EFFECT OF SPLIT BAMBOO SUBSTRATE ON PERIPHYTON AND GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP, *PENAEUS VANNAMEI* (BOONE, 1931) IN LOW SALINE GROUNDWATER CULTURE

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(Received 25th Nov 2021; accepted 25th Feb 2022)

Abstract. An experimental study was undertaken to assess the impact of substrate and periphyton on the growth, survival and feed utilization of *Penaeus vannamei*. This study was conducted in cement tanks with bamboo as substrate. The average submersion depth of the substrates was maintained at 85.16 ± 0.26 cm in the treatment tanks. There was significant difference (P value < 0.05) observed in the values of Chlorophyll a (3.12 ± 0.32 µg/cm^2 minimum and 16.29 ± 1.15 µg/cm^2 maximum values), dry weight (2.49 ± 1.46 mg/cm^2), ash (0.78 ± 0.46 mg/cm^2), ash free dry weight (1.71 ± 1.0 mg/cm^2) and autotrophic index (146 ± 18) in terms of submersion time but there was no significance observed in terms of substrate depth. The bio growth parameters (Average Body Weight - 15.4 ± 4.9 g, Average Daily Growth - 0.165 ± 0.01 g, Specific Growth Rate - 7.67 ± 0.03, Protein Efficiency Ratio - 3.12 ± 0.15 and Food Conversion Ratio - 0.92 ± 0.00018) were also observed to be high in treatments with the substrate. Feed usage in the treatment tank was found to be reduced by 19% compared to the control tank. The Periphyton community recorded on the treatment tanks with split bamboo poles as substrate comprised of 4 groups (Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae). The results on the depth-wise analysis of microbial load on the split bamboo substrate have shown a significant difference between 10 cm and 40 cm depth and 10 cm and 70 cm depth of the substrate (P<0.05), whereas, there was no significance observed between 40 cm and 70 cm depth of the substrate in the microbial load. Under the low saline condition, substrate-based vannamei farming has shown better performance compared to the tanks without substrate.
Keywords: *periphyton, specific growth rate, protein efficiency ratio, food conversion ratio, substrate*

Introduction

Aquaculture continues to grow faster than any other major food sector. Inland aquaculture production contributes to around 64% of the total aquaculture production with different production systems (FAO, 2018). Aquaculture contributes 1.07% to the total GDP and 5.23 % to the agricultural GDP of India. Inland saline ground water-based aquaculture also offers the potential to increase the production of euryhaline and marine

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_24712484

species. In more than 100 countries saline soil occurs in arid regions and the surface water and groundwater in such areas have a salinity of more than 1 ppt (Keren, 2000). Most efforts on the culture of marine shrimp in inland ponds have focused on the use of inland saline groundwater in U.S.A, Israel and India (DattaMunshi, 2010). The commercial farming of shrimps in Inland saline water was done with 2 – 7 ppt in Alabama, Florida, Texas, Arizona and Arkansas. In Texas *Penaeus vannamei* farming in saline, quarry waters started in the 1970s and in Israel culture of finfishes in deep geothermal brackish water aquifers was in practice from the late 1980s and this is known as "desert aquaculture" (Allan et al., 2009). Low saline groundwater refers to the farming of marine shrimps in salinity less than 15 ppt and this inland saline farming of marine shrimp is in practice for more than 10 years in some farms in the states of Florida, Alabama, Arizona and Texas in USA (DattaMunshi, 2010).

Inland saline-based farming of Pacific white shrimp, *Penaeus vannamei* is in trend worldwide, which is native to the pacific coast from Northern Peru to Mexico (Liao and Chien, 2011). The inland-based vannamei farming is more successful compared to seawater or brackish water-based culture. In 2018, the world farmed production of *Penaeus vannamei* was recorded at 4.156 million tonnes, which is 53% of the total farmed crustacean production (FAO, 2018). The Pacific white shrimp is a euryhaline species, it can tolerate a wide range of salinity from 0.5 to 45 g L^{-1} [6,7]. *Penaeus vannamei* can even grow with salinity less than 0.5 g L^{-1} in water (Araneda, 2008; Cuvin-Aralar, 2009). *Penaeus vannamei* has now become the candidate species for low saline inland farming due to its ability to survive and grow in different saline conditions.

Periphyton refers to the total assemblage of sessile or attached organisms on any substrate (Reid and Wood, 1976; Weitzel, 1979). In many water bodies, the contribution of the Periphyton community to production is greater than that of the phytoplankton. In a study that compared the primary productivity of a turbid and clear lake, phytoplankton was found to account for 96% of the total annual production in the turbid lake while epipelon contributed 77% in the clear lake. The contribution of Periphyton to annual primary productivity is as high as 1 kg cm^2 (Azim et al., 2005). The development of periphyton depends on different factors like time, depth and type of substrate used. Based on different studies by different authors have proved bamboo substrate to have better growth of periphyton both quantitatively and quantitatively (Azim et al., 2003; Khatoon et al., 2007; Keshavanath et al., 2017). The present investigation was undertaken to assess the impact of split bamboo substrate on the growth and survival of Pacific white shrimp in low saline groundwater culture conditions.

Materials and methods

Experimental site and design

The experimental study was conducted at the Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India during the months of September 2019. This investigation was done in outdoor circular cement tanks each with 13 tons water holding capacity. The trial was conducted for 90 days. Groundwater with salinity 5 g L^{-1} was used for this investigation. Split Bamboo poles with 4 cm width were used as the substrate for this study, throughout the trial the mean submersion depth of the split bamboo poles was maintained at 85.16 ± 0.26 cm Split bamboos were provided with cement stones as sinkers in the bottom of the pole to maintain it in the vertical hanging position. These substrates were erected inside the circular tanks and one week before the tanks were stocked with

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Penaeus vannamei seed (*Figure 1*). The control tanks were filled with water and maintained without substrate.

Figure 1. *Fabricated split bamboos and their erection in 13 ton circular tanks*

Periphyton sampling

Quantitative and qualitative analysis of periphyton was done every 10 days once from the split bamboo substrate erection in the treatment circular cement tanks, where the substrate samples were analyzed for dry matter (DM), pigment Chlorophyll *a* (Chl *a*), ash free dry matter (AFDM) and autotrophic index (AI) every 10 days once with following standard methods (APHA, 1992). The water sample from the control tank was also analyzed for plankton and Chlorophyll *a* for every 10 days once. From each treatment tank, one substrate was taken and 2 x 2 cm^2 samples of periphyton were taken at three different depths (10, 40 and 70 cm). The area was scrapped carefully using a scalpel blade to remove all the periphyton without affecting the substrate (visually). After sampling, the substrates were replaced in their original position, marked and excluded from subsequent sampling.

Bio growth sampling of Penaeus vannamei

Penaeus vannamei shrimps of Post Larval stage 12 (0.015 gm) were purchased from Coastal Aquaculture Authority (CAA) approved hatchery and stocked in all control and treatment tanks. Stocking density was done as per the norms of CAA, India ($60/m^3$), stocking density was maintained at the same levels for control and treatment tanks also. Initial feeding for the shrimps was done as per the recommendation of the feed company using commercial vannamei feed with 38% protein content. The sampling was done every 10 days once and weekly weight gain and the average of daily growth parameters were recorded.

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Sample analysis

Water quality parameters

Water quality parameters like water temperature, pH and dissolved oxygen were measured daily using YSI professional handheld multi-parameter kit at the water depth of 30 cm. Salinity was checked initially before pumping and checked at an interval of 10 days. Other water quality parameters were checked at an interval of 7 days till the end of the study. The water chlorophyll contents were also determined using the standard method by collecting samples once every 10 days. All the water quality parameters were observed at optimal levels both in control and treatment tanks through out the study (*Figure 2*).

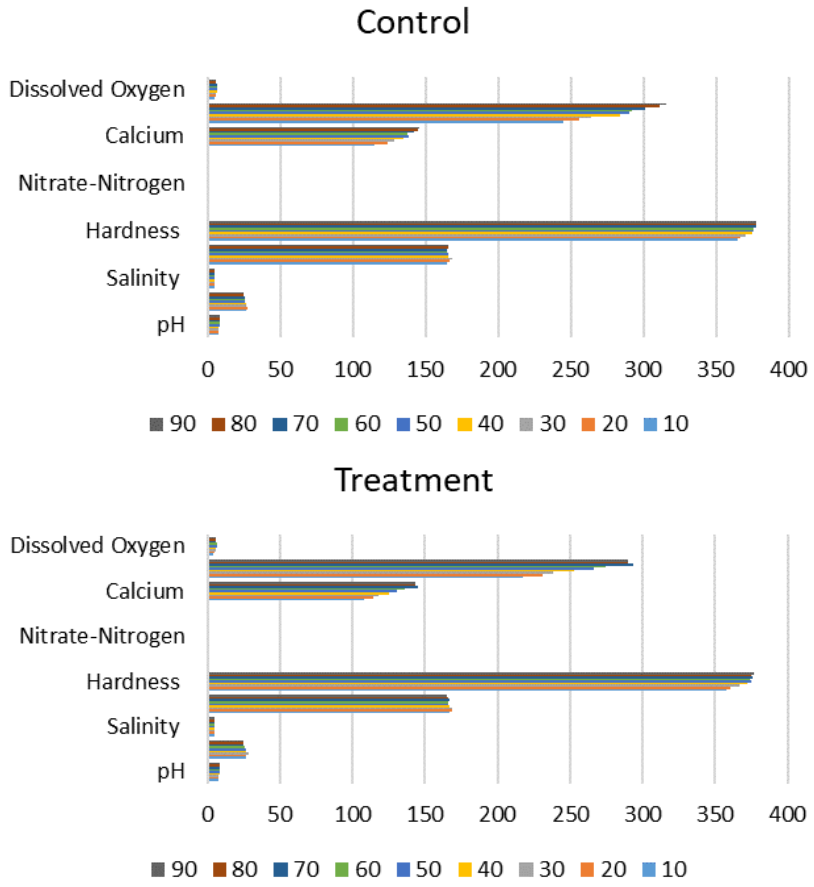

Figure 2. *Water quality parameters*

Chlorophyll a

Periphyton sample collected for estimation of Chl *a* and the sample was transferred into tubes containing 10 ml of 90% acetone, labeled and stored in refrigerator for overnight (APHA, 1992). The next day morning, samples were homogenized for 30 sec in a tissue grinder, again refrigerated for 4 hours and centrifuged for 10 minutes at 2000 rpm. The supernatant was carefully transferred to 1 cm glass cuvettes and absorbance is measured at 663, 645 and 630 nm using an UV spectrophotometer (Perkin Elmer).

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Dry matter and ash free dry matter

From the collected periphyton samples, one sample was used for the determination of total dry matter and ash content of the periphyton. Further, pre-weighed and labeled with aluminum foil and dried at 105 ℃ in a hot air oven (Technico) for 1 hour until constant weight and kept in a desiccator until weighed. The dry matter is transferred to a muffle furnace (Technico) and ashed at 450 ℃ for 6 hours and weighed. Dry matter, ash free dry matter and ash content were determined by weight difference.

Taxonomic analysis of periphyton

The periphyton samples collected were suspended in 50 ml distilled water and preserved in 5% buffered formalin in a sealed plastic vials for taxonomic analysis of periphyton. From the preserved sample 1 ml was transferred to Sedgwick-Rafter cell (S-R cell) divided in 1,000 squares after vigorous shaking. Using Nikon Inverted Microscope (Eclipse TS 100) 10 squares were randomly selected for identification of the algae. Taxa were identified using keys from manual of freshwater biota (DattaMunshi, 2010). Plankton was also determined every ten days once by filtering 5 liters of water from the circular tanks, samples were taken at 2 different locations of the tank using 45µm plankton net and preserved in 5% formalin further analysis of the taxa were done as it was done for the periphyton.

Bio growth parameter analysis of Penaeus vannamei

The weight of the 100 numbers of *Penaeus vannamei* shrimps/sampling from the treatment and control tanks were measured every 10 days once and documented for statistical analysis. The bio growth parameters like Specific Weight Gain (SGR), Protein Efficiency Ratio (PER), Average Daily Weight Gain (ADG) and Food Conversion Ratio (FCR) were calculated after the completion of the trial study following the standard calculation methods.

$$\text{Specific weight gain} = (\text{InFw} - \text{InIw})/t \quad \text{(Eq.1)}$$

where,
InFw is the log value final weight gain;
InIw is the log value Initial weight;
t is the time duration of experiment in days.

$$\text{Protein Efficiency Ratio} = \text{Gain in body mass (g)}/\text{Protein intake (g)} \quad \text{(Eq.2)}$$

$$\text{Average Daily Weight Gain} = \text{Final weight gain (g)}/\text{Days of culture} \quad \text{(Eq.3)}$$

$$\text{Food Conversion Ratio} = \text{Total feed consumed}/\text{Total harvest} \quad \text{(Eq.4)}$$

Statistical Analysis

The normality and homogeneity of chlorophyll *a* data, DW, Ash, AFDW and AI were checked using Shapiro-Wilk and Levene Statistic respectively. ANOVA test was performed for all the variables and evaluated for its significance using SPSS 25.0 statistical analysis software. Chl *a*, dry matter, ash, ash free dry matter, autotrophic index

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depending on time and depth and day wise plankton taxa were also analyzed for its significance using one-way ANOVA. Bio growth parameters were analyzed using descriptive statistics and two-way ANOVA using excel.

Results

Submersion time and periphyton

The overall maximum values of Chl a, DW, Ash, AFDW and AI values were observed as 16.29 ± 1.15 µg/cm^2, 4.28 ± 0.04 mg/cm^2, 1.37 ± 0.01 mg/cm^2, 2.91 ± 0.02 mg/cm^2 and 186 ± 12.8, respectively. The maximum mean values of Chl a, DW, Ash, and AFDW were observed in the experiment tank during the 90th day of sampling and the maximum value of AI was reported on the 80th day of sampling. There was a significant difference (*Figure 3*) observed in the Chl *a* development on the split bamboo substrates with an increase in the submersion time (*P* value <0.05). In the multiple comparisons made for Chl a it was observed that there was no significant difference observed in the levels of Chl *a* in the comparison made between 70th day vs 80th day (P value – 0.559) and 80th day vs 90th day (P value – 0.865). In the case of the multiple comparisons made for DW, Ash, AFDW and AI with submersion time there was a significant difference observed (*Table 1*).

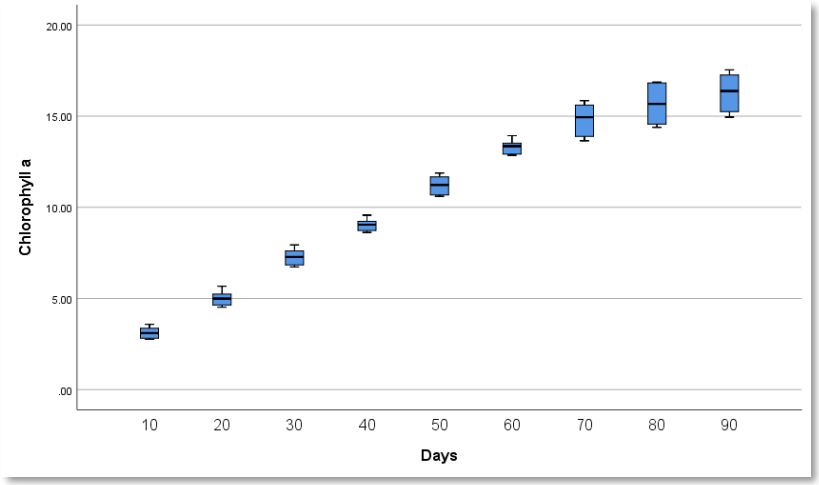

Figure 3. *Submersion time and chlorophyll a concentration in bamboo substrate*

Submersion depth and periphyton

In the present study, the one-way ANOVA analysis on the impact of depth on Chl *a* has not shown any significant differences (*Figure 4*). The mean values observed at different depths of substrate 10 cm, 40 cm and 70 cm were 10.55 µg cm^{-2}, 10.84 µg cm^{-2} and 10.51 µg cm^{-2}, respectively. The highest mean value was observed at 40 cm depth.

Taxonomic composition of periphyton on the split bamboo substrate

This study for periphyton in low saline groundwater using split bamboo substrate has recorded with only eight genera of periphyton taxa which belong to Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae. The overall mean values were

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DOI: http://dx.doi.org/10.15666/aeer/2003_24712484

observed to be higher for genera Euglena (7919 ± 2685) followed by Ankistrodesmus (6291 ± 2270) (*Figure 5*). There was a significant difference observed in the levels of plankton taxa concerning increase in days and between genera (*P* value <0.05).

Table 1. *Turkey HSB multiple comparisons of submersion time (days) showing the significance and non-significance*

Submersion Time (Days)		Dry Weight (P value)	Ash (P value)	AFDW (P value)	AI (P value)
10	20	0.967	0.999	0.936	0.172
10	30	*	*	*	0.517
30	40	*	*	*	0.244
30	50	*	*	*	0.072
40	50	*	*	*	0.986
40	60	*	*	*	0.774
40	70	*	*	*	0.108
40	80	*	*	*	0.054
40	90	*	*	*	0.149
50	60	*	*	*	0.997
50	70	*	*	*	0.350
50	80	*	*	*	0.183
50	90	*	*	*	0.457
60	70	*	*	*	0.703
60	80	*	*	*	0.435
60	90	*	*	*	0.822
70	80	*	0.159	*	1.000
70	90	*	*	*	1.000
80	90	0.967	*	1.000	0.996

AFDW – Ash Free Dry Weight, AI – Autotrophic Index, * P value <0.05 (Significance)

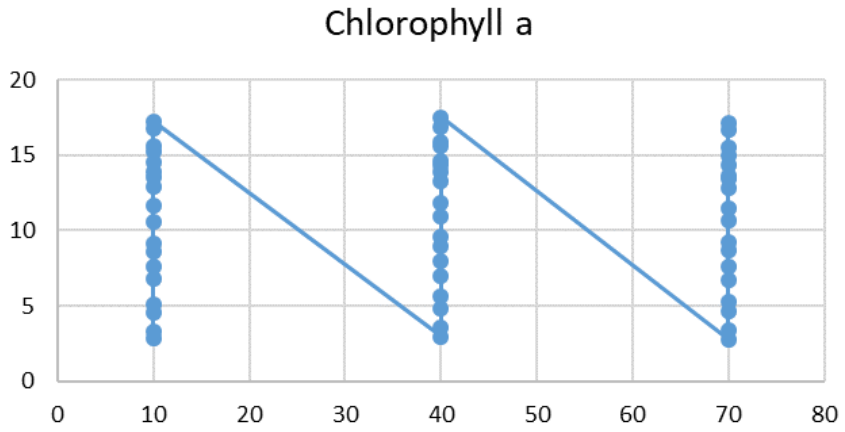

Figure 4. *Chlolophyll a concentration at different depths in the substrate*

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DOI: http://dx.doi.org/10.15666/aeer/2003_24712484

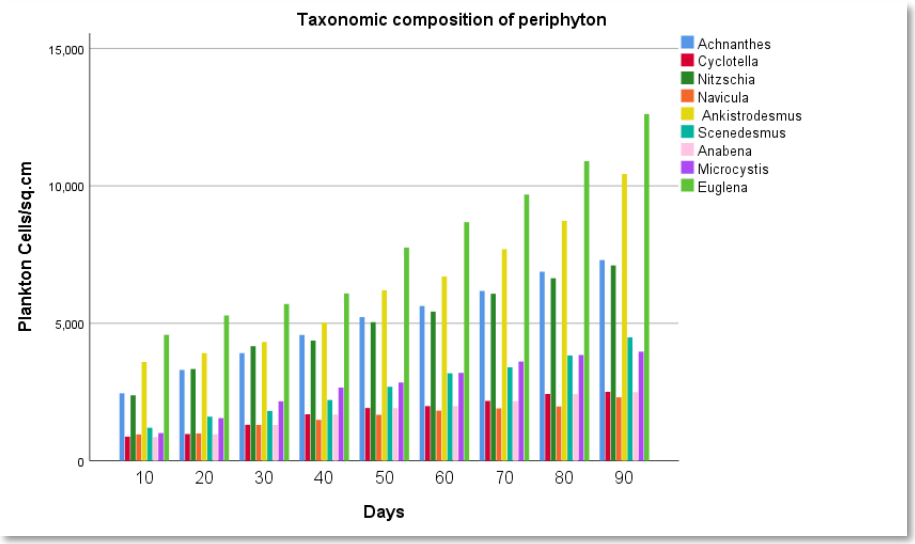

Figure 5. *Taxonomic composition of plankton on split bamboo substrate*

Bio-growth parameters of Penaeus vannamei

The growth performance of *P.vannamei* was observed to be high in treatments with substrate compared to the control tank without substrate (*Table 2*). There was a significant difference observed in growth between treatment and control based on ANOVA test (*P* value < 0.05) (*Table 3*). The growth of *P.vannamei* in the treatment tank was observed to be high by 71% in with substrate compared to the control (*Figure 6*) tank without substrate.

Table 2. *Bio-growth parameters of Penaeus vannamei*

Growth parameters	Control	Treatment
Stocking density (Per m ^{3})	60	60
Total stocking (Numbers)	780	780
Initial weight (g)	0.015	0.015
Days of Culture	90	90
Initial weight	0.015 ± 0.011	0.015 ± 0.011
Initial biomass (g)	11.7 ± 0.43	11.7 ± 0.43
Final weight (g)	8.8 ± 0.14	15.1 ± 0.17
Final biomass (g)	6107 ± 150	11189 ± 225
Feed	12850 ± 250	10244 ± 208
Average Daily Growth (ADG)	0.09 ± 0.0005	0.16 ± 0.007
Specific Growth Rate (SGR)	7.07 ± 0.02	7.67 ± 0.03
Protein Efficiency Ratio (PER)	1.36 ± 0.0003	3.12 ± 0.0006
Food Conversion Ratio (FCR)	2.10 ± 0.0004	0.92 ± 0.00018
Survival rate	89 ± 0.56	95 ± 0.70

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Table 3. *Significant difference observed between control and treatment in growth*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Control x Treatment	41.0368519	2	20.51843	11.13941	0.000932	3.633723
Error	29.4714815	16	1.841968			

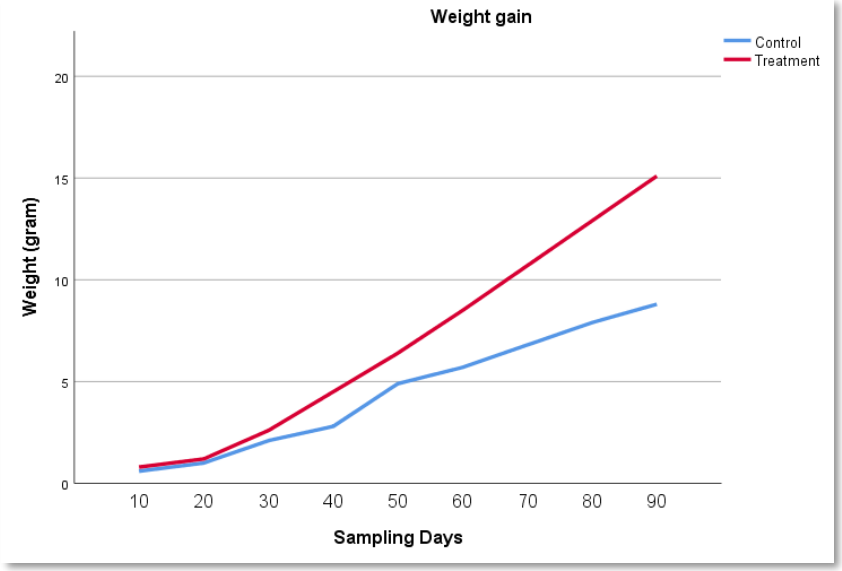

Figure 6. *Penaeus vannamei weight gain in control and treatment tanks*

In the present study, at the end of the 90 days trial, *P.vannamei* in treatment with substrate attained an ABW of 15.4 ± 4.9 and in control, the ABW was 8.8 ± 3.5. Average daily growth gain was observed to be high in treatment tanks with the substrate (0.165 ± 0.01) and relatively less in control (0.09 ± 0.008). In the present trial, the total consumption of feed has been observed to be reduced by 25% (*Figure 7*) in the substrate-based tank (10.24 kg feed) compared to the control tank (12.8 kg feed). The Protein Efficiency Ratio was observed to be high in treatment with the substrate (3.12 ± 0.15) and less in the control tank (1.36 ± 0.12). No specific difference was observed in treatment and control in terms of survival of vannamei shrimp.

Microbiology of split bamboo substrate

The swab samples collected from the split bamboo pole at different depths of 10 cm, 40 cm and 70 cm were analyzed for microbial load by enumerating the total plate count (TPC). The result has shown that there was a significant difference observed between 10 cm and 40 cm depth and 10 cm and 70 cm depth of the substrate ($P<0.05$), whereas, there was no significance observed between 40 cm and 70 cm depth of the substrate in the microbial load. The Shapiro-Wilk analysis on the data has shown the normal distribution of the data, the highest mean value was recorded at 70 cm depth of the split bamboo pole (4.97 ± 0.98 x 10^6 cfu/ml) with minimum and maximum values of 3.6 x 10^6 cfu/ml and 6.6 x 10^6 cfu/ml followed by 40 cm depth with mean value of

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4.76 ± 0.97 x 10^6 cfu/ml and minimum and maximum values of 3.4 x 10^6 cfu/ml and 6.4 x 10^6 cfu/ml. Mean values, minimum and maximum values recorded at 10 cm depth of the substrate was 3.88 ± 1.0 x 10^6 cfu/ml, 2.3 x 10^6 cfu/ml and 5.8 x 10^6 cfu/ml (*Figure 8*).

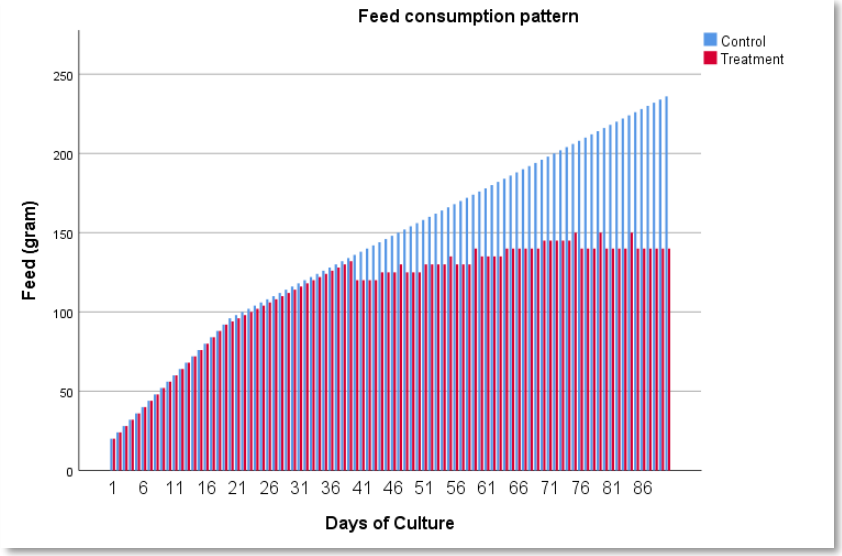

Figure 7. *Comparative feed consumption pattern in control and treatment tank*

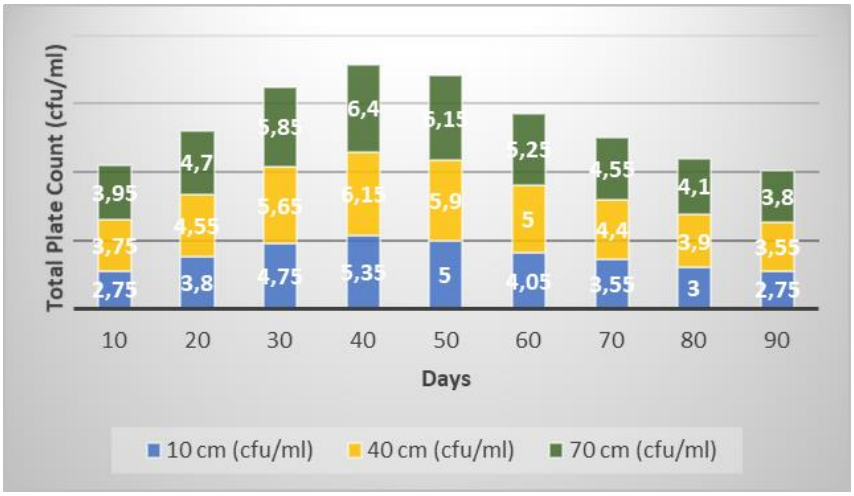

Figure 8. *Total Plate Count (cfu/ml) at different depths of the substrate*

Discussion

Submersion time and periphyton

The mean value of Chl a (10.64 ± 1.59 µg/cm^2) reported from the present study is in line with the Chl a value (11.5 µg/cm^2) reported by Azim et al. (2002a) in hizol substrate and lower than the Chl a value (39.59 µg/cm^2) documented by Keshavanath et al. (2017) in the bamboo substrate from freshwater. Multiple comparisons of AI with submersion time have not shown any significant difference in the increase in AI value with time. The

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significant increase in the levels of periphytic algae in terms of Chl a, DW, Ash and AFDW with submersion time observed in the present investigation in low saline groundwater was is in line with the results reported from other authors in freshwater (Hoagland et al., 1982; Biggs, 1996; Steinman, 1996; Azim et al., 2001, 2002b, 2003), brackish (Khatoon et al., 2007) and marine waters (Richard et al., 2007).

Submersion depth and periphyton

At greater depth, the periphyton standing stock gets reduced due to a reduction in light intensity (Konan-Brou and Guiral, 1994; Azim et al., 2002). According to Kirk (1994), the intensity of light and the spectral composition has a considerable impact on the quality and composition of flora and periphyton with change in depth. Irradiance induced more difference in the epilithic biomass, a higher level of irradiance leading to a higher level of biomass and change in the taxonomic composition of epilithic assemblage (DeNicola and Hoagland, 1996). Similarly, Konan-Brou and Guiral (1994) have reported minimal horizontal heterogeneity of algae in brackish water using Acadja as substrate; Azim et al. (2002b) have reported on the vertical distribution of Chl a in bamboo substrates have no significance with depth in freshwater polyculture system is following the present report of non-significance with the growth of periphyton in terms of depth and the present observation Chl *a* with depth is not accordance with Kirk (1994). The present study is in acceptance with DeNicola and Hoagland (1996). Overall, the irradiance concentration influenced the occurrence of the higher level of periphytic algae throughout the substrate length and also due to the influence of vigorous aeration and water movement in the experiment tank.

Taxonomic composition of periphyton on the split bamboo substrate

In pond habitat, the periphyton taxa are dominated by Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, Zooplankton and Invertebrates. However, in the Estuary and seawater environment, the periphyton taxa are dominated by Bacillariophyceae, Chlorophyceae, Cyanophyceae, Rhodophyceae and Phaeophyceae (Azim et al., 2005). The plankton taxa generic composition observed in this investigation in low saline groundwater was comparatively very less to those reported by other authors in freshwater (Azim et al., 2001; Algarte et al., 2017; Sunil Rai et al., 2018) and marine waters (Richard et al., 2007). The genera of planktons found in low saline groundwater are similar to those reported from freshwater.

Bio-growth parameters of Penaeus vannamei

The difference in growth observed in treatment tanks in the present study with the substrate is in line with Schveitzer et al. (2013), who has reported that the presence of substrate corresponds to shrimp biomass production. Khatoon et al. (2007b) observed that the specific growth rate of shrimp post-larvae increased 28% in the presence of substratum. Ballester et al. (2007) determined that growth and survival of *Farfantepenaeus paulensis* post-larvae did not enhance in the presence of artificial substrata that had their biofilm periodically removed, indicating the importance of biofilm as food. The FCR was observed to be better in tanks with substrates and this matches with the earlier findings in the culture of *P.vannamei* (Audelo-Naranjo et al., 2011) and *F.paulensis* (Ballester et al., 2007). Bratvold and Browdy (2001) have also reported high shrimp production and low feed conversion ratio (FCR) during the culture of *P.vannamei*

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in a high-density culture system with artificial substrata (Aquamats™). The SGR values obtained in the present trial were on par with the results reported by Correia et al. (2014) and Legarda et al. (2018) in a biofloc based nursery rearing of shrimp system.

Microbiology of split bamboo substrate

The Total Plate Count (TPC) values in the present study are in accordance with the other authors (Sanli et al., 2015; Yingshun et al., 2017) report on heterotrophic bacterial composition in submerged substrates. The trial conducted on the growth of *P.vannamei* using bamboo substrate in cement tank has shown an increase in the values of TPC with submersion time which is in line with Haglund and Hillebrand (2005) statement on the impact of grazing on the exponential growth of bacteria.

Conclusion

The substrate-based farming of *P.vannamei* in low saline groundwater has proved with higher production, low FCR, high SGR, higher ABW and higher ADG in substrate-based culture compared to substrate-free culture system. Apart from this, the present trial has also proved the reduction in feed consumption in substrate-based culture compared to substrate-free system. The growth of periphyton in low saline water and its quantitative and qualitative analysis has been reported to be in line with the production reported by other authors in fresh, brackish and marine water culture systems. The taxonomic composition of periphyton was observed to be very limited compared to other reports. Further studies on substrate-based pond culture of *Penaeus vannamei* in low saline groundwater system is essential for field-level validation of this present investigation.

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