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## Evaluation of *Sargassum* sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) culture system

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### Abstract

Effluent water from intensive prawn aquaculture systems typically has a high concentration of dissolved nutrients such as nitrogen and phosphorus. A study was conducted for 42 days to investigate the nutrient flow in a system where brown seaweed (*Sargassum* sp.) was integrated into western king prawn (*Penaeus latisulcatus*) culture. Three treatments namely, western king prawn monoculture ( $5.48 \pm 0.29$  g), *Sargassum* sp. monoculture and seaweed/prawn integrated culture were tested for nutrient flow among feed, water and species cultured. The results showed that by integrating seaweed into prawn culture, the concentrations of total ammonium nitrogen (TAN), nitrite-nitrogen ( $\text{NO}_2^-$ ) and nitrate-nitrogen ( $\text{NO}_3^-$ ), dissolved inorganic nitrogen (DIN), total nitrogen (TN), phosphate ( $\text{PO}_4^{3-}$ ) and total phosphorus (TP) were significantly lower ( $p < 0.05$ ) in the integrated culture system than in the prawn monoculture ( $p < 0.05$ ) and remained within non-toxic limits for the duration of the study. In addition, the integration of *Sargassum* sp. with western king prawn culture did not significantly alter the nitrogen and phosphorus conversion rates from feed into prawns (approximately 17.69-18.99 and 13.79-14.47%, respectively). The specific growth rate (SGR) and survival rate of the prawns in integrated treatment did not significantly differ ( $p > 0.05$ ) from the prawn monoculture. The mean biomass of *Sargassum* sp. in integrated culture increased at the rate of  $3.16 \pm 0.74\%$  g day<sup>-1</sup> after 7 days of the study, which was significantly higher than in the monoculture system ( $5.70 \pm 0.82\%$  g day<sup>-1</sup>). The results suggest that integrating *Sargassum* sp. into western king prawn culture can benefit prawn farming by assisting in the maintenance of optimum water quality and thereby, reducing environmental impacts on surrounding areas.

**Key words:** Brown seaweed, *Sargassum* sp., western king prawn, *Penaeus latisulcatus*, nutrients, nitrogen, phosphorus.

## 1. Introduction

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

In order to improve effluent water quality, assist in maintaining the sustainable development of prawn farming and to mitigate the environmental impacts of prawn farming, various methods have been proposed to address the issue of nutrients discharged from intensive prawn aquaculture (Troell et al., 2003, Neori et al., 2004). One viable approach is to integrate macroalgae with prawn aquaculture where macroalgae are expected to assimilate the nutrients from prawn effluents. This approach is based on the use of macroalgae to remove the dissolved nutrients from aquaculture pond effluents. The concept of developing an “environmentally clean” aquaculture system based on an integrated fish-mollusc and macroalgae system was first proposed by Gordin et al. (1981). The system was further tested by Gordin et al. (1990) and Shpigel et al. (1991). Other authors have also developed systems integrating fish or prawn and macroalgae culture (Liu et al., 1997, Neori et al., 1998, Troell et al., 1999, Jones et al., 2001).

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

## 2. Materials and Methods

Western king prawns (size:  $5.48 \pm 0.29$  g) were collected from the mouth of Swan River in Bicton, Western Australia (32° 40'S 115° 13'E). Prawns were acclimated to the laboratory conditions for 14 days before commencing the study. Brown seaweed (*Sargassum* sp.) was collected from the Cottesloe coast, Western Australia (31° 57'S 115° 05'E).

Three treatments were used, *viz.* prawn monoculture (PM), seaweed monoculture (SM) and integrated seaweed and prawn culture (ISP). Each treatment consisted of four replicates in the form of 0.1m<sup>3</sup> plastic tanks. All 12 tanks were arranged in a completely randomised design. The study was conducted for 42 days under laboratory conditions. Five prawns were placed into each PM and ISP tank and total prawn biomass and survival ( $S_m$ ) in each tank was recorded. The seaweed *Sargassum* sp. was rinsed with ocean water to remove any epiphytes and was then placed into the SM and ISP culture. Seaweed was stocked at the initial biomass of 0.5 kg m<sup>-2</sup> (140 g per tank) in SM and ISP. The feeding rate for the prawns at the commencement of the study was 2.5% of the prawn biomass per day. The feed contained 8.12% of N and 1.29% of P. Prawn mortalities in each tank were removed immediately and weighed and any sign of cannibalism was recorded.

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

Prawns were weighed at the commencement of the study and were re-weighed once a week to obtain the data required to calculate the specific growth rates (SGR %) by using the following formula:

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

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

The survival rate ( $S_m$ ) of the prawns in each tank was also calculated using the formula:

$$S_m = N_m \times 100 / N_i$$

where:  $N_m$ : 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:  $\text{NH}_3^-$  and  $\text{NH}_4^+$ ), nitrite nitrogen ( $\text{NO}_2^-$ ), nitrate nitrogen ( $\text{NO}_3^-$ ), total nitrogen, orthophosphate ( $\text{PO}_4^{3-}$ ) and total phosphorus in all tanks were measured weekly. TAN,  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$  were analysed using standard methods for water and waste water analysis (APHA, 1998).  $\text{NO}_3^-$  was analysed by using a DR/890 Colorimeter

(Hach Company, the United State). Total nitrogen (TN) in water was determined by the indophenol blue method (APHA 1998), after simultaneous persulfate oxidation of unfiltered samples and using Devarda alloy to convert nitrogen into ammonium form (Raveh and Avnimelech, 1979). Total phosphorus was determined by using the ascorbic acid method (APHA, 1998).

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

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

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

$C_p$  ( $\text{mg l}^{-1}$ ) = nutrient concentration in integrated seaweed-prawn treatment

For nitrogen and phosphorous content analysis, samples of feed, prawns, seaweed and sediment wastes (faeces and uneaten feed was collected daily by siphoned method) were oven-dried at  $105^{\circ}\text{C}$  overnight to a constant weight. Nitrogen content was analysed by the Kjeldahl method (AOAC, 1995) using a Tecator 1015 heater block operated by a Tecator autostep 1012 controller, and a Tecator Kjeltex 1030 Auto Analyser. Dried samples were analysed for phosphorus by using the spectrophotometric molybdovanadate method (AOAC, 1995).

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

$$\text{Nutrient inputs} = \text{Nutrient outputs} + \text{Nutrient loss}$$

Where,  $\text{Nutrient inputs} = \text{nutrients in water} + \text{nutrients in stocked prawn and/or seaweed} + \text{nutrients in feed}$

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

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

#### *Survival and growth rates of prawns and seaweed*

The mean growth rate of the prawns was not significantly different ( $p > 0.05$ ) between PM and ISP (Figure 1). The presence of the seaweed in prawn culture did not affect the survival of the prawns ( $p > 0.05$ ), with 55% in the PM and 60% in the ISP (Figure 2).

Culturing *Sargassum* sp. with prawns resulted in a significantly lower ( $p < 0.05$ ) growth rate than the monoculture of the seaweed (Figure 3). However, the *Sargassum* began to die after 7 days of the study and consequently, biomass loss in the subsequent days was observed. Total mortality of the seaweed was recorded by day 28 of the study (thallus deterioration and disintegration) and then dead seaweed was removed from the tanks.

### **Water quality**

Mean water temperature and dissolved oxygen (DO) were 23.60-25.08°C and 5.81-6.16 mg l<sup>-1</sup>, respectively over the studyal period. The pH of water ranged from 7.91 to 8.17, which is within the optimum range for prawn culture (Allan and Maguire, 1992, Wang et al., 2002).

Overall, the mean concentration of nutrients over time was significantly lower ( $p < 0.05$ ) in the ISP and SM than in the PM (Figure 4). The concentration of total nitrogen 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 study. The total phosphorus concentration of ISP was significantly lower ( $p < 0.05$ ) than the PM while seaweed was present in the tanks (that is, until day 28). There was no difference in the concentration of PO<sub>4</sub><sup>3-</sup> between ISP and PM when all seaweed was removed from the tanks at day 28 until the conclusion of the study. The total nitrogen, PO<sub>4</sub><sup>3-</sup> and total phosphorus concentrations in SM were significantly lower than PM and ISP for the duration of the study.

In all treatments, NO<sub>3</sub><sup>-</sup> and total ammonium nitrogen (TAN) were the predominant dissolved inorganic nitrogen forms. Both increased significantly at day 28 and then remained stable until the end of the study (Figure 4). The NO<sub>2</sub><sup>-</sup> concentrations peaked at the end of the study and no significant difference between PM and ISP was observed. Following this, the concentration of TAN decreased significantly ( $p < 0.05$ ) in all treatments and was not significantly different between ISP and PM. The concentration of NO<sub>3</sub><sup>-</sup> decreased slightly in PM, while the NO<sub>3</sub><sup>-</sup> concentration in ISP continued to increase until the end of the study. In contrast, NO<sub>2</sub><sup>-</sup> was generally at the lowest concentration and was always lower in ISP than in PM (Figure 4).

During the study period, nitrogen was the more abundant nutrient when compared to phosphorus (Figure 4). At the end of the study, the total nitrogen had increased significantly ( $p < 0.05$ ) in all treatments. In ISP the concentrations and total nitrogen were lower than in PM. In SM, the concentration of total nitrogen was lower than in PM and ISP. The concentration of PO<sub>4</sub><sup>3-</sup> and total phosphorus increased gradually in all treatments (Figure 4). The maximum

concentration of total phosphorus was recorded at the end of study in PM and SM, while the highest concentration of total phosphorus in ISP was 1.13 mg l<sup>-1</sup> on day 35 of the study.

### ***Nutrient removal***

The removal rates of nitrogen metabolites by *Sargassum* sp. remained constant over the study period, except for NO<sub>2</sub><sup>-</sup> which showed a significant decrease and was not detectable after day 21 (Table 1). The removal rate of NO<sub>3</sub><sup>-</sup> generally increased with increasing NO<sub>3</sub><sup>-</sup> concentration, while the removal rate of TAN decreased with increasing TAN concentration (Figure 4 and Table 1). Overall, the presence of *Sargassum* sp. in prawn culture tanks resulted in more efficient removal of NO<sub>3</sub><sup>-</sup> than TAN. The nutrient removal rate varied over the 1 period, but generally, the removal rate of total nitrogen were higher than those of PO<sub>4</sub><sup>3-</sup> and total phosphorus (Table 1). There was a significant difference between the removal rates of PO<sub>4</sub><sup>3-</sup> over the study period. The highest PO<sub>4</sub><sup>3-</sup> removal rate was observed on day 14 of the study with a 65.9% removal efficiency, but decreased significantly thereafter to 5.6% at day 21 and was not detectable from day 28 onward (Table 1). The removal rate of total phosphorus did not significantly change during the study period. Total phosphorus was removed at an efficiency of 14.5% to 37.0%.

### ***Nutrient budget***

Most of the nitrogen in the tanks containing prawns came from the formulated prawn feed, whereas nitrogen input in SM was primarily from the intake water (Table 3). Within the tank, 17.69% in PM and 18.99% in ISP of the input N from feed was converted to prawn biomass (Figure 5). Wastes, including uneaten feed, faeces and/or dead seaweed, accounted for 27.81% of the nitrogen in PM and 24.42% in ISP and only 8.35% in SM. In the present study, unaccounted nitrogen was detected (5.00% in PM, 9.12% in ISP and up to 50.30% in SM). Phosphorus input in PM and ISP occurred mostly from the prawn feed, while seaweed contributed the largest source of P in SM. Prawns contributed about 32.05% in PM and 24.16% in ISP. In the ISP, there was about 24.57% of P input coming from seaweeds. (Table 3). The phosphorus budget indicated that about 14.47% and 13.79% of the input P as feed was converted to prawn biomass (Figure 2). The contribution of phosphorus from wastes that is uneaten feed, faeces and/or dead seaweed, averaged 42.63% in PM, 35.67% in ISP and 50.92% in SM. Unaccounted input P contributed minor proportions.

### ***Nitrogen and Phosphorus in tissues***

#### ***Nitrogen and Phosphorus in prawn tissue***

Due to the increment in the prawn biomass, the net N and P biomass increased in both PM and ISP. There was no relationship between SGR and the net N biomass gained in tanks of the



prawns ( $r^2 = 0.35$ ,  $p = 0.38$ ; Figure 6). A higher correlation was recorded between the SGR and the net P biomass gained ( $r^2 = 0.60$ ,  $p = 0.02$ ; Figure 6). Over the period of the study, the N:P ratio of prawns in both the PM and ISP treatments did not significantly alter.

#### *Nitrogen and Phosphorus in seaweed tissue*

At the end of the study, the seaweed under both monoculture and integrated culture conditions showed a decrease in the N and P content from the initial concentrations (Table 2). Due to losses of seaweed biomass, the amount of N and P gained in SM and ISP had negative values. From day 7 onward, the thallus of the seaweed began to deteriorate and 100% mortality was observed by day 28. Therefore, neither N and P contents nor N:P ratio could be determined at the end of the study (Table 2).

## 4. Discussion

### *Survival and growth*

Survival, growth and biomass increment are the main concerns in the operation of a commercial aquaculture farm. Survival of the prawns in all of the study tanks was higher than 55% which is higher than another similar study which resulted in 13.64 – 40.91% survival after 30 days of the study (Sang, 2003). However, inclusion of *Sargassum* into prawn culture has no effect on the survival and growth performance of the prawns. Compared with studies on *P. monodon* (Chen et al., 1989, Thakur and Lin, 2003), the SGR of western king prawns in both the monoculture and integrated culture systems in this study was high, possibly as a result of lower stocking densities. In the present study, the stocking density of the western king prawns was 18 prawns per m<sup>2</sup>, while *P. monodon* were stocked at approximately 70 post-larvae per m<sup>2</sup> (PL<sub>25-27</sub>) by Chen et al. (1989) and 20-25 juveniles per m<sup>2</sup> by Thakur and Lin (2003).

The growth rate of *Sargassum* is different under different environmental conditions (Gellenbeck, 1984, Guimaraens, 1999). Integrating seaweed with prawn culture resulted in a lower growth rate of seaweed than in the SM. This was probably due to the excessive increase of dissolved inorganic nutrient concentrations observed during the last days of the study. There was an increase in the nutrient concentration during the last days of study due to decaying and dying seaweed. There is evidence from previous studies, using different species of seaweed, that high nutrient levels can result in an inhibition in the growth rate of seaweed *Ulva* and *Gracilaria* (Waite and Mitchell, 1972, Parker, 1982, Marinho-Soriano et al., 2002), and *Sargassum* (Schaffelke and Klimpp, 1998, Diaz-Pulido and McCook, 2005). Furthermore, the nutrient thresholds for the optimum growth rate of *Sargassum* species are low, ranging from 3 to 5 µM (equivalent to 0.03-0.05 mg l<sup>-1</sup>) for TAN, 6-15 µM (equivalent to 0.06-0.17 mg l<sup>-1</sup>) for (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) and 0.3-0.75 µM (equivalent to 0.014-0.035 mg l<sup>-1</sup>) for PO<sub>4</sub><sup>3-</sup> (Schaffelke and Klimpp,

1998, Ray-Lien Hwang et al., 2004). On day 7 of the present study, concentrations on excess of these values were recorded, specifically, 0.67 mg l<sup>-1</sup> for TAN, 0.845 mg l<sup>-1</sup> for (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) and 0.14 mg l<sup>-1</sup> for PO<sub>4</sub><sup>3-</sup>. It seems that the mechanism(s) may involve a toxic effect due to higher levels of nutrients. Elevated nutrient concentrations, particularly of ammonium, may inhibit the capacity to assimilate nutrients by altering the electron transport chain, and may affect enzyme and membrane functions (Peckol and Rivers, 1995, Kevekordes, 2001).

Realistic growth data of seaweed in the present study could be collected only during the first seven days of the study due to the deterioration and eventual death of the seaweed at day 28, as all thalli began to lose weight. The deterioration of thalli was probably due to differences in the physical environmental conditions in the laboratory compared to natural conditions.

Temperature, salinity and light can play an important role in the growth of marine macroalgae (Lobban et al., 1985). Jones (1999) assumed that the temperature, water flow rate or light availability under laboratory conditions are not adequate for macroalgae growth, resulting in a higher rate of biomass decaying than biomass production. The current study was conducted at a temperature of 25-26°C, 12:12 h light:dark cycle and salinity level of 28-29 ‰. As optimum temperature was maintained in this study, it is assumed that the temperature in this study did not affect the growth performance of *Sargassum* sp. (Hanisak and Samuel, 1987, Ray-Lien Hwang et al., 2004). Therefore, it seems that other factors (e.g. salinity and light) were involved in the performance of *Sargassum* when it was cultivated together with the prawns. Hanisak and Samuel (1987) reported that the optimal salinity range for growth of several *Sargassum* species such as *S. flutans* and *S. natans* is 36-42‰ and a reduction in salinity to 30‰ caused a reduction in the growth rates by almost half. In this study, salinity ranged from 28 to 29 ‰ to optimal salinity for prawn growth that could have affected the performance of the seaweed because the *Sargassum* sp. used in this study was collected from Cottesloe beach, Western Australia where the salinity is at 35-36 ‰. Other laboratory conditions, for example, light duration, intensity and/or excessive handling might have contributed to the mortality of *Sargassum* sp.

#### ***Water Quality and Nutrient Removal***

Different studies have pointed out that species of the *Ulva* and *Gracilaria* are ideal candidates for the development of integrated culture. However, using *Sargassum* sp. to treat waste water from aquaculture has been newly applied by Liu et al. (2004) who designed a system in which *Sargassum enerve* reduced the TAN and NO<sub>3</sub><sup>-</sup> concentrations from 80 µM l<sup>-1</sup> to 20 µM l<sup>-1</sup>. Therefore, the present study assists in a better understanding of the effects of the integration of *Sargassum* sp. species with western king prawn culture in terms of nutrient flows, nutrient removal and the performances of the cultured species.

The nitrogen metabolite analysis in the present study suggests that  $\text{NO}_2^-$  could have been accumulating in the culture system probably due to incomplete nitrification and the kinetic reaction could have been controlled by ammonia oxidation over the study period (Timmons et al., 2002). The increase in the offloading of the metabolite nitrogen at day 28 of the study was probably caused by the decaying thallus of the seaweed (Jones, 1999). This explained the reason of the high concentration of TN from day 28 until the end of the study.

In the present study, *Sargassum* sp. was more efficient at removing  $\text{NO}_3^-$  than TAN. Similarly, green seaweed *Codium fragile* (Hanisak and Harlin, 1978) and *Chaetomorpha linum* (Menéndez et al., 2002), brown seaweed *Laminaria groenlandica* (Harrison et al., 1986), *Laminaria saccharina* (Ahn et al., 1998) and red seaweed *Porphyra yezoensis* (Hafting, 1999) removed  $\text{NO}_3^-$  more efficiently than TAN. There is evidence to suggest that under conditions of high  $\text{NO}_3^-$  and low salinity, seaweed is able to metabolise  $\text{NO}_3^-$  more rapidly (Lartigue and Sherman, 2006, Karmer and Fong, 2000, Karmer and Fong, 2001). In addition, for some macroalgal species, ammonium is a less favourable source of nutrients than nitrate because, during daylight periods, the pH inside the photosynthetic algae increases thereby reducing the pH of the external medium which leads to higher levels of volatile ammonium (Menéndez et al., 2002).

The removal efficiency of total nitrogen (TN) by *Sargassum* in the present study was generally higher than the values previously reported in literature. For example, *Gracilaria tikvahiae* only removed approximately 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).

Although phosphorus does not constitute a danger to fish or prawn culture, it contributes to the eutrophication process. In the absence of *Sargassum*, the  $\text{PO}_4^{3-}$  and TP concentration remained relatively high and was probably caused from the uneaten feed and excretion by the prawns in monoculture systems (Buschmann et al., 1996a). However, integrating *Sargassum* with prawn culture significantly reduced the concentration of phosphorus. Even though all of the *Sargassum* died at day 28 of the study, the concentration of  $\text{PO}_4^{3-}$  and TP in the integrated culture still remained relatively lower than in prawn monoculture.

Compared with the majority of other seaweeds, the performance of *Sargassum* in phosphate removal in this study was relatively high. For example, integrating *Gracilaria chilensis* and salmon culture resulted in the removal of 32% of the  $\text{PO}_4^{3-}$  from the fish farm (Buschmann et al., 1996b), less than 25% of the  $\text{PO}_4^{3-}$  from an integrated system by *Ulva lactuca* and *G. conferta* (Neori et al., 1998) and 27% of the phosphate from salmon cages by *G. chinensis* (Troell et al.,

1997). The finding in the present study therefore shows the potential ability of *Sargassum* to effectively reduce the phosphorus concentration when integrated with prawn culture.

Overall, this study indicates that *Sargassum* probably absorbed dissolved nutrients in the integrated system and thereby significantly reduced the concentrations of nutrients (N and P) in the waste water.

### ***Nutrient budget***

#### *Nitrogen*

Feed is normally the greatest source of nitrogen in fish or prawn ponds, approximately 92% of N as feed in intensive prawn culture (Briggs and Funge-Smith, 1994). In this study, feed was also the major source of nitrogen in both PM and ISP. However, the amount of nitrogen assimilated into prawn biomass is a minor fraction (17.69% and 18.99%) of the total N applied as feed. This result is in close agreement with the findings of Funge-Smith and Briggs (1998) in Thailand, Jackson et al. (2003) in Australia and Lemonnier and Faninoz (2006) in New Caledonia who reported that about 18-27% of input nitrogen was assimilated into prawn tissue in an intensive prawn system.

Differences in study conditions or techniques may explain the differences in contribution of nitrogen sources. Teichert-Coddington et al. (2000) and Shahidul Islam et al. (2004) conducted their studies in ponds, while the present study was conducted in the laboratory. During the study, although no nutrients were added to the SM, the high proportion (28.99%) of nitrogen lost through the water was recorded at the end of the study, probably due to the nitrogen leaching from decomposing dead seaweed. This resulted in a decline in water quality (Qian et al., 1996). Eventually, nitrogen could escape to the atmosphere in gaseous forms after denitrification process was completed.

According to Briggs and Funge-Smith (1994), a significant proportion of the nitrogen output is found in the wastes after each culture cycle. When calculating nitrogen budgets for fish or prawn ponds, denitrification and diffusion process are two potential losses of N to the atmosphere and are rarely measured directly (Briggs and Funge-Smith, 1994, Hopkins et al., 1995, Jackson et al., 2003). Denitrification is the reduction of nitrate ( $\text{NO}_3^-$ ) to gaseous  $\text{N}_2$ . Volatilisation is transmission of  $\text{NH}_3$  from the water column to the atmosphere. Therefore in most studies, including the present one, these factors are estimated indirectly as the difference between the nitrogen inputs and outputs. In the present study, the large amount of nitrogen in the SM was unaccounted for, probably due to nitrogen lost to the atmosphere as  $\text{N}_2$  or ammonia (Funge-Smith and Briggs, 1998). In contrast, only small fraction of the input nitrogen in the PM and ISP treatments was unaccounted for in this study. This is probably due to losing nitrogen to

the atmosphere via denitrification or volatilization of ammonia (Briggs and Funge-Smith, 1994, Martin et al., 1998, Teichert-Coddington et al., 2000, Jackson et al., 2003).

### *Phosphorus*

The contribution of feed to phosphorus input in the present study was observed to be the greatest source of phosphorus to the prawn tanks of both PM and ISP treatments. This is in close agreement with Funge-Smith and Briggs (1998) and Teichert-Coddington et al. (2000) who reported feed as the principal source of phosphorus in prawn culture. At the conclusion of the study, around 13-14% of the phosphorus input as feed was incorporated into prawn biomass in both PM and ISP. These results were comparable with those of Briggs and Funge-Smith (1994) and Shimoda et al. (2005), indicating that only 13% of the feed input of phosphorus to be incorporated into the prawn bodies at harvest.

According to Munsiri et al. (1995), phosphorus accumulates mostly in the wastes over time. In this study, the budget figures have shown that the wastes accounted for a major proportion of P loss in the prawn tanks. In this study, a small proportion of phosphorus in waste water was observed in PM (14.02%) and ISP (22.91%). This was expected, as waste water from aquaculture ponds always contains much less phosphorus than the amount added in feeds because most of the phosphorus is lost to the solid wastes (Boyd and Tucker, 1998). A minor proportion of input phosphorus was unaccounted for in the budget (1-5%), probably because of losing phosphorus through leaching.

### *N, P and N:P ratio in prawn and seaweed tissue*

The results of this study indicate that the presence of *Sargassum* in prawn culture does not alter the assimilation of N and P into prawn biomass. The results of this study are similar to previous studies by Briggs and Funge-Smith (1994) and Teichert-Coddington et al. (2000) who found that the nitrogen and phosphorus contents were 11.5% and 1.19% in black tiger prawns (*P. monodon*), and 11.2% and 1.25% in white leg prawns (*P. vannamei*), respectively. It is expected that, most of the nitrogen and phosphorus stripped from the water within an integrated culture system be accounted for by subsequent gains in macroalgae biomass.

The N:P ratio for optimal seaweed growth is within the range of 13-15. A N:P ratio < 13-15 indicates N limitation and a N:P > 13-15 indicates P limitation. The N:P ratio is also an indicator used to assess the efficiency in the removal of nutrients from the aquaculture system. The initial N:P ratio of *Sargassum* used in this study was 9.87, thereby indicating N limitation. Presumably, this explains the rapid uptake of P observed after day 7 of the study. Unfortunately, in the present study, the tissue N and P content was lost due to the death of the seaweed at day 28, the tissue nitrogen and phosphorus contents were released to the tanks water and the total nitrogen

and phosphorus biomass of seaweed produced was not determined at the end of the study.

Therefore, further studies about this problem should be conducted in the future.

In conclusion, *Sargassum sp.* can be a potential seaweed species in integrating culture with prawns in terms of utilizing nutrient sources and improving the water quality for aquaculture activities. The findings of this study are considered as a reference for further studies about using *Sargassum sp.* for integrating with prawn culture.

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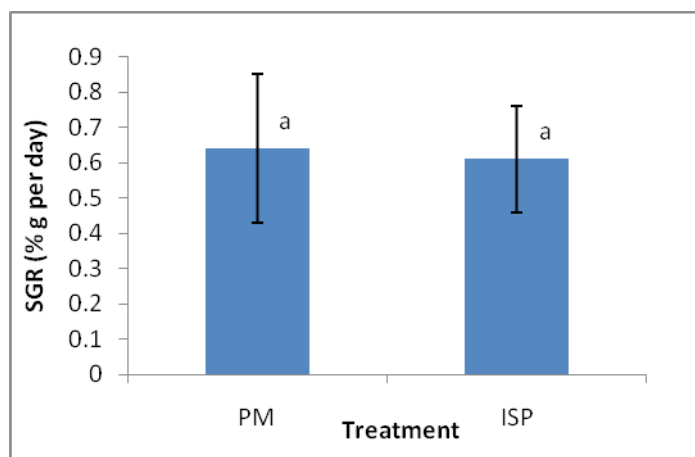


Figure 1. Specific growth rate of prawn biomass in treatments (mean  $\pm$  S.E.).

Note: PM = Prawn monoculture; ISP = Integrated seaweed-prawn culture. *Same letters denote no significant difference ( $p > 0.05$ ).*

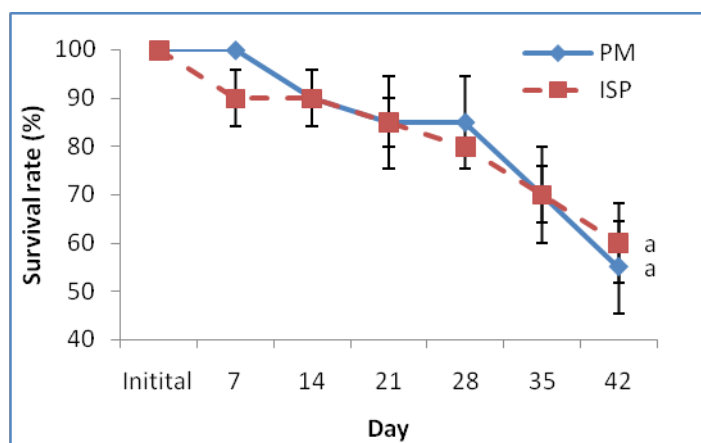


Figure 2. Survival rate of prawn in treatments (means  $\pm$  S.E.).

Note: PM = prawn monoculture; ISP = integrated seaweed-prawn culture. *Same letters denote no significant difference ( $p > 0.05$ ).*

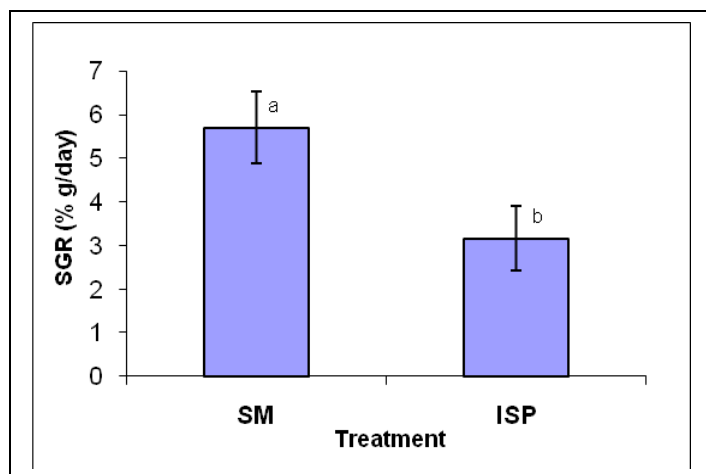


Figure 3. Specific growth rate of seaweed in the treatments (mean  $\pm$  S.E.). Note: SM = Seaweed monoculture; ISP = Integrated seaweed-prawn culture. Different letters (a,b...) denote significant difference ( $p < 0.05$ ). Note: Biomass of seaweed in the first week of the study.

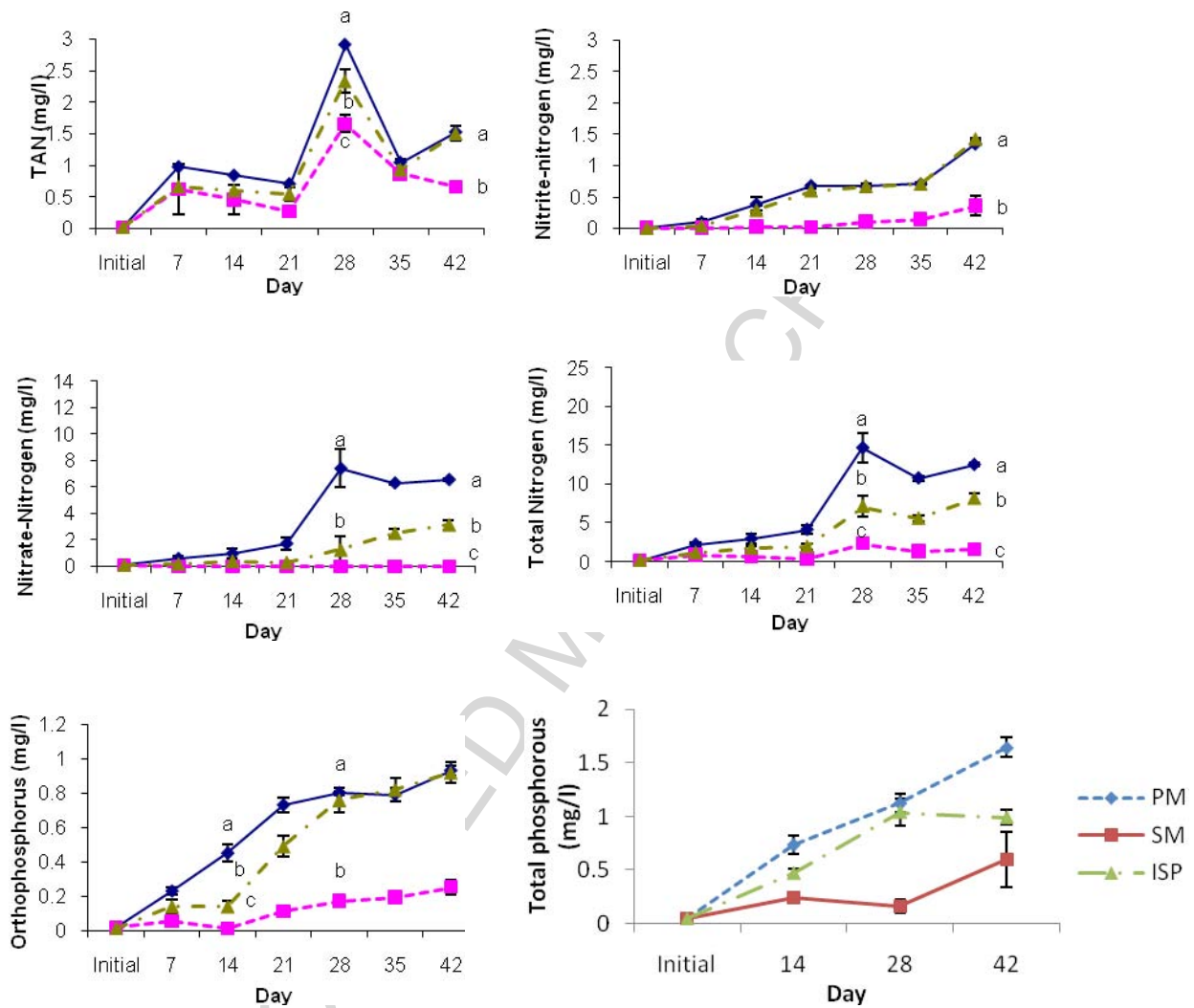


Figure 4. Water quality parameters in treatments recorded over the experimental period (mean  $\pm$  SE).

Note: PM = Prawn monoculture; SM = Seaweed monoculture; ISP = Integrated seaweed-prawn culture. Different letters denote a significant difference between treatments ( $p < 0.05$ ).

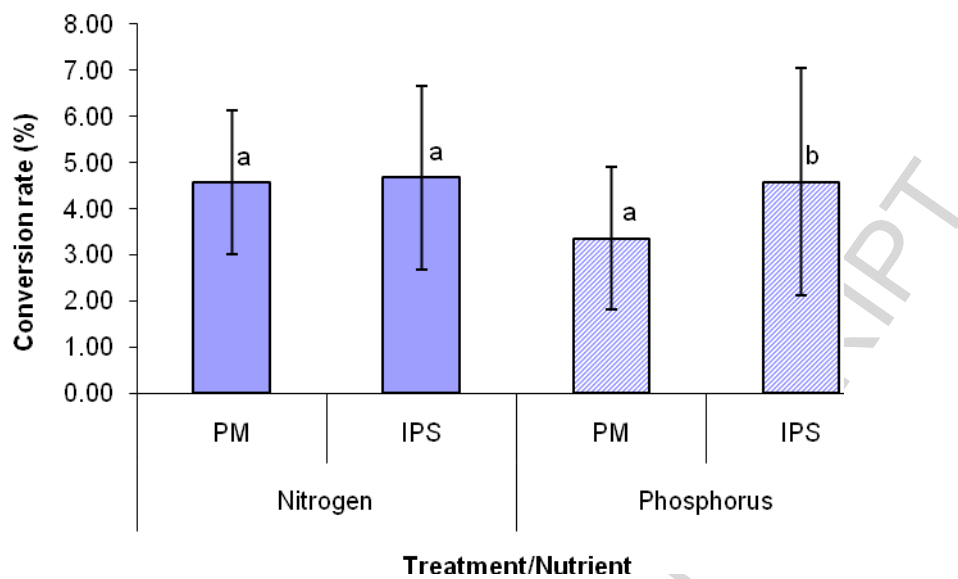


Figure 5. Nitrogen and phosphorus conversion rates from feed into western king prawn (mean  $\pm$  S.E.).

Note: PM = Prawn monoculture. ISP = Integrated seaweed-prawn culture. *Same letters denote no significant difference ( $p > 0.05$ ).*

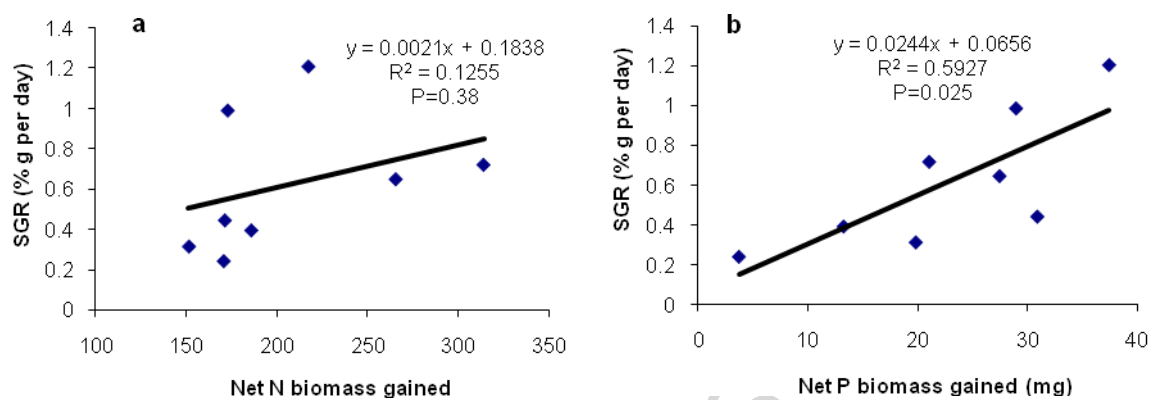


Figure 6. Relationship between specific growth rate (SGR) with net N biomass gained (a) and with net P biomass gained (b) in culture during the experiment.



Table 1. Removal rates (%) of nutrients in integrated western king prawn and *Sargassum* sp. culture systems (mean  $\pm$  S.E.).

Variables	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
TAN	29.21 $\pm$ 7.50 <sup>a</sup>	27.78 $\pm$ 1.30 <sup>a</sup>	24.96 $\pm$ 10.13 <sup>a</sup>	20.75 $\pm$ 9.31 <sup>a</sup>	15.96 $\pm$ 5.12 <sup>a</sup>	1.31 $\pm$ 0.92 <sup>b</sup>
NO <sub>2</sub> <sup>-</sup>	55.58 $\pm$ 6.79 <sup>a</sup>	24.10 $\pm$ 10.53 <sup>b</sup>	12.87 $\pm$ 6.43 <sup>b</sup>	nd	nd	nd
NO <sub>3</sub> <sup>-</sup>	68.10 $\pm$ 7.32 <sup>a</sup>	61.79 $\pm$ 11.03 <sup>a</sup>	72.19 $\pm$ 16.56 <sup>a</sup>	75.04 $\pm$ 10.25 <sup>a</sup>	60.37 $\pm$ 5.30 <sup>a</sup>	52.50 $\pm$ 5.32 <sup>a</sup>
TN	43.76 $\pm$ 6.77 <sup>a</sup>	37.42 $\pm$ 8.53 <sup>a</sup>	48.75 $\pm$ 14.58 <sup>a</sup>	61.94 $\pm$ 6.21 <sup>b</sup>	48.02 $\pm$ 3.17 <sup>a</sup>	34.68 $\pm$ 5.87 <sup>a</sup>
PO <sub>4</sub> <sup>3-</sup>	39.51 $\pm$ 15.67 <sup>a</sup>	65.85 $\pm$ 9.11 <sup>b</sup>	32.70 $\pm$ 9.30 <sup>a</sup>	5.62 $\pm$ 3.54 <sup>c</sup>	nd	nd
TP	24.29 $\pm$ 3.29 <sup>a</sup>	32.77 $\pm$ 11.48 <sup>a</sup>	27.62 $\pm$ 7.72 <sup>a</sup>	20.81 $\pm$ 3.35 <sup>a</sup>	14.47 $\pm$ 3.32 <sup>a</sup>	37.05 $\pm$ 5.57 <sup>a</sup>

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

Note: TAN = Total ammonium nitrogen, DIN = Dissolved inorganic nitrogen, TN = Total nitrogen, TP = Total phosphorus, nd = not detectable.

Table 2. N and P concentrations in prawn and seaweed tissue and N:P ratios at the initial and the end of the study (mean  $\pm$  S.E.).

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

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

Table 3. Nitrogen and phosphorus budget for treatments in means  $\pm$  S.E. (mg per 100L).

Treatments	Prawn monoculture		Seaweed monoculture		Integrated seaweed and prawns	
	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrogen	Phosphorus
<i>Inputs</i>						
Water	223.04 $\pm$ 0.09	3.60 $\pm$ 0.03	223.04 $\pm$ 0.09	3.60 $\pm$ 0.03	223.04 $\pm$ 0.09	3.60 $\pm$ 0.03
(%)	(12.09 $\pm$ 0.57)	(1.39 $\pm$ 0.07)	(59.26 $\pm$ 2.90)	(4.47 $\pm$ 0.45)	(10.11 $\pm$ 0.62)	(1.15 $\pm$ 0.07)
Prawns	475.46 $\pm$ 38.20	83.27 $\pm$ 6.69			436.25 $\pm$ 32.79	76.40 $\pm$ 5.74
(%)	(25.63 $\pm$ 1.81)	(32.05 $\pm$ 2.21)			(19.53 $\pm$ 0.80)	(24.16 $\pm$ 1.31)
Seaweed			156.37 $\pm$ 20.59	80.12 $\pm$ 10.55	499.78 $\pm$ 43.01	76.24 $\pm$ 5.74
(%)			(40.74 $\pm$ 2.90)	(95.53 $\pm$ 0.45)	(22.84 $\pm$ 2.91)	(24.59 $\pm$ 3.54)
Feed	1158.67 $\pm$ 76.75	173.33 $\pm$ 11.48			1074.87 $\pm$ 147.12	160.80 $\pm$ 22.01
(%)	(62.27 $\pm$ 1.99)	(66.55 $\pm$ 2.23)			(47.53 $\pm$ 0.82)	(50.10 $\pm$ 3.13)
Total	1857.16	260.20	379.41	83.72	2233.93	317.03
(%)	(100)	(100)	(100)	(100)	(100)	(100)
<i>Outputs</i>						
Water	570.67 $\pm$ 70.26	36.13 $\pm$ 0.88	113.07 $\pm$ 13.46	24.85 $\pm$ 3.07	463.80 $\pm$ 15.24	72.42 $\pm$ 5.78
(%)	(30.52 $\pm$ 2.95)	(14.02 $\pm$ 0.88)	(28.99 $\pm$ 6.08)	(30.54 $\pm$ 4.63)	(20.93 $\pm$ 1.01)	(22.91 $\pm$ 1.44)
Prawns	681.64 $\pm$ 50.07	108.14 $\pm$ 7.83			642.27 $\pm$ 63.28	97.18 $\pm$ 4.65
(%)	(36.66 $\pm$ 1.64)	(41.74 $\pm$ 3.07)			(28.60 $\pm$ 1.12)	(30.82 $\pm$ 1.07)
Seaweed			49.42 $\pm$ 20.88	12.43 $\pm$ 5.30	388.35 $\pm$ 74.32	18.59 $\pm$ 2.99
(%)			(12.36 $\pm$ 4.51)	(13.45 $\pm$ 3.75)	(17.93 $\pm$ 3.77)	(5.79 $\pm$ 0.63)
Sediment	513.61 $\pm$ 22.60	111.61 $\pm$ 11.52	31.72 $\pm$ 4.48	42.21 $\pm$ 4.67	531.16 $\pm$ 90.59	113.14 $\pm$ 8.98
(%)	(27.81 $\pm$ 1.65)	(42.63 $\pm$ 2.86)	(8.35 $\pm$ 1.13)	(50.92 $\pm$ 3.57)	(23.42 $\pm$ 2.64)	(35.67 $\pm$ 0.94)
Unaccounted	91.24 $\pm$ 12.53	4.32 $\pm$ 1.16	185.20 $\pm$ 31.89	4.23 $\pm$ 0.50	208.34 $\pm$ 45.43	15.72 $\pm$ 4.75
(%)	(5.00 $\pm$ 0.79)	(1.62 $\pm$ 0.40)	(50.30 $\pm$ 10.66)	(5.09 $\pm$ 0.41)	(9.12 $\pm$ 1.38)	(4.81 $\pm$ 1.21)
Total	1857.16	260.20	379.41	83.72	2233.93	317.03
(%)	(100)	(100)	(100)	(100)	(100)	(100)