{-2}) at density of 0.4 fish.m^{-2} could improve productivity, profitability, nutrient utilization and environmental friendliness. However, when tilapia stocked at higher density and larger size up to 1.2 fish.m^{-2} and 42 g, respectively, economic profitability could be negatively affected. Tian et al. (2001) studied on Chinese shrimp *Penaeus chinensis* (2 cm) – red tilapia (150 g) – constricted tagelus *Sinonovacula constricta* (2 cm) polyculture and found the best stocking composition to be 7.2:0.08:14 (m^{-2}) of shrimp, tilapia and constricted tagelus, respectively, while other combinations (0:20, 0.12:10, 0.16:7, and 0.24:0 of tilapia:tagelus.m^{-2}) showed lower yields. Synergistic interactions among cultured species are explained on the basic of two interrelated processes: increase of food resources and improvement of environmental conditions (Milstein, 1992). It is expected that the supplied feed be consumed by both species or consumed by the main species, and the other species feeding on faeces, detritus, plankton and metabolites (Martínez-Porchas et al., 2010), which reduce undesirable wastes and improve water quality.

Inversely, antagonistic interactions occur between incompatible species combinations and when the stocking rates are unbalanced (Milstein, 1992). Wang et al. (1998) reported growth rates of Chinese shrimp co-cultured with tilapia (not in net cages) were lower than those in enclosures with tilapia held in net cages, likely due to the fact that tilapia competed with

shrimp for food and suppressed the shrimp growth. Therefore, the authors suggested that tilapia should be reared in a net cage in a polyculture, or if not, feeding grounds for shrimp were surrounded by a net with a suitable mesh size so that shrimp could enter freely. Similarly, Tidwell et al. (2010) found average harvested prawn weight and production in the polyculture prawn with unconfined tilapia were lower than those in the polyculture prawn with confined tilapia and prawn monoculture in earthen pond. The authors attributed these results were likely derived from competition by tilapia with prawn for food.

In shrimp polyculture, it is not recommended to add species that may be attacked or devoured by the shrimp or vice versa (Martínez-Porchas et al., 2010). Tendencia et al. (2006b) suggested to use seabass and snappers at the same age/size of fish and to enclose the fish in cages or nets when co-cultured with shrimp to prevent the shrimp from being eaten by the fish. Tian et al. (2001) suggested carnivorous fishes (*Labeolabrax japonicus*, *Sparus macrocephalus*, *Pagrosomus major*, etc.) being stocked into shrimp ponds, must be of small size and low density. In another case, Bell et al. (2007) worked co-culture of sandfish *Holothuria scabra* with blue shrimp *Litopenaeus stylirostris* in ponds and concluded that co-culture was not viable because shrimp had a significant negative effect on survival and/or growth of sandfish. According to the authors, all sandfish stocked in co-culture ponds were dead or moribund after a month, while sandfish monocultured in 0.2 ha earthen ponds survived well and grew to mean weights of around 400g within 12 months.

In the previous study (Chapter 4), goldlined rabbitfish *Siganus lineatus* (25.5 g) were stocked into blue shrimp *L. stylirostris* (2.9 g) tanks (15 shrimp.m^{-2}) at densities 1.2 and 2.4 fish.m^{-2} to form polyculture of shrimp and rabbitfish. The results showed that the presence of goldlined rabbitfish had no negative effect on the shrimp growth. On the other hand, rabbitfish adding could improve shrimp survivals and yields in the polyculture treatments, and increase significantly the total production compared with shrimp monoculture. These results indicated that blue shrimp and goldlined rabbitfish seem to be synergistic each other when co-cultured or at least they are not in antagonistic interaction in the culture system. The results of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses showed that blue shrimp and goldlined rabbitfish seemed to have the same sources of carbon (from SOM, POM and the pellet feed) and nitrogen (from the pellet feed and POM and/or SOM) for their growth requirements. However, the growth performances of shrimp and rabbitfish demonstrated a little interspecies competition when co-cultured at experimental densities and sizes. The shrimp – rabbitfish

relationship may be changed when increase stocking size and density (biomass) of shrimp and/or rabbitfish and stocking rates unbalance. Further researches need to be conducted to find out the optimal stocking densities and stocking rates of blue shrimp and goldlined rabbitfish to produce maximum production and benefit in friendly environment in a polyculture system. The optimal stocking density depends on cultural conditions, cultural patterns, initial and final sizes, rearing period, and experiences and techniques (Wang et al., 1998). Besides, the knowledge of cultured species and species-environment quantitative relationships enables choosing adequate combinations of cultured species, stocking rates, input types and rates (Milstein, 1992). Generally, until carrying capacity is reached, yield increases with increasing stocking rate while final size and survival rate decrease as stocking density increases (Wang et al., 1998).

Carrying capacity is defined as the maximum biomass of a farmed species that can be supported without violating the maximum acceptable impacts to the farmed stock and its environment (Stigebrandt, 2011). Maximum acceptable impacts on the farmed stock and the environment are expressed by standards for water quality in the farm and the surrounding environment.

In New Caledonia, most shrimp farms practice an annual production cycle in ponds, which last about 7 months (December to June/July) (SPC, 2010). Shrimp stocking densities are from 20 – 30 PL.m^{-2}, and average yields are 1.74 – 4.04 tonne.ha^{-1}.y^{-1} in the period of 2000 – 2010 (AquaSol, 2011). Meanwhile, goldlined rabbitfish *S. lineatus* is raised in cages and reached marketable size (300 g) in less than a year (SPC, 2008). However, rabbitfish stocking density and yield are not published yet. To get farmed shrimp and rabbitfish products that reach marketable sizes at harvest from a polycultrue system, stocking sizes should be considered for each species and the rearing time should be also regarded. These may be made according to the specific local conditions: climate, quality of water supply and pond fertility, availability of shrimp and rabbitfish seeds, availability of feeds, and market requirements (Milstein, 1992).

In polyculture blue shrimp *L. stylirostris* and goldlined rabbitfish *S. lineatus*, the questions are still given that 1) how is suitable combination in stocking biomass (density) of *L. stylirostris* and *S. lineatus* in a polyculture system, 2) how polyculture shrimp-rabbitfish affects production and environmental quality in the cases of shrimp is a main species and vice versa rabbitfish is a main species, 3) how are production and environmental variations in

shrimp-fish polyculture compared with shrimp/fish monoculture at the same stocking biomass, and 4) how are the sources of carbon and nitrogen for shrimp and rabbitfish requirements when increased stocking biomasses.

To answer these questions, we conducted an experiment in a mesocosm system with different treatments, including shrimp monoculture, shrimp-rabbitfish polyculture, rabbitfish-shrimp polyculture and rabbitfish monoculture, stocked at large sizes and high biomasses in a period of 10 weeks. The objectives of the study were as followed:

- Estimate the effects of high stocking biomass of *L. stylirostris* and *S. lineatus* monoculture on production in a mesocosm system,

- Estimate the effects of high stocking biomass of *L. stylirostris* and *S. lineatus* monoculture on environmental quality and ecological processes in a mesocosm system,

- Estimate the effects of different biomass combinations of *L. stylirostris* and *S. lineatus* polyculture on production in a mesocosm system,

- Estimate the effects of different biomass combinations of *L. stylirostris* and *S. lineatus* polyculture on environmental quality and ecological processes in a mesocosm system,

- Estimate the sources of carbon and nitrogen for shrimp and rabbitfish growth at high stocking biomasses in a mesocosm system.

5.2 Materials and methods

5.2.1 Experimental design

The experiment was conducted for a period of 10 weeks (September – November, 2013) in 16 outdoor circular mesocosm fiberglass tanks as described in the paragraph 2.2.2.2 of Chapter 2. The tanks were filled with fresh seawater up to 75 cm of water level one week before stocking. A daily water exchange of around 10% was applied by regulating individual valves in each tank and water level was maintained at 75 cm (1275 L in volume) during the experiment.

Blue shrimp *L. stylirostris* and goldlined rabbitfish *S. lineatus* were randomly selected and stocked into the experimental tanks to form experimental treatments, including blue shrimp monoculture (SM), shrimp-fish polyculture (SF), fish-shrimp polyculture (FS), and rabbitfish monoculture (FM) treatments at sizes, densities and biomasses as described in table 5.1.

Table 5.1: Stocking sizes, densities and biomasses of blue shrimp and goldlined rabbitfish in monoculture and polyculture treatments.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Rabbitfish monoculture
Shrimp				
Mean weight (g.shr.$^{-1}$)	13.9 ± 0.2	13.9 ± 0.3	13.7 ± 0.6	
Density (shr.m^{-2})	17	11	6	
Biomass (g.m^{-2})	237.1 ± 3.0	155.7 ± 3.9	80.5 ± 3.5	
Fish				
Mean weight (g.fish^{-1})		19.5 ± 0.6	18.8 ± 0.3	19.0 ± 0.1
Density (fish.m^{-2})		4	8	12
Biomass (g.m^{-2})		80.3 ± 2.3	155.1 ± 2.1	234.8 ± 0.6
Combined biomass (g.m^{-2})	237.1 ± 3.0	236.0 ± 4.5	235.6 ± 5.2	234.8 ± 1.6

All treatments were randomly distributed among tanks with four replicates per treatment. Cultured animals in all tanks were fed twice daily at 08:00 am and 16:00 pm with the same commercial pellet feed (35 – 40 % protein, SICA Manufacturer, New Caledonia), and the same feed quantity. Daily feed quantity was calculated following shrimp biomass in the SM treatment and feeding rate of 2.2 – 2.6% of shrimp biomass. Feeding rate was reduced as shrimp size increased, and shrimp biomass was calculated based on shrimp growth rate and survival rate referred from the previous experiment (Chapter 4).

5.2.2 Shrimp and rabbitfish sampling and analyses

Before stocking, 35 shrimp and 35 rabbitfish were randomly collected and individually weighed. The data then were used to calculate the shrimp and rabbitfish mean weight in order to estimate biomass and density for stocking.

At stocking, shrimp and rabbitfish were randomly selected with a number following the calculated stocking biomass and density for each tank and then all shrimp and/ or all rabbitfish stocked per tank was weighed in groups. The data were used to calculate initial shrimp mean weight and/ or initial rabbitfish mean weight for each treatment. At harvesting, all shrimp and/ or all rabbitfish in each tank was counted and individually weighed.

Shrimp and rabbitfish growth performance were evaluated at harvesting in terms of survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), and final biomass.

SR (%) = harvesting number/stocking number*100

DWG ($g.day^{-1}$) = weight gain (g)/time (days)

SGR ($\%.day^{-1}$) = (Ln Wf – Ln Wi)/time (days)*100

Final biomass ($g.m^{-2}$) = total harvested biomass (g)/area of culture tank (m^2)

where Wi: initial mean weight (g), Wf: final mean weight (g)

Food conversion ratio (FCR) was calculated as followed:

FCR = total feed used (dry weight, g)/total shrimp and fish weight gain (fresh weight, g)

5.2.3 Water sampling and analyses

Water temperature and dissolved oxygen (DO) concentrations were recorded twice daily (07:30 am and 15:00 pm) at mid depth of each tank using an OxyGuard meter (Handy Polaris, Birkerod, Denmark). Salinity, turbidity, fluorescence and pH were measured twice a week (08:00 am) using refractometer (Cond 3210, Welheim, Germany); turbidimeter (TN-100, Eutech Instruments, Singapore), Aquafluor (Turner Designs, Sunnyvale, CA. USA), and pH meter (pH 197i, Welheim, Germany), respectively.

Two days before shrimp and rabbitfish stocking and every week thereafter, water samples (2 L) were collected at mid depth of each tank (08:00 am) and filtered through pre-combusted (450^0C, 4 hrs) GF/C Whatman fiberglass filters (47mm). Filtered water was analysed for total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Holmes et al., 1999), soluble reactive phosphorus (SRP) (Murphy and Riley, 1962), nitrite and nitrate nitrogen (NO_2^- + NO_3^-)-N (Wood et al., 1967), and total dissolved nitrogen (TDN) (Raimbault et al., 1999). Urea was analysed every two weeks on thawed samples according the method developed by Mulvenna and Savidge (1992) and adapted by Goeyens et al. (1998). Dissolved organic nitrogen (DON) was expressed as the difference between TDN and total dissolved inorganic nitrogen [(NH_4^+-NH_3)-N + (NO_2^-+NO_3^-)-N]. To estimate chlorophyll *a* (Chl *a*), water sample of 25 mL was filtered through GF/F Whatman fiberglass filters (25 mm) and the filter was immediately frozen until analysing. The filter was analysed using fluorometer (TD 700) with methanol extraction before and after adding HCl 1 % following Holm-Hansen et al. (1965). The ratio

of phaeopigment to total chlorophyll pigments was calculated as (Phaeo)/(Phaeo + Chl *a*) and expressed in %.

Two day before stocking, after that one week, and every two weeks thereafter during the experiment, total suspended solids (TSS) was estimated in two replicates for each treatment. Surface water sample of 250 mL was collected and filtered through pre-dried (60 ^{o}C, 24 hrs) and pre-weighed (Wi) GF/C Whatman fiberglass filters (47 mm). The filter was dried to constant weight (60 ^{o}C, 24 hrs) then weighed again (Wf), and TSS was calculated as followed:

$$TSS\ (mg.L^{-1}) = (Wf - Wi)/V*1000$$

where Wf and Wi: the final weight and initial weight of filter expressed in mg, V: volume of water sample (mL), 1000: index converted from L to mL.

5.2.4 Sediment sampling and analyses

On the day of stocking, one week after that, and every two weeks thereafter during the experiment, sediment samples were collected in all tanks from 1 cm deep cores by using 50 ml cut-off syringes (2.3 cm diameter). The samples were collected at three different points within each tank and combined to provide one sample per tank for the analysis of organic matter content, pH, redox potential, and nutrient concentrations in pore water. The redox potential (Eh) was estimated with a specific electrode (Consort P901, electrochemical analyzer, Beverly, MA, USA) following Hussenot and Martin (1995). pH was directly measured by pushing the glass electrode (pH 197i, Welheim, Germany) into freshly collected sediment in the sample vials. The samples then were dried at 60°C for one week and analysed for loss on ignition (organic matter) in a muffle furnace at 350°C for 8 hrs (Nelson and Sommers, 1996). The initial and final sediment samples were centrifuged at 2000 rpm for 20 minutes. The supernatant parts (pore water) were used to analyse TAN and SRP following the methods described by Holmes et al. (1999) and Murphy and Riley (1962), respectively. Sediment Chl *a* concentration was analysed from three different samples (1 cm core layer) per tank. Frozen sediment samples were freeze-dried (lyophilisated) for 24 hrs, 14 ml of methanol were added to the samples for 20 minutes to extract the pigments. After homogenization, 100 µl of the supernatant were removed and added to 7 ml of methanol. The extracts were analysed before (Fo) and after acidification (Fa) with 50 µl of 10% HCl using a

TD-700 fluorometer (Holm-Hansen et al., 1965). The values were calculated taking into account the dilution and Fo/Fa. The concentration of Chl *a* was expressed by $\mu g.m^{-2}$.

5.2.5 Sedimentation processes

Two days before shrimp and rabbitfish stocking, one week after that and every two weeks thereafter, sedimentation rates were estimated in two replicates per treatment by using sets of sediment trap. A set of sediment trap included a rectangular plastic tray, 6 cm in height, which was covered by a plastic sheet with rows of holes (~ 1 cm diameter for each) to catch settled particulate matter into the tray, and a heavy smooth lead bar to stabilize the tray. The area of the trap (total hole areas) was 0.01425 m^2. The trap was placed on the bottom of each tank at opposite side with water inlet and retrieved after ~ 24 hrs. All settled particulate matter was carefully transferred to bottles. Total solids weight of trapped particulate matter was determined gravimetrically following drying at 60 ^oC to constant weight and sedimentation rate (SdR) was calculated and expressed as $g.m^{-2}.h^{-1}$.

$$SdR = W/(S*T)$$

where W: weight of dry trapped particulate matter (g), S: surface area of sediment trap (m^2), T: setting time (h)

Loss on ignition of deposit was determined in a muffle furnace at 350 °C for 8 hrs following Nelson and Sommers (1996).

5.2.6 Metabolism

On the day before stocking, at the early (4 days after stocking) and at the late (4 days before harvesting) of the experiment, primary productivity (PP) and respiration (R) were estimated in sediments and water columns of two replicates per treatment by using the light and dark bottle method (Strickland and Parsons, 1972). Two pairs of light and dark bottles were incubated in the water column at 20 cm under water surface and 20 cm above the sediment bottom, respectively. Two chambers (light and dark) were placed on bases set up on the bottom sediment. Dissolved oxygen (DO) concentrations were recorded in the bottles and in the chambers every hour, between 10:30 am and 13:30 pm, using an oxygenmeter probe (Fibox 3 LCD – trace, Present, Germany).

Net primary productivity (NPP) and R were assessed as the rates of oxygen variations in the incubated bottles and chambers. NPP, R and gross primary productivity (GPP) were calculated for the water column and sediment using followed formulas.

$$NPP_w = (Slsb + Slbb)/2*H*1000$$

$$R_w = (Sdsb + Sdbb)/2*H*1000$$

$$NPP_s = (Slc – Slbb)* (V/S)*1000$$

$$R_s = (Sdc – Sdbb)*(V/S)*1000$$

$$GPP = NPP + R$$

where NPP_w: net primary productivity of water column ($\mu mol.m^{-2}.h^{-1}$); Slsb: oxygen slope in light surface bottle ($\mu mol.L^{-1}.h^{-1}$), Slbb: oxygen slope in light bottom bottle ($\mu mol.L^{-1}.h^{-1}$); R_w: respiration of water column ($\mu mol.m^{-2}.h^{-1}$), Sdsb: oxygen slope in dark surface bottle ($\mu mol.L^{-1}.h^{-1}$), Sdbb: oxygen slope in dark bottom bottle ($\mu mol.L^{-1}.h^{-1}$); H: the height of the water column (m). NPP_s: net primary productivity of sediment ($\mu mol.m^{-2}.h^{-1}$), Slc: oxygen slope in the light chamber ($\mu mol.L^{-1}.h^{-1}$); R_s: respiration of sediment ($\mu mol.m^{-2}.h^{-1}$); Sdc: oxygen slope in the dark chamber ($\mu mol.L^{-1}.h^{-1}$); V: volume of the benthic chamber (m^3), and S: surface area of the benthic chamber (m^2); GPP: gross primary productivity ($\mu mol.m^{-2}.h^{-1}$)

5.2.7 Stable isotope analysis

Pellet feed samples were collected at three occasions during the experiment, including at the beginning, middle, and close to the end. Sediment organic matter (SOM) and particulate organic matter (POM) were collected on the day before stocking, middle, and at the end of the experiment. At stocking, three shrimps and three rabbitfish were randomly selected and immediately frozen and at harvesting one shrimp and/ or one rabbitfish per tank were randomly sampled and frozen until analyses.

The samples of the pellet feed, SOM, POM, shrimp and rabbitfish were prepared and analysed for stable isotope (^{13}C and ^{15}N) signatures following the methods as described in chapter 4. %N of the pellet feed, shrimp and rabbitfish, and %C and %N of SOM were determined using the same methods of stable isotope analysis. The fractionation factor was calculated following the equation defined by Hobson and Clark (1992).

$$\Delta dt = \delta t - \delta d$$

where δt and δd are the isotopic signature of the consumer tissue and food source, respectively.

5.2.8 Statistical analyses

All data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (HOV, Brown Forsythe test), and statistically analysed using one-way ANOVA with IBM SPSS software version 16.0; with possible differences among data being tested by Duncan's multiple range tests. Percent data were arcsine-transformed before statistical analyses, but non-transformed data are presented in tables and figures. Statistical comparisons of experimental data among treatments were performed for overall mean values and for each time of analyses. Non-parametric test (Kruskal-Wallis test, H test) and Tamhane's T2 (Post-hoc, one-way ANOVA) were used when data were not normally distributed or the variances were heterogeneous. The data of SdR, TSS, and water and sediment metabolism were statistically compared among treatments using a paired Student's t-test in MS-Excel.

5.3 Results

5.3.1 Shrimp and rabbitfish growth performances

Table 5.2: Blue shrimp and goldlined rabbitfish growth performances for monoculture and polyculture treatments. Values are means ± SD.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Rabbitfish monoculture
Shrimp				
SR (%)	32.8 ± 9.1^{a}	36.8 ± 9.6 ^{a}	62.5 ± 32.0^{a}	
Mean weight (g.shr.$^{-1}$)	21.1 ± 1.3^{a}	19.9 ± 3.5^{a}	19.2 ± 1.1^{a}	
DWG(g.d^{-1})	0.10 ± 0.02^{a}	0.09 ± 0.05^{a}	0.08 ± 0.01^{a}	
SGR (%.d^{-1})	0.6 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.1^{a}	
Final biomass (g.m^{-2}.69d^{-1})	116.4 ± 26.4^{a}	81.6 ± 23.1^{ab}	69.5 ± 33.7^{b}	
Fish				
SR (%)		75.0 ± 21.4^{a}	100.0 ± 0.0^{a}	76.2 ± 38.7^{a}
Mean weight (g.fish.$^{-1}$)		47.3 ± 9.1^{a}	47.3 ± 4.9^{a}	42.7 ± 5.3^{a}
DWG (g.d^{-1})		0.40 ± 0.13^{a}	0.41 ± 0.07^{a}	0.38 ± 0.03^{a}
SGR (%.d^{-1})		1.26 ± 0.31^{a}	1.33 ± 0.14^{a}	1.26 ± 0.07^{a}
Final biomass (g.m^{-2}.69d^{-1})		148.7 ± 58.7^{a}	389.5 ± 40.2^{b}	419.4 ± 227.1^{b}
Final combined biomass (g.m^{-2}.69d^{-1})	116.4 ± 26.4^{a}	230.3 ± 40.0^{b}	459.0 ± 53.9^{c}	419.4 ± 227.1^{c}
FCR*			2.36	

Mean values in a same row with different superscript letters are significantly different (P<0.05).
(*): FCR is not possible to calculate for the SM, the SF and the FM treatments due to negative weight gains in one replicate (FM treatment) or in all replicates (SM and SF treatments).

Over the experiment, shrimp gained low survival rates (SR) in all treatments. In the SM and the SF treatments, shrimp SR was lower 40%. Although, shrimp SR got higher value in the FS treatment, it showed a high variability among replicates (CV = 51.2%). There was no significant difference (P>0.05) in shrimp SR among treatments (Table 5.2). Shrimp final mean weight, DWG and SGR were similar among the SM, the SF and the FS treatments (Table 5.2). In the SM and SF treatments, shrimp final biomasses were significantly lower (P<0.05) than shrimp stocking biomasses, indicating significant decreases of shrimp biomasses over the experiment because of low SR. Shrimp final biomass was not significantly different (P>0.05) between the SM and the SF treatments as well as between the SF and the FS treatments. In the SM treatment, shrimp final biomass was significantly higher (P<0.05) than that in the FS treatment (Table 5.2).

Rabbitfish SR reached 100% in the FS treatment, but it was not significantly higher (P>0.05) than those in the SF and the FM treatments (Table 5.2). Rabbitfish final mean weight, DWG and SGR were similar in all treatments (Table 5.2). Rabbitfish final biomasses were significantly higher (P<0.05) in the FS and the FM treatments than that in the SF treatment (Table 5.2). In the FS treatment, rabbitfish final biomass was significantly higher (P<0.05) than stocking biomass while there was no significant difference (P>0.05) between rabbitfish final and stocking biomasses in the SF and FM treatments.

In polyculture treatments, total shrimp and rabbitfish final biomass was slightly lower (P>0.05) than total stocking biomass in the SF treatment whilst it significantly increased (P<0.05) in the FS treatment over the experiment (Table 5.2).

Rabbitfish final biomass in the FM treatment and total shrimp and rabbitfish final biomass in the FS treatment were significantly greater (P<0.05) than those in the SM and SF treatments. Total shrimp and rabbitfish final biomass in the SF treatment was significantly higher (P<0.05) than shrimp final biomass in the SM treatment (Table 5.2).

It was not possible to calculate FCR in the SM, SF and FM treatments due to negative weight gains in one (the FM treatment) or all replicates (the SM and SF treatments). In the FS treatment, although FCR was calculated and showed an acceptable value (Table 5.2), it was derived mainly from rabbitfish weight gain because shrimp biomass slightly reduced over the experiment.

% N of shrimp almost did not change while % N of rabbitfish increased over the experiment

(Table 5.3). In the FS and FM, the increase of %N coupled with high weight gain of rabbitfish led to increase the retention of input N (mainly from the pellet feed) in rabbitfish harvested biomasses. Inversely, the decrease of shrimp biomass in the SM and the SF treatments increased the amount of N releasing to the culture ecosystem (Table 5.3). The quantity of released N from input N (the pellet feed and stocking) was significantly higher ($P<0.05$) in the SM and the SF treatments than those in the FS and the FM treatments (Table 5.3).

Table 5.3: The input N from the pellet feed and stocking, N in harvested biomass, N retention in shrimp and rabbitfish, and N released to the culture ecosystem throughout the experiment in the experimental treatments. Values are means + SD.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
Input N				
N from the pellet feed ($g.m^{-2}$)	28.5	28.5	28.5	28.5
Initial %N of shrimp*	11.2 ± 4.0	11.2 ± 4.0	11.2 ± 4.0	
N from shrimp ($g.m^{-2}$)	6.4 ± 0.1	4.2 ± 0.1	2.2 ± 0.1	
Initial %N of rabbitfish*		7.9 ± 1.9	7.9 ± 1.9	7.9 ± 1.9
N from rabbitfish ($g.m^{-2}$)		1.4 ± 0.0	2.6 ± 0.0	4.0 ± 0.0
Total N from stocking ($g.m^{-2}$)	6.4 ± 0.1[a]	5.6 ± 0.1[b]	4.8 ± 0.1[c]	4.0 ± 0.0[d]
Final % N of shrimp*	11.1 ± 2.6	10.2 ± 1.8	13.3 ± 2.2	
N in harvested shrimp ($g.m^{-2}$)	3.1 ± 0.7	2.0 ± 0.6	2.2 ± 0.1	
Final %N of rabbitfish*		11.0 ± 2.4	10.8 ± 2.8	12.5 ± 1.6
N in harvested rabbitfish($g.m^{-2}$)		3.5 ± 1.4	9.1 ± 0.9	11.3 ± 6.1
Harvested N ($g.m^{-2}$)	3.1 ± 0.7[a]	5.5 ± 0.9[a]	11.3 ± 1.5[b]	11.3 ± 6.1[b]
N retention in animals				
Shrimp ($g.m^{-2}$)	-3.3 ± 0.8	-2.2 ± 0.5	0.1 ± 1.1	
% of pellet feed N				
Rabitfish ($g.m^{-2}$)		2.2 ± 1.4	6.4 ± 0.9	7.3 ± 6.1
% of pellet feed N		7.5 ± 4.4	22.6 ± 3.2	25.7 ± 21.4
Released N ($g.m^{-2}$)	31.8 ± 0.8[a]	28.5 ± 1.0[a]	22.0 ± 1.4[b]	21.2 ± 6.6[b]

Mean values in a same row with different superscript letters are significantly different ($P<0.05$).
(*): % of dry weight of shrimp/rabbitfish.

5.3.2 Environmental variations

5.3.2.1 Water parameters

Mean values of temperature, dissolved oxygen (DO) concentration, salinity, and pH were similar in all treatments over the experiment (Table 5.4). DO concentration, salinity and pH were in suitable ranges for shrimp and rabbitfish growth throughout the experiment. Although, mean temperature was in suitable ranges for shrimp and rabbitfish growth, it fluctuated in relatively wide ranges during the experiment. The temperature changed in a

range of 0.5 - 5.5 ^{o}C between in the morning and the afternoon, fluctuated in the first month and gradually raised from middle to the end of the experiment (Fig 5.1).

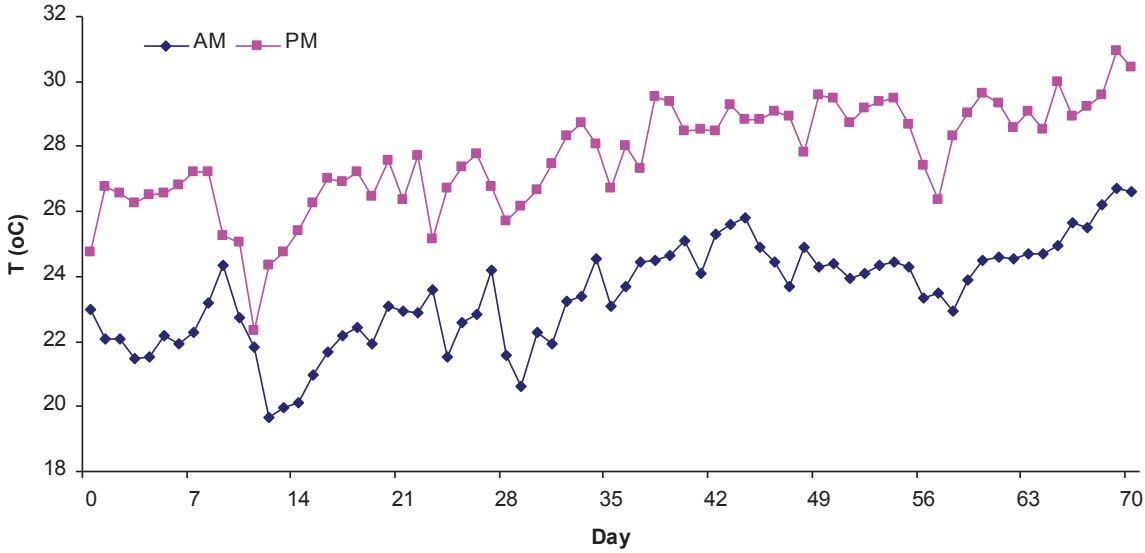


Figure 5.1: Temporal variations of mean temperature in the morning (AM) and in the afternoon ((PM) in all experimental tanks during the experiment (n =16 for each value).

The morning DO gradually decreased in the first month, and then remained stable in suitable ranges for cultured animal requirement to the end of the experiment in all treatments (Fig 5.2).

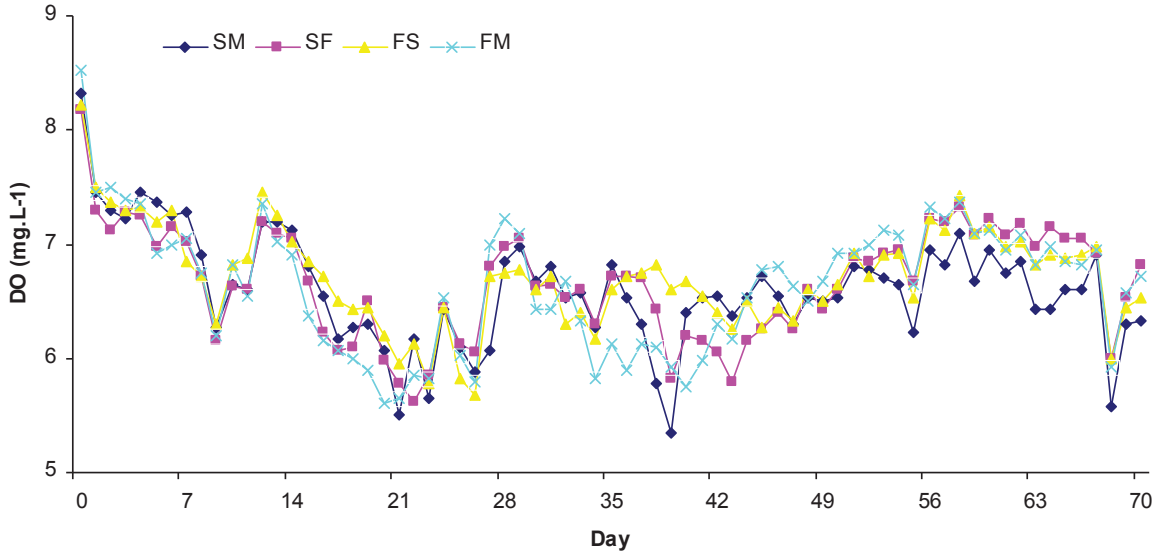


Figure 5.2: Temporal variations of morning DO in the experimental treatments during the experiment. SM: shrimp monoculture, SF: shrimp-rabbitfish polyculture, FS: rabbitfish-shrimp polyculture, FM: rabbbitfish monoculture.

Table 5.4: Water parameters in the experimental treatments throughout the experimental period. Values in parentheses are min – max. Values are means ± SD.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
T (7:30) (^oC)	23.5 ± 0.1 (19.6 – 26.9)	23.5 ± 0.1 (19.6 – 26.9)	23.4 ± 0.3 (19.4 – 26.8)	23.5 ± 0.2 (19.5 – 26.9)
T(15:00) (^oC)	27.7 ± 0.2 (21.5 – 31.2)	27.8 ± 0.2 (22.4 – 31.3)	27.6 ± 0.2 (22.2 – 30.9)	27.7 ± 0.3 (22.2 – 31.3)
DO (7:30) ($mg.L^{-1}$)	6.2 ± 0.4 (4.9 – 7.8)	6.2 ± 0.1 (5.1 – 7.8)	6.3 ± 0.3 (5.2 – 7.8)	6.2 ± 0.4 (5.1 – 8.0)
DO (15:00) ($mg.L^{-1}$)	9.5 ± 0.8 (6.7 - 11.9)	8.7 ± 0.4 (7.2 – 11.7)	8.9 ± 0.3 (7.1 – 12.6)	9.0 ± 0.2 (6.6 – 11.3)
pH	7.9 ± 0.1	7.9 ± 0.0	7.9 ± 0.1	7.9 ± 0.0
Salinity (%)	36.0 ± 0.2	36.0 ± 0.0	36.0 ± 0.2	36.0 ± 0.1
Turbidity (NTU)	27.8 ± 6.4[a]	38.3 ± 13.5[a]	30.0 ± 16.1[a]	20.0 ± 14.5[a]
TSS ($mg.L^{-1}$)	47.4 ± 4.7[a]	58.2 ± 35.8[a]	37.3 ± 12.2[a]	31.3 ± 5.4[a]
Fluorescence ($\mu g.L^{-1}$)	72.7 ± 28.1[a]	82.5 ± 28.5[a]	68.9 ± 20.6[a]	61.1 ± 12.8[a]
Chlorophyll a ($\mu g.L^{-1}$)	67.8 ± 35.6[a]	81.5 ± 37.2[a]	57.6 ± 19.6[a]	54.9 ± 11.3[a]
Phaeopigment (%)	29.0 ± 4.0[ab]	34.3 ± 2.9[a]	26.6 ± 6.9[b]	34.8 ± 4.2[a]
TDN (µM) (n = 11)	48.6 ± 17.5[a]	46.3 ± 8.8[a]	32.8 ± 3.1[a]	34.6 ± 11.3[a]
DON (µM) (n = 11)	40.1 ± 13.9[a]	36.8 ± 5.9[a]	28.5 ± 1.2[a]	31.6 ± 9.2[a]
Urea (µM) (n = 6)	4.9 ± 0.2[a]	6.5 ± 4.8[a]	3.8 ± 0.3[a]	2.8 ± 0.5[a]
(NO_2+NO_3)-N (µM) (n=11)	0.63 ± 0.53[ab]	0.29 ± 0.14[a]	0.20 ± 0.02[a]	0.88 ± 0.48[b]
TAN (µM) (n=11)	8.5 ± 4.1[a]	9.1 ± 2.2[a]	3.8 ± 1.8[b]	3.4 ± 1.1[b]
SRP (µM) (n=11)	0.24 ± 0.03[a]	0.26 ± 0.15[a]	0.20 ± 0.07[a]	0.39 ± 0.27[a]
N/P	177.8 ± 0.7[a]	141.4 ± 112.3[a]	77.3 ± 30.8[a]	42.1 ± 32.0[a]

Mean values in a same row with different superscript letters are significantly different ($P<0.05$).

There was no significant different ($P>0.05$) in mean turbidity and TSS among treatments (Table 5.4). Turbidity had the same trend of temporal variation between the SM and the SF treatments throughout the experiment, which showed a rapid increase in the first three weeks. Meanwhile, turbidity gradually raised within small range in the FM treatment, and fluctuated in relatively wide range in the FS treatment over the experiment (Fig. 5.3).

Trends of temporal variations of TSS were similar between the FS and FM treatments throughout the experiment, which highly increased on the third week. In the SF treatment, TSS dramatically increased on the third week and at the end of the experiment while in the SM treatment it rapidly elevated on the first and the third week (Fig. 5.4).

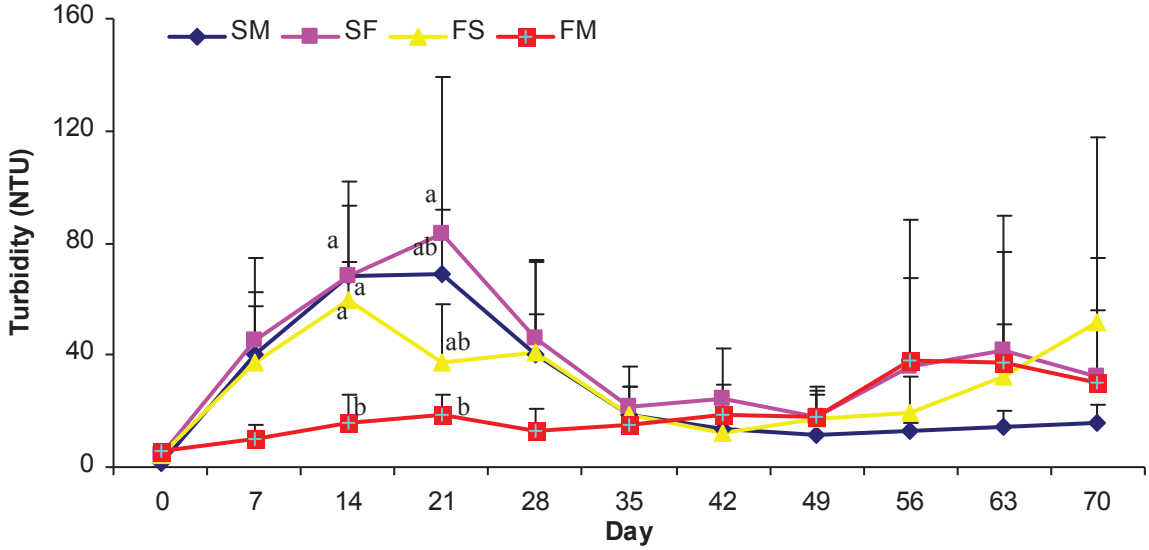


Figure 5.3: Temporal variations of turbidity in the experimental treatments throughout the experiment; SM: shrimp monoculture, SF: shrimp-rabbitfish polyculture, FS: rabbitfish-shrimp polyculture and FM: rabbitfish monoculture. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

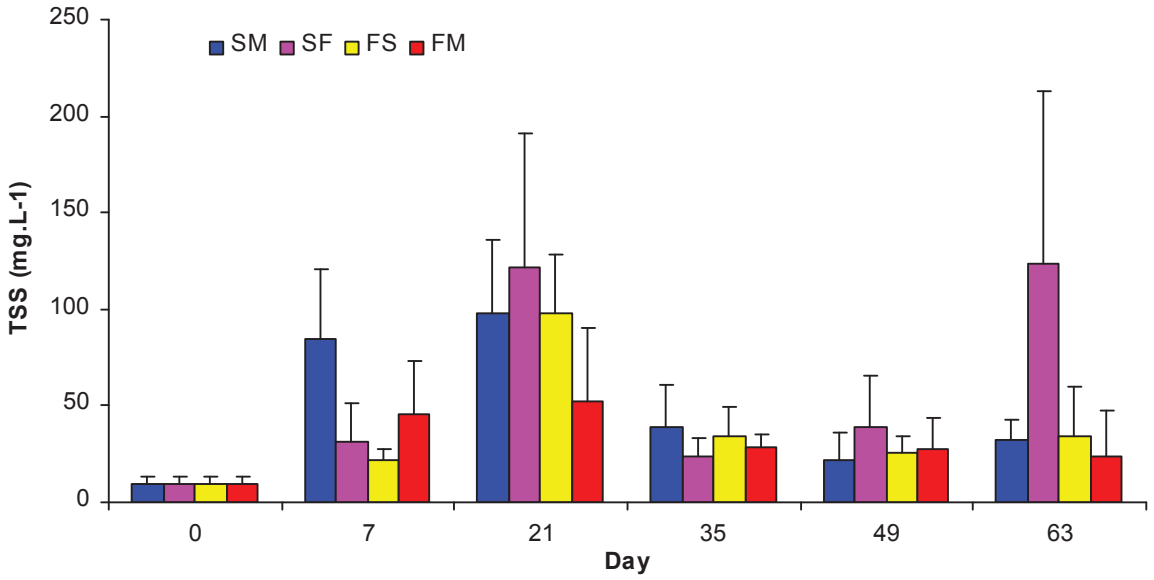


Figure 5.4: Total suspended solids in the experimental treatments during the experiment. Bars present standard deviations. There was no significant difference (P>0.05) in TSS among treatments in the same day.

Mean fluorescence and Chl *a* were not significantly different (P>0.05) among treatments (Table 5.4). Mean phaeopigment ratio was significantly lower (P<0.05) in the FS treatment than those in the SF and the FM treatment (Table 5.4).

Temporal variation of water Chl *a* showed different trends among treatments during the experiment (Fig 5.5). In the SM treatment, Ch *a* highly increased in the first week and

remained stable to the end of the experiment, while it fluctuated within a wide range in the SF treatment during the experiment. In the FS and FM treatments, Chl *a* gradually increased in the first four weeks. From the fifth week to the end of the experiment Chl *a* remained stable in the FS treatment, and gradually decreased in the FM treatment (Fig. 5.5). The variances of Chl *a* fluctuated within small ranges in the SM, the FS and the FM treatments, and in wide range in the SF treatment throughout the experiment (Fig. 5.6).

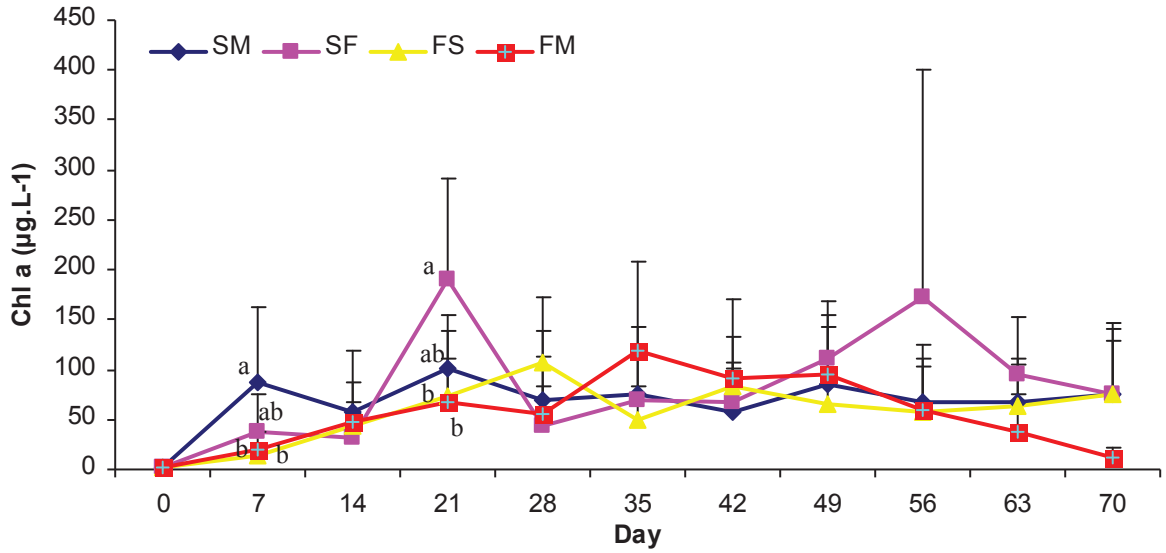


Figure 5.5: Temporal variations of water Chl *a* in the experimental treatments throughout the experiment. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

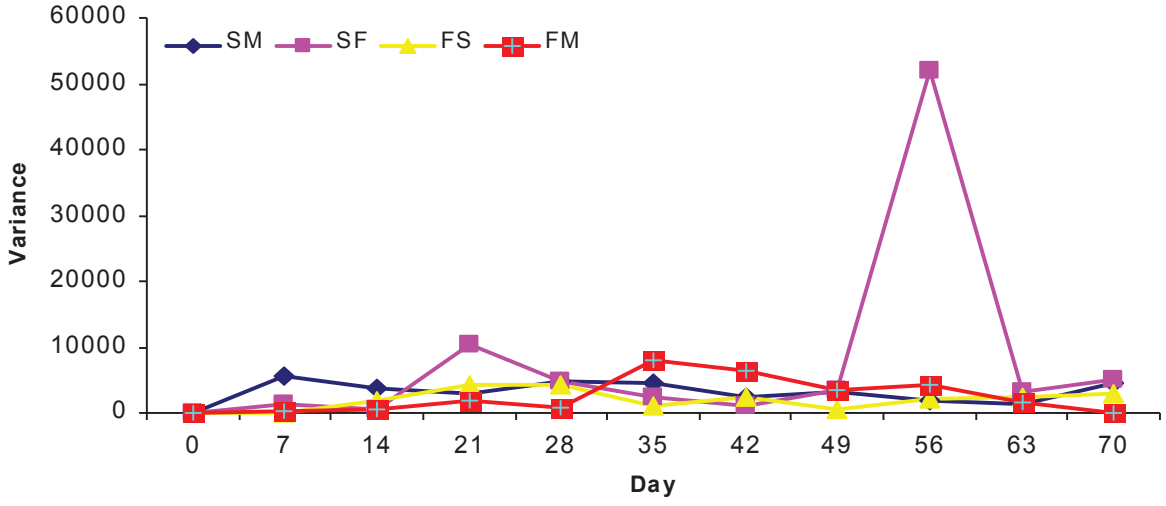


Figure 5.6: Temporal variations of the variances of Chl a in the experimental treatments throughout the experiment.

There were no significant differences (P>0.05) in mean TDN, DON and urea concentrations between all treatments (Table 5.4). Mean nitrite-nitrate nitrogen (NOx-N) concentration was

significantly higher (P<0.05) in the FM treatment than those in the SF and the FS treatments (Table 5.4). NOx-N seemed to be not changed throughout the experiment in the SF and the FS treatments. In the SM treatment, NOx-N gradually increased from beginning to the sixth week, then highly decreased in the next two weeks and remained stable to the end of the experiment. In the FM treatment, NOx-N remained stable until the sixth week, rapidly increased in the next two weeks, decreased in the ninth week and then increased again at the end of the experiment (Fig. 5.7)

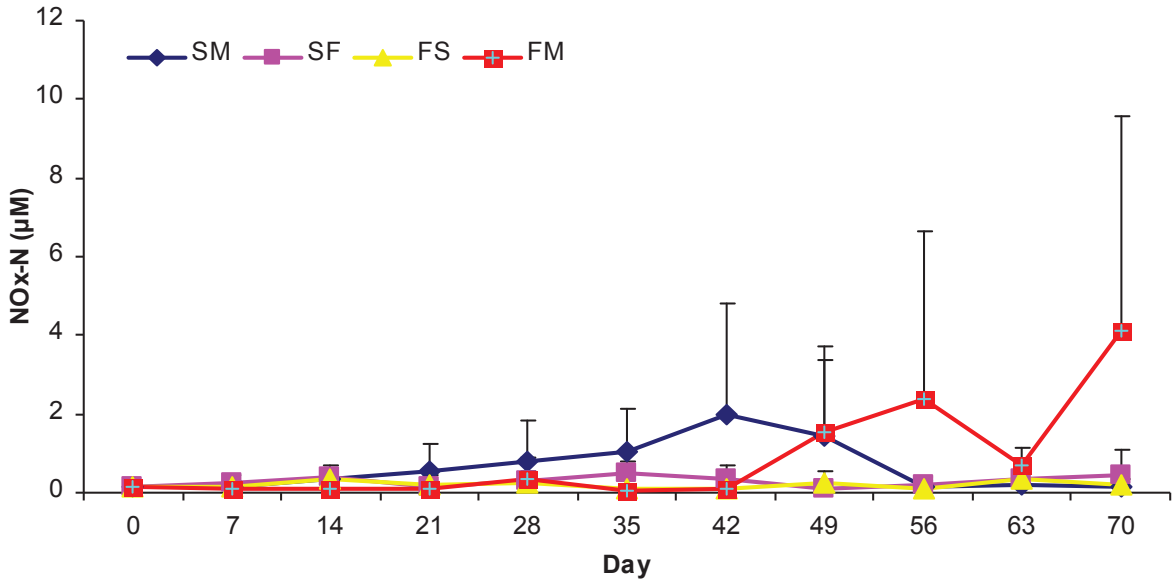


Figure 5.7: Temporal variations of NOx-N in the experimental treatments throughout the experiment. Bars present standard deviations. There was no significant difference (P>0.05) in NOx-N among treatments in the same day.

There was no significant difference (P>0.05) in mean TAN concentration between the SM and the SF treatments as well as between the FS and the FM treatment. However, mean TAN concentrations were significantly higher (P<0.05) in the SM and the SF treatments than those in the FS and the FM treatments (Table 5.4). TAN fluctuated within wide ranges in the SM and the SF treatments during the experiment. In the FS treatment, it highly increased in the first two weeks, then decreased and fluctuated within small range from the fourth week to the end of the experiment. While, TAN gradually increased in the FM treatment throughout the experiment (Fig. 5.8).

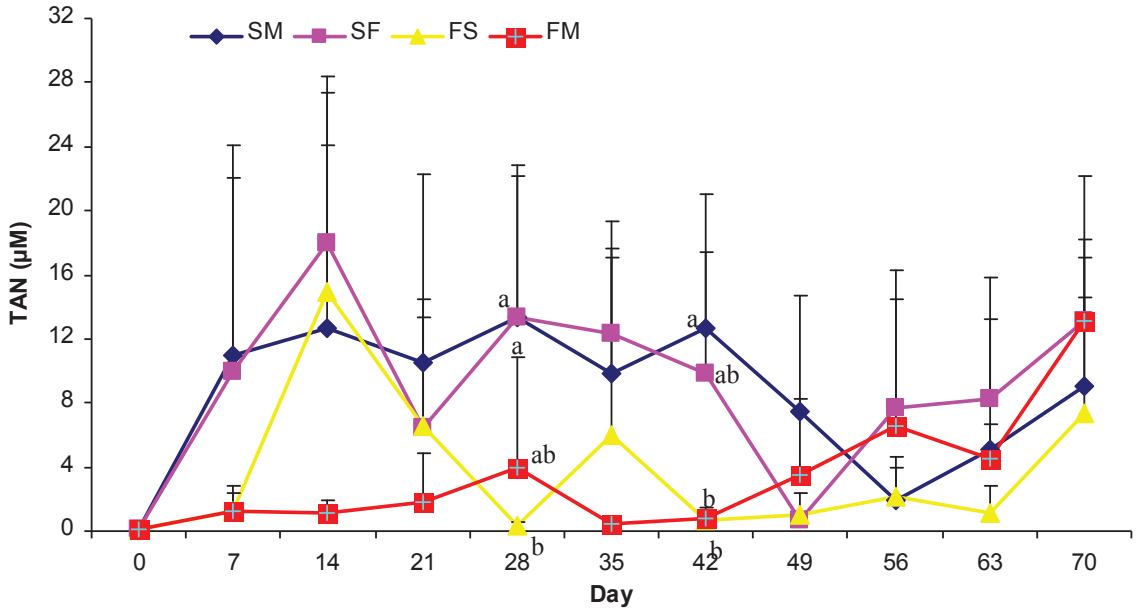


Figure 5.8: Temporal variations of TAN in the experimental treatments throughout the experiment. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

Temporal variation of DON had the same trend in all treatments, which moderately increased in the first half and fluctuated within small ranges in the last three weeks of the experiment (Fig. 5.9).

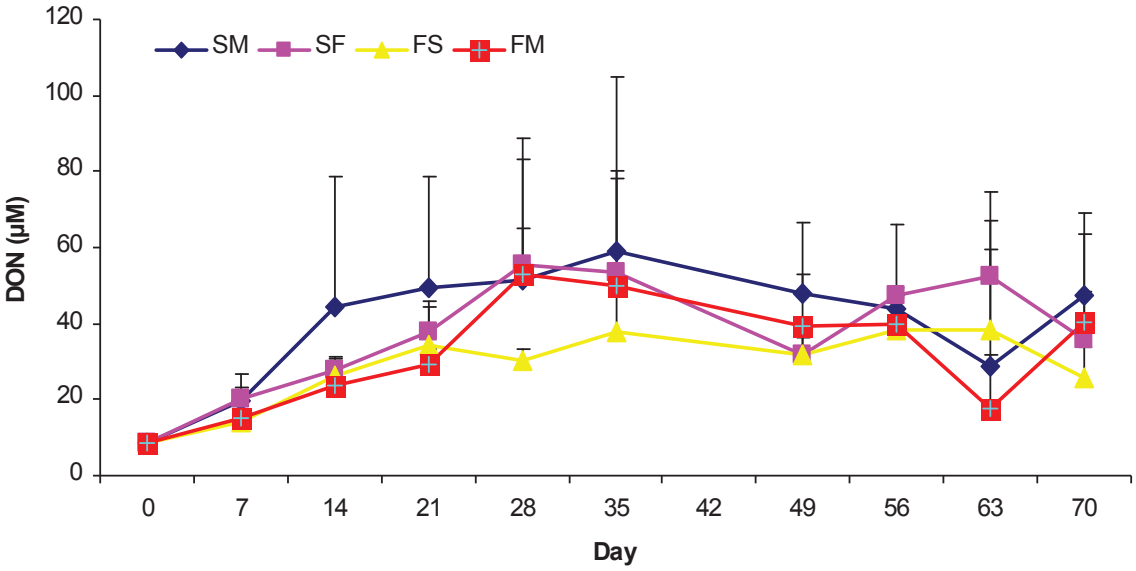


Figure 5.9: Temporal variations of DON in the experimental treatments throughout the experiment. Bars present standard deviations. There was no significant difference (P>0.05) in DON among treatments in the same day.

Mean SRP concentrations were similar among treatments over the experiment (Table 5.4). In the SM and the FS treatments, SRP rapidly increased in the first two weeks, then fluctuated within small ranges to the end of the experiment. Meanwhile, SRP progressively raised in the first four weeks, then fluctuated within wide ranges to the end of the experiment in the SF and the FM treatments (Fig. 5.10).

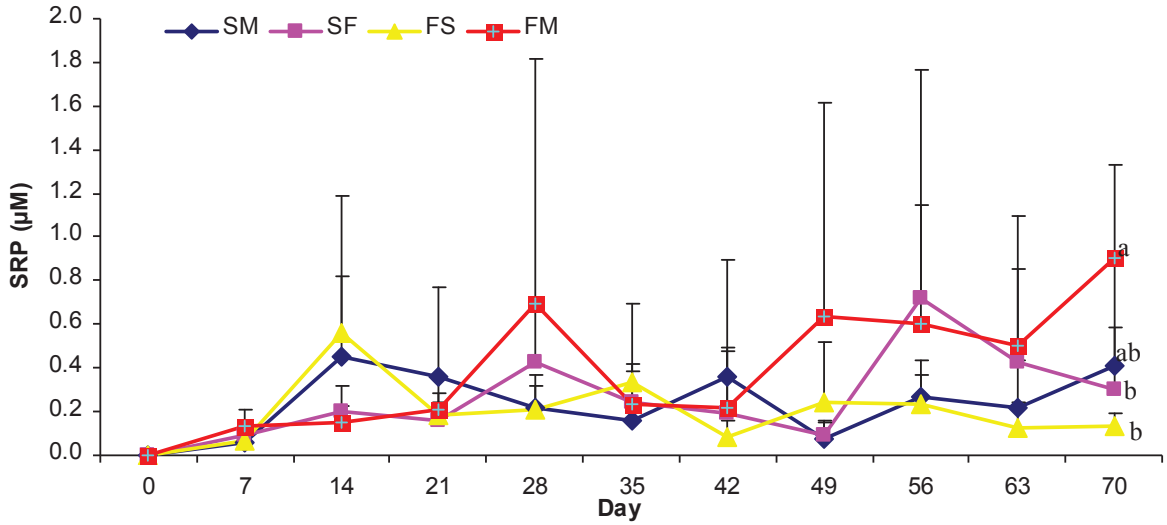


Figure 5.10: Temporal variations SRP in the experimental treatments throughout the experiment. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

5.3.2.2 Sediment parameters

Table 5.5: Sediment parameters in the experimental treatments throughout the experiment. Values are means ± SD.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
pH	7.2 ± 0.1^{a}	7.2 ± 0.1^{a}	7.2 ± 0.1^{a}	7.1 ± 0.1^{a}
Redox potential (mV)	-116.7 ± 8.3^{a}	-102.2 ± 13.6^{a}	-92.3 ± 10.7^{a}	-75.4 ± 30.4^{a}
Chlorophyll a (mg.m^{-2})	87.1 ± 24.7^{a}	88.2 ± 25.5^{a}	118.5 ± 28.9^{a}	114.6 ± 24.4^{a}
Phaeopigment (%)	47.2 ± 4.6^{a}	49.7 ± 3.3^{a}	44.1 ± 6.0^{a}	49.0 ± 3.6^{a}
Loss on ignition (%)	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}
% C	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.2^{a}	1.0 ± 0.2^{a}
% N	0.09 ± 0.02^{a}	0.09 ± 0.01^{a}	0.10 ± 0.02^{a}	0.09 ± 0.01^{a}
C:N	7.6	7.6	7.7	9.5
Final pore water SRP (µM)*	4.3 ± 0.5^{a}	3.1 ± 1.6^{a}	2.0 ± 1.3^{a}	8.8 ± 7.5^{a}
Final pore water TAN (µM)*	886.2 ±155.0^{a}	737.7 ±228.3^{a}	413.0 ±140.0^{b}	313.3 ±149.1^{b}

Mean values in a same row with different superscript letters are significantly different (P<0.05).
(*): The values are the final analyses at the end of the experiment.

Mean values and the trends of sediment pH were similar among treatments during the experiment (Table 5.5). Its range was from 7.0 to 7.3, and was in suitable range for shrimp

and rabbitfish growth. Sediment loss on ignition, percentage of organic carbon (% C) and nitrogen (% N), and C:N ratios were similar among treatments (Table 5.5). Mean redox potential was not significantly different (P>0.05) among treatments (Table 5.5). Range was from -157.0 to -31.5 mV. Redox potential had negative values and had the same trend of temporal variation in all treatments, which gradually decreased during the experiment (Fig. 5.11).

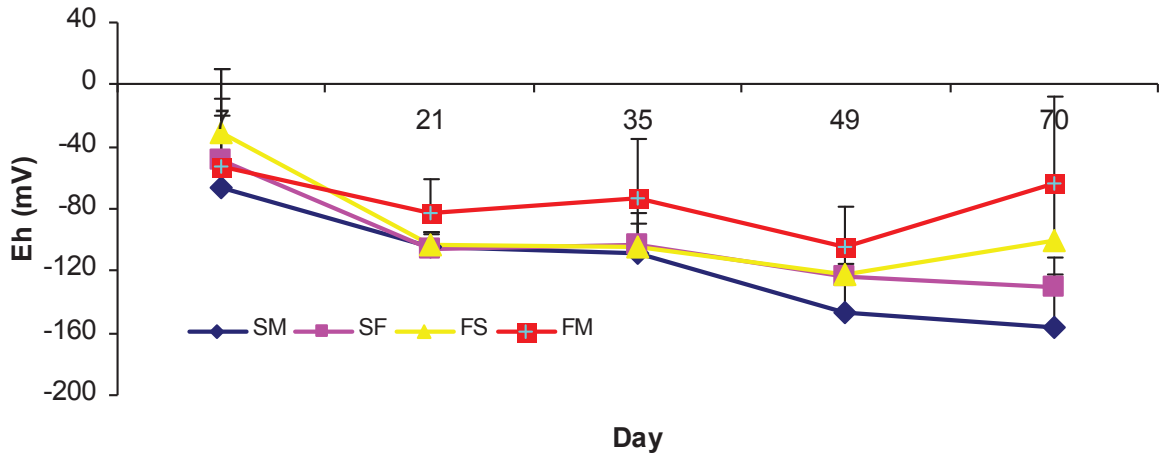


Figure 5.11: Temporal variations of sediment redox potential (Eh) in experimental treatments throughout the experiment. Bars present standard deviations.

There was no significant difference (P>0.05) in sediment Chl *a* and phaeopigment ratio among treatments (Table 5.5). Temporal variations of sediment Chl *a* had the same trend in all treatments throughout the experiment that progressively increased from beginning to the seventh week, and then slightly reduced to the end of the experiment (Fig. 5.12).

Initial pore water SRP was low in all treatments, 0.02 µM. The final values of pore water SRP were not significantly different (P>0.05) among treatments (Table 5.5). Initial pore water TAN was similar among treatments, 108.9 µM. The final values of pore water TAN were significantly higher (P<0.05) in the SM and the SF treatments than those in the FS and the FM treatments (Table 5.5).

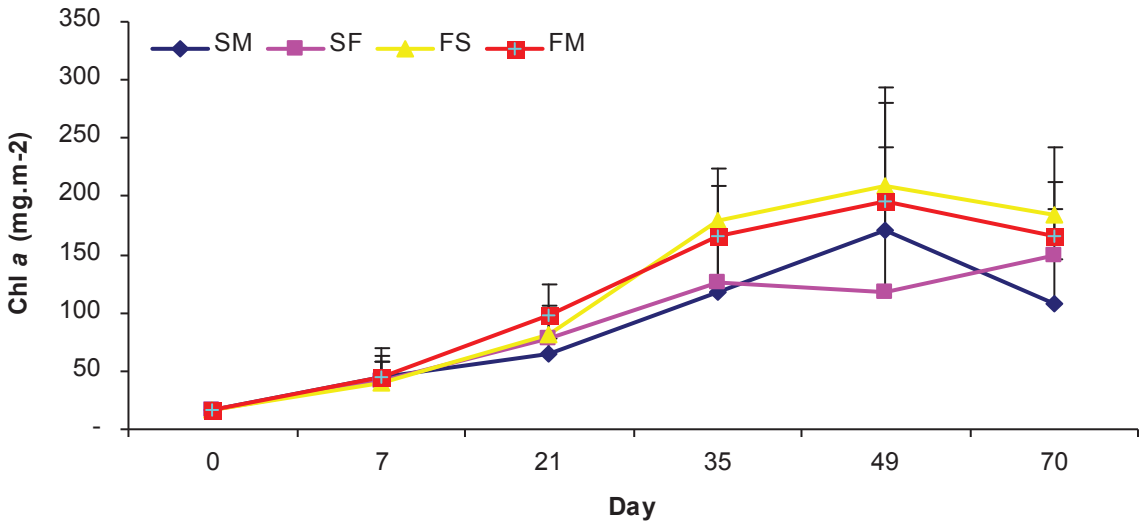


Figure 5.12: Temporal variations of sediment Chl *a* in experimental treatments throughout the experiment. Bars present standard deviations. There was no significant difference (P>0.05) in sediment Chl *a* among treatments in the same day.

5.3.3 Ecological functioning

5.3.3.1 Sedimentation processes

There was no significant difference (P>0.05) in mean sedimentation rate (SdR) and deposit loss on ignition among treatments over the experiment (Table 5.6). After stocking, SdR rapidly increased in all treatments. On the day 7, SdR was significantly higher (P<0.05) in the SF treatment than that in the FM treatment (Fig. 5.13). In the SM treatment, SdR decreased from day 21, while it progressively increased from day 35 to the end of the experiment in the FM treatment. However, the increase of SdR in the FM treatment coincided with increasing variability between replicates (Fig. 5.13). In the SF and the FS treatments, SdR fluctuated within relatively wide ranges over the experiment (Fig. 5.13). Deposit loss on ignition gradually increased in all treatments throughout the experiment (Fig. 5.14).

Table 5.6: Sedimentation processes in the experimental treatments throughout the experiment. Values are means ± SD.

	Treatment			
	Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
Sedimentation rate ($g.m^{-2}.h^{-1}$)	107.1 ± 13.3^{a}	213.2 ± 129.8^{a}	154.7 ± 61.8^{a}	192.9 ± 199.4^{a}
Deposit loss on ignition (%)	4.6 ± 0.4^{a}	4.3 ± 0.1^{a}	5.2 ± 2.4^{a}	4.4 ± 1.0^{a}

Mean values in a same row with different superscript letters are significantly different (P<0.05).

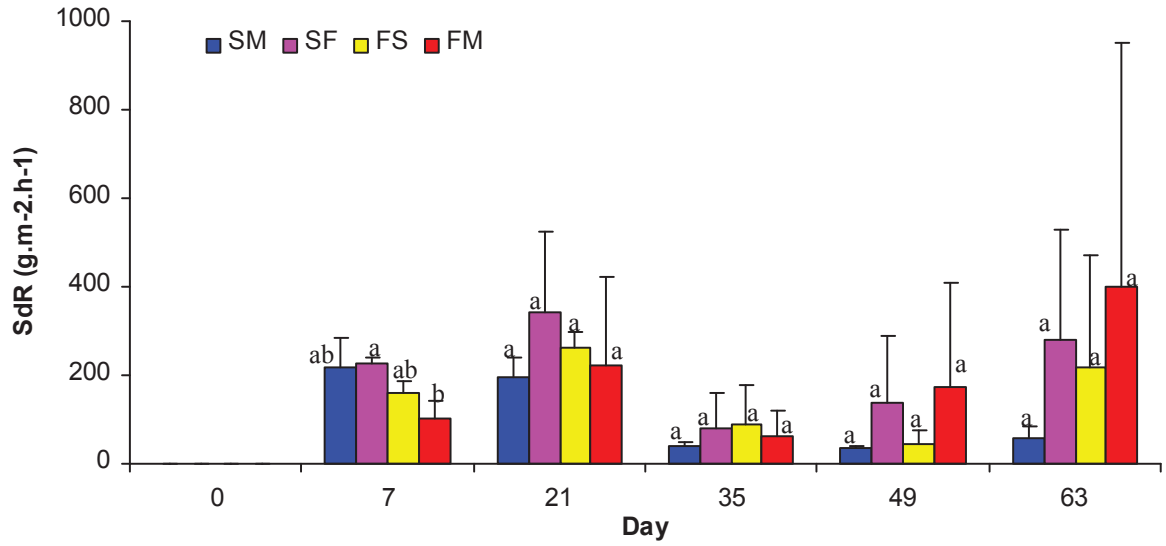


Figure 5.13: Sedimentation process in the experimental treatments during the experiment. Bars present standard deviations. Values in the same day with different letters are significantly different (P<0.05).

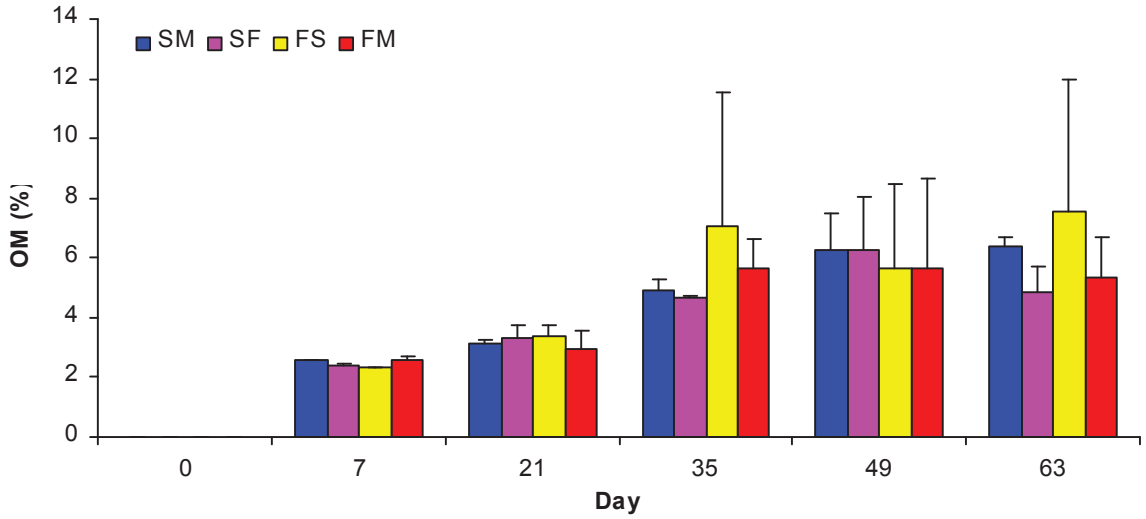


Figure 5.14: Temporal variations of deposit loss on ignition in the experimental treatments during the experiment. Bars present standard deviations. There was no significant difference (P>0.05) in values in the same day among treatments.

5.3.3.2 Water and sediment metabolism

Gross primary productivity (GPP) in the water column and in the whole ecosystem significantly increased (P<0.05) after stocking in all treatments. Meanwhile, sediment GPP reduced in the SM and the FS treatments, and increased in the SF and the FM treatments at the early of the experiment, however, the differences were not significant (P>0.05). The GPP in the water column, sediment and the whole ecosystem were similar between the early and the late of the experiment in all treatments. The entire GPP was significantly greater (P<0.05)

in the SM treatment than those in other treatments at the early, however, there was no significant difference (P>0.05) in the entire GPP among treatments at the late of the experiment (Fig. 5.15). At the early, sediment GPP was not significantly different (P>0.05) among treatments while water GPP was significantly higher (P<0.05) in the SM than those in other treatments. At the late, sediment GPP was significantly greater (P<0.05) in the FM than those in the SM and SF treatments whilst water GPP was not significantly different (P>0.05) among treatments (Fig. 5.15). Water GPP was significant higher (P<0.05) than sediment GPP in the SM, the SF and the FS treatments at the early, and in the SM treatment at the late of the experiment.

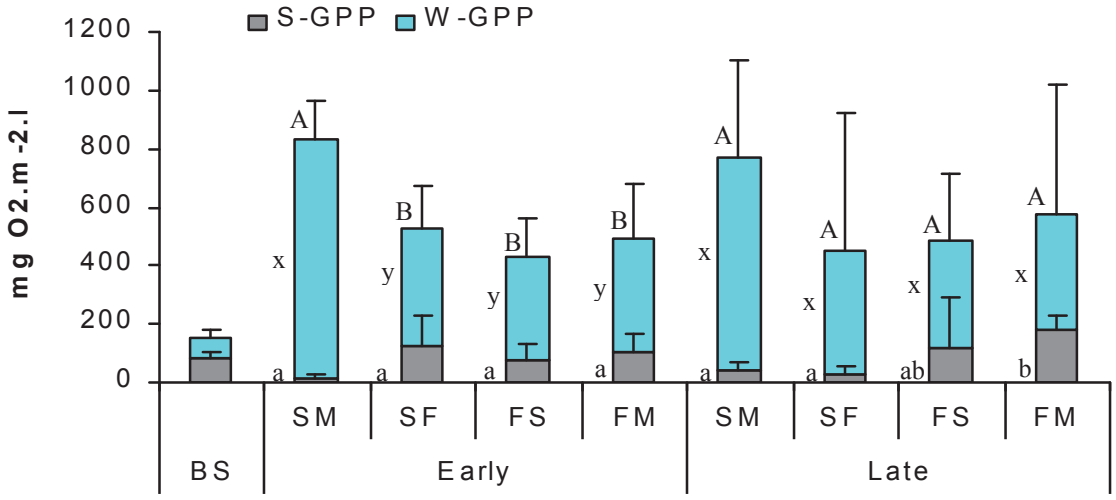


Figure 5.15: Water GPP (W-GPP), sediment GPP (S-GPP) and entire GPP in whole ecosystem (W-GPP + S-GPP) in the experimental treatments before stocking (BS), at the early and the late of the experiment. Bars present standard deviations. In a same day, values with different letters are significantly different (P<0.05). A, B; x, y; a, b: represent for statistical differences of entire GPP, W-GPP and S-GPP, respectively.

In the water column, 1mg Chl *a* produced an average GPP of 31.7 $mgO_2.h^{-1}$ (7.4 – 78.0 $mgO_2.h^{-1}$) through photosynthetic process at the early, which was not significantly higher (P>0.05) than value at the late of the experiment, 17.4 $mgO_2.h^{-1}$ (5.6 – 35.8 $mgO_2.h^{-1}$). In the sediment, the average GPP produced by 1mg Chl *a* was 2.5 (0.6 – 3.5) and 0.6 (0.2 – 1.0) $mgO_2.h^{-1}$ at the early and the late of the experiment, respectively.

Respiration (R) in the water column and the whole ecosystem significantly increased (P<0.05) after stocking in all treatments. Except in the FM treatment, sediment R significantly increased (P<0.05) in the other treatments at the early of the experiment. In the SM and FM treatments, sediment R and the entire R were significantly higher (P<0.05) at the late than those at the early of the experiment. Similarly, sediment R significantly increased

(P<0.05) in the FS treatment between the early and the late of the experiment. Water R were similar between the early and the late of the experiment in the SF, the FS and the FM treatments while it significantly increased (P<0.05) in the SM treatment.

Sediment R and the entire R in the SM treatment were significantly higher (P<0.05) than those in the FM treatment at the early of the experiment. Meanwhile, there was no significant difference (P>0.05) in water R among treatments (Fig 5.16). At the late of the experiment, the entire R was significantly greater (P<0.05) in the SM treatment than those in the other treatments (Fig. 5.16). Water R was significantly higher (P<0.05) in the SM than those in the FS and the FM treatments while sediment R were significantly higher (P<0.05) in the SM and the FM treatments than those in the SF and the FS treatments (Fig 5.16).

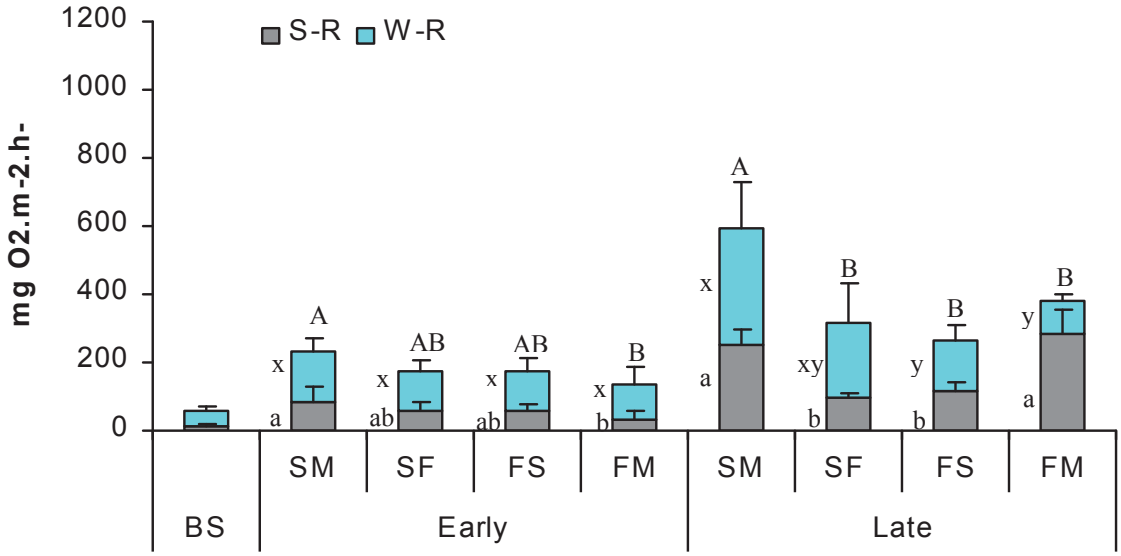


Figure 5.16: Respiration (R) in the water column (W-R), sediment (S-R) and the whole ecosystem (W-R + S-R) in the experimental treatments before stocking (BS), at the early and the late of the experiment. Bars present standard deviations. In a same day, values with different letters are significantly different (P>0.05). A, B; x, y; a, b: represent for statistical differences of entire R; W-R and S-R, respectively.

There was no significant difference (P>0.05) between water and sediment R in all treatments at the early of the experiment whist sediment R was significantly higher (P<0.05) than water R in the FM treatment at the late of the experiment.

Before stocking, the ratio of primary productivity and respiration (P/R) in the water column was below 1.0. It increased up to 1.5 – 3.0 at the early and reduced to 1.1 – 1.8 at the late of the experiment (Fig 5.17), indicating the trophic status changed from autotrophic to heterotrophic situation. In the sediment, the P/R had a high value (>3.0) before stocking,

except in the FM treatment at the early, it decreased to below 1.0 at the early and the late of the experiment, indicating heterotrophic sediments (Fig 5.17).

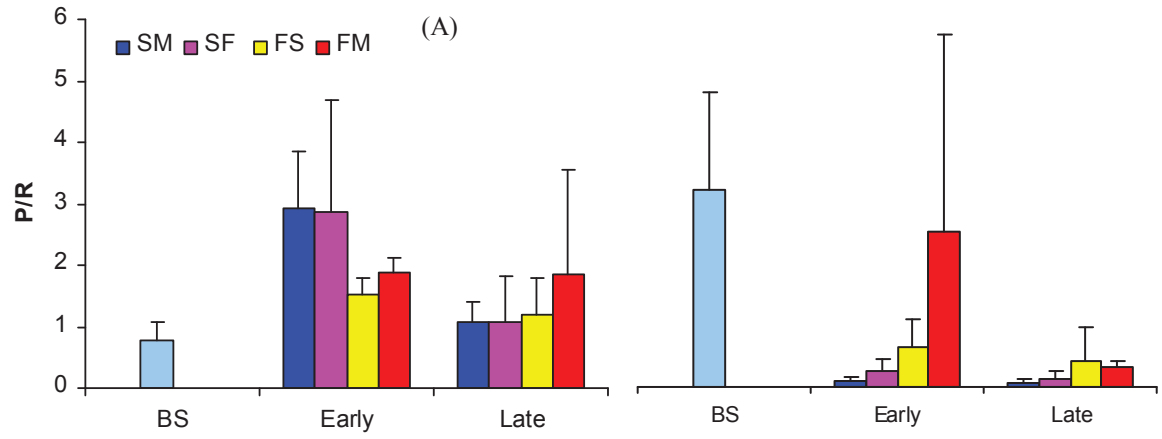


Figure 5.17: Primary Productivity and Respiration ratio (P/R) in the water column (A) and sediment (B) of the experimental treatments before stocking (BS), at the early and the late of the experiment. Bars present standard deviations.

5.3.4 Stable isotope analyses

The same pellet feed was used during the experiment, so stable isotope signatures were similar in all analysed feed samples and the average values were -22.54 ± 0.13 ‰ for $\delta^{13}C$, and 7.31 ± 0.11 ‰ for $\delta^{15}N$. At beginning of the experiment, stable isotope signatures of particulate organic matter (POM) were higher than the pellet feed for $\delta^{13}C$ value, but lower for $\delta^{15}N$ (Table 5.7). Throughout the experiment, the final POM $\delta^{13}C$ value was significantly higher ($P<0.05$) than initial value in the FS treatments. Meanwhile, there was no significantly difference ($P>0.05$) between the final and initial values in other treatments (Table 5.7). The final POM $\delta^{15}N$ values were lower than the initial value in the SM, SF and FS treatment, and higher than the initial value in the FM treatment. However, the differences were not significant ($P>0.05$) (Table 5.6). The $\Delta\delta^{13}C$ POM-feed changed from 1.03 ‰ at the beginning to 0.36 – 2.51 ‰ at the end of the experiment. The $\Delta\delta^{15}N$ POM-feed was from -2.01 to - 0.77 ‰ at the end of the experiment while the initial value was - 0.85 ‰.

Isotope signatures of sediment organic matter (SOM) were higher than the pellet feed $\delta^{13}C$ value ($\Delta\delta^{13}C$ = 3.83 ‰), and lower than the pellet feed $\delta^{15}N$ value ($\Delta\delta^{15}N$ = -1.96 ‰) at the beginning of the experiment (Table 5.7). During the experiment, the SOM $\delta^{13}C$ reduced in all treatments, but there was no significant difference ($P>0.05$) between the final and initial $\delta^{13}C$

values (Table 5.7). The SOM $\delta^{15}N$ significantly increased in the SF treatment over the experiment while the final SOM $\delta^{15}N$ values were not significantly higher (P>0.05) than the initial one in the other treatments (Table 5.7). The final $\Delta\delta^{13}C$ SOM-feed was 2.27 – 2.69 ‰ and the final $\Delta\delta^{15}N$ SOM-feed was from -0.95 to -0.7 ‰.

Table 5.7: Stable isotope ($\delta^{13}C$, $\delta^{15}N$) values in particulate organic matter (POM) and sediment organic matter (SOM) at the beginning and at the end of the experiment in the experimental treatments. Values are means ± SD, expressed in ‰.

	Initial	Final			
		Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
POM - $\delta^{13}C$	-21.51 ±0.32^{ab}	-22.18 ± 1.94^{a}	-20.85 ±1.37^{ab}	-20.03 ± 0.57^{b}	-20.50 ±0.21^{ab}
- $\delta^{15}N$	6.45 ± 1.09^{a}	6.11 ± 2.03^{a}	5.46 ± 1.59^{a}	5.30 ± 1.72^{a}	6.53 ±0.62^{a}
SOM - $\delta^{13}C$	-18.71 ± 0.88^{a}	-19.85 ± 1.45^{a}	-19.91 ± 1.14^{a}	-20.27 ± 1.23^{a}	-20.01 ± 0.73^{a}
- $\delta^{15}N$	5.74 ± 0.27^{a}	6.45 ± 0.63^{ab}	6.60 ± 0.80^{b}	6.38 ± 0.29^{ab}	6.35 ± 0.47^{ab}

Mean values in a same row with same superscript letters are not significantly different (P>0.05).

At stocking, the $\Delta\delta^{13}C$ of shrimp to the pellet feed, POM and SOM were high, 8.62, 7.59 and 4.79 ‰, respectively, and the $\Delta\delta^{15}N$ of shrimp to the pellet feed, POM and SOM were low, -0.44, 0.42 and 1.13 ‰, respectively. Over the course of the experiment, the shrimp $\delta^{13}C$ values were significantly lower (P<0.05) than that at stocking in all treatments. Meanwhile, the shrimp $\delta^{15}N$ became significantly enriched (P<0.05) for all treatments (Table 5.8). The $\Delta\delta^{13}C$ of shrimp to the pellet feed, POM and SOM reduced to 4.48 – 5.58, 1.97 – 5.02, and 2.21 – 2.95 ‰, respectively, while the $\Delta\delta^{15}N$ of shrimp to the pellet feed, POM and SOM increase up to 0.41 – 1.33, 2.03 – 3.34, and 1.12 – 2.26 ‰, respectively, at harvesting in all treatments.

At stocking, stable isotope signatures of rabbitfish were higher than those of the pellet feed, POM and SOM. Over the experiment, the rabbitfish $\delta^{13}C$ values significantly decreased (P<0.05) in the SF and FS treatments while the rabbitfish $\delta^{15}N$ values significantly lower (P<0.05) than the stocking value in all treatments (Table 5.8).

At harvesting, the $\Delta\delta^{13}C$ of rabbitfish to the pellet feed and POM were 3.14 – 3.58 and 0.63 – 1.54 ‰, respectively, which were smaller than those at stocking. Meanwhile, the $\Delta\delta^{13}C$ of rabbitfish to SOM, 0.59 – 1.65 ‰, was higher than value at stocking, 0.52 ‰. The $\Delta\delta^{15}N$ of rabbitfish to the pellet feed, POM and SOM were 3.67 – 3.88, 4.65 – 5.67, and 4.47 – 4.83 ‰, respectively, which were smaller than those at stocking, 4.8, 5.65, and 6.36 ‰, respectively.

Table 5.8: Stable isotope ($\delta^{13}C$, $\delta^{15}N$) values of shrimp and rabbitfish at stocking and harvesting in the experimental treatments. Values are means ± SD, expressed in ‰.

	Stocking	Harvesting			
		Shrimp monoculture	Shrimp-fish polyculture	Fish-shrimp polyculture	Fish monoculture
Shrimp $\delta^{13}C$	-13.92 ±1.72^{a}	-17.16 ±0.86^{b}	-16.96 ±0.54^{b}	-18.06 ±0.77^{b}	
$\delta^{15}N$	6.87 ± 0.43^{a}	8.14 ± 0.65^{bc}	7.72 ± 0.13^{b}	8.64 ± 0.38^{c}	
Rabbitfish $\delta^{13}C$	-18.19 ±0.85^{a}		-19.32 ±0.37^{b}	-19.40 ±0.28^{b}	-18.96 ± 1.1^{ab}
$\delta^{15}N$	12.11 ± 0.11^{a}		11.07 ± 0.06^{b}	10.97 ± 0.47^{b}	11.18 ± 0.42^{b}

Mean values in a same row with same superscript letters are not significantly different (P>0.05)

There were little differences in the $\delta^{13}C$ and $\delta^{15}N$ values of POM, SOM, shrimp and rabbitfish among treatments at the end of the experiment. Therefore, the data from all treatments were calculated for average values for ease of comparison between the beginning and the end of the experiment. In general, the $\delta^{13}C$ and $\delta^{15}N$ values of SOM, shrimp and rabbitfish changed over the experiment following the direction that to be closer to the pellet feed $\delta^{13}C$ and $\delta^{15}N$ values, while the $\delta^{13}C$ and $\delta^{15}N$ values of POM changed in the opposite direction (Figure 5.18).

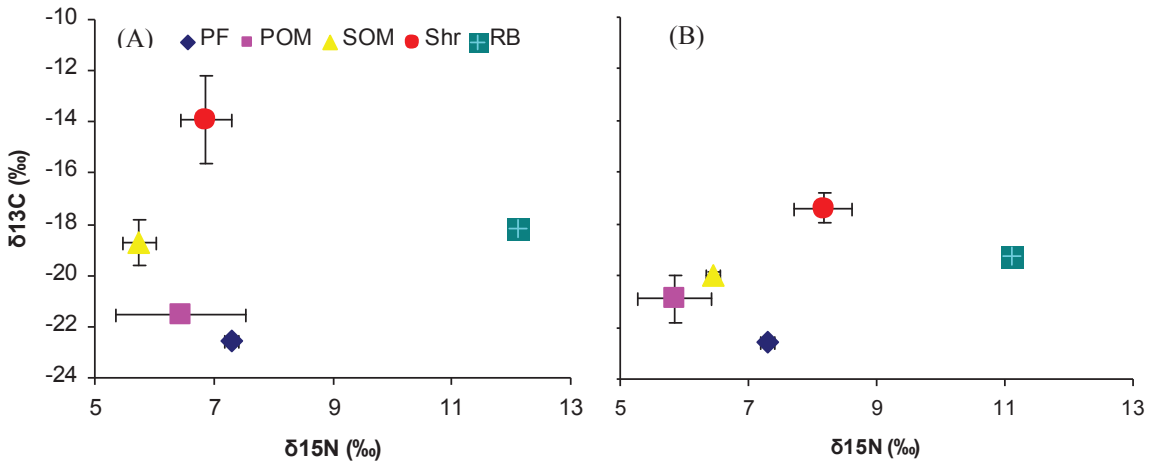


Figure 5.18: The changes of the stable isotope (δ13C, δ15N) values of the pellet feed (PF), particulate organic matter (POM), sediment organic matter (SOM), shrimp (Shr) and rabbitfish (RB) between the beginning (A) and the end (B) of the experiment. Bars present standard deviations.

5.4 Discussion

5.4.1 Blue shrimp and goldlined rabbitfish growth performances

Low shrimp survival, especially in the SM and the SF treatments, and low growth rate were

possibly derived from shrimp stress caused by some environmental variables, including low temperature (Table 5.4), bad conditions of bottom sediment (as high concentration of final pore water TAN, low redox potential, black sludge accumulated) (Table 5.5, Photo 5.1), and the early eutrophication (rapid increases of Chl *a* and nutrient variables) (Table 5.4 & Fig. 5.5, 5.8, 5.9, 5.10 & 5.12).

Like the previous experiment (Chapter 4), low water temperature was probably one of factors that affected low growth and survival of shrimp in this study. Mean water temperature in the morning was 23.5 ^{o}C that be well lower than optimal temperature for *L. stylirostris,* 28 ^{o}C (Díaz et al., 2004; Bondad-Reantaso et al., 2005). Furthermore, the lowest temperature both in the morning and afternoon were similar with the low edge of the thermo-preferendum for *L. stylirostris*, 20-22 ^{o}C that likely caused considerable stress for shrimp in the culture tanks (Wabete et al., 2008). Thermal stress of *L. stylirostris* would increase as temperatures decreased or increased with respect to the optimum temperature of 28 ^{o}C (Díaz et al., 2004), and at 20 to 22 ^{o}C shrimp were thermally stressed and often died after 2 days (Wabete et al., 2008). In our study, water temperature gradually increased in the course of the experiment (Fig. 5.1), but at the beginning, morning temperature was as low as thermal stressed level for shrimp that might negatively affect shrimp growth and survival (Wyban et al., 1995).

Ammonia in un-ionized form (NH_3) is toxic to shrimp and aquatic animals, at low concentrations of ammonia could damage gills, reduce growth and cause mortality (Lazur, 2007). In this study, water TAN concentrations were lower by far safe levels of ammonia recommended for rearing penaeid shrimp, 4.26 mg.L^{-1} (304.3 µM) TAN and 0.08 mg.L^{-1} (5.7 µM) NH_3-N (Chen et al., 1990); or for grow-out ponds, NH_3-N < 0.15 mg.L^{-1} (10.7 µM) (Lazur, 2007). Besides, the (NO_2+NO_3)-N concentrations were also well below safe levels of nitrite for rearing penaeid shrimp, 10.6 mg.L^{-1} (757.1 µM) (Chen et al., 1990); or for grow-out pond, 4.5 mg.L^{-1}(321.4 µM) (Lazur, 2007). So, TAN and (NO_2+NO_3)-N were probably not factors that considerably stressed shrimp in all treatments. However, final concentrations of pore water TAN in the SM, the SF and the FS treatments, 886.2, 737.7 and 413.0 µM, respectively, were well higher than safe level for rearing penaeid shrimp (Chen et al., 1990), and also higher than stressful level for *L. stylirostris* recorded in a shrimp pond in New Caledonia, 607.9 µM (Mugnier et al., 2006). Elevated pore water ammonia concentrations during the experiment were mostly of concern with respect to the growth and survival of cultured shrimp with benthic feeding and burrowing habits (Hargreaves, 1998). High TAN

concentrations were expected in chemically reduced sediments due to the effects of low oxygen on both organic nitrogen mineralization and nitrification. The effects of anoxic conditions on TAN accumulation might have a negative impact on shrimp growth and the accumulation of ammonia may lead to shrimp mortality (Joyni et al., 2011).

Pond bottom conditions are more critical for shrimp than for other aquaculture species because shrimp spend most of their time on the bottom, burrow into the soil and ingest pond-bottom soil (Dall et al., 1990; Avnimelech and Ritvo, 2003). Shrimp significantly decreased or even stopped feeding in areas with sulphide containing sediment or covered by reduced sediment (Avnimelech and Ritvo, 2003). In intensive shrimp culture ponds, the accumulated black sludge on the pond bottom leads to reducing conditions and negative redox values (Muralidhar et al., 2014). In our study, black sludge was established in the SM and the SF treatments (Photo 5.1), along with negative redox values (Table 5.4) indicated reduction processes in sediment, possibly including iron reduction ($Fe^{+3} \rightarrow Fe^{+2}$) and sulphate reduction ($SO_4^{2-} \rightarrow S^{-2}$) (Avnimelech and Ritvo, 2003, Muralidhar et al., 2014), which might produce sulphide toxicity to fish and shrimp even at a very low concentration (Lazur, 2007). In this study, sulphides were not analysed. However, regardless of sulphides, the sediment characterized by high pore water TAN, negative redox potential and accumulated black sludge in the SM and the SF treatments probably was not suitable for shrimp growth and activity.

Shrimp survivals in the SM and the SF treatments were similar with the results of those in disease shrimp farms recorded by Lemonnier et al. (2006) in New Caledonia, during summer grow-out seasons or by Mermoud et al. (1998) during winter grow-out seasons. The first authors stated that high stocking densities increased the risk of transmission of the disease within the pond, and an early eutrophication of water could play a role by directly or indirectly inducing a stress for shrimp and/or a growth and/or virulence factors of pathogen, *Vibrio penaeicidae* (Goarant et al., 2004) or *V. nigripulchritudo* (Goarant et al., 2006). Moreover, these two pathogens were found regularly at the aquaculture station of Saint Vincent in ponds or in some experimental tanks. In this study, high biomasses and densities at stocking possibly induced eutrophication due to high loading of nutrients from feed used and animal metabolic products, as expressed by relatively high concentrations and rapid increases of Chl *a* and nutrient variables (Table 5.4, 5.5, and Fig. 5.5, 5.8, 5.9 & 5.10). Neither shrimp disease nor pathogen was studied during the culture period, but disease might

not be excluded in involving to shrimp mortality. It was not surprising that early eutrophication might have a certain role of causing stress for shrimp that possibly brought to shrimp disease and ultimate mortality. However, the results likely indicated that the synergistic effects of temperature, bad sediment conditions and early eutrophication, and/or pathogen caused stress for shrimp that led to low growth and survival in the culture tanks.



Photo 5.1: The view of bottom sediments at harvesting in the culture tanks of shrimp monoculture (SM), shrimp-fish polyculture (SF), fish-shrimp polyculture (FS), and rabbitfish monoculture (FM).

The results of shrimp growth and survival in the SM, the SF and the FS treatments showed that the presence of rabbitfish did not negatively affect shrimp growth performance. On the other hand, shrimp survival in the FS treatment was higher than those in the SM and SF treatments. This might be resulted from better environmental conditions in the FS treatment than those in the SM and the SF treatments. As high biomass of rabbitfish in the FS treatment, feed consumption could be more efficient and so could prevent the further degradation of the environment caused by uneaten feed.

Significantly negative weight gain of shrimp in the SM and the SF treatments indicated that shrimp biomasses were beyond carrying capacity of the culture system. The results suggested that high shrimp biomass at stocking in this experiment was not suitable for shrimp production in the culture system of both monoculture and polyculture with rabbitfish. However, the shrimp biomasses at the end of the experiment in this study were still lower than the results in the previous experiment, in which shrimp yields were 143 $g.m^{-2}$ in

monoculture, and 145 – 179 g.m^{-2} in polyculture with rabbitfish (Chapter 4). The reason was probably that as high stocking biomass due to high individual shrimp weight (> 13g), high amount of food supplied to the culture tanks, lead to high organic matter loaded in the environment from feed and animal metabolites immediately at the beginning of the experiment. As a consequence, concentrations of some environmental variables, such as Chl *a* unexpectedly increased and early eutrophication occurred along with degradation of sediment conditions (TAN accumulation, negative redox potential, established black sludge) caused by uneaten feed and accumulated organic matter decomposition. Thus, the high variability of environmental parameters likely caused shrimp stressed and/or sensitive to pathogen that resulted in low shrimp growth, survival and yields.

The similarity in rabbitfish final mean weight and growth rate at all different biomasses and densities in both monoculture and polyculture showed that there was little intraspecies and interspecies competition. This result consistent with the results in the previous experiments (Chapter 3 & 4), and that confirmed rabbitfish could be stocked at relatively high density in captivity for production. High survival, growth rate and weight gain of rabbitfish in the SF, the FS and the FM treatments indicated that rabbitifish could well tolerate the environmental variations that caused stress for shrimp in the culture tanks. In the same conditions in the SF and the FS treatments, while shrimp biomass reduced, rabbitfish biomass significantly increased over the experiment.

Rabbitfish mortality was observed in some tanks of the FS and the FM treatments from middle to near the end of the experiment. Like the results of the rabbitfish monoculture study (Chapter 3), mortality closely coincided with phytoplankton bloom in the culture tanks (Fig. 5.19). At that time, Chl *a* concentration highly increased (> 150 µg.L^{-1}), water became dark, rabbitfish glassily swam on the surface and exhaustedly breathed, and eventually mortality happened after 1 - 3 days. The floating dead rabbitfish were collected and checked under microscope, but no clinical sign, which can explain mortality, was found.

A massive die-off of wild juveniles *Siganus argenteus* and *S. spinus* that simultaneously occurred with a bloom of cyanobacteria at Tumon Bay, Guam was recorded (Nagle and Paul, 1998). The researchers analysed that mortality might be from starvation due to available food sources for juvenile rabbitfish depleting as cyanobacteria bloomed and possible role of toxicity associated with planktonic cyanobacterial blooms. In our study, rabbitfish mortality likely was not derived from starvation because the supplied pellet feed was the major source

of nutrition for rabbitfish and shrimp requirements (Chapter 4). The composition of phytoplankton and the toxicity and chemical ecology associated with phytoplankton blooming was not studied. During phytoplankton blooming, DO became supersaturated and dissolved organic matter (not shown), likely derived from massive die-off phytoplankton, was very high in the water column. It probably was hypothesized a synergistic effect of possible toxicity associated with phytoplankton bloom, DO supersaturation and high organic content causing rabbitfish mortality. However, to assess the actual factors and conditions that induce rabbitfish mortality, additional researches should be conducted.

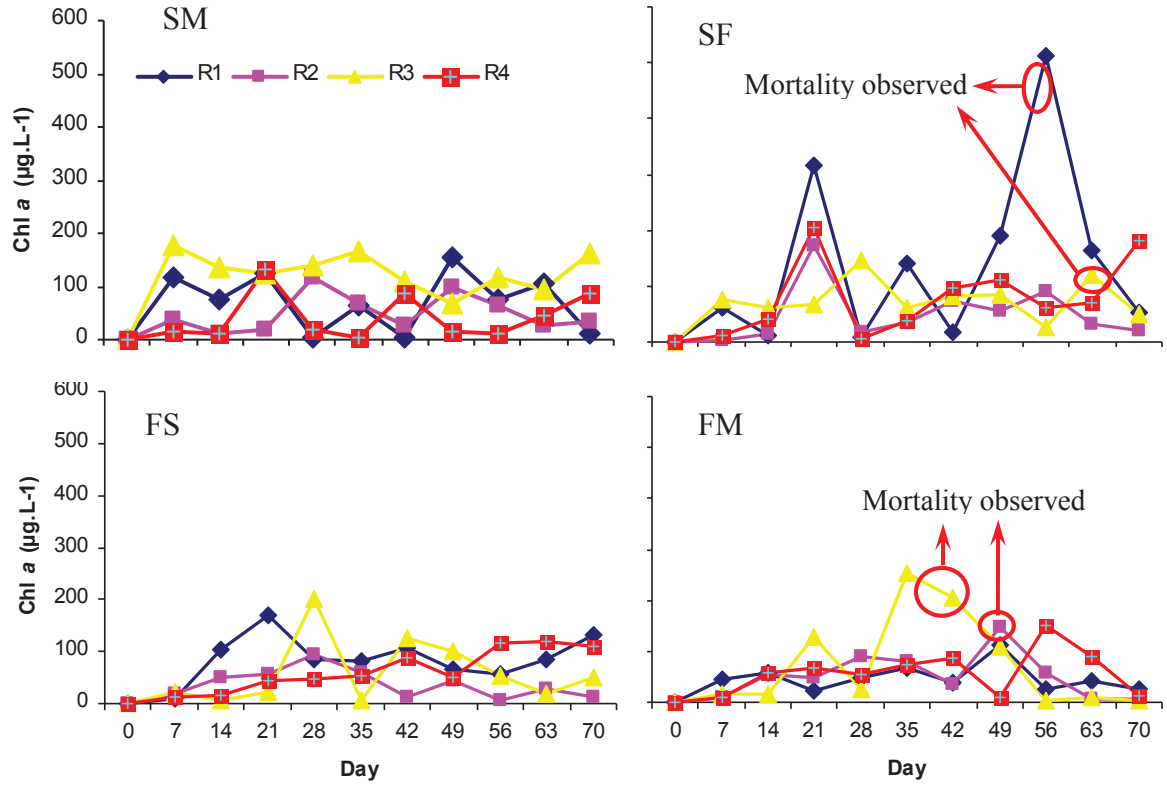


Figure 5.19: Temporal variations of Chl *a* in replicates of each treatment, and the Chl *a* concentrations, at which rabbitfish mortality was observed in the SF and FM treatments; R1, R2, R3, R4: replicate 1, 2, 3 and 4.

The significantly higher biomasses of rabbitfish in the FS and the FM treatments than that in the SF treatment suggested that rabbitfish biomass was well within the carrying capacity of the culture system. Increasing rabbitfish stocking biomasses in the SF, the FS and the FM treatments remarkably increased the rabbitfish biomasses at harvesting. This result suggested that polyculture with higher rabbitfish stocking biomass effectively produced higher production than that of higher shrimp stocking biomass. However, further studies

investigating the optimal ratio of shrimp to rabbitfish and studying the implications under pond conditions are needed.

5.4.2 Environmental characteristics

Although total harvested biomasses were significantly higher in the FS and the FM treatments than those in the SM and SF, mean values of TSS were lower in the FS and the FM than those in the SM and the SF treatments. The mean values and temporal variations of TSS (Table 5.4, Fig. 5.4) indicated that TSS did not follow increasing of cultured animal biomass in the tanks. TSS was weakly correlated with Chl *a* concentration ($R^2 = 0.38$, $P = 0.01$). In general, TSS was in the ranges for aquaculture ponds (10 – 50 $mg.L^{-1}$, Boyd, 1998).

There was a highly positive correlation between turbidity and TSS for all treatments ($R^2 = 0.89$, $P < 0.0001$). In the culture system, suspended clay particles and phytoplankton were the major sources of turbidity (Boyd, 1998). Management activities, such as feeding and aeration also tended to increase turbidity (Ritvo et al., 1997). However, turbidity seemed to be not correlated with phytoplankton (as Chl *a*) ($R^2 = 0.16$, $p = 0.007$) for all treatments. Temporal variations of turbidity were different among treatments (Fig. 5.3), likely due to differences in shrimp and rabbitfish sizes and biomasses among treatments during the experiment. In the FM treatments, turbidity gradually increased over the course of the experiment because elevated rabbitfish size and biomass that increased suspended sediment particles from raising movement and feeding activity of the larger rabbitfish (Ritvo et al., 1997). Rapid increase of turbidity in first three weeks in the SM, SF and FS treatments was probably derived from shrimp activity. Shrimp have behaviour that spend most of their time on the bottom and burrow into the sediment (Dall et al., 1990), which strongly increased suspension of sediment particles as stocking at large shrimp size (Ritvo et al., 1997). Over the course of the experiment, as shrimp mortality occurred and density reduced, turbidity might be negatively affected by decrease of suspended and resuspended sediment particles caused by reduced bioturbation.

As high nutrient source from the daily pellet feed applied and accumulated in the culture tanks, which, in turn, caused an increase of eutrophication in the culture ecosystem (Thomas et al., 2010). Consequently, Chl *a* rapidly increased and remained stable during the experiment, especially in the SM and SF treatments. Large growth of phytoplankton stably maintained ammonia at low concentrations during the experiment by absorbing for its

nutritional requirement. Semi-intensive aquaculture ponds often develop dense phytoplankton populations (Chl *a* >250 μgL^{-1}) in response to a high rate of nutrient input (Hargreaves, 1998). In our study, mean concentrations of Chl *a* were higher than concentration that was regularly observed in shrimp ponds in New Caledonia (50 $\mu g\ L^{-1}$, ranged from 4.4 to 84.6 $\mu g.L^{-1}$) (Thomas et al., 2010). At the time of phytoplankton blooming, Chl *a* concentration increased up to higher than 150 to 512 μgL^{-1}.

Phosphorus released by decomposition of organic matter in pond bottoms is rapidly absorbed by sediment and little of it enters the water (Boyd et al., 2002). The temporal variation of SRP, generally, had the trend that gradually increased during the experiment, indicating the increasing decomposition of organic matter in tank sediment. The high development of phytoplankton could absorb large amount of SRP (Boyd, 1998), and maintained it at low concentration in all treatments. High N/P ratios (> Redfield ratio, N/P = 16) (Table 5.4) suggested that P seemed to be limited for phytoplankton growth in the culture tanks. However, phytoplankton community have a high very plasticity to adapt their growth to change of their nutrient environment, and have the ability to decrease their cellular phosphorus content when phosphorus in their environment is scarce (Van Mooy et al., 2009). A study conducted at Saint-Vincent station reported that adjusting the N/P ratio using fertilizer from 5 to 45 in the experimental outdoor mesocosms did not result in major change in Chl *a* concentration and phytoplankton composition. Mean maximum values were 22.4, 27.4 and 36.7 $\mu g\ L^{-1}$ for N/P: 45, 16 and 5, respectively (Ifremer LEAD, unpublished data). Geider and Roche (2002) reviewed that the N/P ratio of algae is very plastic in nutrient-limited cells, ranging from < 5 mol N: mol P (P in excess of nitrate or ammonium) to > 100 mol N: mol P (N in excess of P). Geider and Roche (2002) reported the critical N/P that marks the transition between N- and P- limitation of phytoplankton growth lies in the range 20 – 50 mol N: mol P. In our study, the N/P ratios were so higher than this range, especially in the SM and the SF treatments, that to be assumed P limitation for phytoplankton growth. Phosphorus effluxed from sediment to the water column may be adsorbed by mineral particulate that reduced the P availability for phytoplankton.

During the experiment, the tanks were supplied daily with equal quantity of the pellet feed to feed shrimp and/or rabbitfish. In the SM and the SF treatment, the decrease of shrimp biomasses over the experiment that increased N loaded from the input (the pellet feed and stocking) to the tank ecosystem, resulted in concentrations of TDN, TAN, DON, and final

TAN in pore water of sediment were higher than those in the FS and the FM treatments (Table 5.4 & 5.5). In our study, DON accounted for a large proportion of TDN (79.5 – 86.9 %) in all treatments because it leached from the pellet feed and faeces, seemed to be less effectively utilized by the microbial community and was likely to accumulate in tank water (Burford and Williams, 2001). Inversely, urea concentrations were low and accounted small proportions of DON (9.8 – 17.7% in this study), because urea leached from animal faeces was rapidly utilized by the microbial community in tank water (Burford and Williams, 2001).

The concentration and temporal variation of NOx-N indicated the nitrification process that converted ammonia to nitrite-nitrate by bacterial action was stronger in the SM and the FM treatments than those in the SF and the FS treatments (Boyd, 1998; Lazur, 2007). It also showed that the nitrification increased from the day 21 following the increasing concentration of TAN in both the SM and the FM treatments (Fig. 5.7 & 5.8). This process could reduce TAN concentration, thus decrease the toxicity of ammonia to cultured animals. However, nitrite is also stressful to shrimp at low concentration (Lazur, 2007). Meanwhile, nitrate is generally not toxic and can be used by phytoplankton. Low concentrations of NOx-N in all treatments might be due to low nitrification process and/or nitrate uptake by phytoplankton.

Ammonia in ponds comes from feed and nutrients entering with the water. If feed is uneaten, then more ammonia is present than if it is consumed by shrimp (Lazur, 2007). Higher quantity of released N from the pellet feed and stocking input (Table 5.3) was likely the main cause of TAN concentrations were higher in the SM and the SF treatments than those in the FS and the FM treatments. Phytoplankton can absorb large amounts of ammonium as a food source, and they are a dominant factor controlling the concentration of ammonia nitrogen in the culture system (Boyd, 1998; Lazur, 2007). Low concentration of TAN might be resulted from nitrification that converted to NOx-N and absorption by phytoplankton. Ammonia is the preferred N substrate for phytoplankton, and only after it has been depleted <0.03 $mg.L^{-1}$ (2.1 μM) will significant quantities of nitrate be assimilated (Hargreaves, 1998).

The high TAN production in the sediment was presumably a result of the high waste N loads resulting from feeding, which would be a large source of organic N for decomposition and mineralization on the sediment bottom that released ammonia and recycled to the water (Boyd et al., 2002). In the SM and SF treatments, the decrease of shrimp biomasses over the experiment suggested that a large portion of the pellet feed supplied was uneaten and accumulated in the culture tanks. Much of the resultant uneaten feed most likely settled on

the sediment floor where it was subject to remineralization processes (Buford and Longmore, 2001). As a result, the pore water TAN highly increased at the end of the experiment.

Organic matter from uneaten feed settled on the tank sediment might lead to the development of anoxic and reducing conditions in the sediment and the production of toxic gases (e.g. ammonia, methane and hydrogen sulphide) (Wu, 1995). In the SM and SF treatments, the accumulated black sludge on the bottom sediment might bring about reducing conditions and negative redox values (Muralidhar et al., 2014). However, Eh values were not significantly different among treatments. This was likely due to the method of redox measurement from 1 cm core sediment sample, while black sludge accumulated on the sediment surface. High final pore water TAN and black sludge accumulation indicated the degradation of the sediments in the SM and the SF treatments was higher than in the FS and the FM treatments.

Sediment organic carbon were compatible with sediment loss on ignition in all treatments, but were lower than optimal range (1.5 - 2.5%) for pond production (Boyd, 1998). The high C/N ratios in sediments indicated that the organic matter was more highly degraded in comparison with that of settling particles (Kassila and Hussenot, 2006; Fernandes et al., 2009).

5.4.3 Ecological functioning

Mean values and temporal variations of sedimentation rate (SdR) (Table 5.6, Fig 5.13) indicated that sedimentation process seemed to be independent of increasing biomasses of shrimp and rabbitfish in the experimental treatments. SdR was positively correlated to TSS in the SM treatment (R^2 = 0.93, P = 0.02) and in the SF treatment (R^2 = 0.76, P = 0.025). Meanwhile, there was a weak correlation between SdR and TSS in the FS treatment (R^2 = 0.56 P = 0.085), and no correlation in the FM treatment (R^2 = 0.27, P = 0.75). On the other hand, in the FM treatment, SdR showed a high variability between replicates on the day 49 and the day 63 of the experiment (Fig. 5.13). This was due to the low values of SdR in a replicate where high rabbitfish mortality occurred. Although rabbbitfish harvested size, density and biomass in the FS and the FM treatments were greater than those in the SM and the SF treatments, there was no significant difference in SdR between all treatments. The results indicated that shrimp might strongly affect sediment disturbance compared with rabbitfish. Shrimp are characterized as benthic feeding animals and burrow in the soil (Dall et al., 1990), causing strong resuspension of sediment particles that increased material source of

sedimentation (Avnimelech et al., 1999). The potential of brackish water finfish (*Etroplus suratensis*, pearlspot, *Mugil cephalus,* mullet, *Chanos chanos*, milk fish, and *Oreochromis mossambicus*, tilapia) to improve bottom soil was assessed (Joyni et al., 2011). The researchers reported that tilapia digs site-specific holes and mixes the various bottom soil layers only during nest building activities, and pearlspot is very efficient by stirring a continuous resuspension of bottom material. Therefore, finfish may be a natural control of bottom soil characteristics, may improve shrimp production and add a valuable product to shrimp production. Further studies investigating bioturbation caused by rabbitfish and the effect of bioturbation on bottom sediment properties and shrimp production as co-cultured are needed.

The increases of GPP and P/R ratio in the water column were likely derived from strong photosynthesis processes of phytoplankton, indicating the autotrophic water at the early of the experiment. Rapid growth of phytoplankton in the SM treatment (as Chl *a*, Fig. 5.5) after stocking was probably a major reason of the water GPP and, thus, the entire GPP was higher than those in the other treatments (Fig. 5.15). Sediment GPP did not increase while sediment R significantly increased, leading to reducing of P/R and indicating heterotrophic sediments at the early of the experiment, especially in the SM, the SF and the FS treatments. As high stocking biomass, high quantity of feed offered to the culture tanks, bringing about high loaded organic matter from feed input and excretion accumulating on the sediment bottoms and causing heterotrophic sediments at the early and also at the late of the experiment. Sediments were enriched with nutrients and organic matter, and became a favorable site for microbial development due to the availability of organic matter (Avnimelech and Ritvo, 2003). The decomposition processes by microbial community increased continuously, leading to increasing sediment oxygen demand (Fig. 5.16). Heterotrophically dominated sediments had the potential to degrade water quality, whereas photoautotrophy in the sediments improved this impact (Torres-Beristain, 2005). Microphytobenthos can also ameliorate water quality by stabilizing sediments and altering sediment-water nutrient fluxes.

The similarity of water and sediment GPP between the early and the late of the experiment in all treatments indicated the maintenance of photosynthetic processes by phytoplankton and microphytobenthos (MPB). However, the yield of GPP produced by MPB was low at both the early (2.5 $mgO_2.h^{-1}$/mg Chl *a*) and at the end (0.6 $mgO_2.h^{-1}$/mg Chl *a*), indicating the production by MPB could be limited by insufficient conditions, for example lack of light on the sediment bottom. The yield of GPP in the water column was lower at the late (17.4

$mgO_2.h^{-1}$/mg Chl *a*) than at the early (31.7 $mgO_2.h^{-1}$/mg Chl *a*) indicated the reduction of photosynthetic intensity of phytoplankton caused by a certain factor. For instance, the light intensity reduced by increasing turbidity, or the limitation of a nutrient when requirement for nutrients of phytoplankton increased, such as limited P as expressed by high N/P ratio.

The increases of water and sediment R at the late led to reducing the P/R ratio in both the water column and sediment. However, the P/R ratio in water column was still higher than 1.1 (Fig. 5.17), indicating the excess oxygen produced by primary productivity during light hours could support the respiration of organisms and decomposition in the system. Meanwhile, the P/R ratio in sediment was below 1.0 at both the early and the late of the experiment (except in the FM treatment at the early) (Fig. 5.17) showed the intensive organic matter decomposition and sediment oxygen demand exceeded the oxygen renewal rate from primary productivity. This might lead to the development of anoxic conditions in the sediments and at the sediment–water interface (Avnimelech and Ritvo, 2003). In this case, a series of anaerobic processes took place and generated a large amount of potentially toxic materials, such as organic acids, reduced organic sulfur compounds, reduced manganese and sulfides (Avnimelech and Ritvo, 2003). To maintain adequate conditions for production, our results suggested that extra oxygen sources, such as aeration, need to be supplied to the culture system, especially to the sediment-water interface layer.

5.4.4 Nutrient sources for reared shrimp and rabbitfish

The stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of POM had different trends of variations among treatments throughout the experiment. Additionally, the initial $\delta^{13}C$ and $\delta^{15}N$ values of POM were similar with the values of the pellet feed, but the isotope fractionation of POM to the pellet feed increased at middle and late of the experiment. These results suggested that the pellet feed did not largely influence stable isotope signatures of POM over the experiment. Stable isotope ratios of primary producers can be a function of chlorophyll *a*, carbon dioxide concentrations, light intensity, dissolved organic and inorganic C and N, and bacterial carbon sources (Jardine et al., 2003). In the culture tanks, water well mixed under continuous aeration, phytoplankton were surrounded by an abundance of available CO_2 and N diffused from the air, which was favour for photosynthetic process of phytoplankton rather than CO_2 and N released from the pellet feed. Besides, the source of N and CO_2 generated form the pellet feed might have insufficiently provided for phytoplankton growth demand, which well

developed over the course of the experiment. Moreover, organic matter loading from the pellet feed partly accumulated on the sediment, as expressed by high SdR and deposit loss on ignition and another part was decomposed in the water column by biochemical processes. Organic matter loading from the pellet feed was likely retained in the water column with small amount that had not significantly affected stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures of POM.

The change of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures of SOM throughout the experiment (Table 5.7) suggested that the pellet feed had largely influenced stable isotope values of SOM. It was attributed that nutrients and organic matter loading from the pellet feed accumulated on the sediment were the main materials for photosynthetic process of MPB after being mineralized and decomposed by microbial communities. MPB well developed (as Chl *a*, Table 5.5 & Fig. 5.12) during the experiment. In contrast with phytoplankton, MPB were CO_2 and N (diffused from the air) limited (Jardine et al., 2003), and therefore preferentially took up CO_2 and N released through mineralizing and decomposing processes. Besides, organic matter loading from the pellet feed and uneaten feed continuously accumulated on the sediment. Thus, stable isotope signatures of SOM were largely affected by the pellet feed, as their $\delta^{13}C$ and $\delta^{15}N$ values were closer to the pellet feed stable isotope signatures at the end than at the beginning of the experiment.

Although the shrimp $\delta^{13}C$ values significantly reduced and the $\delta^{13}C$ fractionations of shrimp to the pellet feed decreased over the experiment, the pellet feed had a sufficiently different $\delta^{13}C$ value from shrimp to suggest that it was not a major source of carbon for shrimp. Assuming a 1 ‰ increase in the $\delta^{13}C$ value of consumer to the diet (De Niro & Epstein, 1978), the results indicated that POM and SOM also were not main sources of carbon for shrimp. Unlike the previous experiment (Chapter 4), which resulted that SOM was a major source of carbon and the pellet feed was a secondary one for shrimp growth, in this study neither the pellet feed nor SOM appeared as a major carbon source for shrimp. This was likely due to the differences in natural biota composition (not completely studied), which might be another major source of carbon for shrimp as stocked at high biomass. Persson and Hansson (1999) determined that three months were required for the isotopic composition of new prey items to be detectable in consumer tissue. The culture period of this study, 10 weeks, was quite enough for assimilation process stable isotopes from prey to consumer tissues. Thus, the carbon source for shrimp might be a combination of the pellet feed, SOM,

POM and another potential diet in natural biota, such as zooplankton and macrobenthos (Burford et al., 2004). Further researches need to be conducted on combination stable isotope analysis with traditional methods of diet determination (Persson and Hansson, 1999) to confirm potential sources of carbon for shrimp in the culture system.

The final $\delta^{13}C$ fractionation of rabbitfish to SOM and POM suggested that SOM and POM appeared to be main sources of carbon for rabbitfish nutrition. The pellet feed seemed not to be a major carbon source for rabbitfish. This result was agreed with those from the previous study (Chapter 4). However, stable isotopes of SOM varied following the pellet feed isotopic values throughout the experiment. Thus, the pellet feed might be an indirect source of carbon for rabbitfish requirement. It was not possible to calculate the relative contribution of potential food sources to rabbitfish growth due to high $\delta^{13}C$ fractionation presented by rabbitfish.

The ^{15}N is excreted in lower amounts than the more abundant ^{14}N, so that consumers become enriched in $\delta^{15}N$ compared with their food (Peterson and Fry, 1987). Throughout the experiment, although the $\delta^{15}N$ fractionation of shrimp to the pellet feed, POM and SOM increased, the values were in the range of recognized increase in $\delta^{15}N$ of consumer to the trophic level, 3 – 5 ‰ (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Minagawa and Wada, 1984). The results suggested that the pellet feed seemed to be a main nitrogen source for shrimp nutrition, and SOM and POM have provided at least some of the nitrogen requirements of shrimp. This result was similar with the previous experiment (Chapter 4), which estimated pellet feed contribute 70 – 85% of nitrogen to the shrimp growth and remainder from natural biota, 15 – 30 %. The other studied also indicated that commercial feed supplied most of the nitrogen (70 – 80%) to shrimp, and 20 - 30% of nitrogen requirements for shrimp deriving from pond biota (Epp et al., 2002; Abreu et al., 2007). In this study, it was not possible to calculate the nitrogen contribution of the pellet feed and SOM and/or POM to shrimp growth due to the high $\delta^{15}N$ fractionation presented by shrimp.

The final $\delta^{15}N$ fractionation of rabbitfish to the pellet feed, POM and SOM suggested that the pellet feed was probably a main source of nitrogen and SOM and POM were secondary sources for rabbitfish nutrition. This result was consistent with the previous study as described in chapter 4. However, it was impossible to calculate the relative contribution of potential food sources to rabbitfish growth due to $\delta^{15}N$ fractionation presented by rabbitfish.

5.5 Highlights and limits of this experiment

5.5.1 Highlights

- The results of this study show that blue shrimp *Litopenaeus stylirostris* (14.0 g) stocked at 6 and 11 shrimp.m^{-2} (80 and 155 g.m^{-2}) coupled with goldlined rabbitfish *Siganus lineatus* (19.0 g) stocked at 8 and 4 fish.m^{-2} (155and 80 g.m^{-2}), respectively do not mutually affect their growth and survival in polyculture system.

- Polyculture of shrimp-rabbifish and rabbitfish-shrimp produce significantly higher production than shrimp monoculture at the same stocking biomass (235 g.m^{-2}) in culture period of 70 days.

- Production of *S. lineatus* monoculture (stocked at 235 g.m^{-2}) is similar with that of polyculture combined *S lineatus* and *L. stylirostris* (stocked at 155 and 80 g.m^{-2}, respectively), and both these productions are significantly higher than those of polyculture of *L. stylirostris* and S. *lineatus* (stocked at 155 and 80 g.m^{-2}, respectively) and of *L. stylirostris* monoculture (stocked at 237 g.m^{-2}) in culture period of 70 days.

- Increase shrimp stocking biomass from 80 to 237 g.m^{-2} (6 – 17 shrimp.m^{-2}), mortality increases and correspondingly biomass reduces. Shrimp stocking biomasses from 80 to 237 g.m^{-2} are beyond carrying capacity of the culture system.

- Increase rabbitfish stocking biomass from 80 to 235 g.m^{-2} would increase harvested biomass. Rabbitfish harvested biomasses, 149 – 419 g.m^{-2}, are still within the carrying capacity of the culture system.

- The environmental quality is better in rabbitfish-shrimp polyculture and rabbitfish monocuture than those in shrimp-rabbitfish polyculture and shrimp monoculture during the culture period. Increase shrimp stocking biomass (density), the environmental deterioration increase.

- *L. stylirostris* could be more sensitive to variations of environmental parameters than *S. lineatus*, leading shrimp stressed and high mortality occurrence, whereas rabbitfish get high survival in the same culture system.

- The factors associated with hypereutrophication, such as phytoplankton bloom, oxygen supersaturation and high dissolved organic matter, could be possible factors that can cause

rabbitifish mortality.

- Low bioturbation caused by rabbitifsh in rabbitfish-shrimp polyculture and rabbitfish monoculture is compared with those in shrimp-rabbitfish polyculture and shrimp monoculture.

- There are no strong differences, except bioturbation intensity, in ecological processes among shrimp and rabbitfish monoculture and polyculture of shrimp and rabbitfish. The water column is autotrophic from the early to the late of the culture period, while the sediment and the entire ecosystem become to be heterotrophic at the late of culture period in all culture systems.

- The pellet feed used is not a major source of carbon for both shrimp and rabbitfish. Natural biota, sediment organic matter (SOM) and particulate organic matter (POM), is a main source of carbon for rabbitfish, while it is not a primary carbon source for shrimp.

- The pellet feed used is a major nitrogen source for both shrimp and rabbitfish, and natural biota also contributes some of nitrogen requirements for both shrimp and rabbitfish.

5.5.2 Limits

- The main cause that induces shrimp and rabbitfish mortality during the culture period is not determined, and the resistance of cultured animal to mortality causative agents, if any, is also not assessed in this study.

- Exception of SOM and POM, other potential food sources in natural biota (periphyton, zooplankton and macrobenthos) are not analyzed if to be nutrient sources for cultured animal.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Goldlined rabbitfish *Siganus lineatus* culture was carried out in a closed system (Chapter 3), and the feasibility of blue shrimp *Litopenaeus stylirostris* and *S. lineatus* polyculture was investigated in a mesocosm system (Chapters 4 & 5). It was shown that *S. lineatus* would be a highly suitable species for commercial culture in brackish earthen ponds in the forms of monoculture or polyculture with *L. stylirostris*. Adding *S. lineatus* to *L. stylirostris* culture system did not affect shrimp growth performance. Polyculture *L. stylirostris* and *S. lineatus* could produce valuable secondary crop and increase total combined production without further degradation of the environment. At high stocking biomass, rabbitfish monoculture and rabbitfish-shrimp polyculture could yield higher benefits in production and the environment than shrimp monoculture and shrimp-rabbitfish polyculture.

6.1 Goldlined rabbitfish - a potential species for commercial culture in earthen ponds

Siganus lineatus, cultured in a closed system, had a low growth rate at low water temperature, average 19.7 – 23.0 ^{o}C (Chapter 3). However, the growth remarkably increased as water temperature increased near to its optimal temperature, 27 ^{o}C (Saoud et al., 2008b) (Chapters 4 & 5). In addition, *S. lineatus* could survive at low water temperature around 16 ^{o}C (Chapter 3). Naturally, rabbitfishes are eurythermal and tolerate a wide range of water temperature variation (Lam, 1974; Duray, 1998), but the scientific literature indicated a stop of feeding at 14 and 36 ^{o}C (Saoud et al., 2008b).

In New Caledonia, the water temperature in the earthen ponds of the shrimp farming varies during the year with a cool season (May to October) with average 19 ^{o}C, and a warmer season (November to April) with average 29 ^{o}C. The water temperature is suboptimal for production of *L. stylirostris* several months of the year, and imposes a major limitation on growth of shrimp and the lowest water temperatures cause considerable stress to farmed shrimp, as shown in chapters 4 and 5. It should be considered that *S. lineatus* is an importantly alternative species for stocking in the earthen ponds in the cold season. In the earthen ponds, deeper than the experimental tanks (0.5 m), the heat would be well stored and water temperature could resist changing during brief cold-snaps, thus growth of rabbitfish might be less affected by low temperature as cultured in the tanks.

Rabbitfishes are schooling fishes (Lam, 1974), and have tolerance of overcrowding (Ben-Tuvia et al., 1973). In culture system, *S. lineatus* showed little competitive behaviour among individuals reared at high densities (Chapters 3 & 5). *S. lineatus* (5.7g) stocked at high

density (21 fish.m^{-2}, Chapter 3), or stocked at high biomass (235 g.m^{-2}/12 fish.m^{-2}, 19.0 g.fish^{-1}, Chapter 5), its growth and survival was not negatively affected by density. In aquaculture, an increase in number of fish per culture unit is desirable since high stocking densities generally reduce production costs per unit of fish (Huguenin, 1997). The optimal stocking density depends on cultural conditions (ponds, instruments and climates), cultural patterns (monoculture or polyculture and intensity), initial and final sizes (marketable size), rearing period and experiences and techniques (Wang et al., 1998). Generally, until carrying capacity, defined as the maximum biomass of a farmed species (Stigebrandt, 2011), is reached, yield increases with increasing stocking rate while final size and survival rate decrease as stocking density increases (Wang et al., 1998). A rabbitfish biomass of 390 g.m^{-2} in polyculture with blue shrimp or 420 g.m^{-2} in monoculture appeared to be within carrying capacity of a mesocosm culture system (Chapter 5). This suggested *S. lineatus* can be stocked at a high density in a semi-intensive or intensive culture system, and correspondingly produce a high yield. However, as density (biomass) increases, so does the quantity of feed offered, resulting in potential environmental problems and oxygen concentration depletion. In a closed system, high stocking density (24 fish.m^{-2}, 4.8 g.fish^{-1}) might lead to rapid water oxygen depletion in a short-term and cause rabbitfish mortality (DO < 2 mg.L^{-1}) (Chapter 3). A stocking density at 21 fish.m^{-2} (5.7 g. fish^{-1}) in a closed system (Chapter 3) or a stocking biomass at 235 g.m^{-2} in a low water exchange (10 %.d^{-1}) mesocosm (Chapter 5) might lead to hypereutrophied environment at certain times of culture period as accumulated nutrients increasing. Rabbitfish mortality was observed simultaneously during these events in the culture system (Chapters 3 & 5). Factor(s) associated with this mortality was unknown. This is an important subject for further research and a useful note for managing rabbitfish farming.

Managing eutrophication and so oxygen and nutrient concentrations are important daily tasks for farmers in aquaculture to support maximum growth of reared animals, and thus harvest the highest production. In pond culture system, these factors could be controlled, such as by properly adjusting water level, supplying aeration, using inflow of new water, and strategically managing of feeding (Boyd and Tucker, 1998; Martin et al., 1998; Lemonnier et al., 2006; Lazur, 2007).

High stocking density in a closed system easily lead to degradation of the environmental quality by nutrients loading from food input and rabbitfish excreta and accumulated organic matter (Chapter 3). Exchange water may maintain adequate environmental quality in culture

system, but cause pollution for receiving water when discharged nutrients and organic matter exceed capacity of assimilation of the water body. So, stocking density of rabbitfish for commercial culture should be considered depending on rearing conditions, fish size, experience and technique. Our results indicated that density from 7 – 14 fish.m^{-2} (5.7 g.fish^{-1}) seem to be suitable for stocking in earthen ponds for production.

6.2 Polyculture of *L. stylirostris* with *S. lineatus* – possibilities

6.2.1 Production benefit

Polyculture *L. stylirostris* with *S. lineatus* showed that the presence of rabbitfish had no negative effect on shrimp growth performance. Polyculture produced significantly higher combined production than that in shrimp monoculture (Chapters 4 & 5). As rabbitfish (25.5 g) added to the shrimp culture system at 1.2 and 2.4 fish.m^{-2}, total production increased by 47% and 106%, respectively compared with shrimp monoculture (Chapter 4). Polyculture of shrimp with fishes was also widely practiced with various results of production. Akiyama and Angawati (1999) performed a polyculture of *Penaeus monodon* and red tilapia, and reported shrimp production was improved by 10%. García-Pérez et al. (2000) reported that prawn-tilapia polyculture increased economic return by 21% when stocking at 7 prawns/ 1 tilapia in earthen ponds. Yuan et al. (2010) found an addition of red tilapia at 0.4 fish.m^{-2} and 13.7 g size improved shrimp biomass at harvest by 6%, but any increase in tilapia density or size could significantly reduced the shrimp production. Inversely, carnivorous fishes, such as sea perch (*Lateolabrax japonicus*), black porgy (*Sparus macrocephalus*), genuine porgy (*Pagrosomus major*) (Tian et al., 2001), seabass (*Lates calcarifer*) and snapper (*Lutjanus argentimaculatus*) (Tendencia et al., 2006a) can negatively affect the shrimp survival by eating. Benthic-feeding fishes, such as striped mullet (*Mugil cephalus*), redeye mullet (*Liza haematocheila*) would impact the shrimp growth as polycultured with shrimp at high density (Tian et al., 2001). Another our result also showed that polyculture *L. stylirostris* with mullet (*Mugil* spp.) obtained lower shrimp survival and yield compared with shrimp monoculture (see appendix 2). Tilapia represents a suitable candidate for polyculture with shrimp due to high tolerance to adverse conditions, commercial demand and feeding habits (Tian et al., 2001; Martínez-Porchas et al., 2010). However, free-swimming tilapias compete with shrimp for food and suppress the shrimp growth (Wang et al., 1998; García-Pérez et al., 2000; Yuan

et al., 2010). Our results suggested that *S. lineatus* could consume uneaten feed offered for shrimp (Chapter 4), which prevent further deterioration of the environment, and thus increase shrimp survival and production.

S. lineatus added to the *L. stylirostris* culture gained survival rate (SR) of 100% (Chapter 4), and as polycultured with shrimp it obtained SR from 75 to 100% (Chapter 5). In addition, *S. lineatus* had high growth rate, and growth performance was similar at all stocking densities in polyculture with shrimp (Chapters 4 & 5). The results suggested that rabbitfish could get benefit for its growth when stocking with shrimp in a polyculture system at lower or higher density (biomass) compared with shrimp. In polyculture, as *S. lineatus* stocking density (biomass) increased, combined production shrimp and fish significantly increased (Chapters 4 & 5). For example, when rabbitfish added to shrimp tanks increased from 1.2 to 2.4 fish.m^{-2}, combined production increased by 38.5%, from 213 to 295 g.m^{-2} (Chapter 4).

Stocking biomasses of shrimp at 237 g.m^{-2} in monoculture, and at 81 and 156 g.m^{-2} in polyculture with *S. lineatus* appeared to be over carrying capacity of the experimental mesocosm system, expressed by shrimp biomasses reduced over the experiment. The decrease of shrimp biomass in polyculture was lower than that in shrimp monoculture, might be due to the environment was less degradation by organic wastes (uneaten feed, excretion, organism metabolites) in polyculture compared with what in monoculture. Meanwhile, at the same stocking biomass in monoculture and polyculture with *L. stylirostris*, *S. lineatus* biomass highly increased over the experiment (Chapter 5). This indicated that the rabbitfish biomasses at harvest were probably well within carrying capacity of the experimental mesocosm system. Although, the combined productions in polyculture were significantly higher than that in shrimp monoculture, and significantly increased following increasing stocking biomass of rabbitfish (Chapter 5), these increases were mainly derived from increases of rabbitfish biomasses.

Polyculture of *L. stylirostris* and *S. lineatus* showed a synergistic effect that increase food utilization, provide a profitable secondary crop, and improve total production.

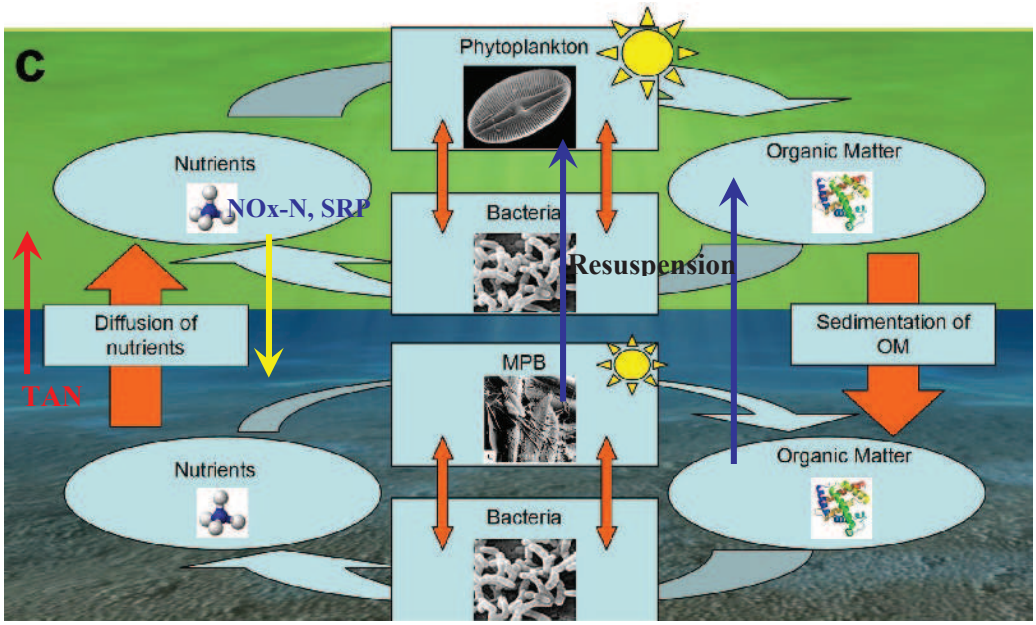
6.2.2 Pond ecology

In polyculture, feed consumption by rabbitfish led to minimizing uneaten feed accumulation in the culture tanks, thus prevented further degradation of the environment. In addition, a

higher retention by rabbitfish of input nutrients (Chapter 5), in turn, positively influenced the water and sediment quality (Bosma and Verdegem, 2011). Our results showed that the addition of *S. lineatus* at 1.2 and 2.4 fish.m^{-2} to the shrimp tanks significantly increased total combined production and at the same time reduced FCR by 31.6% and 47.4%, respectively. The lower FCR in polyculture meant higher efficiency of food utilization that minimized uneaten feed accumulation and waste in the culture system. Lin et al. (1993) estimated 15% of feed used remains uneaten in intensive shrimp aquaculture systems. Organic matter from uneaten feed settled on the tank sediment lead to the development of anoxic and reducing conditions in the sediment and the production of toxic gases (e.g. ammonia, methane and hydrogen sulphide) (Wu, 1995). The results described in Chapter 5 indicated that the environmental deterioration in shrimp monoculture and shrimp-fish polyculture was due to large decreases of shrimp biomasses leading to increases of uneaten feed and nutrients loading (from shrimp stocking, inflow water, senescent plankton, etc.) in the culture system. Conversely, the remarkable increases of rabbitfish biomasses in the fish-shrimp polyculture and rabbitfish monoculture minimized uneaten feed and elevated nutrient retention in harvested rabbitfish biomass, which led to maintenance of the environmental quality. Particularly, the increase of nitrogen retention by rabbitfish would reduce nitrogenous wastes that were further converted into toxic metabolites (ammonia, nitrite) in the culture system.

Intensive shrimp farming rely on high protein feed pellets to produce high growth rate, but a large proportion of the pellets is not assimilated by shrimp (Primavera, 1994), and, thus, excreted as metabolic waste, producing large amounts of gaseous, dissolved and particulate wastes (Lin et al., 1993). An increase in environmental waste production may lead to occurrence of early water eutrophication, which could induce directly or indirectly a stress for shrimp and/or a growth and/or virulence factors of the pathogen that eventually cause shrimp mortality (Lemonnier et al., 2006). In addition, high enriched nutrient and organic matter effluent discharged from shrimp farm may cause environmental pollution in receiving waters (Phillips et al., 1993, Phillips, 1995; Páez-Osuna, 2001 a & b; Lewis et al., 2003, Thomas et al., 2010). Reducing negative environmental impacts from shrimp aquaculture activities is a key issue for ensuring long-term sustainability of the industry (Troell et al., 2003). The feasible methods have been widely applied are polyculture and integrated aquaculture (Casalduero, 2001).

The benefits of polyculture as already determined include the mitigation of environmental impacts and improvement of yield and water quality (Chien and Liao, 1995; Martínez-Porchas et al., 2010; Yuan et al., 2010; Bosma and Verdegem, 2011). Oysters and seaweeds have been shown to remove suspended particles and dissolved nutrients from water in shrimp farming that diminish the pollution associated with shrimp farm effluent (Jones et al., 2001; Martínez-Porchas et al., 2010). Polyculture of penaeid shrimps and tilapias showed an improvement in water quality as fish feed on organic wastes (Akiyama and Angawati, 1999; Cruz et al., 2008), and an increase of nutrient retention in fish biomass (Wang et al., 1998; Yuan et al., 2010). Our study showed that *S. lineatus* is capable to reduce organic wastes and nutrient release by consumption uneaten feed and retention input nutrients in biomass that contribute to maintenance the environmental quality and mitigation the ecological impacts in culture system.



Fgure 6.1: Scheme of exchange between sediment and water column in pond ecosystem (Hochard et al., 2001, 2002).

The organic matter sedimentation in the culture system steadily increases during the culture cycle (Chapters 4 & 5), mainly because the daily feed portion increases in concordance to the growing animal biomass (Torres-Beristain, 2005). Sedimentation rate increased in polyculture of *L. stylirostris* with *S. lineatus* and *L. stylirostris* monoculture as described in chapter 4 might be resulted from increased shrimp and/ or rabbitfish size and increased nutrient input led to higher suspended solids in the water column (Avnimelech et al., 1999). However, sedimentation rate described in chapter 5 varied independently of increased shrimp

and/ or rabbitfish size and increased food input in all experimental treatments. This might be results of decreased shrimp and/or rabbitfish density due to mortality occurrence and/ or alteration of bioturbation process caused by shrimp and/ or rabbitfish. Massuda and Boyd (1994) reported that sedimentation dynamics and recycling of organic mater accumulated in the pond varied greatly along the grow-out period. The cycling of organic matter in the culture system was influenced by sedimentation and resuspension processes (Fig 6.1). Resuspension brings organic matter and nutrients back into the oxygen rich water column where organic matter decomposition occurs much more efficient, yielding less toxic components than in the sediment (Jiménez-Montealegre et al., 2002; Torres-Beristain, 2005; Joyni et al., 2011). Resuspension of microphytoplankton (MPB) attached to sediment particles also contributes significantly to the dominant of primary productivity in water columns compared with sediment as described in chapters 4 &5 (MacIntyre et al., 1996). Resuspension can represent a major mechanism of material and nutrient transfer between sediment and water (Boyd, 1995; Avnimelech et al., 1999). Avnimelech et al. (1999) estimated resuspension of organic matter accounted for 60 – 90% of the total solids flux in fish ponds. In shrimp ponds in New Caledonia, the resuspension of total solids was estimated around 80% (Hochard, personal communication).

Low rates of nutrient fluxes across the sediment-water interface were found in both polyculture *L. stylirostris* with *S. lineatus* and *L. stylirostris* monoculture (Chapter 4). High MPB development (as Chl *a*) in sediments might have effects on the low rates of nutrient fluxes (Sundback et al., 2000). Sediments dominated by MPB have lower rates of ammonium, phosphate, and nitrate + nitrite release to the water column, and in many cases become sinks for nutrients rather than sources (Torres-Beristain, 2005). In our study, sediments represented as sources of total ammonia nitrogen and sinks of nitrite-nitrate nitrogen and soluble reactive phosphorus (Chapter 4, Fig 6.1).

Increased concentrations of organic matter in sediment and large amount of feed input supplied to culture system led to increasing concentration of TAN in pore water (Chapters 4 & 5) (Massuda and Boyd, 1994). Elevated ammonia concentrations in pore water are mostly of concern with respect to the growth and survival of cultured species with benthic feeding or burrowing habits, particularly crustaceans (Hargreaves, 1998). In our study, the ecosystems became heterotrophic near the end of the culture period as illustrated in Fig. 6.2, which expressed by accumulated TAN in pore water, increased sediment respiration and decreased primary production concomitantly cultured animal mortality was observed. The heterotrophy

of ecosystem was estimated by the day 80 of culture cycle (Fig. 6.2) or as daily feed input provided to the tanks was over 6 $g.m^{-2}.d^{-1}$ (Fig. 6.3). After stocking, total GPP and primary productivity/respiration (P/R) ratio (trophic status) highly increased and highest P/R ratio (maximum autotrophy) get by the day 45 of the culture period or as daily feed input was around 4 $g.m^{-2}.d^{-1}$.

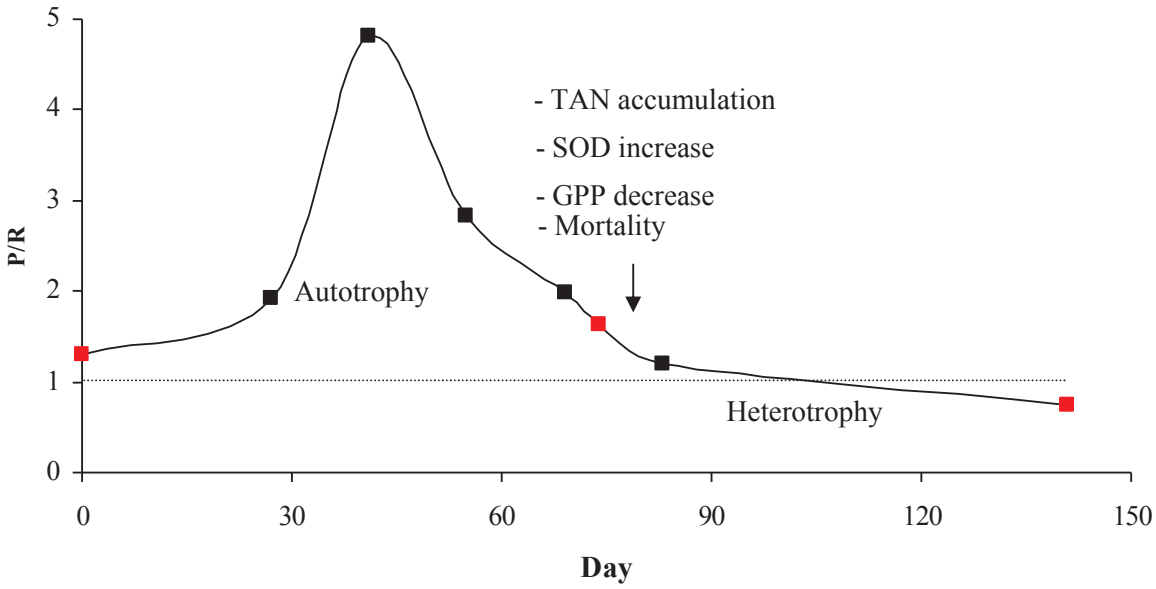


Figure 6.2: Temporal variation of the trophic status of the pond ecosystem. Black point: data from the former experiment (Chapter 4), red point: data from the last experiment (Chapter 5).

Oxygen was consumed in culture systems during aerobic bacteria break down of organic matter (Torres-Beristain, 2005). As increasing organic matter loads accumulated in the culture systems, bacteria decomposition processes increased continuously, leading to increasing respiration throughout the culture period in the water columns as well as in sediments (Chapters 4 & 5). Suplee and Cotner (1996) found an increase in the sediment oxygen demand (SOD) throughout the shrimp grow-out season, and Ellis (1992) reported that sediment oxygen demand consisted of more than 50% of the total shrimp pond oxygen demand at the end of the rearing cycle. In our study, sediment respiration accounted for 19.6 – 36.3 % (Chapter 4), and 30.5 – 74.3 % (Chapter 5) of total respiration in the ecosystem at the end of the culture period. The increase of food input following high cultured animal biomass (Chapter) led to increasing accumulated organic matter on sediment and subsequently the sediment respiration elevated. In addition, the sediment respiration in shrimp monoculture was lower than those in shrimp-rabbitfish polyculture and rabbitfish monoculture (Chapters 4 & 5), indicating higher bioturbation caused by shrimp compared

with rabbitfish. The increased organic matter resuspension resulted in decrease of sediment respiration and increase of water respiration. Dissolved oxygen concentration is one of the critical factors affecting processes and conditions at the sediment–water interface. SOD is an indicator of the intensity of the mineralization process and benthic community metabolism (Avnimelech and Ritvo, 2003). When the ecosystem became heterotrophic, SOD increased while total GPP decreased, leading to depletion of oxygen budget produced by metabolism process. In intensive and semi intensive systems more oxygen is consumed than provided through photosynthesis and surface diffusion, hence aeration is needed (Torres-Beristain, 2005). Our results also suggested that extra oxygen would be needed to satisfy the animal's growth requirements, mainly when P/R ratio reduced after the day 50 of culture cycle.

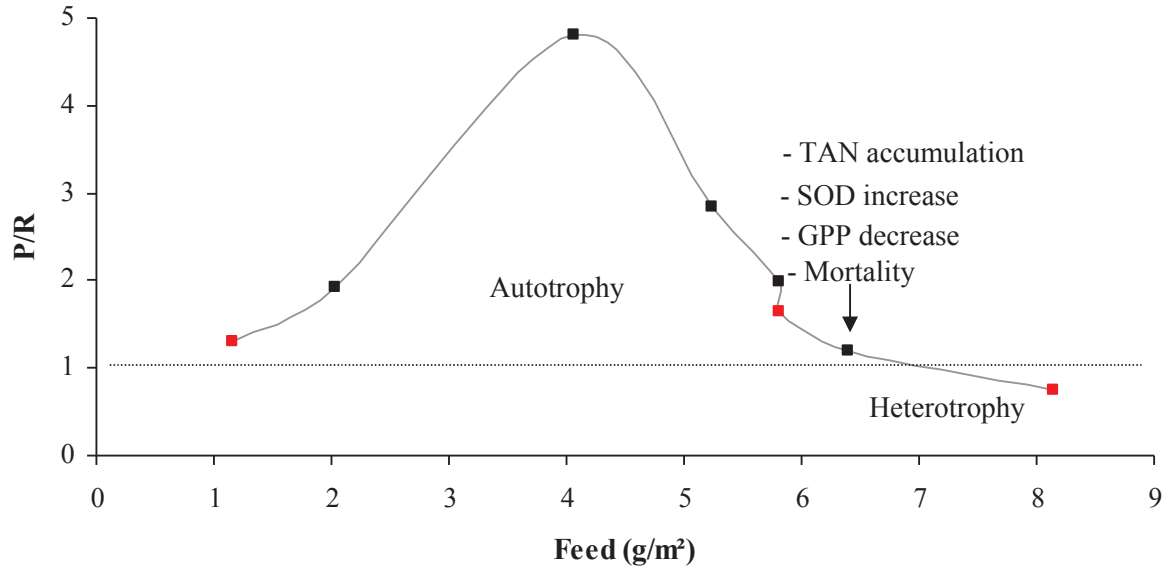


Figure 6.3: Temporal variation of the trophic status of the pond ecosystem following feed quantity supplied. Black point: data from the former experiment (Chapter 4), red point: data from the last experiment (Chapter 5).

At the beginning of the rearing we observed no or few flux of nutrients and few particulate exchanged across the sediment-water interface (Chapter 4). The ecological process in the water column and in sediment seemed to be independent. The highest P/R ratio in sediment get before the day 30 after stocking, while it was later in the water column, over the day 35. Sediment became heterotrophic around the day 70 whereas the water column was autotrophic during the culture period. The P/R ratio of the sediment was an important control on the rates, direction (uptake, efflux) and composition dissolved inorganic N (DIN), dissolved organic N (DON), and N_2 of N fluxes across the sediment–water interface, with an efflux below P/R =

1.5 and an uptake above P/R = 1.5 (Eyre et al., 2010). In our study, NOx-N was uptake when P/R ratio was above 1.5. The net uptake of TAN occurred as P/R ratio above 3, and net efflux of TAN was as P/R ratio below 2.0. The net efflux of DON and SRP were when P/R ratio lower 1.

In general, the nutrient fluxes across the sediment-water interface in our study were in low rates, so sediments did not highly impact on the availability of nutrients in the water column. However, what would happen when the ecosystem become high heterotrophy in the second phase of culture cycle (Chapter 5), as accumulated TAN in pore water increase. It is supposed that the nutrient fluxes, especially TAN, would occur with much higher rates as described by Burford and Longmore (2001), in hypereutrophic shrimp ponds (around 182 mmol.m^{-2}.d^{-1}).

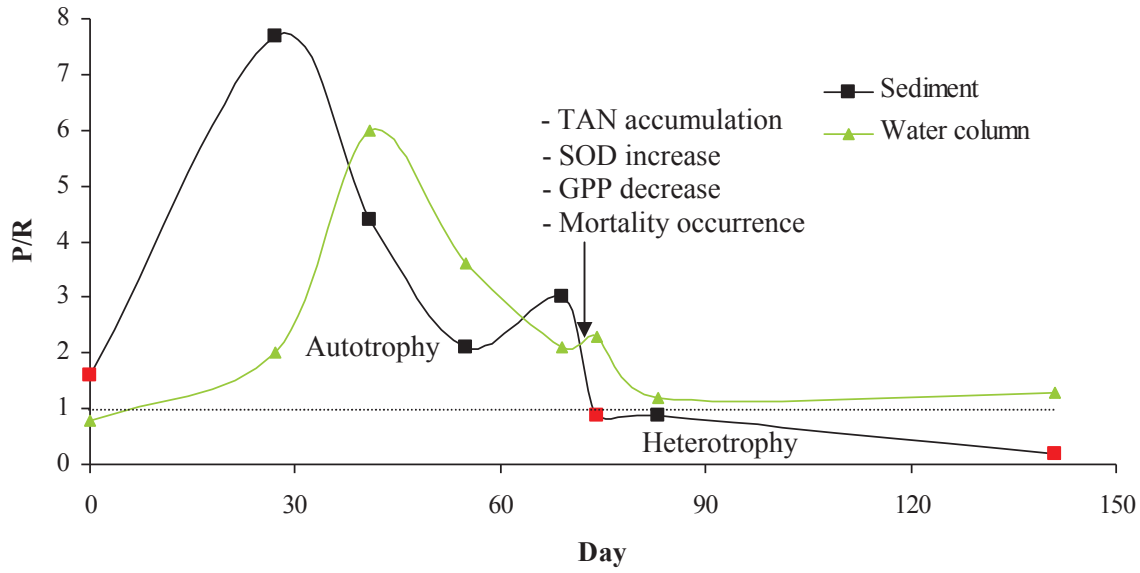


Figure 6.4: Temporal variation of the trophic status in the water column and sediment of pond ecosystem. Black point: data from the former experiment (Chapter 4), red point: data from the last experiment (Chapter 5).

6.2.3 Nutrient sources for reared *L. stylirostris* and *S. lineatus*

L. stylirostris is omnivorous, eating on small marine invertebrates (e.g. worms, small crustaceans, etc.), detritus, plant material and formulated feed (Bondad-Reantaso et al., 2005; Martínez-Córdova and Pena-Messina, 2005). The ability to use a wide range of prey items would allow shrimp to be cultured with a number of other species (Jackson and Ozbay, 2008). Rabbitfishes are primarily herbivores, but may turn to other diets readily (Ben-Tuvia et al., 1973; Lam, 1974) and in commercial culture, they are mainly fed with the artificial

food pellets (Parazo, 1990; Ghanawi, 2011; Xu et al., 2011). Regarding feeding habits, *L. stylirostris* and *S. lineatus* should be 'ideal' species to co-culture together due to their feeding niches do not entirely overlap.

Polyculture can improve the nutrient recovery in ponds as long as the feeding niches of the different species overlap partially and antagonistic encounters between the different species are minimal (Bosma and Verdegem, 2011). Ideally, co-cultured species occupy different niches and possess feeding habits, which are different from or complementary to each other, thus are able to utilize food available in the pond more efficiently than single species (Yuan et al., 2010). In polyculture systems, only a proper combination of ecologically different species at adequate densities will utilize the available resources efficiently, maximize the synergistic interaction and minimize the antagonistic ones (Milstein, 1992).

In our study, the culture tanks were supplied the same pellet feed at the same daily feed quantity throughout the experiments. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis was used to estimate the potential food sources for *L. stylirostris* and *S. lineatus* in two experiments (Chapters 4 & 5). In the former experiment (Chapter 4), the results showed that sediment organic matter (SOM) was a major carbon source for both shrimp and rabbitfish and the pellet feed was a secondary source of carbon for shrimp, whereas particulate organic matter (POM) was another one for rabbitfish. The pellet feed was an important nitrogen source for both species, and POM was a secondary nitrogen source for shrimp, whilst POM and SOM were other sources for rabbitfish. The pellet feed contributed 29 – 40% of carbon, and 70 – 85% of nitrogen to shrimp nutrition, and the remainders were attributed for natural biota (POM and SOM).

However, in the last experiment (Chapter 5), the results showed that neither SOM nor the pellet feed was a major carbon source for shrimp requirement. Meanwhile, the source of carbon for rabbitfish and the source of nitrogen for both species were similar with those in the former. Reason(s) of change in carbon source for shrimp between two experiments was unknown. It was possibly that shrimp might ingest and assimilate other potential diets available in natural biota, such as zooplankton and macrobenthos (Burford et al., 2004). However, additional researches combination stable isotope analysis with traditional methods of diet determination should be conducted.

In any culture system, the pellet feed was an important nitrogen source for both *L. stylirostris* and *S. lineatus*. The same source of nutrient (food) might led to interspecies competition and suppress the growth of shrimp and/ or rabbitfish. However, there was no difference in growth performance of shrimp and rabbitfish between monoculture and polyculture. Beside the pellet feed, POM and SOM were also the nutrient sources for shrimp and rabbitfish, indicating that the animal consumed natural food available in the culture tanks. The interspecies competition might stimulate the cultured animal to increase efficiency of food consumption, thus reduce uneaten feed accumulation. The pellet feed was not a major source of carbon and SOM and POM had high importance to supply the carbon and nitrogen sources for *L. stylirostris* and *S. lineatus*. This suggested that feed management should be geared toward supporting the food web to produce food items that animals prefer, rather than relying on the direct consumption of the pellet feed.

6.3 Conclusions and perspectives

6.3.1 Conclusions

Siganus lineatus is an excellent candidate for commercial culture in brackish earthen ponds, practice in semi-intensive or intensive systems as it can well adapt and grow in a closed system, even at low temperature (ca. 20 ^{o}C) and stocked at high density (21 fish.m^{-2}, 5.7 g.fish^{-1}). High stocking density has no negative effect on *S. lineatus* growth performance and produce high harvested yield; however it can lead to oxygen depletion and hypereutrophy that would cause *S. lineatus* mortality. For production in ponds, *S. lineatus* can be stocked at densities from 7 to 14 fish.m^{-2} and management should be noted on maintenance suitable water temperature and oxygen and preventing hypereutrophy occurrence.

S. lineatus is an important species for co-culture with *L. stylirostris*. The presence of *S. lineatus* at a density is as high as 8 fish.m^{-2} (19 g.fish^{-1}) has no negative effect on shrimp growth and survival. Adding *S. lineatus* to *L. stylirostris* culture system or co-stocking both species at adequate ratios can improve shrimp production and produce a remarkable secondary crop, and thus increase total production. High stocking biomasses of *L. stylirostris* in polyculture with *S. lineatus* has not success in both production and environmental stability, but inversely, high stocking biomass of S. *lineatus* in polyculture with *L. stylirostris* can get benefits in both production and environmental stability. Stocking biomasses of *L. stylirostris*

from 80 to 155 g.m^{-2} in polyculture with *S. lineatus*, and at 237 g.m^{-2} in monoculture are beyond the carrying capacity of the culture system. Harvesting biomasses of *S. lineatus* from 150 to 390 g.m^{-2} in polyculture with *L. stylirostris*, and 420 g.m^{-2} in monoculture are well within the carrying capacity of the culture system. In polyculture with *S. lineatus* and monoculture, *L. stylirostris* could be stressed and mortality occurs by synergistic effects of low temperature, early eutrophication and bad sediment conditions. In polyculture with *L. stylirostris* and monoculture, *S. lineatus* could die by factor(s) associated with hypereutrophied environment.

Polyculture *L. stylirostris* and *S. lineatus* do not largely change the tank ecology and had no or little effect on the environmental quality in comparison with *L. stylirostris* monoculture. However, *S. lineatus* can consume uneaten feed and incorporate a certain portion of input nutrients into biomass, and thus prevent further deterioration of the environment in culture systems. Increase stocking biomass of *L. stylirostris* from 80 to 237 g.m^{-2} in polyculture with *S. lineatus* and in monoculture, the environmental quality tend to decrease, but increase stocking biomass of *S. lineatus* from 80 to 237 g.m^{-2} in polyculture with *L. stylirostris* and in monoculture, stability of the environmental quality is improved.

In both polyculture and monoculture of *L. stylirostris* and *S. lineatus*, sediment oxygen demand increases throughout the experiment, while gross primary productivity and primary productivity/respiration ratio decrease from middle to the end of the culture period, leading to oxygen budget produced by metabolism reduces. In polyculture of *L. stylirostris* with *S. lineatus* oxygen amount self-produced by photosynthesis process in ecosystem is lower than shrimp oxygen demand at the end of the culture period, so it needs an extra oxygen source to fulfill cultured animals' requirement and maintain the production. Small rates of nutrient fluxes across sediment-water interfaces are found in both polyculture of *L. stylirostris* with *S. lineatus* and *L. stylirostris* monoculture. Sediment represents as a source of total ammonia nitrogen and a sink of nitrite-nitrate nitrogen and soluble reactive phosphorus in both systems. During the culture period, gross natural production produced through photosynthetic process in entire ecosystem is higher than daily external organic carbon source from the pellet feed supplied in both polyculture of *L. stylirostris* with *S. lineatus* and *L. stylirostris* monoculture. This source would partly provide nutrients for cultured animals and remainder lose in the environment.

The pellet feed used is a main nitrogen source for *L. stylirostris* and *S. lineatus*, but it is not a major source of carbon for both these species. *L. stylirostris* and *S. lineatus* can use natural biota (sediment organic matter and particulate organic matter) as a main source of carbon and a secondary source of nitrogen for their requirements.

6.3.2 Perspectives

S. lineatus can be commercial cultured in earthen ponds, stocked at relatively high density (e.g. 7 – 14 fish.m^{-2}) and fed with formulated feed. Particularly, it can be stocked in the cold season when farms stop shrimp stocking to avoid shrimp disease problems associated with low temperature in New Caledonia.

Factor(s) associated with hypereutrophy that possibly cause *S. lineatus* mortality should be an interesting area for next study. In addition, tolerance of *S. lineatus* to principal environmental parameters (e.g. dissolved oxygen, toxic gasses, etc.) in culture system should be investigated, and then possible total biomass per unit volume calculation could be based on *S. lineatus* requirements and the metabolic parameters.

Polyculture of *L. stylirostris* with *S. lineatus* should be practiced in earthen ponds with *L. stylirostris* or *S. lineatus* as a main species or *vice versa* to confirm the present results. The capacity of *S. lineatus* to reduce shrimp mortality caused by disease should be prioritized for next researches.

L. stylirostris and *S. lineatus* can use natural biota available in culture system as nutrient sources for their requirements. An important area for further study is using artificial substrates to promote preferable biotic community (phytoplankton, zooplankton and periphyton) development, which could provide an extra food source and favor environment for both *L. stylirostris* and *S. lineatus*. Development natural food source can reduce pellet feed providing to the culture system and the loss of nutrients, leading to prevent environmental degradation caused by uneaten and accumulated nutrients, increase production and profitability.

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APPENDIX 1

Feasibility of polyculture of blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* in a mesocosm system

Luong, C.T., Hochard, S., Royer, F., Lemonnier, H., Letourneur, Y., 2014. Feasibility of polyculture of blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* in a mesocosm system. Aquaculture 433, 340 – 347.

APPENDIX 2

Feasibility of polyculture blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* or mullet *Mugil* spp. in New Caledonia

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Feasibility of polyculture blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* or mullet *Mugil* spp. in New Caledonia

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Summary

A study was conducted to estimate the effects of polyculture blue shrimp with fish (goldlined rabbitfish or mullet) on production performance and environmental quality. Results indicated that goldlined rabbitfish and mullet do not affect shrimp production performance, and the combined shrimp and fish yields in shrimp-rabbitfish polyculture were significantly greater than shrimp yield in shrimp monoculture. Most of the water and sediment parameters were similar among treatments. The results showed that the polyculture of blue shrimp with goldlined rabbitfish is technically possible without additional environmental degradation.

Introduction

Blue shrimp *Litopenaeus stylirostris* farming has been practised for over 30 years in New Caledonia, applied semi-intensive grow-out technique, and produced an annual average production of around 2000 tonnes. Shrimp industry, however, is affected by seasonal mortalities, either during the winter and cold seasons ("Syndrome 93") or during the warm season ("Summer Syndrome"). These two pathologies reduce the profitability and affect the sustainability of the industry and are therefore its main concerns.

Shrimp-fish Polyculture

Shrimp-fish polyculture has demonstrated as an ecologically and economically sound method of sustainability of shrimp culture and shown an effective choice for solving and/or minimizing some of the problems that shrimp industry has encountered presently. Some studies on polyculture penaeid shrimps with omnivorous fishes showed a remarkable improvement in water quality due to fishes helped to reduce organic wastes and selective phytoplankton, clean the pond bottom and increase nutrient uptake into cultured animals.

From literature, siganids, herbivorous fish, and mullet, omnivorous fish, could be excellent candidates for polyculture with shrimp because fishes help to decrease the impact of the disease, to prevent deterioration of the environment and to increase the production of the ponds. So we conducted an experiment on polyculture blue shrimp with fish, goldlined rabbitfish *Siganus lineatus* or mullet *Mugil sp*, in a mesocosm system to evaluate the effects of polyculture on production performance and environmental quality and to find suitable fish for polyculture with shrimp in practical development that contribute to sustaining the shrimp industry in New Caledonia.

Experimental design

The study was conducted in 16 outdoor circular mesocosm fibreglass tanks (1.7 m^2). Each tank contains a 15 cm layer of sediment and a 75 cm high water column (1275L in volume). A daily water exchange of around 10% was applied.

Shrimp juvenile (2.9g) were randomly stocked into the experimental tanks at density of 15shrimp.m^{-2}. One month later, rabbitfish or mullet were added into the shrimp tanks to form polyculture treatments. Rabbitfish (25.5g), hatchery-reproduced, were stocked into shrimp tanks at either low density (LDRB) of 1.2 fish.m^{-2} or high density (HDRB) of 2.4 fish.m^{-2}. Mullet (ML) (20.5g), wild-caught, were added into shrimp tanks at density of 1.2 fish.m^{-2}. And a control treatment was without fish. All treatments were randomly distributed among tanks with four replicates per treatment. Shrimp were fed with commercial pellet feed at feeding rate 3-5% of shrimp biomass, and the same feed amount was supplied into all tanks daily. At the beginning and at the end of 12 weeks of culture period, shrimp and fish were sampled and individually weighted to evaluate the growth performance.

Environmental survey

Environmental parameters were regularly sampled and analyzed daily (T^o, DO), weekly (pH, salinity, turbidity, chlorophyll a, TAN, SRP) and biweekly (TDN, NO_x-N, DON and sediment parameters) to estimate the environmental variation.

Result and discussion

Production performance

Shrimp survivals were lowest in the mullet polyculture (62.5%) and highest in high density rabbitfish polyculture (80.8%). In addition, the standard deviation of shrimp survival rate decreased from the control (20.7%) to the high rabbitfish density treatment (HDRB) (7%). Shrimp yield were not significantly different among treatments, although higher values obtained in rabbitifish polyculture treatments (145.4-170.3 $g.m^{-2}$), and slightly lower value was in the mullet treatment (130.3 g.m-2), compared with the control (143.2 $g.m^{-2}$). These results showed that the presence of fishes had no negative effect on shrimp growth. Shrimp growth obtained in this study was similar to the results recorded by other study on blue shrimp reared in earthen ponds in New Caledonia.

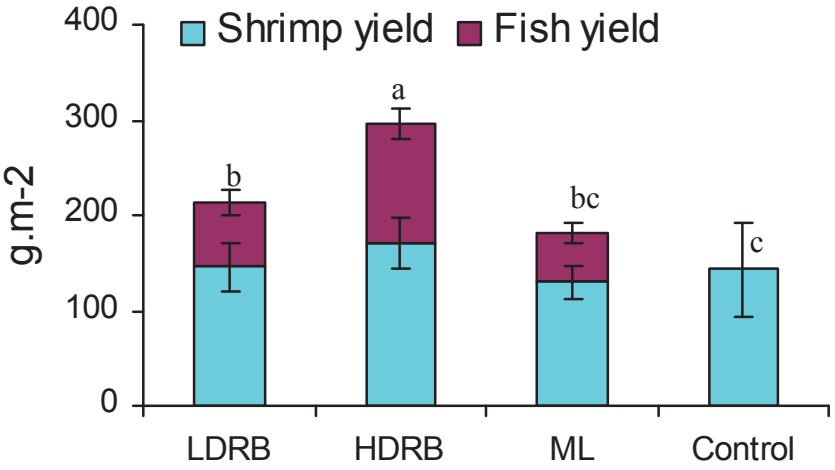


Figure 1: Shrimp yield, fish yield and total combined shrimp and fish yield in low density rabbitfish polyculture (LDRB), high density rabbitfish polyculture (LDRB), mullet polyculture (ML) and shrimp monoculture (control). Bars present standard deviations. Different letters are significantly different ($P<0.05$).

Fish survival gained 100%, and fish growth rate was similar in all polyculture treatments. The fish yield, however, was significantly higher in the HDRB than those in the other polyculture treatments. The total combined shrimp and fish yield were significantly higher in two rabbitfish polyculture treatments than shrimp production in the control (Fig. 1). The results of fish growth performances here indicated that conditions in the shrimp tanks were suitable for rabbitfish and mullet.

The overall food conversion ratio (FCR) was significantly lower in the HDRB polyculture than those in the mullet polyculture and the control (Fig. 2).

Polyculture blue shrimp with rabbitffish produced a remarkable secondary crop and significantly increaed total production. Furthermore, rabbitfish polyculture decreased significantly FCR by 31.6% and 47.4% in the LDRB and HDRB treatments, respectively,

compared with the control. Overall lower FCR in rabbitfish polyculture possibly contributed to increasing productive benefit.

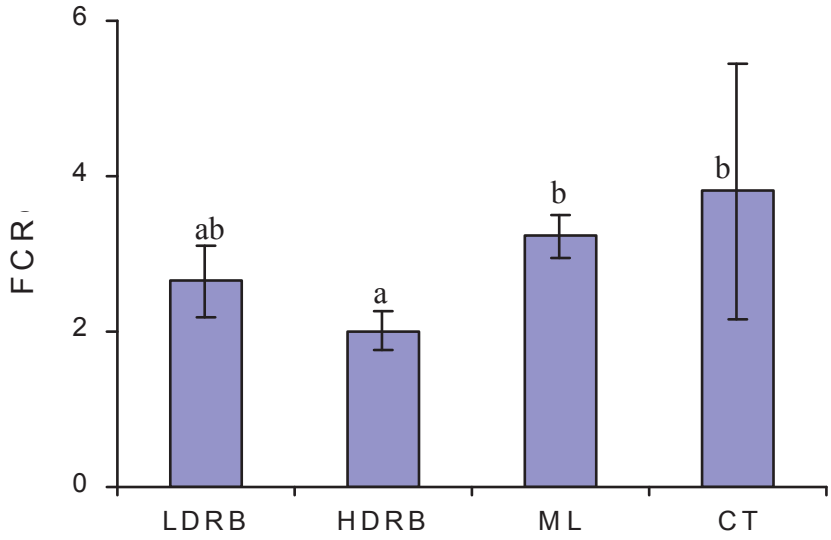


Figure 2: Overall food conversion ratio (FCRc) in low density rabbitfish polyculture (LDRB), high density rabbitfish polyculture (LDRB), mullet polyculture (ML) and shrimp monoculture (control). Bars present standard deviations. Different letters are significantly different ($P<0.05$).

Environmental parameters

The temperature (T), dissolved oxygen (DO), salinity, and pH were similar in all treatments, and in general, their variations were within suitable ranges for shrimp and fish growth (T: 22.4 – 26.5 ^{o}C; DO: 5.5 – 9.4 mg.L^{-1}, Salinity: 36.1 – 36.3 and pH 8.1 – 8.2).

Almost water parameters were little different among treatments (turbidity: 8.3 – 10.3 NTU, Chl *a*: 18.3 – 31.6 µg.L^{-1}, total dissolved nitrogen: 21.5 – 26.1 µM, total ammonia nitrogen: 0.96 – 1.84 µM, Nitrite-nitrate nitrogen: 0.19 – 0.20 µM). Although overall mean soluble reactive phosphorus concentration was significantly higher in the LDRB polyculture than those in the other treatments, its values maintained low in all treatments, 0.15 – 0.26 µM.

No significant difference was observed in all sediment parameters among polyculture treatments and the control. pH ranged from 6.8 to 6.9. Trends in redox potential were similar across all treatments, from -27 to -37 mV. Sediment loss on ignition was 1.5 – 1.6%. Sediment Chl *a* ranged 143.6 – 180.0 mg.m^{-2}. TAN and SRP in pore water ranged 261.3 – 318.7 µM, and 1.40 – 2.32 µM, respectively.

All the results indicated that there were little changes in the environmental quality in all treatments throughout the culture period.

Perspective

Rabbitfishes, herbivorous fish, have fast growth, high environmental and crowding tolerance, good flavour and high demand and market prices, and are considered as important candidates for polyculture with shrimps. The results of this study indicate that polyculture blue shrimp with goldlined rabbitfish produce a remarkable secondary crop and increase pond production as well as improve food utilization without adverse effect on shrimp growth performance and further environmental degradation compare with shrimp monoculture. Although shrimp-mullet polyculture did not get high result on production and environmental improvement, mullet also are potential species for polyculture with shrimp due to the fish are omnivorous that could help to control organic matter in culture system.

Further researches need to be conducted to (1) find the optimum density and biomass of fish and shrimp at stocking in order to achieve consistently high production and maximum environmental efficiency and to (2) study pathological conditions (with vibrio) to test the effect of co-culture on vibriosis.

Abstract

Blue shrimp *Litopenaeus stylirostris* farming is a major and profitable activity of aquaculture industry in New Caledonia. However, it is facing two seasonal bacterial diseases, which decrease reared shrimp yield and threaten the sustainability of the aquaculture development. As reported by the literature, polyculture with fish would have the potential to decrease the impact of these kind of diseases, prevent the deterioration of the environment and to increase the production of the ponds. The feasibility of *L. stylirostris* and *Siganus lineatus* polyculture in earthen ponds was carried out in this study. The main objectives were 1) to estimate the adaptive capacity and growth performance of *S. lineatus*, and the environmental variations in a closed culture system, 2) to estimate the technical feasibility of *L. stylirostris* and *S. lineatus* polyculture, and 3) to estimate the effects of *L. stylirostris* and *S. lineatus* polyculture on zootechnical performances and pond ecology in comparison with monoculture of these species.

To answer to the first objective, *Siganus lineatus* (5.7g) was stocked at 7, 14 and 21 fish.m^{-2} in a closed culture system. After 8 weeks of culture period, *S. lineatus* growth performance was similar between all densities. *S. lineatus* could well adapt and grow in a closed system, even at low temperature, ca. 20^{o}C and high stocking density, 21 fish.m^{-2}. High stocking density did not have negative effects on *S. lineatus* growth performance, but could cause the environmental deterioration due to increased nutrient input and accumulated organic wastes in the culture system. As consequence, *S. lineatus* could die when water oxygen depleted to below 2 mg.L^{-1}.

To reach the second and the third objective, a second experiment showed that adding *S. lineatus* (25.5 g) to *L. stylirostris* (2.9 g) culture system (15 shrimp.m^{-2}) at 1.2 and 2.4 fish.m^{-2} did not affect *L. stylirostris* zootechnical performance during the first stage of the rearing. *S. lineatus* gained 100% of survival and similar growth performance in all densities. The polyculture system increased total combined production by 47 – 106 % and reduced FCR by 31.6 – 47.7% compared with those in shrimp monoculture. The addition of *S. lineatus* to the culture system did not have significantly effects on the gross primary productivity, the respiration, the nutrient and particulates fluxes at the water-sediment interface of the pond ecosystem.

In a third experiment, using higher stocking biomasses of *L. stylirostris* (14 g) at 156 g.m^{-2} in a polyculture with *S. lineatus* (19 g) (80 g.m^{-2}) and at 237 g.m^{-2} in shrimp monoculture, we observed a high shrimp mortality. Conversely, stocking biomasses of *S. lineatus* (19 g) at 155 g.m^{-2} in a polyculture with *L. stylirostris* (14 g) (81 g.m^{-2}) and at 235 g.m^{-2} in monoculture resulted in increases of *S. lineatus* biomasses. This study showed that polyculture using *S. lineatus* is able to reduce organic wastes and nutrient release by consumption uneaten feed and retention input nutrients in biomass that contribute to limit the impact of the pond ecosystem eutrophication.

Results of stable isotope analyses showed that the used pellet feed was not a major carbon source, but was an important nitrogen source for both *L. stylirostris* and *S. lineatus*. Natural biota, including sediment organic matter and particulate organic matter, was a main carbon source and represented as a secondary nitrogen source for both species.

It may be concluded that *S. lineatus* is a suitable candidate for commercial culture in both monoculture and polyculture with *L. stylirostris* in earthen pond. Polyculture should be a proper approach that could partly contribute to sustainable development of aquaculture, but needs specific research to optimize the trophic status of the reared species.

Keywords: Polyculture, Penaeidae, Siganidae, integrated production, pond ecology

Résumé

L'aquaculture de la crevette bleue *Litopenaeus stylirostris* représente une activité profitable en Nouvelle-Calédonie. Cependant, elle doit faire face à deux maladies bactériennes saisonnières qui diminuent les rendements et menacent le développement aquacole. D'après la littérature, la polyculture avec des poissons pourrait diminuer l'occurrence des maladies, prévenir la dégradation de l'environnement et au final augmenter la production des bassins. Cette étude porte sur la faisabilité d'élever en bassins *L. stylirostris* avec *Siganus lineatus*. Les objectifs majeurs ont été 1) d'estimer la capacité de *S. lineatus* à s'adapter aux bassins et d'évaluer les performances de croissance, 2) d'estimer la faisabilité technique de la polyculture de *L. stylirostris* avec *S. lineatus* et 3) d'estimer les effets de cette polyculture sur les performances zootechniques des deux espèces et sur le fonctionnement écologiques des bassins par comparaison avec leur monoculture.

Pour répondre au premier objectif, *Siganus lineatus* (5.7g) a été mis en culture à des densités de 7, 14 et 21 poissons.m^{-2} en système clos. Après 8 semaines de culture, les performances de croissance de *S. lineatus* ont été similaires quelles que soient les densités. *S. lineatus* peut donc bien s'adapter et croître dans ces systèmes clos, même à des températures basses, proches de 20°C, et à forte densité. Les fortes densités n'ont pas eu d'effets négatifs sur la croissance, mais sont à l'origine d'une détérioration des conditions environnementales suite à un apport plus marqué en aliment générant davantage de déchets organiques. En conséquence, une mortalité de *S. lineatus* peut apparaître lorsque la concentration en oxygène descend en dessous des 2 mg.L^{-1}.

Pour atteindre le 2ème et le 3ème objectif, une seconde expérience a montré que l'ajout de *S. lineatus* (25.5 g) à une culture de crevettes (2.9 g; 15 crevettes.m^{-2}) à des densités de 0, 1.2 et 2.4 poissons.m^{-2} n'a pas affecté les performances zooetchniques de *L. stylirostris* sur cette première phase d'élevage. La survie de *S. lineatus* a été de 100% et sa croissance a été similaire quelle que soit sa densité. La production combinée totale pour les traitements polyculture a augmenté de 47 – 106 % et les indices de conversions diminuent de 31.6 – 47.7% en comparaison avec le traitement "monoculture de crevettes". L'ajout de *S. Lineatus* n'a pas eu d'effets significatifs sur la production primaire, la respiration, les flux dissous et particulaires à l'interface eau-sédiment de l'écosystème bassin.

Dans une troisième expérience, avec des biomasses initiales plus élevées de *L. stylirostris* (14 g) de 156 g.m^{-2} en polyculture avec *S. lineatus* (19 g) (80 g.m^{-2}) et de 237 g.m^{-2} en monoculture, nous avons observé une forte mortalité des crevettes. Inversement, avec des biomasses initiales de *S. lineatus* (19 g) de 155 g.m^{-2} en polyculture avec *L. stylirostris* (14 g) (81 g.m^{-2}) et de 235 g.m^{-2} en monoculture, nous avons montré une augmentation de la biomasse de *S. lineatus*. En utilisant l'aliment pour sa propre croissance non utilisé par les crevettes, *S. lineatus* est ainsi capable de limiter l'impact dû à l'eutrophisation de l'écosystème bassin.

Les résultats des analyses isotopiques montrent que l'aliment n'est pas une source majeure de carbone pour *L. stylirostris* et *S. lineatus* alors qu'il l'est pour l'azote. Le biotope, en y incluant la matière organique des sédiments et la matière organique particulaire dans la colonne d'eau est la principale source de carbone pour les deux espèces et une source secondaire pour l'azote.

Pour conclure, *S. lineatus* est un candidat potentiel pour la culture commerciale que ce soient sous forme de monoculture ou de polyculture avec *L. stylirostris* en bassin de terre. Cette polyculture est une approche adaptée qui pourrait contribuer au développement durable de l'aquaculture. Toutefois, des recherches spécifiques doivent être menées pour maximiser le statut trophique des espèces élevées.

Mots clés : Polyculture; Penaeidae; Siganidae; Production intégrée, Ecologie des bassins