

Removal of nutrients 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 nutrients such as nitrogen and phosphorus. An experiment was conducted for 42 days to investigate the nutrient flow where seaweed (*Sargassum* sp.) was integrated into western king prawn (*Penaeus latisulcatus*) culture. Three treatments were used, each consisting of four, 0.1 m³ plastic tanks. Treatment 1 and 2 were the monocultures of western king prawns (5.48 ± 0.29 g) and 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 results showed that the concentration of DIN, total nitrogen (TN) and total phosphorous (TP) in the integrated culture system was significantly lower ($p < 0.05$) at the termination of the experiment than at the prawn monoculture system. Mean removal rates of DIN and total nitrogen ranged from 35.8 to 52.6% and from 34.7 to 61.9%, respectively. Total phosphorus was removed at an efficiency of 14.5% to 37.0%. The concentration of DIN, total nitrogen, PO_4^{3-} and total phosphorous 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 $3.16 \pm 0.74\%$ g day⁻¹ after 7 days of the experiment, which was significantly lower ($p < 0.05$) than the growth rate of the seaweed in the monoculture ($5.70 \pm 0.82\%$ g day⁻¹). 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, phosphorous, removal nutrient, *Sargassum* sp., *Penaeus latisulcatus*, western king prawn.

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 & Selvaraj 1987). To increase prawn productivity, the management practices have been intensified by using high quality and quantity of feed (Brzeski & Newkirk 1997, Shepherd & Bromage 1988, Seymour & Bergheim 1991) which accounts for more than 95% of the nutrient input (Krom & Neori 1989). However, less than one third of nutrients are assimilated into the prawn biomass (Briggs & 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 prawns 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, Philips et al. 1986). The aim of this study was to evaluate the efficacy of *Sargassum sp.* in assimilating nutrients 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 ($32^{\circ} 40''\text{S}$ $115^{\circ} 13''\text{E}$). 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 ($31^{\circ} 57''\text{S}$ $115^{\circ} 05''\text{E}$). 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 $0.5 \text{ kg}/\text{m}^2$ (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 42 days.

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

2.2 Analytical procedures

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

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

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^- and NH_4^+), nitrite nitrogen (NO_2^-), nitrate nitrogen (NO_3^-), total nitrogen, orthophosphate (PO_4^{3-}) and total phosphorus in all tanks were measured biweekly. TAN, NO_2^- and PO_4^{3-} were analysed using standard methods for water and waste water analysis (APHA 1998). NO_3^- was analysed by using a DR/890 Colorimeter. Total nitrogen (TN) in water was determined by indophenol blue method (APHA 1998), after simultaneous persulfate oxidation of unfiltered samples and using Devarda alloy to convert nitrogen into ammonium form (Raveh & Avnimelech 1979). Total phosphorus was determined by using the ascorbic acid method (APHA 1998).

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

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

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.

3. Results and discussion

3.1 Water quality parameters

Overall, the mean concentration of nutrients over time was significantly lower ($p < 0.05$) in the ISP and SM than in the PM (Figure 1). The concentration of total nitrogen and DIN in the ISP was significantly lower ($p < 0.05$) than the PM, even when no seaweed was present in ISP for the last 14 days of the experiment. The concentration of nitrogen metabolites peaked by day 28 of the experimental period in all treatments, 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 day 28 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).

Similarly, the orthophosphorus (PO_4^{3-}) and total phosphorus (TP) concentrations of ISP were significantly lower ($p < 0.05$) than the PM while seaweed was present in the tanks. The high concentration of PO_4^{3-} and TP observed in the prawn monoculture was probably caused from the uneaten feed and excretion by the prawns (Buschmann et al. 1996a). However, the concentration of PO_4^{3-} in the ISP was the same ($p > 0.05$) at both ISP and PM when all seaweed was removed from the tanks at day 28 until the conclusion of the experiment. This probably resulted in the decaying thallus of the seaweed.

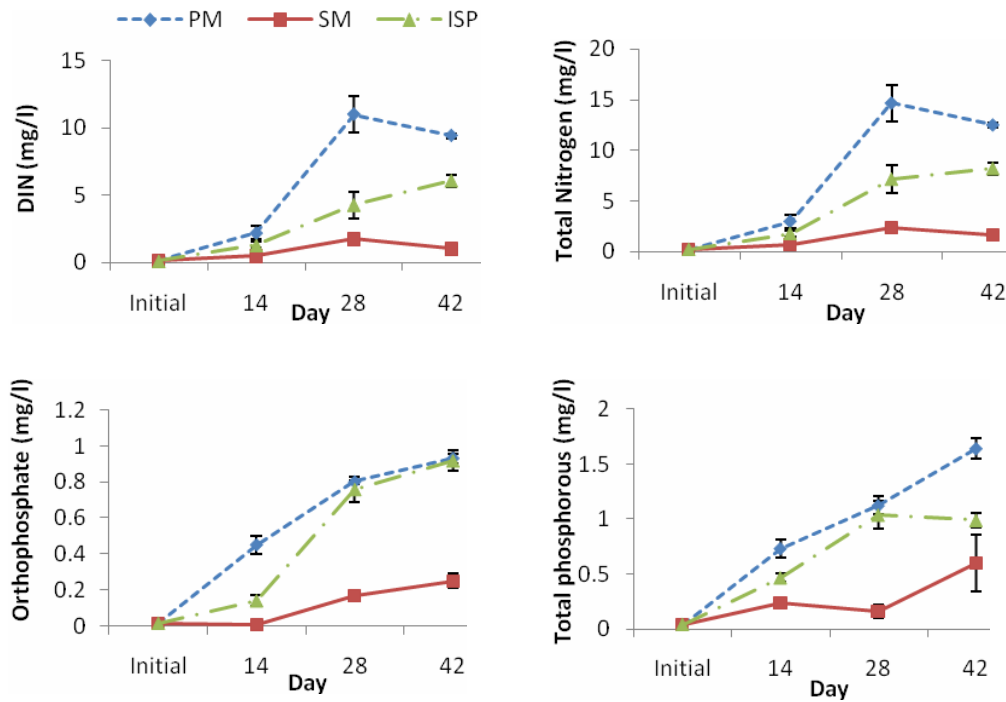


Figure 1: Concentrations of water parameters in different systems over 42-day experiment (PM = Prawn monoculture, SM = Seaweed monoculture, ISP = Integrated seaweed & prawn)

3.2 Nutrient removal

The removal rates of nitrogen and phosphorus from water when *Sargassum* sp. was present in prawn culture were not significantly different over the period of the experiment, except for PO_4^{3-} which showed a significant decrease and TN which showed a various removal rates (Table 1). The removal efficiency of both DIN and TN by *Sargassum* in the present study were generally higher (35.82-52.57% and 34.68-61.94%, respectively) than the values previously reported in literature. For instance, *Gracilaria longissima* removed only 17% of DIN when integrated with fish (*Sparus auratus*) culture (Hernández et al. 2005). *Gracilaria tikvahiae* removed around 10-14% of the nitrogen in the effluent pond which was used for the intensive culture of the Pacific white prawns (*Litopenaeus vannamei*) (Kinne et al. 2001). This indicates that *Sargassum* sp. has a potential to act as a nitrogen sink when integrated with western king prawn culture.

In contrast, few studies have addressed the efficiency of phosphorus removal. Recently, Jones et al. (2001) reported that *G. edulis* was able to remove up to 95% of PO_4^{3-} when cultivated in prawn effluents. In the present experiment, *Sargassum* was able to remove maximum of 65.85% of the PO_4^{3-} by day 14 of the experiment. Compared with the majority of other seaweeds, the performance of *Sargassum* in phosphate removal in this study was relatively high. For instance, integrating *Gracilaria chilensis* and salmon culture resulted in the removal of 32% of the PO_4^{3-} from the fish farm (Buschmann et al. 1996b). Studies on other seaweed species have also shown relatively low removal efficiency for PO_4^{3-} (DeBoer et al. 1978, Neori et al. 1996). Neori et al. (1998) reported that *Ulva lactuca* and *Gracilaria conferta* removed less than 25% of the PO_4^{3-} from an integrated system. Troell et al. (1997) showed that *G. chinensis* would be capable of removing 27% of the phosphate from salmon cages. Similarly, the removal rate of total phosphorus was recorded at high level with the mean removal rate of 30.21%. The finding in the present study therefore shows the potential ability of *Sargassum* to effectively reduce the phosphorus concentration when integrated with prawn culture, and thus the quality of water for prawn culture.

Table 1: Removal rate of nutrients over the experimental period

Variable	Day 14	Day 28	Day 42	Mean
DIN (%)	37.89 ± 8.45 ^a	52.57 ± 3.73 ^a	35.82 ± 4.07 ^a	42.09 ± 5.27
TN (%)	37.42 ± 8.53 ^a	61.94 ± 6.21 ^b	34.68 ± 5.87 ^a	44.68 ± 8.67
PO_4^{3-} (%)	65.85 ± 9.11 ^a	5.62 ± 3.54 ^b	nd	35.74 ± 30.11
TP (%)	32.77 ± 11.48 ^a	20.81 ± 3.35 ^a	37.05 ± 5.57 ^a	30.21 ± 4.86

Values in any one row not followed by the same superscript letters are significantly different at $p < 0.05$; nd = not detectable (DIN = Dissolved inorganic nitrogen, TN = Total nitrogen, PO_4^{3-} = orthophosphorus, TP = Total phosphorus)

3.3 Survival and growth performance of prawns and seaweed

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 significant 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 & 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 prawns per tank), while *P. monodon* were stocked at approximately 70 postlarvae per m^2 (PL₂₅₋₂₇) 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 55% survival in prawn monoculture and 60% survival in integrated prawn and seaweed culture.

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
<i>Prawns</i>			
SGR (% g day ⁻¹)	0.64 ± 0.21 ^a	-	0.61 ± 0.15 ^a
WG (g)	3.99 ± 0.98 ^a	-	3.31 ± 0.77 ^a
Survival (%)	55.00 ± 9.57 ^a	-	60.00 ± 5.75 ^a
<i>Seaweed</i>			
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 $p \leq 0.05$

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

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 & Mitchell 1972, Parker 1982, Lignell & Pedersen 1987, Marinho-Soriano et al. 2002).

4. Conclusions

The seaweed *Sargassum* can be cultivated in prawn culture and can function as an effective biofilter for prawn culture. The findings of this study suggest the use of *Sargassum* for the improvement in water quality in prawn culture. Furthermore, the growth and survival of the prawns did not differ between the monoculture or the integrated culture of the prawns. The biofiltering potential of *Sargassum* may thus encourage future polyculture systems to be adopted by farmers as an environmentally friendly way of recycling waste waters from aquaculture systems.

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