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

Trung Cong Luong[a, c], Sebastien Hochard[a], Florence Royer[b], Hugues Lemonnier[b, *], Yves Letourneur[a]

[a] Université de la Nouvelle-Calédonie, Laboratoire LIVE, BP R4 - 98851 Nouméa Cedex, New Caledonia
[b] IFREMER, Lagons, Ecosystèmes et Aquaculture durable (LEAD/NC) BP 2059, 98846 Nouméa Cedex, New Caledonia
[c] Nha Trang University, 02 Nguyen Dinh Chieu Str., Nha Trang, Viet Nam

*: Corresponding author : Hugues Lemonnier, tel.: + 687 28 51 71 ; fax: + 687 28 78 57 ;
email address : hugues.lemonnier@ifermer.fr

Abstract:

The study was conducted to (1) estimate the effects of polyculture of blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* on production, environmental quality and water and sediment metabolism and (2) to determine if blue shrimp and goldlined rabbitfish polyculture is possible. The experiment was carried out for 12 weeks in a mesocosm system that consisted of 12 circular outdoor mesocosm fiberglass tanks (1.7 m^2, 1275 L water volume). Shrimp (2.9 g) were randomly stocked at density of 15 shrimp.m^{-2} without rabbitfish (control), with rabbitfish (25.5 g) at low density (1.2 fish.m^{-2}) (LDRB) and high density (2.4 fish.m^{-2}) (HDRB). Results indicated that goldlined rabbitfish *S. lineatus* did not affect shrimp growth performance. At the end of the experiment, the combined shrimp and rabbitfish yields in the LDRB (213 g.m^{-2}) treatment and the HDRB (295 g.m^{-2}) treatment were significantly greater ($P < 0.05$) than the shrimp yield in the control (143 g.m^{-2}). Most of the water and sediment parameters were similar among treatments. There was no significant difference ($P > 0.05$) in gross primary productivity and respiration between the HDRB treatment and the control. It was concluded that the polyculture of blue shrimp with goldlined rabbitfish is technically possible without degradation of the environment in the culture system.

Highlights

► *Siganus lineatus* does not affect *Litopenaeus stylirostris* growth in a polyculture system. ► Polyculture *L. stylirostris* and *S. lineatus* significantly increases pond production. ► Adding *S. lineatus* to *L. stylirostris* culture does not impact environmental quality. ► Adding *S. lineatus* to *L. stylirostris* culture does not affect pond metabolism.

Keywords : Polyculture ; Penaeidae ; Siganidae ; integrated production ; environment ; pond ecology

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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). However, shrimp aquaculture has faced many challenges during this time including disease, high production costs, fluctuating market prices and environmental management. The expansion of intensive shrimp culture has also increased environmental pollution due to enriched nutrient (total nitrogen: 34.6 – 133.1 μM, and total phosphorus: 0.95 – 8.32 μM) and particulate matter (TSS: 15.2 – 109.3 $mg.L^{-1}$) effluent discharged from shrimp farms (Thomas et al., 2010). Currently, the adverse impacts of these challenges and environmental pollution are so great that they are a threat to the sustainability of shrimp farming.

Shrimp polyculture has been shown to be an ecologically and economically sound method to increase the sustainability of shrimp culture (Akiyama and Angawati, 1999; Tian et al., 2001; Martínez-Córdova and Martínez-Porchas, 2006; Cruz et al., 2008), and shown to be 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). In recent years, research on shrimp polyculture has increased, showing in some cases, it can be successful and sustainable, both in freshwater and marine systems (García-Pérez et al., 2000; Martinez-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 el at., 2010), mollusks (*Sinonovacula constricta, Crassostrea gigas* and *Chione fluctifraga*) (Tian et al., 2001; Martínez-Córdova and Martínez-Porchas, 2006) and fish (tilapias, mullets and milkfish) (Eldani and Primavera, 1981; Wang et al., 1998; García-Pérez et al., 2000; Yuan et al., 2010; Biswass et al., 2012) have been co-cultured with penaeid shrimps. 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 el., 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 fishes such as tilapia showed an improvement in water quality as fish fed on organic wastes (Akiyama and Angawati, 1999; Cruz et al., 2008), selectively fed on larger unicellular algae and larger zooplankton that increase the dominance of beneficial phytoplankton (Tian et al., 2001; Cruz

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et al., 2008), and increased nutrient retention in fish biomass (Wang et al., 1998; Yuan et al., 2010). Several fish species (tilapia, snapper, seabass and siganids) 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., 2006a, 2006b, 2006c; Cruz et al., 2008).

Monoculture shrimp farming in New Caledonia is affected by seasonal mortalities, both during the winter cold season ("Syndrome 93") (Mermoud et al., 1998), and during the warm season ("Summer Syndrome") (Goarant et al., 2006; Lemonnier et al., 2006). These two pathogens reduce the profitability of the industry and are therefore of great concern. In both cases, mortalities are related to septicemic vibriosis.

As reported by Tendencia et al. (2006a), siganids, herbivorous fish, are excellent candidates for shrimp polyculture and considered as species with high potential to decrease the impact of the disease, prevent the deterioration of the environment and to increase the production of the ponds. In this study, we conducted an experimental polyculture of blue shrimp *Litopenaeus stylirostris* with goldlined rabbitfish *Siganus lineatus* in a mesocosm system. The aims of our study were (1) to estimate the effects of blue shrimp and goldlined rabbitfish polyculture on production, environmental quality, and water and sediment metabolism and (2) to determine if blue shrimp and goldlined rabbitfish polyculture is possible.

2. Materials and Methods

2.1. Experimental system

The experiment was conducted for a period of 12 weeks, from shrimp stocking (August to November, 2012) at the Saint-Vincent Aquaculture Research Station, New Caledonia (21°58'N, 165°57'E). The experiment was carried out in 12 circular outdoor mesocosm fibreglass tanks (1.7 m^2). Sediment taken from earthen shrimp pond located at the research 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 sediment surface, from which aeration was continuously supplied to the tank. The tanks were filled with sand-filtered seawater one week before shrimp stocking. A daily water exchange of around 10% was applied by regulating individual valves in each tank

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and water height was maintained at 75 cm (1275 L in volume) above the sediment surface during the experiment.

2.2. Experimental design

Blue shrimp (*L. stylirostris*) juveniles (2.9 ± 1.1 g) were randomly selected and stocked to the experimental tanks at density of 15 shrimp.m^{-2} (26 shrimp.tank^{-1}). One month later, rabbitfish (*S. lineatus*) were added to the shrimp tanks to form polyculture treatments. Rabbitfish (25.5 ± 2.9 g, 11.2 ± 0.4 cm in total length), hatchery-reproduced, were stocked to 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 shrimp monoculture tanks 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 8:00 am and 16:00 pm, with a feeding rate of 3 - 5% of shrimp biomass per day during the experiment. Feed quantity was adjusted 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 on 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 into the experimental tanks.

2.3. Shrimp and fish sampling and analysis

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 rabbitfish growth performances were evaluated at harvesting in terms of survival, daily weight gain (DWG), specific growth rate (SGR), and yield.

$$\text{Survival (\%)} = \text{harvesting number/stocking number}*100$$

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

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

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

where Wi, Wf: shrimp/rabbitfish initial and final mean weight (g)

Coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean.

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

FCR = 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)

2.4. Water sampling and analysis

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). Salinity, turbidity and pH were measured three times a week (08:00 am) using a refractometer (Cond 3210, Welheim, Germany); turbidimeter (TN-100, Eutech Instruments, Singapore), and pH meter (pH 197i, Welheim, Germany), respectively. On the day of 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 (450^0C, 4 hours) GF/C Whatman fiberglass filters (47 mm). Filtered water were analysed for total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Koroleff, 1976), soluble reactive phosphorus (SRP) (Murphy and Riley, 1962), nitrite and nitrate (NO_2^--NO_3^-)-N (Wood et al., 1967) and total dissolved nitrogen (TDN) (Raimbault et al., 1999). 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, water sample of 25 mL was filtered through GF/F Whatman fiberglass filters (25 mm) and analysed by fluorometric methods (Holm-Hansen et al., 1965).

2.5. Sediment sampling and analysis

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

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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 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 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).

2.6. Water and sediment metabolism

Primary productivity (PP) and respiration (R) were measured in the HDRB treatment and the control to compare between polyculture and monoculture treatments. On the day prior to rabbitfish stocking and every two weeks thereafter, PP and 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 set up on the bottom sediment. DO concentrations were recorded in the bottles and in the chambers every hour, between 10:30 am and – 13:30 pm, using an Oxygenmeter (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.

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

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

$$NPP_s = (Slc – Slbb)* (V/S)*1000$$

$$R_s = (Sdc – Sdbb)*(V/S)*1000$$

$$GPP = NPP + R$$

where NPP_w: net primary productivity of 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 ($\mu mol.L^{-1}.h^{-1}$); R_w: respiration of water column ($\mu mol.m^{-2}.h^{-1}$), Sdsb: oxygen slope in dark surface bottle

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($\mu mol.L^{-1}.h^{-1}$), 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 ($\mu mol.m^{-2}.h^{-1}$)

Oxygen self-produced budget from oxygen metabolism in whole tank 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 ($mgO_2.m^{-2}.h^{-1}$): gross primary productivity in whole tank; R ($mgO_2.m^{-2}.h^{-1}$): respiration in whole tank; 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 ($mgO_2.g^{-1}.d^{-1}$) 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 GPP, as follow:

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

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

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

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 16.0; with possible differences among data being tested by Duncan's multiple range tests. Percent data were arcsine-transformed before statistical analyses. Statistical comparisons of experimental data among treatments were performed for overall mean values and for each time of analyses. Non-parametric tests (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.

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The water and sediment PP and R between the HDRB treatment and the control were statistically compared by using a paired Student's t-test in MS-Excel.

3. Results

3.1. Shrimp and fish growth performances

Shrimp final mean weight, survival, DWG, SGR, and yield were not significantly different ($P>0.05$) among treatments. The variability in shrimp survival and yield among replicates of each treatment reduced from the control to the HDRB treatment (Table 1).

Rabbitfish survival was 100 % in all polyculture treatments. Rabbitfish final mean weight, DWG, and SGR were similar between the LDRB and the HDRB treatments (Table 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 production in polyculture treatments was significantly greater ($P<0.05$) than shrimp production alone in the control (Table 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 (Table 1).

3.2. Water quality parameters

Mean values and temporal variation trends (not shown) in temperature, DO, salinity, and pH were similar for all treatments. Salinity and pH ranged from 36 to 36.1 and from 8.1 to 8.2, respectively. In general, temperature and DO fluctuated within suitable ranges for shrimp and rabbitfish growth (Table 2). Mean turbidity and Chl *a* were not significantly different ($P>0.05$) among treatments (Table 2). However, Chl *a* temporal variations showed slightly different trends among treatments (Fig. 1). Large standard deviations 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 2).

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Except for SRP, mean nutrient concentrations (TDN, TAN, etc) were similar for all treatments (Table 2). Different trends in TAN temporal variations were observed among treatments (Fig. 1). Mean SRP concentration was significantly higher ($P<0.05$) in the LDRB treatment than 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 showed a similar trend with a slow increase at the end of the experiment (Fig. 1).

3.3. Sediment parameters

No significant differences were observed in all sediment parameters among polyculture treatments and the control (Table 3). Trends in redox potential were similar across all treatments. However, there was high variability among replicates of each treatment. Polyculture treatments and control treatment had the same trend in sediment Chl *a* variation throughout the experiment (Fig. 2), with fluctuation over the first four weeks and a gradual increase during the last four weeks.

3.4. Water and sediment metabolism

There was no significant difference ($P>0.05$) in GPP and R between the HDRB treatment and the control. The trend of GPP temporal variation was different between two treatments (Fig. 3). 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. 3).

The trend of R temporal variation was different between the HDRB and the control (Fig. 4). Within each treatment, R was significantly higher ($P<0.05$) in the water column than in sediment (Fig. 4). Sediment R temporal variation had same trend in both treatments (Fig. 4).

4. Discussion

Shrimp growth performance, including final mean weight, DWG and SGR, were not significantly different among 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 fish is that competition for food could potentially negatively affect shrimp growth (Wang et al., 1998; García-Pérez et al., 2000; Yuan et al., 2012)

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because fish are faster swimmers than shrimp and quickly monopolize the feed. Rabbitfish are herbivorous and gregarious fishes (Lam, 1974), and are thought to exhibit low competitive behaviour even when reared at high densities (Saoud et al., 2007). In captivity, rabbtifish 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). When polycultured with shrimp, rabbitfish consume uneaten feed, and thus prevent further deterioration of the environment (Tendencia et al., 2006a). In our study, it was expected that goldlined rabbitfish 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 their 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 monocultured in tanks (0.17 – 0.33 $g.d^{-1}$; Kumaraguru vasagam et al., 2009). Quite low temperatures (Table 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 lower 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 and often died after 2 days. 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 (Table 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 shrimp survival observed between replicates. This suggested that rabbitfish activity caused a possible increase in the stability of environmental conditions in tanks subsequently reducing shrimp mortality in some tanks. However, this hypothesis is not yet well supported 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 2 and Fig. 1) that were unlikely to cause shrimp mortality.

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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 growth-out pond, 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 ponds, 4.5 $mg.L^{-1}$ (321.4 µM) (Lazur, 2007). pH in sediment and the water column varied within narrow 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 mortality. However, mean TAN in pore water in this study were lower than stressful values recorded in a shrimp pond in New Caledonia (288.6 – 607.9 µM, Mugnier et al., 2006) and were below safe levels of TAN defined for rearing penaeid shrimp, 304.3 µM TAN (Chen et al., 1990).

Rabbitfish gained survival of 100% and had high growth rate (0.5 – 0.6 $g.d^{-1}$), 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 rabbitfish *S. lineatus* resulted in a significant increase in total production compared with shrimp monoculture. Furthermore, polyculture significantly decreased overall FCR by 31.6% and 47.4% in the LDRB and the HDRB treatments, respectively, compared with the control. Higher total production and lower overall FCR in rabbitfish polyculture contributed to increasing benefit.

Mean Chl *a* concentrations were not significantly different among polyculture treatments and the control. This might be due to equal nutrient inputs provided into the tanks from pellet food supplied and water intake daily. Furthermore, rabbitfish, in juvenile and adult stages, were unlikely to be feeding on phytoplankton (Lam, 1974).

Most nutrient concentrations were low and varied within small ranges during our experiment. This indicated that water quality remained stable over the course of the experiment in all tanks. 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 concentration was likely due to absorption by phytoplankton. This

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would also explain the decrease of TAN in the control tanks coinciding with a rapid increase of Chl *a* at the end of the experiment.

The changes of GPP in the water column and in sediment followed opposite trends in the HDRB polyculture and control treatments. As phytoplankton biomass increased, light availability at the bottom was reduced, resulting in limited photosynthesis of microphytobenthos. This process might also be enhanced by resuspended particulate matter caused by rabbitfish and/ or shrimp activities. Furthermore, in well-mixed or turbulent environments, resuspension of benthic microalgae attached to sediment particles also contributed significantly to GPP in water columns (MacIntyre et al., 1996). As increasing organic matter loads accumulated in the tanks, bacterial decomposition processes increased continuously, leading to increasing oxygen demand throughout the experiment in the water columns as well as in sediments in both treatments (Fig. 4). 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.

In general, total GPP of water and sediment tended to reduce from middle to the end of the experiment (Fig. 3), while total R in entire tank increased throughout the experiment (Fig. 4) in both treatments. These processes led to the differential oxygen amount between daily GPP and R in entire tank tended to decrease close to the end of the experiment (Fig. 5). In the HDRB treatment, oxygen budget generated from daily GPP and R 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. 5). 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 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.

GNP, in term of organic matter, produced by algae in the tanks was higher than organic carbon sources from food ration provided in both treatments (Fig. 6). Most of produced GNP might transfer to the bottom through sedimentation and to the surrounding environment

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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, 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.

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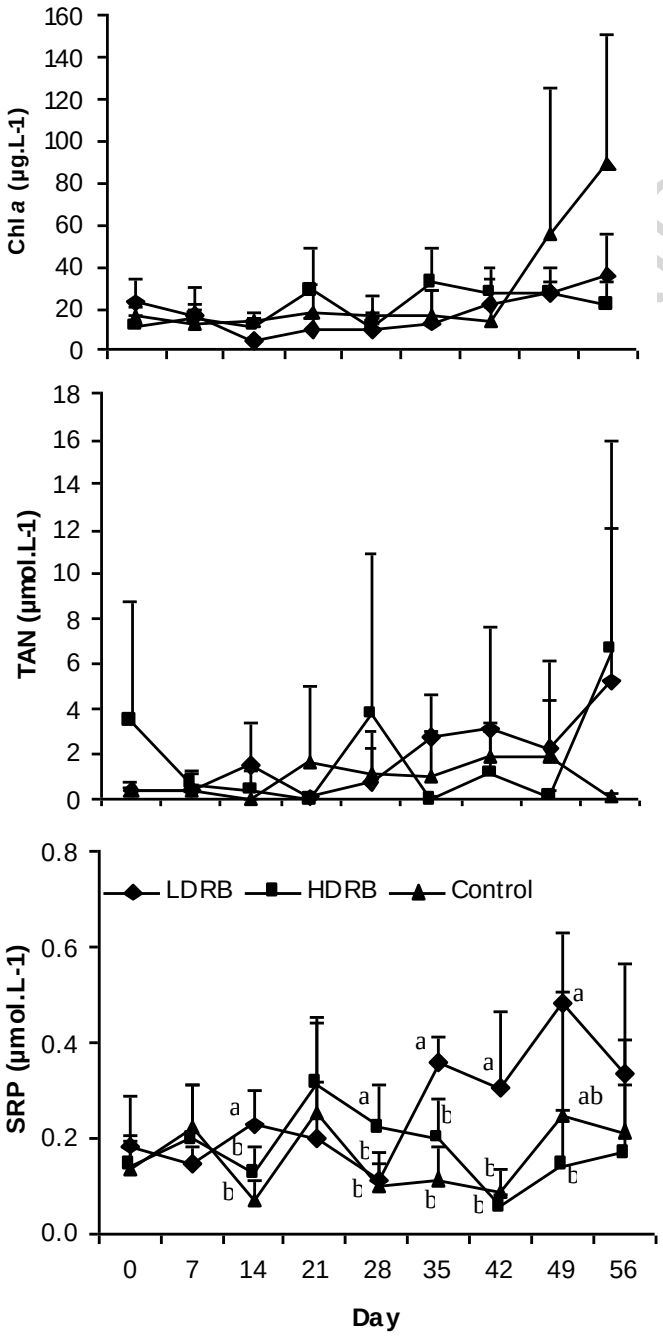

Figure 1: Temporal variation of water Chl *a*, TAN, and SRP in the LDRB and the HDRB polyculture and the control throughout the experimental period. Bars presented standard deviations. Values in the same day with different letters are significantly different ($P<0.05$)

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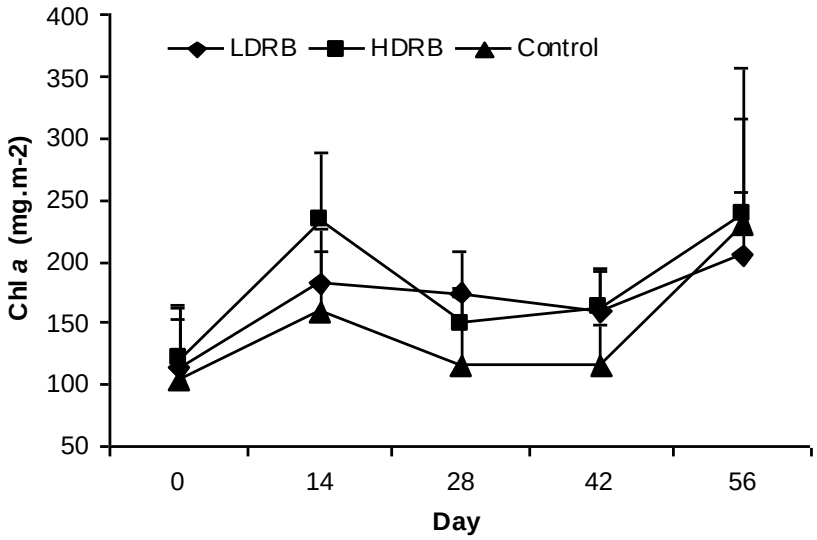

Figure 2: Temporal variations of sediment Chl *a* in the LDRB and the HDRB polyculture and the control throughout the experimental period. Bars presented standard deviations.

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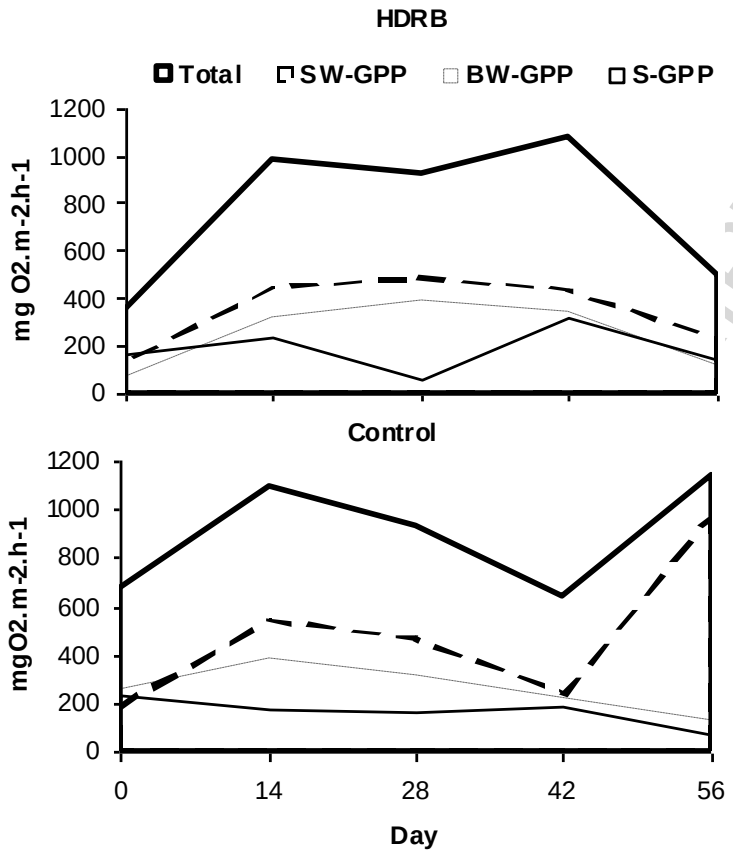

Figure 3: Temporal variations of GPP in the HDRB polyculture and the control; total: gross primary productivity in whole tank, SW-GPP: surface water gross primary productivity, BW-GPP: bottom water gross primary productivity, and S-GPP: sediment gross primary productivity,

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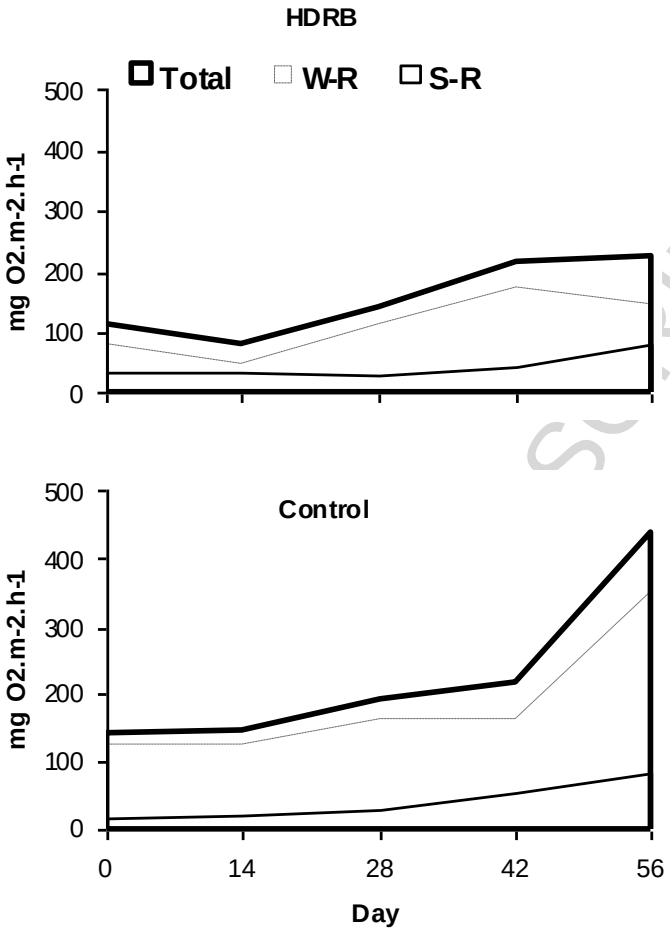

Figure 4: Temporal variations of respiration in the HDRB polyculture and the control; total: respiration in whole tank, W-R: water respiration, S-R: sediment respiration

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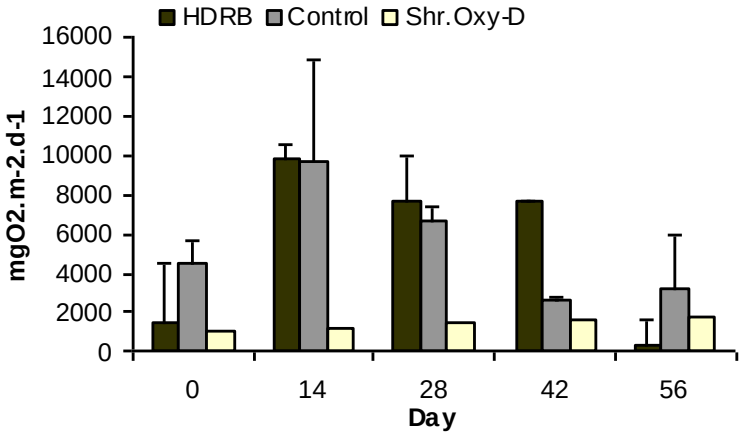

Figure 5: Oxygen budget produced by oxygen metabolism in the HDRB polyculture and the control in comparison with shrimp oxygen demand (Shr.Oxy-D) throughout the experiment. Blue shrimp, *L. stylirostris*, consume an average amount of 11.3 $mgO_2.g^{-1}.d^{-1}$ at stage used for the experiment (Wabete et al., 2008)

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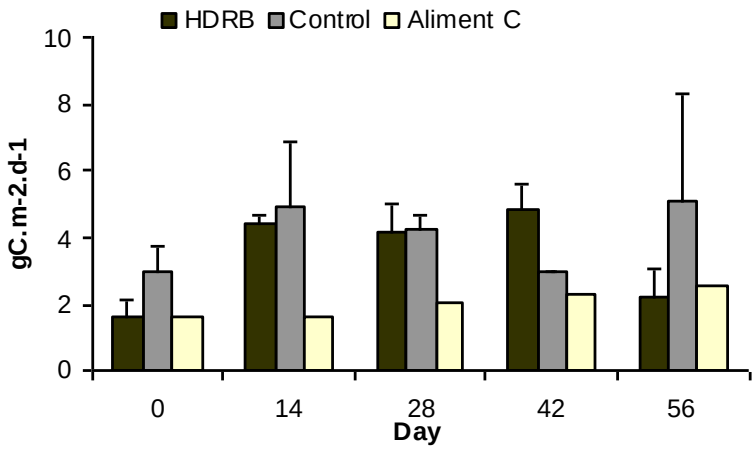

Figure 6: Gross natural production produced by algae photosynthesis in the HDRB polyculture and the control in comparison with organic carbon daily supplied from pellet feed (Aliment C).

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Table 1: Blue shrimp and goldlined rabbitfish growth parameters for polyculture of blue shrimp with goldlined rabbitfish at different stocking densities and shrimp monoculture. Values are mean ± SD

	Treatment		
	Control	Low density rabbitfish	High density rabbitfish
Shrimp			
Final mean weight (g.shrimp^{-1})	14.0 ± 0.7^{a}	13.4 ± 0.9^{a}	13.9 ± 0.2^{a}
DWG (g.d^{-1})	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.02^{a}
SGR (%.d^{-1})	1.89 ± 0.06^{a}	1.83 ± 0.08^{a}	1.88 ± 0.12^{a}
Survival (%)	66.3 ± 20.7^{a}	71.2 ± 13.1^{a}	80.8 ± 7.0^{a}
CV in survival (%)	31.2	18.5	8.7
Yield (g.m^{-2}.84d^{-1})	143.2 ± 48.6^{a}	145.4 ± 24.7^{a}	170.3 ± 27.0^{a}
CV in yield (%)	33.9	17.0	15.9
FCR	3.8 ± 1.6^{a}	3.7 ± 0.7^{a}	2.9 ± 0.5^{a}
Fish			
Final mean weight (g.fish^{-1})		57.5 ± 11.4^{a}	53.1^{a} ± 6.5
DWG (g.fish^{-1}.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}.56d^{-1})		67.7 ±13.5^{a}	125.0 ±15.4^{a}
Combined			
Total yield (g.m^{-2})	143.2 ± 48.6^{a}	213.1 ± 34.3^{b}	295.3 ± 24.4^{c}
FCRsf	3.8 ± 1.6^{a}	2.6 ± 0.5^{ab}	2.0 ± 0.3^{b}

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

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Table 2: Water parameters in polyculture treatments and in the control throughout the experimental period. Values in parentheses are min - max. Values are means ± SD

	Treatment		
	Control	Low density rabbitfish	High density rabbitfish
T (07:30) (^{0}C)	22.4 ± 0.3 (17.5 - 28.2)	22.5 ± 0.1 (17.6 - 28.0)	22.4 ± 0.2 (17.7 - 28.1)
T (15:00) (^{0}C)	26.5 ± 0.3 (22.3 - 31.7)	26.4 ± 0.1 (22.4 - 31.4)	26.4 ± 0.5 (21.7 - 32.8)
DO (07:30) (mg.L^{-1})	5.7 ± 0.2 (1.6 - 7.6)	5.5 ± 0.2 (2.4 - 7.6)	5.5 ± 0.1 (2.1 - 7.4)
DO (15:00) (mg.L^{-1})	9.3 ± 0.5 (4.0 - 15.3)	9.2 ± 0.1 (5.0 - 15.0)	9.4 ± 0.1 (4.3 - 16.1)
Turbidity (NTU)	8.3 ± 2.0^a	9.8 ± 1.8^a	9.9 ± 1.6^a
Chlorophyll *a* (µg.L^{-1})	28.5 ± 15.3^a	18.3 ± 4.3^a	20.7 ± 4.5^a
Phaeopigment (%)	30.2 ± 2.6^a	35.0 ± 2.8^a	32.8 ± 5.0^a
TDN (µM) (n = 5)	21.5 ± 2.1^a	26.1 ± 4.3^a	26.4 ± 5.1^a
TAN (µM) (n = 9)	0.96 ± 0.70^a	1.84 ± 1.35^a	1.83 ± 1.05^a
(NO_2 + NO_3)-N (µM) (n = 5)	0.20 ± 0.04^a	0.19 ± 0.06^a	0.25 ± 0.08^a
DON (µM) (n = 5)	20.6 ± 2.2^a	23.7 ± 1.9^a	23.1 ± 3.4^a
SRP (µM) (n = 9)	0.16 ± 0.07^a	0.26 ± 0.04^b	0.17 ± 0.03^a

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

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Table 3: Sediment parameters in polyculture treatments and in the control throughout the experimental period. Values are means ± SD.

	Treatment		
	Control	Low density rabbitfish	High density rabbitfish
pH	6.9 ± 0.1	6.9 ± 0.0	6.8 ± 0.1
Redox potential (mV)	-34.0 ± 11.9[a]	-37.6 ± 11.4[a]	-27.1 ± 23.8[a]
Loss on ignition (%)	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
Chlorophyll *a* ($mg.m^{-2}$)	144.7 ± 15.8[a]	166.3 ± 20.7[a]	180.0 ± 41.8[a]
Phaeopigment (%)	25.6 ± 3.7[a]	26.6 ± 1.9[a]	25.8 ± 3.6[a]
TAN (µM) in pore water	261.3 ± 40.6[a]	277.6 ± 96.5[a]	283.3 ± 206.0[a]
SRP (µM) in pore water	2.3 ± 1.1[a]	2.4 ± 0.8[a]	1.4 ± 0.4[a]

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

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Research highlights

- *Siganus lineatus* does not affect *Litopenaeus stylirostris* growth in a polyculture system.

- Polyculture *L. stylirostris* and *S. lineatus* significantly increases pond production.

- Adding *S. lineatus* to *L. stylirostris* culture does not impact environmental quality.

- Adding *S. lineatus* to *L. stylirostris* culture does not affect pond metabolism.

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