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Bioremediation Potential of the Brackishwater Macroalga *Gracilaria tenuistipitata* (Rhodophyta) Co-cultured with Pacific White Shrimp *Penaeus vannamei* (Boone)

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ABSTRACT

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The rapid development of intensive shrimp culture has aroused increased concerns about its impact on coastal waters. Practicing propensity of a balanced ecosystem based culture approach is the best way towards sustainability. In this context, seaweed based co-culture finds significance but evaluation of its symbiotic nature needs to be ascertained. Accordingly, an outdoor trial was conducted to arrive at the effective biomass intensity of seaweed *Gracilaria tenuistipitata* for efficient bioremediation as well as growth and survival of *Penaeus vannamei*. An experiment with five treatments (different biomass intensity, (0, 0.5, 1.5, 2.5 and 3.5 g L^{-1}) revealed that at a biomass intensity of 3.5 g L^{-1}, NH_4 – N and PO_4 – P significantly ($p < 0.05$) reduced by 95.71% and 95.74%, respectively in three weeks. It was also observed that turbidity was significantly lowest (0.94 NTU) and specific growth rate was significantly higher (2.86% d^{-1}) at the same biomass intensity (3.5 g L^{-1}). Average body weight and specific growth rate of *P. vannamei* were not significantly increased but survival (99.17%) was significantly ($p < 0.05$) higher. Total bacterial count was also significantly ($p < 0.05$) reduced. This study revealed that seaweed (3.5 g L^{-1}) – shrimp when co–cultured, improves the water quality and has bioremedial benefits in the culture system.

ADDITIONAL INDEX WORDS: *Biomass, bioremediation, brackishwater, co-culture,* Gracilaria tenuistipitata, Penaeus vannamei.

INTRODUCTION

Shrimp aquaculture has expanded rapidly in the entire world, especially in tropical areas. Shrimp ponds have traditionally been constructed near estuarine zones, creating a concern related to environmental impacts (Lombardi *et al*., 2006). Cost-effective technologies for high shrimp yield with minimal environmental impact are necessary for the sustainable expansion of the shrimp aquaculture (Tacon *et al*., 2002). In India, shrimp culture has been on an increase since 2009, after the introduction of the species *Penaeus vannamei* with production levels of 10–12 tonnes/ha/crop within 135 days duration (Laxmappa, 2016). The production of this species has reached to a level of 4,06,018 tonnes during 2015–16 (MPEDA, 2016). This rapid expansion of intensive mariculture system has led to conduct research on mitigation of environmental impacts including seaweed-based integrated techniques. Seaweeds can significantly absorb waste nutrients, control eutrophication and consequently improve the health and stability of marine ecosystems and promote a sustainable aquaculture (Buschmann, Troell, and Kautsky, 2001; Chopin *et al*., 2001; Fei, 2004; Neori *et al*., 2004; Mao, Yang, and Wang, 2005; Troell *et al*., 2003; Yang *et al*., 2005).

The physiological responses and the biofiltering ecological functions of seaweeds have been studied in different culture systems, namely shrimp culture ponds (Jones, Dennison, and Preston, 2001; Nelson *et al*., 2001), fish cage farms (Hayashi *et al*., 2008; Troell *et al*., 1997; Zhou *et al*., 2006;) and Integrated Multitropic Aquaculture (IMTA) systems containing finfish, shellfish and seaweed (Chow *et al*., 2001; Neori, Shpigel, and BenEzra, 2000; Shen *et al*., 2007; Shpigel and Neori, 1996). However, the determination of the exact role of seaweed for enhancement of production in shrimp farms is still in the experimental phase (Portillo-Clark *et al*., 2012).

Rhodophyta (red seaweed) are particularly efficient at absorbing nutrients rapidly and have mechanisms for storing large reserves of nutrients (Vergara, Niell, and Torres, 1993). The red seaweed, *Gracilaria* is distributed worldwide, but grows mostly in tropical and subtropical water bodies. The genus *Gracilaria* includes more than 100 species (Bird, Hanisak, and Ryther, 1984). *Gracilaria edulis* rapidly assimilates NH_4^+ (Jones, Stewart, and Dennison, 1996).

Apart from bioremediation potential; *Gracilaria* has economic importance as an agarophyte and as a food for humans

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and marine animals (Yang *et al*., 2015). Several *Gracilaria* spp. have formed the basis of multi-million dollar agar and agarose phycocolloid industry throughout the world (Johnson *et al*., 2014). In India, 32 marine *Gracilaria* spp. have been recorded so far, and the main species cultivated is *Gracilaria edulis* (Kalimuthu *et al*., 1990).

Gracilaria tenuistipitata, which is available in tropical brackishwater bodies of Chennai region, such as Muttukadu lagoon, was taken as a component for the experiment with *Penaeus vannamei*. The aim of this study was to evaluate the effect of seaweed *G. tenuistipitata* on the water quality and growth of *P. vannamei* and to determine the optimum seaweed biomass intensity in an intensive system.

METHODS

Seaweed (*G. tenuistipitata*) was collected from Muttukadu lagoon (12°48′42.1″N and 80°14′39″E). After collection, the macroalgae was transferred to the laboratory and cleaned to remove epiphytes and encrusting organisms (Tsutsui *et al*., 2010). Thereafter, they were placed in a container containing filtered saline water until the start of the experiment. *P. vannamei* was obtained from a local shrimp culture farm. Prior to start of the experiment, the shrimps were acclimatized to experimental water salinity condition.

Experimental Design

A 21-day experiment was carried out using juveniles of *P. vannamei* from November to December 2018 at Muttukadu Experimental Station, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India. The experiment in a completely randomized design included different seaweed biomass intensity as treatment in five intensities (0 (control), 0.5, 1.5, 2.5 and 3.5 g L^{-1}) with triplicates. Fifteen 100 litre rectangular FRP tanks were filled with saline water (25 ppt), filtered through a 0.5-µm sieve and maintained under constant aeration through perforated air stones. The experiment was conducted under transparent plastic shades where the intensity of light was 3000 lux with a 12-h light/dark photoperiod.

Stocking and Feeding Management for Shrimp

Seaweed was stocked in the tanks according to the biomass intensity for five treatments in 100-litre water. Forty shrimps (stocking density - 120/m^2) with an average body weight of 0.25 ± 0.08 g were kept in each replicate tank for all treatments. Each tank was covered with a small mesh net structure to avoid escape of shrimps from tanks. Shrimp were fed with a commercially available crumble feed (composition: 43% crude protein, 6% ether extract, 6% moisture, 3% crude fibre, 12% mineral matter, 3% calcium and 1.45% phosphorus) and equally distributed in all experimental tanks thrice daily at 0800, 1200 and 16:00 h. Feed was provided initially at 10% of the biomass of shrimp and adjusted daily based on the estimated shrimp consumption and left over feed.

Assessment of Water Quality Parameters

Water quality of the experimental system was checked at weekly intervals in the morning and afternoon. Water temperature (using mercury thermometer), pH and dissolved oxygen (DO) were determined and recorded using probes (Orion 9107 BNMD for pH and Lutron DO – 5510 for dissolved oxygen). Salinity was checked with a hand refractometer and was maintained as 25 ppt throughout the experimental period. The water samples (in triplicate) were collected and analyzed for turbidity (Thermo Scientific Eutech, TN – 100 turbidimeter), ammonium (NH_4 – N) (Phenol hypochlorite method), nitrite (NO_2 – N), nitrate (NO_3 – N) and orthophosphate (PO_4 – P) following APHA (1998).

Estimation of Bacterial Biomass

Total heterotrophic bacterial count of water was determined at the end of the experiment. Water samples were collected in sterile polypropylene bottles from the centre of the tank. The samples were maintained at 4°C and immediately brought to the laboratory.

Tenfold serial dilution was made in normal saline solution (NSS) and 0.1 ml of appropriate dilution was plated in triplicate on Zobell marine agar (HiMedia, Mumbai, India) for total count. Plates were incubated at room temperature for 24 to 48 hours and colonies were counted and expressed as bacterial colony forming unit (cfu) (Musa and Wei, 2008).

Estimation of Nutrient Removal Percentage and Growth Parameters

Nutrient removal percentage (NR%) for ammonium (NH_4 – N) and orthophosphate (PO_4 –P) in the systems was estimated by the following equation (Zhou *et al*., 2006):

$$\text{Nutrient Removal Percentage (NR \%)} = 100 \times (C_{cnl} - C_p) / C_{cnl} \quad (1)$$

Where, C_{cnl} = nutrient concentration in the control treatment (mg L^{-1});
C_p = nutrient concentration in the seaweed treatment (mg L^{-1}) at particular time since beginning.
At the end of the experiment, the seaweed in each tank was weighed, and their growth was estimated using the formula after Rosenberg, Probyn, and Mann, 1984;

$$\text{Specific Growth Rate (SGR \%)} = \text{In} (W_f / W_0) / t \times 100 \quad (2)$$

Where, SGR = specific growth rate (% d^{-1});
W_0 is initial wet weight (g);
W_f is final wet weight (g) at time t since the beginning.

Statistical Analysis

The data were analyzed by statistical package, SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). To determine the difference among treatments for turbidity, NH_4 – N, PO_4 – P throughout the experimental period and total bacterial count, nutrient removal percentage, growth parameters for last week of the experiment, among different treatments the parameters were subjected to ANOVA and if found significant, Tukey's HSD test was deployed at 5% level of significance. Pearson correlation between different biomass intensity and NH_4 – N, PO_4 – P concentrations and its significance ($\alpha = 0.05$) was studied.

RESULTS

This experiment helped to arrive at the results on four important aspects, namely water quality improvement, control of

microbial population, growth of seaweed with nutrient assimilation, growth and survival of shrimp in the co–culture system with seaweed.

Water Quality Parameters

Temperature and salinity in the experimental tanks ranged from 27.9-29ºC and 25-28 ppt, respectively. Dissolved oxygen (DO) did not vary among the treatments (with the concentration ranging between 6-7 mg L^{-1}) because continuous aeration was provided to the experimental tanks. The pH ranged between 8.20-8.45 and mean pH value was found slightly higher in seaweed tanks compared to control tanks (0 g L^{-1}), however, there was no significant difference between treatments for this parameter (Table 1). Turbidity was significantly reduced by co-culture. It was observed that higher the seaweed biomass intensity, lesser was the turbidity value but optimum reduction of turbidity was obtained between 2.5–3.5 g L^{-1} biomass intensity. The effect was prominent after two weeks, as average turbidity was observed to be the highest (> 30 NTU) at 0 g L^{-1} group (Figure 1).

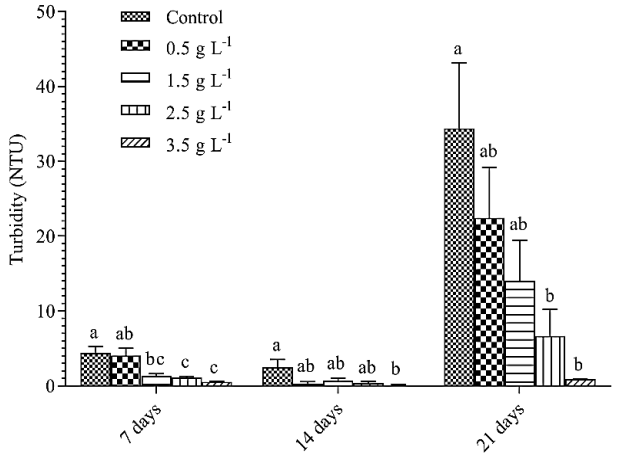

Figure 1. Turbidity levels (mean±SE, n=3) during the co–culture experiment with different seaweed biomass intensity. (Means with superscripted letters indicate significant difference (p <0.05) between treatments for different seaweed biomass intensity).

The concentration of two nutrients tested weekly (NH_4-N and PO_4-P) were analyzed at different biomass intensity levels every week. The NH_4 - N level was significantly different between seaweed biomass treatments. It was also observed that there exists a negative relation between biomass intensity and NH_4-N level (Figure 2). The NH_4-N concentration (< 0.5 mg L^{-1}) was observed to be significantly lower at 3.5 g L^{-1} biomass intensity within 7 days, whereas in control it was > 2.5 mg L^{-1}. A similar trend was noticed at 14 and 21 days for the same biomass intensity of 3.5 g L^{-1} with the lowest concentration (< 0.2 mg L^{-1}), whereas in control it was > 3 mg L^{-1} and > 2 mg L^{-1}, respectively (Figure 2). The concentration of NO_2 - N and NO_3 - N was found to increase after 7 days of the experiment and was significantly lower in the treatment of higher biomass intensity (2.5 to 3.5 g L^{-1}) compared to other treatments.

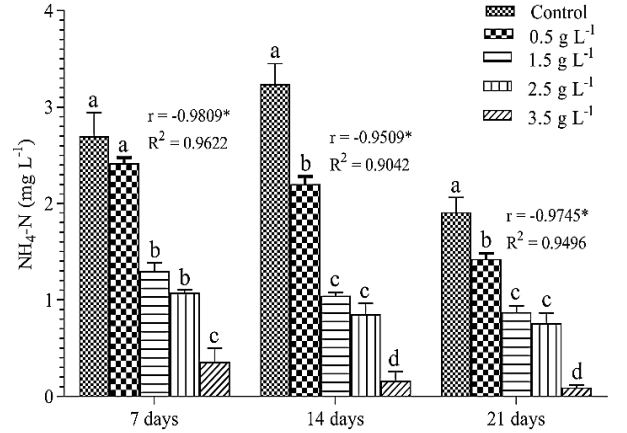

Figure 2. NH_4 – N concentration (mean±SE, n=3) during the co–culture experiment with different seaweed biomass intensity (Means with superscripted letters indicate significant difference (p <0.05) between treatments for different seaweed biomass intensity) (Pearson correlation r is given and * indicates p <0.05).

At 14 days, concentration of both nutrients was not significantly different for varying biomass intensities (Table 2). The PO_4 – P concentration (> 0.5 mg L^{-1}) increased after 7 days of culture and by 14 days, it was at its peak (> 1 mg L^{-1}) and thereafter, it reduced (< 1 mg L^{-1}) by 21st day. Weekly values revealed that PO_4 – P concentration significantly reduced as seaweed biomass increased and highest assimilation was obtained at 3.5 g L^{-1} biomass intensity. It was observed that PO_4 – P concentration on 7th, 14th and 21st days was > 0.5, > 1 and < 1 mg L^{-1} in control, respectively, whereas it was < 0.2 mg L^{-1} at 3.5 g L^{-1} treatment (Figure 3).

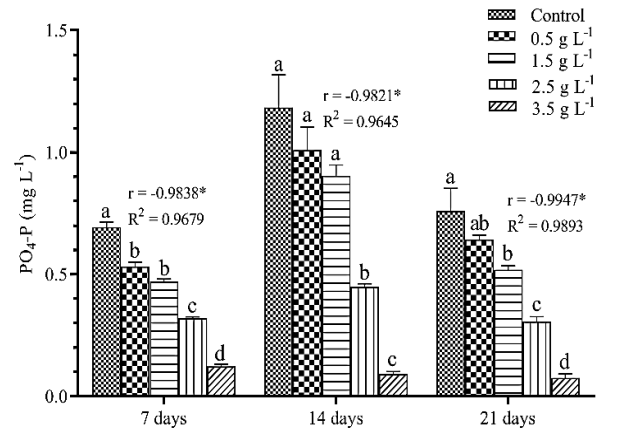

Figure 3. PO_4 – P concentration (mean±SE, n=3) during the co–culture experiment with different seaweed biomass intensity (Means with superscripted letters indicate significant difference (p <0.05) between treatments for different seaweed biomass intensity) (Pearson correlation r is given and * indicates p <0.05).

Table 1. *Water pH, total bacterial loads and nutrients removal % of seaweed (G. tenuistipitata) in the co–culture experiment with different initial seaweed biomass intensity*.

Treatments	pH	TBC (Log cfu)	NH_4 – N removal %	PO_4 – P removal %
Control (0 g L^{-1})	8.20±0.07^a	4.85±0.12^a	-	-
0.5 g L^{-1}	8.22±0.06^a	4.33± 0.20^{ab}	16.80± 2.78^a	25.50± 4.07^a
1.5 g L^{-1}	8.44± 0.02^a	4.42± 0.06^{ab}	51.40± 7.38^b	32.84± 4.40^a
2.5 g L^{-1}	8.44± 0.05^a	4.10± 0.06^b	71.72± 5.24^b	54.22± 3.97^b
3.5 g L^{-1}	8.45± 0.15^a	3.56± 0.07^c	95.71± 2.45^c	95.74± 3.70^c

Data represents mean ± S.E, n=3. Means with different superscript letters in the column differ significantly (p <0.05).

Table 2. *NO_2– N and NO_3– N concentration during the co–culture experiment with different initial seaweed biomass intensity*.

Days of culture (DOC)	Parameters (mg L^{-1})	Control (0 g L^{-1})	0.5 g L^{-1}	1.5 g L^{-1}	2.5 g L^{-1}	3.5 g L^{-1}
7	NO_2-N	0.064±0.004^a	0.053±0.009^a	0.066±0.016^a	0.057±0.007^a	0.004±0.002^b
	NO_3-N	0.222±0.020^a	0.363±0.067^a	0.301±0.057^a	0.276±0.053^a	0.001±0.0003^b
14	NO_2-N	0.754±0.194^a	0.959±0.028^a	0.978±0.010^a	0.858±0.121^a	0.975±0.010^a
	NO_3-N	2.562±0.063^a	2.537±0.057^a	2.513±0.008^a	2.028±0.423^a	1.426±0.491^a
21	NO_2-N	0.743±0.157^a	0.728±0.140^a	0.842±0.014^a	0.002±0.001^b	0.002±0.001^b
	NO_3-N	2.124±0.307^a	2.396±0.127^a	2.050±0.252^a	0.004±0.002^b	0.003±0.0006^b

Data value represents mean ± S.E, n=3. Means with different superscript letters in the row differ significantly (p <0.05).

Table 3. *Growth performance of seaweed (G. tenuistipitata) and shrimp (P. vannamei) in the co–culture experiment with different initial seaweed biomass intensity*.

Treatments	Seaweed	Shrimp		
	SGR (% d^{-1})	Final ABW (g)	SGR (% d^{-1})	Survival %
Control (0 g L^{-1})	-	1.13± 0.13^a	6.94± 0.96^a	70.67± 0.93^a
0.5 g L^{-1}	0.85± 0.18^a	1.34± 0.14^a	7.95± 0.22^a	75.00± 1.44^a
1.5 g L^{-1}	0.96± 0.02^a	1.37± 0.19^a	8.03± 0.44^a	85.00± 1.44^b
2.5 g L^{-1}	1.27± 0.15^a	1.07± 0.10^a	6.90± 0.09^a	92.33± 1.45^c
3.5 g L^{-1}	2.85± 0.35^b	1.15± 0.12^a	7.17± 0.48^a	99.17± 0.44^d

Data represents mean±SE, n=3. Means with different superscript letters in the column differ significantly (p <0.05).

Microbial Analysis

Total bacterial count was significantly higher in control and reduced successively with increase in biomass intensity. Similar results were observed between 0.5 and 1.5 g L^{-1} groups, the total bacterial count being significantly higher in 2.5 g L^{-1}. Bacterial count was found to be significantly lower in 3.5 g L^{-1} biomass intensity during the 3rd week of the experiment (Table 1).

Efficiency of Nutrients Removal (%) and Growth Performance of Seaweed

The NH_4 - N removal % was about 51.40 and 71.72% for 1.5 and 2.5 g L^{-1}, respectively in 21 days and significantly higher removal efficiency was found at 3.5 g L^{-1} (95.71%) biomass intensity compared to other treatments. This shows that the NH_4 - N removal increased with seaweed biomass intensity. Similar result was observed for PO_4 - P removal but significantly higher removal (> 50%) was obtained at 2.5 g L^{-1} biomass intensity compared to 0.5 - 1.5 g L^{-1} range. Optimum PO_4 - P removal (> 90 %) found at 3.5 g L^{-1} biomass treatment (Table 1). The SGR of seaweed was significantly higher (2.85% d^{-1}) at 3.5 g L^{-1} biomass intensity, whereas similar SGR was observed at 0.5 to 2.5 g L^{-1} biomass range (Table 3).

Growth Performance of Shrimp

The average body weight and specific growth rate of *P. vannamei* was not affected by different seaweed biomass intensities. Mean ABW and SGR of shrimp ranged between 1.13-1.37 g and 6.94-8.03% d^{-1}, respectively (Table 3). The survival of shrimp increased proportionately to the seaweed biomass intensity and significantly higher survival (99.17%) was obtained at 3.5 g L^{-1} biomass intensity compared to 0 g L^{-1} (70.67%) (Table 3).

DISCUSSION

During the experimental period, oxygen concentration remained above 6 mg L^{-1}, temperature above 27^{o}C, salinity above 25 ppt and pH above 8, and these factors did not limit the growth of *P. vannamei* (Van Wyk and Scarpa, 1999). In this study, pH was slightly higher in seaweed tanks during afternoon, but it had no effect on water quality for different seaweed biomass intensities ranging from 0 to 3.5 g L^{-1}. A similar observation was reported by Neori *et al.* (1996) in seaweed tanks where in the pH was nearly 0.5 units above that in the fish tanks and this difference was larger in the afternoon than in the morning. Turbidity was mainly observed at 3rd week of the experiment and it was very high in control due to phytoplankton and bacterial growth. Increment of seaweed biomass could effectively control turbidity and it was almost nil at 3.5 g L^{-1} biomass intensity because of least level of phytoplankton and bacterial growth. In this study, waste generated from shrimp was utilized by seaweed resulting in the control of growth of microalgae. Therefore, optimum proportion of biomass of *G.*

tenuistipitata could effectively utilize dissolved nutrients (mainly NH_4 - N and PO_4 - P) from the environment. A previous study revealed high nutrient bioremediation and assimilative capacity of *G. lemaneiformis* and its effectiveness to reduce nutrient loading from bivalve culture (Mao *et al*., 2009). This study revealed that shrimp aquaculture could greatly increase nutrient (NH_4 - N and PO_4 - P) levels in the water column. In the shrimp control tanks, the NH_4 - N and PO_4 - P concentrations in water increased from the initial day to 14 days of culture and at 21 days, it was reduced. Although, weekly analysis of water revealed higher levels of NH_4 - N and PO_4 - P in control, the concentration of both nutrients were significantly less in seaweed tanks. There was a negative relationship between biomass and NH_4 - N and PO_4 - P (Figure 2 and 3) concentrations in water and 3.5 g L^{-1} seaweed biomass could be appropriate to reduce significant quantity of nutrients among other treatments. An earlier study revealed that higher seaweed stocking could increase the potential for nutrient uptake because of proper proportion of biomass and the higher surface area of seaweed (DeBusk, Blakeslee and Ryther, 1986; Lapointe and Tenore, 1981; Neori *et al*., 2004). Another study was conducted by Anaya-Rosas *et al*. (2019), who reported that *G. vermiculophylla* @ 2 - 4 g L^{-1} stocking with *P. vannamei* could reduce remarkable quantities of NH_4 - N compared to control (without seaweed). The excretion of NO_2 - N and NO_3 - N has commonly been neglected or found to be insignificant during 14 days but at 21 days of culture, it was found significantly lesser for NO_2 - N and NO_3 - N concentrations at 2.5 - 3.5 g L^{-1} seaweed biomass range than in other treatments. Similar results have been reported by Mao *et al*. (2009) for seaweed (*G. lemaneiformis*) and scallop (*Chlamys farreri*) for integrated multi-trophic aquaculture and by Anaya-Rosas *et al*. (2019). They reported that co-culture of shrimp (*P. vannamei*) with seaweed (*G. vermiculophylla* and *D. dichotoma*) @ 2 - 4 g L^{-1} biomass reduced significant amounts of NO_2 - N compared to control (without seaweed) but there was no significant difference of NO_3 - N concentration between control and treatments.

Heterotrophic microbial biomass was minimal at 3.5 g L^{-1} treatment and decreased generally with the seaweed intensity in all treatments. This could be explained by the NH_4 - N concentration in water. Lesser the concentration of NH_4 - N, lowers the heterotrophic bacterial counts in the water column. The lowest NH_4 - N concentration was obtained in 3.5 g L^{-1} treatment resulting in the lowest microbial load in water. This phenomenon was explained by Francis-Floyd *et al*. (2012) in their study on ammonia in aquaculture system. Chances of bacterial infections get enhanced in the fishes (shrimps) exposed to low levels of ammonia over time which may favor the growth of bacterial biomass in water. Ammonia when present in higher concentrations in water is toxic to the animals.

The NH_4 - N and PO_4 - P removal increased with the seaweed intensity within 3 weeks of cultivation. Both NH_4 - N and PO_4 - P removal efficiency reached its peak in treatment 3.5 g L^{-1}. Many studies have shown that seaweeds can assimilate ammonium and phosphate generated by fish culture (Zhou *et al*., 2006). For instance, *Gracilaria edulis* removed around 95% of ammonium originating in shrimp farming (Jones, Dennison, and Preston, 2001). Buschmann *et al*. (1996) reported that *G. chilensis* was capable of removing 95% ammonium and 32% orthophosphate from integrated system (seaweed/salmon). Neori, Ragg and Shpigel (1998) found that *G. corticata* removed 34% of ammonium and about 25 % of orthophosphate from a polyculture system (mollusc/fish/seaweed). Martinez-Aragon *et al*. (2002) reported that *G. gracilis* removed around 93 % and 62.2% of ammonium and orthophosphate, respectively. In this study, maximum NH_4 - N and PO_4 - P removal efficiency were 95.71% and 95.74%, respectively during 21 days of co-culture.

In this study, the SGR of the thallus in the co-culture treatments showed no significant difference in 0.5 to 2.5 g L^{-1} treatments, whereas maximum SGR was obtained at 3.5 g L^{-1} biomass intensity and it was 3% d^{-1}. Due to the highest level of nutrients (NH_4 - N, NO_2 - N, NO_3 - N and PO_4 - P) assimilation in treatment 3.5 g L^{-1}, optimal growth was obtained. Many workers reported similar observations in an integrated aquaculture system. Rodrigueza and Montano (2007) evaluated the growth of *Kappaphycus alvarezii* in tanks containing fish (*Chanos chanos*) effluent and observed an increase in the growth rate compared to control (cultivated only in seawater). Lombardi *et al*. (2006) also observed an increase in biomass of *K. alvarezii* when co-cultivated with *P. vannamei* in cages, thereby demonstrating that shrimp effluents cause no damage in seaweed development. Zhou *et al*. (2006) reported increasing growth rate (3.73 - 4.49% d^{-1}) in an integrated system with seaweed (*Gracilaria lemaneiformis*) and fish (*Sebastodes fuscescens*) in a laboratory experiment in tanks.

The average body weight and specific growth rate of *P. vannamei* were similar in control and co-culture treatments. Seaweed biomass of 1.5–3.5 g L^{-1} range was not a hindrance for growth of shrimp during the entire period of the study rather it helped to improve survival of shrimp even at a high stocking density. In this study, maximum survival (99.2 %) was observed at 3.5 g L^{-1} co-culture treatment. An almost similar survival rate (98 %) was reported in an integrated multi-trophic aquaculture system developed with seaweed *G. tikvahiae* and *P. vannamei* (Samocha *et al*., 2015). It was recommended by Wu *et al*., 2015 that the high-temperature adapted macroalgae such as *Gracilaria tenuistipitata* should be introduced into integrated system with culture of marine aquatic animals for removing nutrients released by these mariculture practices. Thus, the species can be used to balance nutrients produced by shrimp farming in an IMTA system when it is in the estimated optimal co-cultivation proportion.

CONCLUSIONS

The results of the study suggest that *G. tenuistipitata* had remarkable nutrient bioremediation efficiency and assimilative capacity and its co-culture with shrimp *P. vannamei* could be an effective and environment-friendly method to reduce nutrient loads from shrimp culture. The seaweed biomass produced as a by-product of the bioremediation could be efficiently utilized to obtain an economic advantage. It could, therefore, be concluded that the optimal biomass intensity of *G. tenuistipitata* is 3.5 g L^{-1} based on this study. However higher biomass intensity may give better results which is to be investigated further. Besides, co-cultivation of macroalgae and shrimp provides them with adequate environmental conditions to reduce stress due to the low concentration of ammonium-nitrogen. The study also

revealed that co-culture with seaweed proved to be a rather symbiotic relationship because it had no negative effect on growth of shrimp but helped to improve survival of shrimp and also facilitated optimal utilization of the available space in the culture system.

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