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

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Abstract

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

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

1. Introduction

Prawn farming has developed steadily over the last decades in response to increasing world market demand. The western king prawn (*Penaeus latisulcatus,* Kishinouye 1896*)* is considered as one of the candidate species for culture and has been widely cultured in several Asian countries (Kathirvel and Selvaraj, 1987). To increase prawn productivity, the management practices have been intensified by using high quality and quantity of feed (Brzeski and Newkirk, 1997, Shepherd and Bromage, 1988, Seymour and Bergheim, 1991) which accounts for more

than 95% of the nutrient input (Krom and Neori, 1989). However, less than one third of nutrients are assimilated into the shrimp biomass (Briggs and Funge-Smith, 1994) and the remainder is lost to the system (Wu, 1995, Piedrahita, 2003). In addition, aquatic species excrete to the water 70-80% of their ingested protein, the majority of which (80%) are composed of dissolved nitrogen in ammonium forms (Porter et al., 1987).

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

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

2. Materials and Methods

2.1 Materials and experimental design

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

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

2.2 Analytical procedures

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

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

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

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

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

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

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

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

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

2.3 Statistical analysis

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

3. Results and discussion

3.1 Water quality parameters

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

In this study, the concentration of TAN in all treatments remained below 1.0 mg/l until day 21 of experiment and then significantly increased to 2.92 mg/l in prawn monoculture, 2.34 mg/l in integrated culture and 1.66 mg/l in seaweed monoculture. The concentration of NO_2^- in prawn monoculture and integrated culture increased to nearly 0.7 mg/l by day 21 and remained at this level until the conclusion of the experiment. TAN and NO_2^- levels remained within the known acceptable concentration for successful prawn culture (3.0 mg/l and 1.0 mg/l, respectively) in both prawn monoculture and integrated culture treatments (Timmons et al., 2002).

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

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

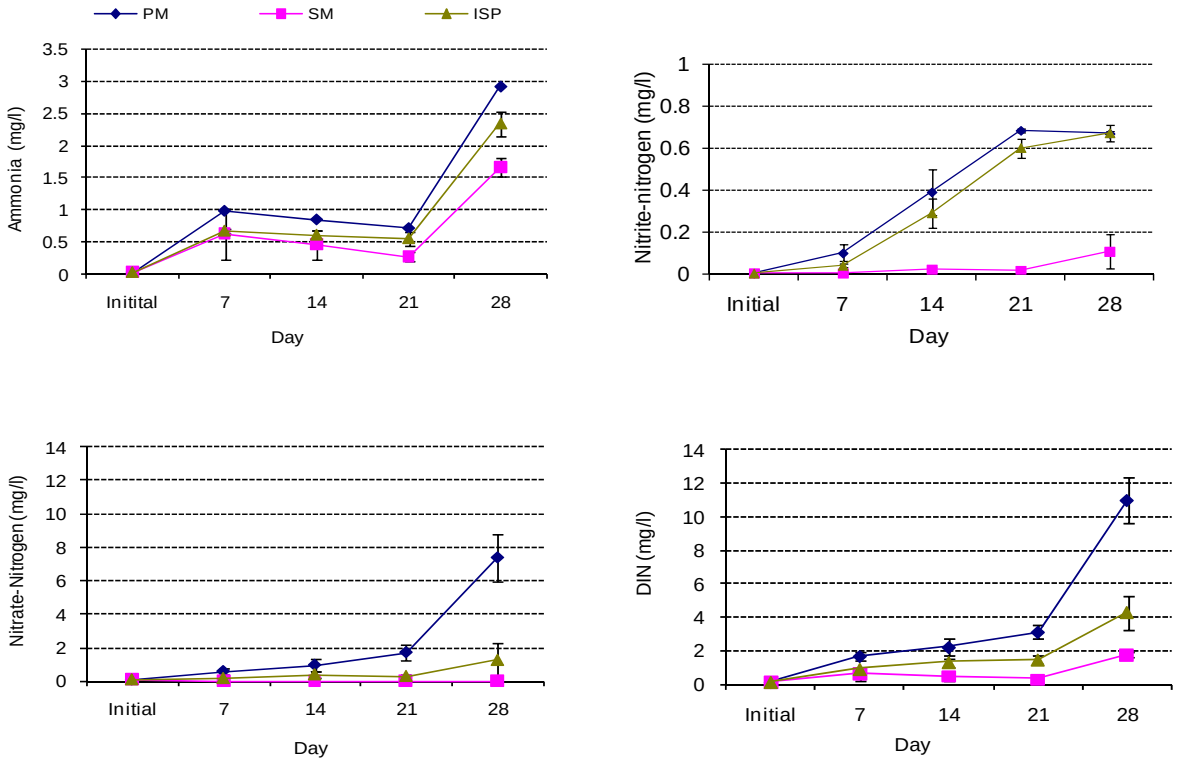

Figure 1: Concentrations of TAN, NO_2^-, NO_3^- and DIN in different systems over 28-day experiment
(PM- prawn monoculture, SM - seaweed monoculture, ISP- integrated seaweed & prawn)

3.2 Nitrogen removal

The removal rates of nitrogen metabolites from water when *Sargassum sp.* was present in prawn culture were not significantly different over the period of the experiment, except for NO_2^- which showed a significant decrease (Table 1). The removal rate of NO_3^- generally increased with increasing NO_3^- concentration, while the removal rate of TAN decreased with increasing TAN concentration (Figure 1, Table 1). The presence of *Sargassum sp.* resulted in more efficient at removal of NO_3^- than TAN, with removal rates of 69.28% and 28.25%, respectively.

Similarly, previous research has shown that seaweeds, such as green seaweed *Codium fragile* (Hanisak and Harlin, 1978), brown seaweed *Laminaria groenlandica* (Harrison et al., 1986) and red seaweed *Porphyra yezoensis* (Hafting, 1999) removed NO_3^- more efficiently than TAN. However, other research has shown *Ulva pertusa*, *Gelidum amansii* and *Sargassum enerve* to be more useful in removal of TAN than NO_3^- from water (Liu et al., 2004). In this study, *Sargassum sp.* was able to remove a maximum of 52.57% of DIN. This is higher than the values reported for red seaweed *Gracilaria longissima* where such seaweed removed only 17% of DIN when integrated with fish (*Sparus auratus)* culture (Hernández et al., 2005). This indicates that *Sargassum sp.* has a potential to act as a nitrogen sink when integrated with western king prawn culture.

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

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

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

3.3 Survival and growth performance of prawns and seaweed

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

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

Values in any one row not followed by the same superscript letters are significantly different at p≤0.05
** Biomass of live seaweed after 7 days of the experiment*

Integrating *Sargassum sp.* with prawn culture did not alter the SGR or weight gain of prawns (Table 2). Similarly, Lombardi et al. (2006) reported no signifcant differences in weight gain between monoculture and integrated culture when seaweed (*Kappaphycus alvarezii*) was integrated into Pacific white prawn (*Litopenaeus vanamei*) culture. Compared with studies on *P. monodon* (Chen et al., 1989, Thakur and Lin, 2003), the growth rate of western king prawns in both the monoculture and integrated culture of this study was higher, possibly as a result of lower stocking densities. In the present study, the stocking density of western king prawn was 18 prawns per m^2 (5 prawn per tank), while *P. monodon* were stocked at approximately 70 postlarvae per m^2 (PL_{25-27}) by Chen et al. (1989) and 20-25 juveniles per m^2 by Thakur and Lin (2003). Mean prawn survival rate was not significantly affected by the presence of seaweed, with 85% survival in prawn monoculture and 80% survival in integrated prawn and seaweed culture.

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

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

4. Conclusions

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

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