Pakistan J. Zool., pp 1-10, 2024. DOI: https://dx.doi.org/10.17582/journal.pjz/20221007111040

Effect of Various Densities of Natural Substrate in *Penaeus vannamei* Production System with Different Stocking Densities on Water Quality, Growth Performance and Overall Outcome

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ABSTRACT

This study was conducted with *Penaeus vannamei* to evaluate the effect of stocking density with various densities of natural substrate (coconut coir) on water quality and growth performance and overall outcomes from the *P. vannamei* culture system. Totally, three trials were conducted and two earthen lined ponds were used for each trial, one was control pond and another one was treatment pond. In trial I, 63 coconut coirs (6.17 m^2- 20.57 % of the total pond area) were used in the treatment pond and the stocking density of *P. vannamei* was 55/m^2. In trial II, 36 coconut coirs (2.64528 m^2- 11.75 % of the total pond area) were used and the stocking density of *P. vannamei* was 55/ m^2. In trial III, 36 coconut coirs (2.64528 m^2- 11.75 % of the total pond area) were used and the stocking density of *P. vannamei* was reduced to 45 / m^2. In trial I, significant differences ($P < 0.05$) were observed in all the growth parameters of *Penaeus vannamei* and growth parameters in control pond was little higher than the treatment pond. In trial II and III, no significant differences ($P > 0.05$) were observed in growth parameters of *P. vannamei* culture between control and treatment ponds. The mean weight gain, specific growth rate, feed conversion efficiency, feed efficiency ratio and average daily growth showed higher values in the treatment pond of the trial II and trial III than trial I. Physico-chemical water quality parameters did not show significant differences ($P > 0.05$) between control and treatment pond in trial I, trial II and trial III. Among the three trials conducted, the better outcome was obtained from trial II and trial III. Trial II had high revenue as high stocking density was used than trial III. Finally, trial II with less substrate surface area is suggested from this study for commercial application of natural feed based system for *P. vannamei* production.

Article Information
Received 07 October 2022
Revised 05 February 2024
Accepted 13 February 2024
Available online 25 July 2024 (early access)

Authors' Contribution
AAVS conducted this research, written this paper. SA had helped the study and guided till completion of research.

Key words
Substrate surface area, Coconut coir, *Penaeus vannamei*, Earthen lined pond, Growth parameters

INTRODUCTION

The rapid growth of shrimp aquaculture resulted mainly in severe impacts on the climate and reliance on fishmeal as a major protein ingredient for shrimp feed (Tacon and Forster, 2003; Porchas-Cornejo *et al*., 2011). Today, the commercial shrimp community is dominated by intense and semi-intensive cultural systems. A lot of water exchange is inevitable in such a raising scheme in order to preserve the water quality. Operations of this type of facility involve eutrophication of the marine underwater habitats, not just wastewater resources (Audelo-Naranjo *et al*., 2011). Aquaculture is increasingly being considered as the answer for food security issues the world over, in the face of declining marine capture fisheries. At the same time, there is a great concern on the possible environmental impacts of aquaculture.

Since 2000, research has focused on alternative raising systems with eco-friendly, sustainable and low-cost characteristics in the cultivation of shrimps. This has made the substratum-based method of aquaculture a possible rearing system for future cultivation of shrimp (Keshavanath *et al*., 2001; Azim *et al*., 2002; Van Dam *et al*., 2002). The alternative technology based on the use within the cultivation system of dissolved waste through the use of autotrophic bacteria and algae or by the direct heterotrophic conversion into microbial biomass of organic and inorganic nitrogen species which improve water qualities while at the same time making microbial biomass an important natural food source for far off (Nunes and

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0030-9923/2024/0001-0001 $ 9.00/0

Parsons, 2000; Burford *et al*., 2004; Ebeling *et al*., 2006; Linares and Sundbáck, 2006). It can be obtained either by using active suspension tanks, where heavy aeration and blending cause microbial flocks to shape and develop into water pillars or by adding submerged substrate tanks that promote the growth of mixed algae bacterial mats (periphyton) (Avnimelech, 2006; Crab *et al*., 2007).

The existence of a plentiful periphyton for fish in semi-intensive systems may decrease the amount of additional feeding supplies since fish feed is partially satisfied by the periphyton ingestion. Fully removing additional feed, however, can negatively influence fish growth (Milstein *et al*., 2009). The greater the region, the greater the periphyton benefits, such as more food and better water quality is widely agreed (Uddin, 2007). The amount of supplementary feed supplied to fish is therefore crucial to viability or the feeding rate followed by the manufacturer. Apart from the feeding volume, the size of the submerged area available for periphyton development is also a deciding factor in the performance of substrates based aquaculture systems (Asaduzzaman *et al*., 2010). While the individual effects of periphytones on fish production are relatively common, they are still poorly understood in terms of their joint activity with various feeding speeds.

In this context, the analysis examined the best form and the benefit of the periphyton for overcoming the negative impact of stocking density on shrimp growth parameters and water quality by adding coconut coir substrates in various density.

MATERIALS AND METHODS

Study area and pond facilities

The experiment was carried out in the Mariculture Research Farm Facility (MRFF), Tharuvaikulam, Thoothukkudi, South India. The outdoor experiments were carried out in 2 earthern lined ponds, each having water spread area of 30 m^2. The ponds were well exposed to sun light with water supply facilities. The water depth was constantly maintained as 1.2 m throughout the study period.

Experimental design and tanks

Out of two earthen lined ponds used, one was considered as a control pond and another one was considered as a treatment pond. These two ponds were allowed to dry and top soil was removed. Then, the coconut coir substrates were installed into the treatment pond alone. Totally, three trials were conducted to compare the growth of *P. vannamei*.

The first growth trial was carried out for a period of 84 days. In the first trial, 63 numbers of coconut coirs were used as substrate in the treatment pond. Coconut coirs were allowed to hang in seven rows vertically in the treatment pond. Each row consists of nine numbers of coconut coirs. The end of the coconut coir touched bottom of the pond (Supplementary Fig. 1). The total surface area of the coconut coir used in the first trial was 6.17 m^2. It occupies 20.57 % of the total pond area. The stocking density of *P. vannamei* was 55/m^2 for both the control and treatment pond in the first trial studied.

The second growth trial was carried out for a period of 84 days. In the second trial, 36 numbers of coconut coirs were used as substrate in the treatment pond. Coconut coirs were allowed to hang vertically in four rows in the treatment pond. Each row consists of 9 numbers of coconut coir. In this study, the end of the coconut coir not touch the bottom of the pond. The coconut coir was hanged 30 cm above from the bottom of the pond (Supplementary Fig. 2). The total surface area of the coconut coir used in the second trial was 2.64 m^2. It occupies 11.75 % of the total pond area. The stocking density of *P. vannamei* was 55/ m^2 for both the control and treatment pond in the second trial.

The third growth trial was carried out for a period of 84 days. In the third trial, 36 numbers of coconut coirs were used as substrate in the treatment pond. Coconut coirs were allowed to hang vertically in four rows in the treatment pond. Each row consists of 9 numbers of coconut coir. Similar to second growth trial, the end of the coconut coir did not touch bottom of the pond. The coir was hanged 30 cm above from the bottom of the pond (Supplementary Fig. 2). The total surface area of the coconut coir used in the third trial was 2.64 m^2. It occupies 11.75 % of the total pond area. The stocking density of *P. vannamei* was reduced to 45/m^2 from 55/m^2 for both the control and treatment pond in the third trial.

Shrimp stocking and management

All the ponds were dried and limed before stocking. Water is pumped from the sea with the help of motors and provided with filter bags. Application of inorganic fertilizers and chlorination process were done before stocking the post larvae. Healthy and disease free post larvae (PL 15) were procured from Dolphin Shrimp Hatchery, Munthal, Sayalkudi, Thoothukudi city, Tamil Nadu, India. Upon the experiment was commenced, the mean body weight of shrimp was 0.015 g. After the proper acclimatization, post larvae were released to both treatment and control pond. Six air stones were used in each pond to aerate the pond by air pump (2.2 kW) throughout day and night.

For all the three growth trials, the total culture period was 84 days. Formulated pellet feed containing 35 % crude protein (CP Aquaculture India Pvt Ltd) was used to feed the shrimps three times/day at 09:30 am, 12:30

pm and 03:30 pm. The amount of feed was adjusted daily according to feeding performance of shrimp by using check trays monitoring. The daily feeding rates of shrimp were maintained at 10 % of total body weight at the beginning of the experiment, and reduced gradually to 2 % of the total body weight by referring the feed chart based on shrimp biomass from sampling and the estimated survival in the pond.

Water quality management

Water quality parameters were continually measured throughout the experiment. DO, temperature, salinity, transparency and pH were measured on three days interval between 10 am and 11am. Water quality parameters such as temperature by mercury thermometer, salinity by hand refractometer, transparency by secchi disc and pH by pH pen were measured directly from these two ponds and dissolved oxygen was measured following Winkler Method (APHA, 1995) at lab. The water samples were filtered through Whatman glass fibre filter (GF/C) and the filtrate was analyzed for Nitrate - N, Nitrite - N and total ammonia nitrogen (TAN) by using the standard procedures given by APHA (1995). Non-filtered water column samples were analyzed for Chlorophyll 'a' by following standard methods as given by APHA (1995). Biological oxygen demand (BOD) and alkalinity of water samples was estimated following APHA (1995).

Growth performance analysis

Sampling was started from 30th DOC onwards. Every week, 20 shrimps were randomly collected from each experimental pond for growth measurement. The body weight of individual shrimp was measured by an electronic scale accurate to 0.01 g. At each sampling, weight Increments were recorded. Average daily gain (ADG), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated from the sampling data. After the experiment was completed, the remaining shrimp in each pond was weighed for total biomass. After harvesting from the two experimental ponds, total yield, survival rate and total feed consumption were recorded and the following growth parameters were calculated for studying the growth performance of *Penaeus vannamei* at different stocking densities.

Mean weight gain (MWG) (g) = Mean final weight – mean initial weight

Average daily growth (ADG) (g/day) = Mean final weight – mean initial weight/ days of culture

Specific growth rate (SGR) (%/day) = ln (final weight) – ln (initial weight)/ number of days × 100

Survival rate (SR) (%) = Initial total number stocked/ final numbers obtained x 100

Feed conversion ratio (FCR) = Dry feed fed/wet weight gain

Feed conversion efficiency (FCE) (%) = Wet weight gain/dry feed fed x 100

Feed efficiency ratio (FER) = 1/FCR

Protein efficiency ratio (PER) = Wet weight gain/ dry protein fed

Proximate composition of periphyton sample

At the end of the culture, periphyton samples were collected from different substrate and analysed for their proximate composition from Animal Feed Analytical and Quality Assurance Laboratory, Namakkal, Tamil Nadu.

Statistical analysis

Data Collected from this study were analyzed statistically by the biostatistical method of Christenson (1996) and One-way ANOVA (Duncan's multiple range test) using SPSS 20.0.

RESULT

In the present study, as per the one way ANOVA and Duncan Multiple Range Test analysis, the data collected in trial I were clearly affirmed that significant differences (P < 0.05) were observed in all the growth parameters such as MBW, ADG, and SGR of *P. vannamei* culture between control and treatment pond. In trial I, the MBW of *P. vannamei* in control and treatment pond were 7.38 ± 0.065 and 5.55 ± 0.04, respectively. The ADG of *P. vannamei* in control and treatment pond were 0.09 ± 0.0006 and 0.07 ± 0.0005, respectively. The SGR (%/ day) of *P. vannamei* in control and treatment pond were 17.7 ± 0.010 and 17.4 ± 0.008, respectively (Table I).

As per the one way ANOVA and Duncan Multiple Range Test analysis, the data collected in trial II and trial III were clearly affirmed that no significant differences (P > 0.05) were observed in growth parameters, MBG, ADG, and SGR (Table I).

In the present study, the lower FCR was obtained in treatment pond than control pond in all the three trials conducted (Table I). The FCR of *Penaeus vannamei* obtained from control and treatment ponds were 1.66 and 1.58, respectively in trial I. In trial the FCR of *P. vannamei* obtained from control and treatment pond were 1.4 and 1.24, respectively whereas in trial II, these values were 1.34 in control and 1.16 in treatment pond. Comparatively, the lowest FCR was recorded in the treatment ponds of the trial III conducted.

In the present study, the higher survival rate (SR) of *P. vannamei* was recorded in treatment pond thancontrol pond in all the three trials conducted (Table I).

Table I. Bio-growth parameters of *Penaeus vannamei* culture in trial I, trial II and trial III.

Parameters	Trial I		Trial II		Trial III	
	Control	Treatment	Control	Treatment	Control	Treatment
Stocking density (numbers/m^2)	55	55	55	55	45	45
Mean initial weight (g)	0.015	0.015	0.015	0.015	0.015	0.015
Mean final weight (g)	7.38 ± 0.07^a	5.55 ± 0.04^b	9.4 ± 0.04^a	9.1 ± 0. 24^a	9.6 ± 0.46^a	9.3 ± 0.26^a
Mean weight gain (g)	7.37 ± 0.07^a	5.54 ± 0.04^b	9.39 ± 0.04^a	9.09 ± 0.24^a	9.5 ± 0.46^a	9.2 ± 0.26^a
Days of culture (days)	84	84	84	84	84	84
Average daily growth (g/day)	0.09 ± 0.0006^a	0.07 ± 0.0005^b	0.11 ± 0.000^a	0.10 ± 0.003^a	0.11 ± 0.006^a	0.11 ± 0.002^a
Specific growth rate (%/day	17.7 ± 0.01^a	17.4 ± 0.008^b	18.02 ± 0.005^a	17.98 ± .03^a	18.05 ± 0.06^a	18.01 ± 0.03^a
Survival rate (%)	76.36	88.60	84	90	86	92
Total feed consumed (kg)	15.53	12.85	18.24	16.75	14.92	13.36
Net weight gain (kg)	9.3	8.12	13.03	13.51	11.15	11.55
FCR	1.66	1.58	1.4	1.24	1.34	1.16
FCE %	59.88	63.15	71.43	80.67	74.69	86.45
FER	0.60	0.63	0.71	0.81	0.75	0.86

Values in the same row with different superscript in each trial differ significantly ($P < 0.05$) for each parameters. One way ANOVA was used following Duncan multiple ranges testing SPSS 20.0. Values are presented as mean ± Standard Error. FCR, feed conversion ratio; FCE, feed conversion efficiency; FER, feed efficiency ratio. Trial I: 63 coconut coir (6.17m^2, 20.57% of the total pond area) with stocking density of 55/m^2 of *Penaeus vannamei*. Trial II: 36 coconut coir (2.64528 m^2, 11.75% of the total pond area) with stocking density of 55/m^2 of *P. vannamei*. Trial III: 36 coconut coir (2.64528m^2, 11.75% of the total pond area) with stocking density of 45/m^2 of *P. vannamei*.

The survival rate of *P. vannamei* obtained from control pond and treatment pond were 76.36 % and 88.60 %, respectively in trial I, 84 % and 90 %, respectively in trial II, and 86 % and 92 %, respectively in trial III. Comparatively, the highest survival rate was recorded in the treatment pond of the trial III conducted.

Table II. Total quantity of artificial feed intake in each trial during the experiments.

Trial	Feed consumption		Decrease in quantity of feed after in treatment (%)
	Control	Treatment	
Trial I	15.53 Kg	12.85 Kg	17.26
Trial II	18.24 Kg	16.75 Kg	8.17
Trial III	14.92 kg	13.36 kg	10.46

In the present study, the artificial feed consumption by the *P. vannamei* was reduced in treatment pond compared to control ponds in all the three trials conducted (Table II). In trial I, 17.26 % of artificial feed consumption was less in treatment pond as compared to control pond with a stocking density of 55/m^2. In trial II, the artificial feed consumption was less by 8.17 % in treatment pond as compared to control pond with a stocking density of 55/m^2. In trial III, 10.46 % of artificial feed consumption was less in treatment pond as compared to control pond with a stocking density of 45/m^2. Comparatively, the quantity of natural food availability was high in the treatment pond of the trial I because of higher substrate surface area compare to treatment pond of the trial II and trial III.

Proximate compositions of periphyton biomass in the growth trial are given in Figure 1. The dried periphyton contained 12.21 % crude protein, 1.31 % crude lipid, 2.71 % crude fibre, 8.16 % moisture and 58.39 % total ash. The gross energy of the dried periphyton biomass was 1611 kcal/kg.

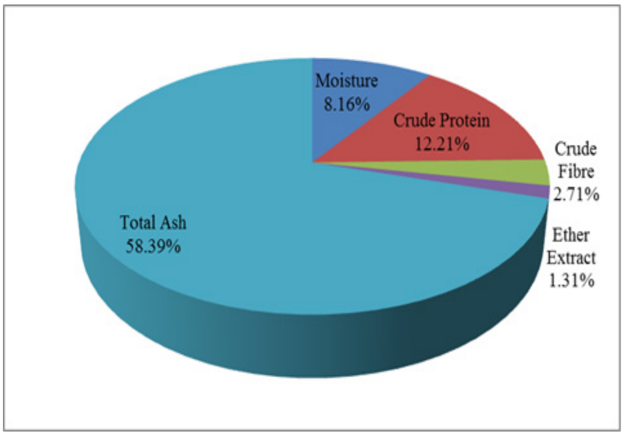

Fig. 1. Quantity of artificial feed consumption in *Penaeus vannamei* culture pond.

Table III. Physico-chemical parameters of seawater in earthen lined pond during *Penaeus vannamei* culture period in trial I, trial II and trial III.

Water parameters	Trial I		Trial II		Trial III	
	Control	Treatment	Control	Treatment	Control	Treatment
Transparancy (cm)	46.2 ± 21.20^a	44.2 ± 14.83^a	43.08 ± 17.23^a	41.50 ± 16.81^a	42.92 ± 12.18^a	41.25 ± 12.66^a
Water temperature (˚C)	29.9 ± 0.89^a	29.9 ± 0.89^a	30.58 ± 1.44^a	30.58 ± 1.44^a	31.08 ± 0.79^a	31.08 ± 0.79^a
Salinity (ppt)	37.0 ± 1.87^a	37.4 ± 1.34^a	34.91 ± 0.99^a	34.83 ± 1.11^a	34.75 ± 0.45^a	35.42 ± 0.79^a
pH	7.72 ± 0.08^a	7.74 ± 0.05^a	7.84 ± 0.12^a	7.83 ± 0.13^a	8.01 ± 0.20^a	8.04 ± 0.19^a
Dissolved oxygen (mg / l)	7.2 ± 0.75^a	7.2 ± 0.58^a	6.87 ± 0.70^a	6.97 ± 0.51^a	6.77 ± 0.82^a	6.86 ± 0.97^a
BOD (mg / l)	1.72 ± 0.13^a	1.76 ± 0.25^a	2.12 ± 0.52^a	2.35 ± 0.55^a	1.68 ± 0.27^a	1.85 ± 0.32^a
Ammonia (µg.at.NH_3-N / l)	0.15 ± 0.05^a	0.14 ± 0.04^a	0.11 ± 0.03^a	0.09 ± 0.03^a	0.08 ± 0.01^b	0.07 ± 0.01^a
Nitrite (µg.at.NO_2-N / l)	0.48 ± 0.04^a	0.45 ± 0.07^a	0.36 ± 0.03^a	0.36 ± 0.02^a	0.46 ± 0.05^a	0.43 ± 0.02^a
Nitrate (µg.at.NO_3-N / l)	0.06 ± 0.04^a	0.07 ± 0.03^a	0.03 ± 0.01^a	0.04 ± 0.00^b	0.04 ± 0.01^a	0.05 ± 0.02^b
Total alkalinity (mg / l)	134.4 ± 10.01^a	138 ± 25.62^a	105.50 ± 3.32^a	108.0 ± 3.72^b	105.25 ± 3.49^a	107.50 ± 3.12^b
Chlorophyll a (mg / m^3)	77.63 ± 17.11^a	78.96 ± 18.54^a	73.78 ± 19.48^a	75.05 ± 19.25^a	67.8 ± 20.78^a	69.4 ± 20.96^a

Values are expressed as (Mean ± SD). Values with same superscripts in a row do not differ significantly at P > 0.05. See Table II for details of trials.

The physico-chemical water quality parameters in trial 1 culture did not show significant differences (P > 0.05) over the experimental periods between control and treatment pond (Table III). In both the trial II and trial III culture, water transparency, water temperature, water salinity, water pH, BOD, nitrite, ammonia and chlorophyll 'a' did not show significant differences (P > 0.05) over the experimental periods between control and treatment pond, while, nitrate and alkalinity were shown significant differences (P < 0.05) over the experimental periods. Significantly, higher value of Nitrate - N was observed in treatment pond (0.04 ± 0.01 µg.at.NO_3 - N/l) of the trial II as well as treatment pond (0.05 ± 0.02 µg.at.NO_3 - N / l) of the trial III. Significantly, higher value of total alkalinity was observed in treatment pond (108.0 ± 3.72 mg / l) of the trial II as well as treatment pond (107.50 ± 3.12 mg / l) of the trial III.

DISCUSSION

In the present study, growth parameters performance of the *P. vannamei* was better in substrate added treatment ponds in trial II and trial III when compared to treatment pond of the trial I. Furthermore, the mean body weight, SGR, FCR, FCE, FER, and ADG attained also showed higher values in the treatment pond of the trial II and trial III. Anand *et al*. (2015) reported that better growth performance of tiger shrimp was found in substrate based system with the average body weight of 25.85 ± 2.62 g compared to control pond with the average body weight of 22.00 ± 2.83 g. In the present study also, the data collected in trial II and trial III affirmed that no significant differences (P > 0.05) were observed in growth parameters such as MBW, ADG and SGR of *P. vannamei* culture between control and treatment ponds. This result indicates the full utilisation of available natural food and natural productivity as periphyton by cultured shrimps. Rebouças *et al*. (2012) reported that there were different effects on animal growth performance and negative effects on final body weight and SGR of the animal when the substrate surface area was increased in the system. The same findings were observed in the trial I treatment pond with more substrate surface area, where, the substrate surface area was less in the trial II and trial III treatment ponds. In the present study, total substrate surface area used was 6.17211 m^2 (20.57 % of the total pond area) in trial I and 2.64528 m^2 (11.75 % of the total pond area) in trial II and trial III treatment pond. The results indicated that better growth performances were recorded in trial II and trial III than trial I. These results are in agreement with Tortolero *et al*. (2016) who evaluated the Influences of substrates at 3 different densities (10, 20, 30 %) on periphyton development and growth performance of Jaraqui (*Semaprochilodus insignis*) and reported that higher fish biomass was recorded in 20 % and 10 % substrate treatments. In trial II and trial III of the present study, there was no significant difference observed in SGR of *P. vannamei* culture between control pond (without substrate) and treatment pond (with substrate). Wahab *et al*. (1999) evaluated the potential of periphyton based system for aquaculture and found that SGR of a native major carp calbasu, *Labeo calbasu* (Hamilton) was higher in treatment pond as compared to control pond without substrate. Hasan *et al*. (2012) reported that specific growth rate of the animal was significantly higher in treatment pond than control.

Schveitzer *et al*. (2013) found in their study indicated that shrimp performances are higher due to reduced stress level of shrimp in proper relative stocking density of substrate and shrimp in the system. The present results are also in agreed with the result of Schveitzer *et al*. (2013). In the present study, less performance of shrimp growth parameters were recorded in treatment pond due to high relative stocking density of substrate and shrimp in trial I. High relative stocking density of substrate surface area and shrimp appears to increase the stress level of shrimp. But, in trial II and trial III, no significant difference was recorded between control and treatment pond due to less relative stocking density of substrate surface area and shrimp. The presence of the substrates altered the relative stocking density by adding more surface area for the shrimp. Considering the negative effect of intensification of the production system on the growth of shrimp, the increase in the relative stocking density in trial I explained clearly the poorer performance of the shrimp for this treatment. On contrary, the shrimp from trial II and trial III exhibited better growth in the experiments than trial I due to the lower relative stocking density than trial I.

In the present study, the lower FCR was obtained in treatment pond than control pond in all the three trials conducted. The present study results coincided with the result of Naranjo *et al*. (2012) and Anand *et al*. (2013) who highlighted that improved feed conversion ratio were observed in substrate based penaeid shrimp culture. Zhang *et al*. (2014) indicated that FCR was reduced effectively in the indoor shrimp culture system with artificial substrates. The addition of artificial substrates increases shrimp growth and reduce FCR (Arnold *et al*., 2006, 2009; Audelo-Naranjo *et al*., 2011). Uddin (2007) reported that FCR was 13 % lower in periphyton based ponds compared to substrate free fed ponds.

In the present study, the higher SR of *P. vannamei* was recorded in treatment pond than control pond in all the three trials conducted. Many trials have demonstrated that fish production from the ponds provided with substrate for periphyton is higher than that of substrate free ponds (Hem and Avit, 1994; Wahab *et al*., 1999; Keshavanath *et al*., 2004; Uddin *et al*., 2006). Experiments conducted in Bangladesh highlighted that addition of vertical substrate in prawn and tilapia polyculture, improved the survival increment of 75 % (Uddin, 2007). Similarly, the higher SR of *P. vannamei* was also recorded in treatment pond than control pond in all the three trials conducted in the present study. Browdy *et al*. (2001) reported that 5 % increase in survival rate in *P. vannamei* grow out systems with buoyant aquamats substrate at Belize Aquaculture Limited (BAL). Schveitzer *et al*. (2013) indicated that the tanks without substrates had significantly lower survival than the tanks with substrates. The increase in the stocking density only reduced the survival rate in the tanks without substrates. Arnold *et al*. (2006) and Abdussamad and Thampy (1994) highlighted that higher survival rate was observed in substrate treatment which was attributed to increase in shelter that enabled newly molted animals to escape from cannibalism.

In the present study, the artificial feed consumption by the *P. vannamei* was reduced in treatment pond compared to control ponds in all the three trials conducted. Comparatively, the quantity of natural food availability was high in the treatment pond of the trial I because of higher substrate surface area compare to treatment pond of the trial II and trial III. The variation in artificial feed reduction between trial II and trial III was due to various stocking density of animals in trial II (55/m^2) and trial III (45/m^2). The added surface area provided by the substrates enhance the colonization of epiphytic biota, which in turn provides a natural food supplement for the shrimp (Moss, 1998; Burford *et al*., 2004). Uddin (2007) reported that FCR was 13 % lower in periphyton fed based ponds compared to substrate free fed ponds because of the reduced artificial feed supplement in periphyton fed based ponds. Similarly, substrate introduction in the present study provided a natural food supplement and reduced the artificial feed consumption in the treatment pond of all the trials studied in the experiment. Many studies indicated that artificial and natural substrates could increase the natural food supplement for shrimp, improve the water quality of pond and control the disease bacteria as biofilms (Thompson *et al*., 2002; Burford *et al*., 2004; Preto *et al*., 2005; Zarain- Herzberg *et al*., 2006; Arnold *et al*., 2009). When substrates are installed in the pond, inorganic nutrients can also follow the extra periphyton loop. This adds a third natural food source existing of periphytic microorganisms that can be consumed by the fish.

In the present study, the dried periphyton contained 12.21 % crude protein, 1.31 % crude lipid, 2.71 % crude fibre, 8.16 % moisture and 58.39 % total ash in growth trial. An average protein content of 15 % was estimated in periphyton collected from coral reef (Polunin, 1988). Dempster *et al*. (1995) reported 28 to 55 % protein and 5 to 18 % lipid in some algal species of periphytic nature. Ledger and Hildrew (1998) recorded as low as 2 to 3 % protein, 0.04 to 0.29 % lipid and 29 to 33 % carbohydrate in periphyton grown on stones. Ash content of periphyton is known to increase as the community grows older (Huchette *et al*., 2000). The nutrient quality and availability on periphyton varies with several factors like grazing pressure, algal and bacterial taxonomic composition, nutrient level of environment, environmental purity and most significantly to substrate type (Makarevich *et al*., 1993; Azim *et al*., 2002).

In trial I culture, physico-chemical water quality parameters did not show significant differences (P > 0.05) over the experimental periods between control and treatment pond (Table III). In both the trial II and trial III culture, water transparency, water temperature, water salinity, water pH, BOD, nitrite, ammonia and chlorophyll 'a' did not show significant differences (P > 0.05) over the experimental periods between control and treatment pond, while, nitrate and alkalinity were shown significant differences (P < 0.05) over the experimental periods. Significantly, higher value of Nitrate - N was observed in treatment pond (0.04 ± 0.01 µg at NO_3 - N/l) of the trial II as well as treatment pond (0.05 ± 0.02 µg at NO_3 - N/l) of the trial III. Significantly, higher value of total alkalinity was observed in treatment pond (108.0 ± 3.72 mg/l) of the trial II as well as treatment pond (107.50 ± 3.12 mg / l) of the trial III. Schveitzer *et al*. (2013) reported that alkalinity of substrate tank was greater compared with those without substrates because of increasing feed quantity in the substrate tank. The nitrate was increased in the substrate tank due to the process of nitrification and was proportional to the contribution of nitrogen from the feed.

In the present study, growth parameters performance of the *P. vannamei* was better in substrate added treatment ponds in trial II and trial III when compared to treatment pond of the trial I. Furthermore, the mean body weight, SGR, FCR, FCE, FER, and average daily growth attained also showed higher values in the treatment pond of the trial II and trial III. The differences of shrimp growth parameters were affected mainly by the living space added with the addition of artificial substrates (Azim *et al*., 2005; Arnold *et al*., 2009). In trial II and trial III, the results indicate that the better growth and survival rate in the tanks were likely due to the reduction in the relative stocking density provided by the less substrates surface area, which could have been determinant for the shrimp to express their growth. According to Milstein *et al*. (2013), the ideal density of substrate is very important in culture systems of the pond area as it reduce the stress of the animal and reduces the requirement of feed by increasing the natural food in the systems. Considering the negative effect of intensification of the production system on the growth of shrimp, the increase in the relative stocking density in trial I explained clearly the poorer performance of the shrimp for this treatment. On contrary, the shrimp from trial II and trial III exhibited better growth in the experiments than trial I due to the lower relative stocking density than trial I. Tidwell and Coyle (2008) found that there was no improvement in prawn production or feed conversion efficiencies, as surface area was increased. Reductions in antagonistic interactions between prawns have been shown to reduce stress, improve growth and thereby improve feed conversion efficiency (Karplus *et al*., 1992) and it appears that the primary benefit of substrate is to provide the prawns the ability to physically separate themselves from each other, thus reducing prawn – prawn interaction and stress.

CONCLUSION

The aim of aquaculture is to maximize production of cultured organism in a sustainable manner. The experiment carried out involves substrates periphyton shrimp environment relationship. Periphyton grown on coconut coir substrate favored the natural food for shrimp species (*Penaeus vannamei*) and supports to further enhance the growth and survival of *P. vannamei*. Manipulation of relative stocking density by the addition of substrate surface area in shrimp culture system facilitated reduce the stress of the animal and increase the natural food in the culture system, thus making the shrimp farming more ecologically sustainable by reducing inorganic nitrogen production and economically viable by minimizing the requirement of artificial feed. From this study, it was concluded that growth parameters performance of the *P. vannamei* was better in substrate added treatment ponds in trial II and trial III when compared to treatment pond of the trial I. Among the three trials conducted, the better outcome was obtained from trial II and trial III. However, the stocking density of the *P. vannamei* is varied between trial II (55/m^2) and trial III (45/m^2) with same substrate surface area (2.64528 m^2). Considering the revenue, out of trial II and III conducted, trial II had high revenue as high stocking density was used than trial III. Finally, trial II [high stocking density (55/m^2) with less substrate surface area (2.64528 m^2 – it occupies 11.75% of the total pond area)] is suggested from this study for commercial application of natural feed based system for *P. vannamei* production. Earlier review works also indicated that periphyton based aquaculture is a new novel approach to shrimp culture also, the technology is appropriate under all circumstances from the nursery to the grow out systems, from the commercial level to resource, poor and marginal shrimp farmers. Further research should also focus on the application, practicality and durability of different natural substrate materials under their pond site conditions.

DECLARATIONS

Acknowledgement

The authors are thankful to the University Grant

Commission (UGC), New Delhi, India for providing financial support. The authors are grateful to Dean, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India for providing facilities and encouragement.

Funding

The funds for this study were provided by University Grant Commission (UGC), New Delhi, India.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20221007111040.

Statement of conflict of interest

The authors have declared no conflict of interests.

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Pakistan J. Zool., pp 1-10, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221007111040

Supplemenatry Material

Effect of Various Densities of Natural Substrate in *Penaeus vannamei* Production System with Different Stocking Densities on Water Quality, Growth Performance and Overall Outcome

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Supplementary Fig. 1. Experimental set up in treatment pond for growth trial I.

Supplementary Fig. 2. Experimental set up in treatment pond for growth trial II and III.

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0030-9923/2024/0001-0001 $ 9.00/0