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Substrate based black tiger shrimp, *Penaeus monodon* culture: Stocking density, aeration and their effect on growth performance, water quality and periphyton development

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ABSTRACT

Growth performance of black tiger shrimp *Penaeus monodon* juveniles were evaluated in two outdoor experimental trials as a function of two stocking densities and aeration system in substrate based shrimp culture. The experiment 1 had 2 × 2 factorial design with two levels of stocking density and two management systems (with and without substrate addition) resulting in four treatment, each with three replicates. The 12 FRP tanks (1000 L) were stocked with *P. monodon* juveniles (1.08 ± 0.12 g) at 10 and 20 m^2 as first factor, and with substrate (10 + S and 20 + S) and without substrate (10-S and 20-S) as second factor. In experiment 2, effect of aeration in substrate system was evaluated in 1000 L FRP tanks with *P. monodon* (3.89 ± 0.12 g) in four treatments: provision of aeration (A) with substrates (S + A), without substrate (C + A), and absence of aeration with (S-A) or without substrate (C-A). In both experiments, bamboo substrates (5 × 2 × 1 cm) were installed in substrate based groups which generated an additional 540 cm^2 surface area for periphyton development. Formulated feed containing 38% crude protein was provided in both the experiments. In trial 1, stocking density did not significantly affect water quality parameters, whereas provision of bamboo substrate significantly reduced nitrate-N level ($P < .05$). Both stocking density and substrate addition played significant role in shrimp survival by 14–30% improvement due to substrate addition while 18–34% changes in survival due to stocking density. The substrate addition improved FCR significantly ($P < .05$) by 21–33% compared to without substrate based treatments. In experiment 2, lack of aeration significantly affected the dissolved oxygen level ($P < .05$) in the water column, while it does not significantly affected the growth parameters, and substrate addition alone improved 21% of survival rate of shrimp. Estimation of periphyton biomass revealed that stocking density and aeration effects resulted 60 and 40% significant ($P < .05$) difference in ash free dry matter in periphyton biomass. From the present trials, it can be concluded that stocking density of shrimp plays an important role in optimum utilization of periphyton developed over submerged substrates. Also, insignificant growth performance of shrimps reared in low density substrate based systems without aeration indicated that the system has the potential to adopt by small scale shrimp farmers for sustainable ecofriendly farming.

1. Introduction

Shrimp farming in India remains the commercial face of brackishwater aquaculture due to high rate of return over investment within a short period of culture duration. As the world shrimp farming shifted towards the intensive culture due to technological advents with high investments, many shrimp farmers in developing countries became unable to cope up with high investment oriented technologies. Also, large scale expansion of intensive shrimp culture started to face challenges like increase in price of commercial feed, disease outbreaks, environmental sustainability issues etc. This unplanned intensification with limited resources urges the scientific communities to explore ecofriendly alternatives which can be easily adopted by small traditional shrimp famers without much investment. Moreover, though effluent treatment ponds are becoming highly mandatory to control heavy nutrient discharge by shrimp farms, majority of Indian small shrimp farmers (> 90%) with < 1 ha water area are yet to adopt effluent treatment ponds (CAA, 2005). In these circumstances, it is

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advantageous to harness the excess nutrient load in the pond ecosystem via natural community which in turn can positively improve growth performance of cultured shrimps without causing eutrophication. In recent years, eco-friendly faming technologies like periphyton or biofloc based farming techniques are gaining importance based on these principles.

Periphyton is a complex of microalgae-microorganism based community develops over the submerged substrate provided in aquaculture ponds. Being a quality natural food, provision of substrate in culture pond for periphyton development is reported to have multiple benefits (Azim et al., 2005), and is well documented in finfish culture (Wahab et al., 1999; Keshavanath et al., 2001; Biswa,s, et al., 2017). Similarly, better growth performance and FCR are recorded in *Litopenaeus vannamei* (Moss and Moss, 2004; Zhang et al., 2010; Audelo-Naranjo et al., 2011; Kumar et al., 2017) and *Penaeus monodon* (Arnold et al., 2009; Anand et al., 2013a) when cultured in periphyton based systems. Apart from being a source of continuously available quality natural food for shrimp grazing, better nitrification with lower inorganic N or P metabolites were also recorded in these periphyton based systems (Ramesh et al., 1999; Thompson et al., 2002; Kumar et al., 2017). Also, in nursery rearing of penaeid shrimps, substrate provision improves survival by alleviating the crowding stress in high density system (Arnold et al., 2009) due to the generation of additional surface area for living.

Though periphyton based farming is reported to have several benefits, autotrophic or heterotrophic community developed in this system can consume dissolved oxygen in water which can lead to additional oxygen demand at night hours. This high energy demand in the later phase of culture generally reduces the acceptability of this farming system among shrimp farmers. In this context, it is highly speculative whether small shrimp farmers can adopt this farming system in their traditional farms with minimum aeration requirements. Furthermore, optimization of stocking density of cultured shrimps also found to be pertinent as over/under development of periphyton biomass as insufficient grazing of periphyton by shrimps can lead to degradation and disturbance to nitrifying cycle. Though periphyton based farming system has much applicability, there is a dearth of scientific data regarding differential development of periphyton biomass or shrimp growth and water quality parameters in systems with different density of shrimps, and with or without aeration system. Against this background, the present experiments were conducted to study the effect of aeration and different shrimp densities in substrate based systems with *P. monodon* juveniles.

2. Materials and methods

Two sets of experiments were carried out at Kakdwip Research Centre, ICAR-Central Institute of Brackish water Aquaculture, Kakdwip (Lat. 21°51′15.01″–21°51′30.77″N, Long. 88°10′58.44″–88°11′12.09″E), South 24 Parganas, West Bengal, India.

2.1. Experimental design

2.1.1. Experiment 1 (Exp 1)

The on-station experimental trial 1 had a 2 × 2 factorial design with two levels of stocking density in presence and absence of substrate resulting in four treatments: stocking density 10 (no. m^{-2}) with substrate (10 + S) and without substrate (10–S), stocking density 20 (no. m^{-2}) with substrate (20 + S) and without substrate (20–S). Twelve FRP tanks of 1000 L were used for the study, and each treatment with three randomly assigned replicate tanks were stocked with *P. monodon* juveniles (1.08 ± 0.12 g) at 10 and 20 number m^{-2}. Experimental duration was 75 days.

2.1.2. Experiment 2 (Exp 2)

The second experiment was conducted as 2 × 2 factorial design in triplicate in randomly assigned 1000 L FRP tanks with or without aeration (A) as first factor and, with and without substrate (S) for periphyton development, as second factor. The treatments without substrates (control, C) are referred to as C + A and C-A, while the treatments with substrates are referred as S + A and S-A. Randomly assigned tanks under the treatments were stocked with *P. monodon* juveniles (3.89 ± 0.12 g) at 8 number m^{-2} and the experiment was carried out for a period of 60 days.

2.2. Experimental tank preparation

Before start of experiments, brackishwater from nearby creek was treated with bleaching powder (60 ppm) in reservoir tanks and was filled to all the experimental tanks after vigorous aeration. On day 1, agricultural lime was applied to all the tanks @10 g m^{-3} and on 4th day, urea and triple super phosphate (TSP) were added at the rate of 2.5 and 2.5 g m^{-3}, respectively. On 6th day, in each tank of Exp 1(10 + S and 20 + S) and Exp 2 (S + A and S-A), 27 dried split bamboos (5 × 2 × 1 cm) were suspended in the water column, and arranged in three horizontal rows and three vertical columns at 10 cm apart. This resulted in an additional surface area of 540 cm^2 for periphyton development. The experiment was conducted in outdoor units under transparent roof shed which provided natural photoperiod (12L: 12D) for development of periphyton over submerged substrate. After fertilization, the tanks were left undisturbed for 10 days for periphyton development on submerged bamboo substrate.

2.3. Shrimp stocking and management

In both the experiments, formulated pellet feed containing 38% crude protein was used in all the treatment tanks. Composition of the feed ingredients is given in Table 1 and the proximate composition of the diet is given in Table 2. In Exp 1, the daily feeding rate was 8% of body weight at the start of experiment, and declined gradually to 4% of body weight at the end, where as in Exp 2, the daily feeding rate was started with 6%, and reduced to 3.5% based on the body weight as initial size of the animals were higher in second experiment. Feed was distributed equally to shrimps in all the experimental tanks, twice daily at 10:00 and 18:00 h during experimental period. Left over feed and faecal matter were siphoned daily and 25% water exchange was done every 5th day. In Exp 1 and aeration based treatment groups in Exp 2, continuous aeration was provided by two sand air stones, fixed in peripheral alignment and each air stone was diffusing 3.75 m^3 air/tank/min.

Table 1
Composition of the ingredients used for experimental diet in *Penaeus monodon* juvenile growth Experiment 1 and 2.

Ingredients	Test diet
Fish meal	380
Shrimp meal	150
Soyabean meal	207.6
Wheat flour	172.9
Soya oil	15
Cod liver oil	20
Lecithin	10
Cholesterol	1
Vitamin–Mineral mix[a]	23
BHT	0.5
Guar gum	20
Total	1000

[a] Composition of vitamin mineral mix (Supplevite-M) (quantity kg^{-1}): Vitamin A, 20,00,000 IU; Vitamin D3, 400,000 IU; Vitamin B2, 800 mg; Vitamin E, 300 unit; Vitamin K, 400 mg; Vitamin B6, 400 mg; Vitamin B12, 2.4 mg; Calcium Pantothenate, 1000 mg; Nicotinamide, 4 g; Choline Chloride, 60 g; Mn, 10,800 mg; Iodine, 400 mg; Fe, 3000 mg; Zn, 6 g; Cu, 800 mg; Co, 180 mg; Vitamin C, 1000 mg.

Table 2
Proximate composition (%) of experimental diet (mean ± SD) used in *P. monodon* experiment 1 and 2.

Nutrients	Percentage (%)
Organic matter[a]	82.12 ± 0.10
Moisture	8.10 ± 0.42
Crude protein (CP)	37.88 ± 0.03
Crude lipid (EE)	7.67 ± 0.26
Ash	17.88 ± 0.10
Crude fiber (CF)	3.16 ± 0.08
Nitrogen-free extract[b]	25.32 ± 0.73
Gross energy[c]	401.23 ± 0.71

[a] Organic matter = 100–Ash.
[b] Nitrogen free E = 100 – (CP + EE + CF + ash + moisture).
[c] Gross energy (GE) = (CP × 5.6) + (EE × 9.44) + (CF × 4.1) + (NFE × 4.1) Kcal/100 g.

2.4. Determination of water quality parameters

The water quality parameters were measured at fortnightly intervals between 09:00 and 10:00 h. Salinity, temperature and pH were measured using salinometer, thermometer and pH meter, respectively. Water nutrient parameters like total ammonia-N (TAN), nitrite-N (NO_2-N), nitrate-N (NO_3-N) and phosphate-P (PO_4-P) were analyzed immediately after sample collection following the procedures described by Strickland and Parsons (1972). Total alkalinity and dissolved oxygen were determined as described in APHA (1998).

2.5. Determination of periphyton biomass

The periphyton biomass, in terms of dry mater (DM), ash free dry matter (AFDM) and chlorophyll *a* were determined at 15-day intervals. From each tank, three bamboo pieces were randomly selected and 2 × 2 cm^2 samples of periphyton were collected. After sampling, the bamboo stick were replaced in their original positions, marked and excluded from subsequent samplings. The materials from each bamboo were then transferred into a pre-weighed and labeled crucible, dried at 105 °C, and kept in a desiccator until weighed. Dry samples from each tank were ashed at 450 °C for 6 h in a muffle furnace and weighed. The dry matter (DM) and ash free dry matter (AFDM) were determined by weight differences (APHA, 1998). To determine chlorophyll concentration, collected materials were immediately transferred to centrifuge tubes containing 10 mL of 90% acetone, sealed and stored overnight in a refrigerator. The samples were homogenized, centrifuged (10 min at 2000 rpm) and the OD of the supernatant was measured at 750 nm and 664 nm, 647 nm and 630 nm using a spectrophotometer (Systronic UV-VIS spectrophotometer, model 118). Chlorophyll concentration was calculated using the trichromatic equation (APHA, 1998). The autotrophic index (AI) was calculated using the following formula (APHA, 1998): AI = AFDM in μg cm^{-2}/ Chlorophyll a in μg cm^{-2}.

2.6. Estimation of yield parameters

At the end of each experiment, the final weight of the shrimp was recorded and specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were calculated as follows:

$$SGR = (\ln \text{ final weight} - \ln \text{ initial weight}) \times 100/\text{days of experiment}$$

$$FCR = \text{Feed intake/live weight gain}$$

$$PER = \text{Gain in body mass/protein intake}$$

$$\text{Survival} = \text{Total animal survived/Total number of animal stocked} \times 100$$

2.7. Statistical analysis

Growth performance and water quality parameters in both the experimental trials were analyzed by two-way ANOVA with substrate (with and without addition) and stocking density as main factors for Exp 1, and aeration (with and without) and substrate (with and without addition) as main factors for Exp 2. One-way ANOVA was used to determine the significance of periphyton biomass like DM, AFDM, chlorophyll *a* between substrate added systems in both the trials. All analysis was performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). If a main effect was significant, the ANOVA was followed by Tukey's test at $P < .05$ level of significance.

3. Results

3.1. Effect on water quality parameters

The water quality parameters of Exp 1 and 2 are presented in Tables 3 and 4. In Exp 1, water quality parameters, namely temperature, salinity, alkalinity, and PO_4-P were not influenced by stocking density or substrate addition ($P > .05$). However, provision of substrate in high density system (20 + S) significantly reduced ($P < .05$) the level of nitrite–N (NO_2-N), 29.30 μg L^{-1} compared to without substrate, 20-S (57.13 μg L^{-1}). Similarly, 8.5 and 10% non-significant reduction ($P < .05$) in total ammonia nitrogen (TAN) and nitrate-N were noticed in 20 + S group compared to without substrate, 20-S. At low stocking density, there was 8.4 % significant reduction nitrate −N (NO_3-N) levels in 10 + S compared with 10-S while stocking density does not found to play significant role.

In Exp 2, the factorial analysis of water quality parameters revealed that substrate provision had a significant effect ($P < .01$) on total ammonia −N, nitrite −N and nitrate–N. The mean values for total ammonia-N were 313.35 ± 30 and 337.60 ± 110 μg L^{-1} in control groups, C + A and C-A respectively which significantly reduced to 179.19 and 194.26 μg L^{-1} in treatments provided with substrates, S + A and S-A, respectively. Absence of aeration did not result any significant changes in water nutrient parameters, whereas substrate addition played significant role. For example, when substrate incorporated with aeration groups (S + A) recorded 55.6 and 34% significantly lower ($P < .01$) NO_2-N and NO_3-N compared with C-A, treatments with substrate even in the absence of aeration (S-A) also significantly reduced ($P < .01$) NO_2-N, NO_3-N by 50% and 22%, respectively compared with C-A treatment. Hence, absence of aeration did not found to result any significant changes in water nutrient parameters, whereas substrate addition played significant role. Dissolved oxygen (DO) content in the experimental tanks during 05:00 and 15:00 h was recorded twice in a week (Fig. 1). Substrate addition did not found to affect the DO level significantly among the experimental groups, but aeration factor played a significant variation in DO levels among the groups. Though water chlorophyll *a* levels were not significantly different among the treatments, groups without aeration had 38–40% lower level of Chl a compared to treatment with aeration (Fig. 2).

3.2. Effect on periphyton biomass

The periphyton biomass in terms of dry matter (DM) and ash free dry matter (AFDM); autotrophic index (AI) and chlorophyll *a* (chl a) content per unit surface area of the two experimental trials are presented in Table 5. In Exp 1, the periphyton dry matter showed a significant difference ($P < .05$) between the substrate based treatments, with mean value 12.77 ± 1.47 and 8.70 ± 0.90 mg cm^{-2} in 10 + S and 20 + S treatments, respectively. Similarly, low stocking density treatments recorded 60% significant increase ($P < .05$) in ash free dry matter content of the periphyton developed over the substrate compared to high density system. Similarly, autotrophic index level in the periphyton biomass was 19.2% lower in shrimps reared at higher density compared to lower density, 10 + S.

Table 3
Effects of substrate and shrimp stocking density on water quality parameters (mean ± standard error) in *Penaeus monodon* Experiment 1 based on two- way ANOVA.

Variables	With Substrate		Without substrate		Level of significance		
	10 + S	20 + S	10 – S	20 – S	Substrate	Density	Substrate × Density
pH	7.65 ± 0.18 (7–8.1)	7.64 ± 0.07 (7.24–8.1)	7.91 ± 0.20 (7.5–8.7)	7.98 ± 0.34 (7.5–9.2)	**	NS	NS
Salinity (ppt)	10.34 ± 0.55 (9.3–12)	10.02 ± 0.34 (9.3–11)	10.90 ± 1.13 (9.3–12)	10.4 ± 0.39 (9.6–12)	NS	NS	NS
Alkalinity (mg CaC_3)	90.57 ± 16.03 (56–132)	86.83 ± 15.48 (52–120)	88.08 ± 12.01 (64–112)	86.5 ± 13.06 (52–112)	NS	NS	NS
Nitrite-N (μg L^{-1})	27.53 ± 6.45 (11–63)	29.30 ± 8.25 (22–73)	28.73 ± 7.70 (20–48)	57.13 ± 30.20 (21–145)	NS	NS	NS
Total Ammonia Nitrogen (TAN) (μg L^{-1})	147.9 ± 34.9 (101–267)	150.17 ± 35.11 (103–272)	150.53 ± 44.08 (95–291)	164.07 ± 43.45 (103–281)	NS	NS	NS
Phosphate-P (μg L^{-1})	100.64 ± 9.6 (64–118)	111.28 ± 6.12 (96–127)	112.93 ± 6.87 (65–134)	118.28 ± 11.49 (65–135)	NS	NS	NS
Nitrate-N (μg L^{-1})	122.89 ± 6.87 (102–143)	122.03 ± 8.07 (103–145)	134.12 ± 4.17 (123–148)	135.37 ± 4.69 (123–148)	**	NS	NS

Ranges are in parenthesis. If the effects were significant, two-way ANOVA was followed by Tukey test. NS, not significant.
** $P < .01$.

Table 4
Effects of substrate and aeration on water quality parameters (mean ± standard error) in *Penaeus monodon* Experiment 2 based on two way ANOVA.

Variables	With aeration		Without aeration		Level of significance		
	S + A	C + A	S – A	C – A	Aeration	Substrate	Aeration × Substrate interaction
Temperature (°C)	29.09 ± 0.25 (28.5–29.70)	28.94 ± 0.20 (28.5–29.4)	28.85 ± 0.22 (28.4–29.5)	29.07 ± 0.23 (28.4–29.5)	NS	NS	NS
pH	7.82 ± 0.07 (7.65–8.01)	7.84 ± 0.11 (7.7–7.95)	7.36 ± 0.05 (7.15–7.74)	7.38 ± 0.11 (7.1–7.81)	**	NS	NS
Salinity (ppt)	18.82 ± 0.34 (18.2–19.7)	18.83 ± 0.39 (18.2–19.9)	18.79 ± 0.36 (18.2–20)	18.75 ± 0.33 (18–19.4)	NS	NS	NS
Alkalinity (mg $CaCo_3$)	92.33 ± 8.9 (68–108)	83.67 ± 7.3 (56–104)	84.00 ± 10. (64–100)	86.15 ± 8.23 (68–104)	NS	NS	NS
Nitrite-N (μg L^{-1})	25.06 ± 7.21 (12.2–47.8)	52.25 ± 8.07 (20.18–98.59)	28.51 ± 15.89 (14.62–50.59)	56.53 ± 14.50 (28.66–88.62)	NS	**	NS
Total ammonia nitrogen (TAN) (μg L^{-1})	202.44 ± 73.22 (117.79–465.25)	331.35 ± 30 (250.44–564.24)	189.91 ± 67.51 (133.64–326.67)	337.60 ± 109.09 (190.06–807.76)	NS	**	NS
Phosphate-P (μg L)	193.31 ± 60.72 (62.16–368)	200.71 ± 37.05 (81.21–302.69)	138.47 ± 46.38 (67.29–212.57)	185.65 ± 50.66 (101.26–323.48)	NS	NS	NS
Nitrate-N (μg L^{-1})	159.26 ± 24.40 (119.42–248)	245.29 ± 24.43 (173.21–358.91)	187.53 ± 41.57 (109.36–243.38)	239.97 ± 57.42 (149.43–440.50)	NS	**	NS
Dissolved oxygen level (5 am)	6.89 ± 0.16 (6.6–7.2)	6.96 ± 0.25 (6.3–7.3)	2.02 ± 0.57 (1.1–3.9)	2.28 ± 0.74 (1.4–4.6)	**	NS	NS
Dissolved oxygen level (3 pm)	7.71 ± 0.20 (7.4–8.2)	7.37 ± 0.22 (7–8.1)	4.08 ± 1.28 (1.6–7.7)	3.45 ± 0.86 (2.5–4.6)	**	NS	NS

Ranges are in parenthesis. If the effects were significant, two-way ANOVA was followed by Tukey test; NS, not significant.
** $P < .01$.

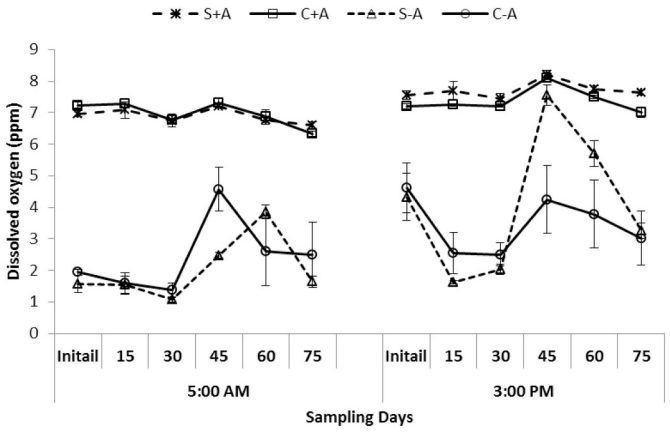

Fig. 1. Diurnal variation in dissolved oxygen level (mean ± standard error) in substrate and aeration based treatments in *Penaeus monodon* culture Experiment 2.

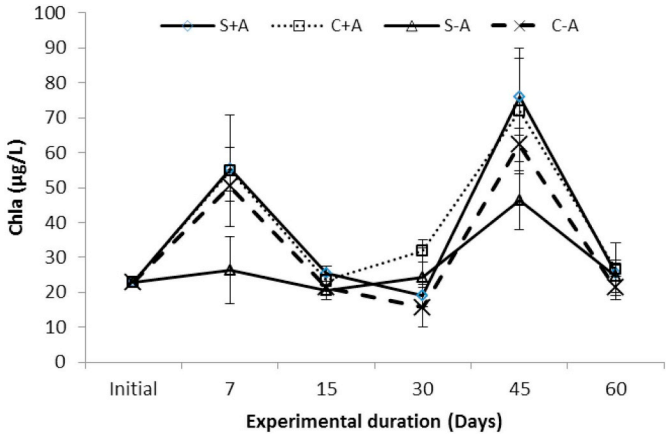

Fig. 2. Water chlorophyll *a* level (mean ± standard error) in substrate and aeration based *Penaeus monodon* culture Experiment 2.

In Exp 2, lack of aeration did not result any significant difference in periphyton dry matter content (mg cm^{-2}) in S + A (4.07 ± 0.39) and S-A (3.29 ± 0.33). However, significantly lower level of ash free dry matter content, 1.88 ± 0.21 mg cm^{-2} was recorded in S-A compared with S + A (2.64 ± 0.29 mg cm^{-2}). Treatments without aeration (S-A and C-A) recorded insignificantly ($P > .05$) lower level of water chlorophyll *a* compared to treatment with aeration (S + A and C + A).

3.3. Growth performance

Average growth of *P. monodon* juveniles over the time period in Exp 1 and 2 are presented in Figs. 3 and 4, and the growth performance of

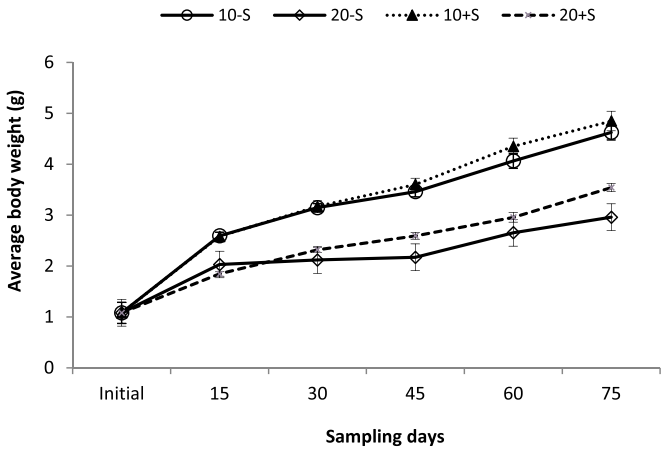

Fig. 3. Average biweekly growth of *Penaeus monodon* juveniles with different stocking density and substrate in Experiment 1. Error bar indicates ± standard error.

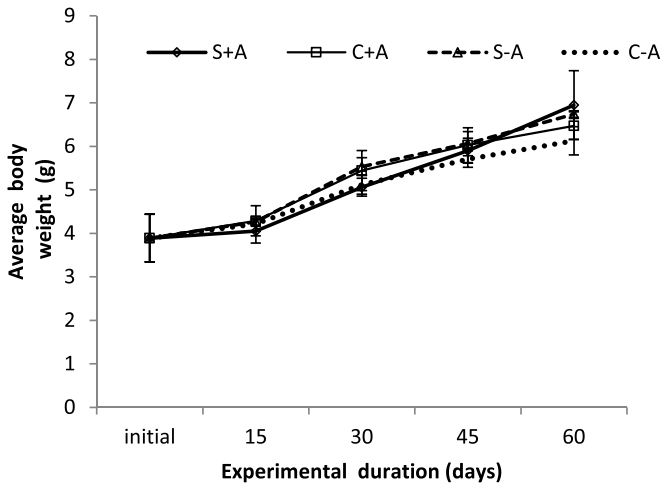

Fig. 4. Average biweekly body weight of *Penaeus monodon* juveniles with aeration (S + A and C + A) and substrate addition (S-A and C-A) in Experiment 2. Error bar indicates ± standard error.

shrimps are presented in Tables 6 and 7. In Exp 1, survival of the shrimp was significantly improved by 14 and 30% due to substrtae addition in high (20 + S) and low density groups (10 + S) respectively aginst respective groups without substrate addition (20-S and 10-S). Similarly, stocking density also resulted 34 and 17.6% significantly higher ($P < .05$) survival in 10 + S against 20 + S, and 10-S against 20-S respectively. Stocking density also found to have a significant role in final body weight, and SGR. About 37 and 56% significantly higher

Table 5
Autotrophic index (AI), chlorophyll *a* (Chl a) concentration and periphyton biomass (DM and AFDM) per unit surface area during the experimental period in Experiment 1 and 2 based on one way ANOVA.

Parameters	Experiment 1			Experiment 2		
	CN 10 + S	CN 20 + S	Level of significance	A + S	A – S	Level of significance
DM (mg cm^{-2})	12.77 ± 1.47 (3.4–22.7)	8.7 ± 0.91 (3.8–14.1)	*	4.07 ± 0.39 (1.9–7.6)	3.29 ± 0.33 (1.22–6.45)	NS
AFDM (mg cm^{-2})	4.31 ± 0.63 (0.6–8.2)	2.69 ± 0.41 (0.7–5.9)	*	2.64 ± 0.29 (1.02–5.4)	1.88 ± 0.21 (0.7–3.9)	*
Chl a (µg cm^{-2})	11.77 ± 1.05 (4.7–18)	9.28 ± 1.04 (5–18)	NS	13.84 ± 2.46 (6.8–41.4)	9.12 ± 1.04 (3.2–18.4)	NS
AI	345.57 ± 42.88 (111.2–594)	279.26 ± 29.08 (131.3–481)	NS	250.87 ± 42.26 (80.4–610)	224.49 ± 22.36 (87.8–348)	NS

Values are presented as mean ± standard error. DM, dry matter, AFDM, ash free dry matter, NS, not significant.
* $P < .05$.

Table 6
Effect of shrimp stocking density and addition of substrate on growth performance parameter (mean ± SE) of *Penaeus monodon* juveniles reared in Experiment 1 based on two way -ANOVA.

Yield parameters	Low density		High density		Level of significance		
	10 + S	10 – S	20 + S	20 – S	Substrate, S	Density, D	Substrate (S) × Density (D)
FINAL ABW (g)	4.85 ± 0.49	4.63 ± 0.40	3.54 ± 0.12	2.96 ± 0.03	NS	**	NS
SGR (% d^{-1})	1.99 ± 0.14	1.93 ± 0.11	1.58 ± 0.05	1.34 ± 0.02	**	**	NS
FCR	2.37 ± 0.22	3.16 ± 0.20	2.26 ± 0.01	2.73 ± 0.16	**	NS	NS
PER	1.13 ± 0.10	0.84 ± 0.06	1.17 ± 0.01	0.97 ± 0.05	*	NS	NS
Survival (%)	75 ± 2.89	57.67 ± 1.45	55.83 ± 2.20	49 ± 2.08	*	**	*

Values are presented as mean ± standard error. If the effects were significant, ANOVA was followed by Tukey test. NS, not significant. S × D, Interaction effect between substrate and density, ABW, Average body weight, SGR, Specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.
* $P < .05$.
** $P < .01$

final ABW were recorded in 10 + S and 10-S compared to 20 + S and 20-S respectively. Also, substrate installation improved the food conversion ratio of cultured shrimp by 21 and 33% in 20 + S and 10 + S, respectively against 20-S and 10-S. Better PER values, 1.13 and 1.17 were observed in substrate based treatments, 10 + S and 20 + S, against 0.84 in 10-S and 0.97 in 20-S treatments. In Exp 2, neither aeration nor substrate provision was found to have a significant effect on growth parameters like ABW, FCR, SGR etc. among the experimental groups. Absence of aeration did not significantly affect the growth performance of shrimps both in presence of substrate, S + A (6.96 ± 0.79) and absence of aeration, S-A (6.13 ± 0.32). However, factors like substrate addition, aeration and their interaction effects were found to play a significant role in survival rate of shrimp. Highest survival, 81.25 ± 3.60% was noticed in substrate with aeration (S + A), which was 25–35% higher compared to all other treatments.

4. Discussion

Periphyton based culture systems are documented to have multiple benefits in aquaculture systems in terms of growth performance and water quality improvement. However, there is apprehension about the wide adoption of these systems among shrimp farmers about aeration requirement and appropriate grazing pressure by shrimp on periphytion. Present studies were undertaken to have better understanding on these aspects.

Optimization of shrimp density in substrate based shrimp culture is highly pertinent as higher or lower shrimp density leads to either over grazing or under grazing. Experimental trial 1 revealed that stocking density of shrimps had a significant effect on the periphyton biomass developed over the substrate as lower periphyton biomass (DM and AFDM) was recorded in high density system in contrast to low density system which indicates increased grazing by the shrimps in higher density system. In general, higher periphyton biomass was recorded in ungrazed ponds as grazing selectively removes more digestible periphyton species leaving less palatable communities or trapped inorganic material (Azim et al., 2005; Asaduzzaman et al., 2010). Apart from stocking density, other factors like type of cultured species, their food habit, age and substrate density also plays an important role in periphyton grazing (Azim et al., 2005). Absence or lack of proper grazing results in self-shading of periphyton and eventual sloughing and dislodgement of the periphyton results in its low productivity (Huchette et al., 2000; Keshavanath et al., 2001; Ballester et al., 2007). Amount of periphyton developed over substrate varies with type of culture condition or grazing pressure by cultured animals. An average periphyton dry matter of 4.5 mg cm^{-2} in freshwater fish ponds (Huchette et al., 2000; Azim et al., 2005; Keshavanath et al., 2001; Asaduzzaman et al., 2008), 9.9 mg cm^{-2} in marine ecosystem (Richard et al., 2010) and 5.07 to 8.83 mg cm^{-2} in C:N ratio manipulated substrate integrated brackishwater systems (Anand et al., 2013a) were reported. Higher values, 12.77 ± 1.47 recorded in the 10+ S treatments can be attributed to lower grazing pressure by shrimps compared to 20 + S groups. This indicate optimization of stocking density of cultured species and substrate density becomes highly imperative for best possible utilization of natural resources developed in the system.

In the experiment 2, aeration effects were found to have a significant influence on AFDM content of periphyton, while no significant effects on dry matter biomass were noticed. Aeration determines the amount of periphyton biomass developed as it affects the development of different algal community in the periphyton. Choi et al. (1999) reported that periphyton algal community seems to change with artificial aeration in lake compared to natural lakes without aeration. The AFDM indicates the viable periphytic algal biomass excluded from inorganic solids or dead algal cells. Hence, the lower AFDM noticed in absence of aeration treatments can be attributed to accumulation of more inorganic components or dead cells compared to aerated systems. Though, it is reported that the absence of oxygen also can change the

Table 7
Effect of aeration and addition of substrate on growth performance (mean ± SE) of *Penaeus monodon* juveniles in Experiment 2 based on two way ANOVA.

Yield parameters	With aeration		Without aeration		Level of significance		
	S + A	C + A	S – A	C – A	Aeration, A	Substrate, S	Substrate × Aeration
FINAL ABW (g)	6.96 ± 0.79	6.48 ± 0.32	6.74 ± 0.07	6.13 ± 0.32	NS	NS	NS
SGR (% d^{-1})	0.95 ± 0.18	0.85 ± 0.08	0.92 ± 0.02	0.75 ± 0.09	NS	NS	NS
FCR	2.04 ± 0.13	2.55 ± 0.19	2.61 ± 0.12	2.62 ± 0.12	NS	NS	NS
PER	1.30 ± 0.09	1.04 ± 0.07	1.01 ± 0.04	1.01 ± 0.05	NS	NS	NS
Survival (%)	81.25 ± 3.60	64.58 ± 2.08	60.42 ± 2.08	64.58 ± 4.17	**	NS	**

Values are presented as mean ± standard error. If the effects were significant, ANOVA was followed by Tukey test. NS, not significant. S, Substrate, A, aeration, S × A, Interaction effect between substrate and aeration, ABW, Average body weight, SGR, Specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.
** $P < .01$.

periphyton community (Choi et al. 1999), no significant differences in periphyton biomass were noticed in Exp 2 which indicates that periphyton can flourish without much oxygen demand like natural system when shrimp are provided at low density systems. Microbial community develops over submerged substrates being a mix of nitrifying bacteria, and its consortium varies with type of substrate or culture environment used (Kumar et al., 2017). This suggests qualitative analysis of periphyton community need to be further explored as aeration or grazing effects can definitely alter the composition of microbiota developed over substrate.

Autotrophic index (AI), the relative autotrophic and heterotrophic dominance in the periphytic community (Azim et al., 2005) decreases when autotrophic community in periphyton decreases. Insignificantly higher AI recorded in low density treatments compared to higher density groups in Exp 1 can be ascribed to higher dominance of heterotrophic organism or slower pace of rejuvenation of autotrophic community due to lesser grazing of periphyton by shrimps. It is reported that grazing enhances rejuvenation of periphyton community which results in higher productivity (Wetzel, 1983). An AI index below 200 is reported in a well grazed periphyton community developed in fish ponds (Azim et al., 2005; Asaduzzaman et al., 2010) and C:N ratio manipulated shrimp culture system (Anand et al., 2013b) while an AI value above 200 is reported from marine culture systems (Richard et al., 2009). Our results represent much higher value compared to earlier reports which may be due to various factors like under grazing of biomass by shrimps in relation to amount of periphyton biomass due to higher nutrient load in the tank system which hastes the biomass development or dominance of heterotrophic systems in periphyton community.

In the present Exp 2, absence of aeration in substrate based groups resulted in significant variation in DO levels. The lowest DO level (< 4 ppm) noticed in the early morning was due to the respiratory activity of both shrimps and algal or microbial community developed in the substrate (Azim et al., 2005; Keshavanath et al., 2001., Kumar et al., 2017). However, due to the higher rate of photosynthesis, DO level in the later afternoon was higher in substrate based tanks compared to control. The pH of the periphyton and control groups was found to be in optimum range for shrimp farming (Anand et al 2015b; Biswa,s, et al., 2017). Periphyton community developed over the submerged substrates enhances the nitrification cycle and improves the water quality parameters (Ramesh et al., 1999). Although submerged substrates were reported to be effective in removal of TAN or other dissolved nutrients, no significant differences were noticed in experimental trial 1 except nitrate-N while In Exp 2, substrate found to significantly reduced the nitrogenous metabolites. The present observation corroborate with the findings of Audelo-Naranjo et al. (2011) who also could not find significantly better water quality parameters when submerged substrates provided in *L. vannamei* zerowater exchange mesocosm culture. In contrast, in our earlier substrate based shrimp culture pond trial, significant differences in water nutrient parameters were recorded (Anand et al., 2015b). These contrasting results may be probably due to variation in type culture management system with respect to variation in nutrient load generated in the substrate based system. Recently, integration of substrates in biofloc based systems are reported to trap the higher total suspended solids generated in water column and there by further enhances the water quality parameters.

Substrate provision in shrimp culture enhances the growth performance of shrimp through providing additional quality natural food in the form of periphyton community. Considerable growth improvement and reduction in feed conversion ratio were noticed when shrimps were reared with submerged substrates (Moss and Moss, 2004., Ballester et al., 2007; Abreu et al., 2007; Anand et al., 2013a). Microalgal communities are excellent source of essential nutrients (Zhukova and Kharlamenko, 1999). In the present trial, variation in shrimp density resulted in significant variation in growth rate as lower density of shrimp had better growth increment and survival rate compared to higher density system. It is well reported that, for all species cultured, including *L. vannamei*, production output is limited by reduced growth and/or survival as stocking densities increase (Bratvold and Browdy, 2001; Moss and Moss, 2004). Hence, to increase better growth from high intensity systems, and to mitigate the negative effects of high stocking density, it is required to explore specific design features. It is reported that artificial substrates installation improves growth and survival of juvenile *P. monodon* even at densities of 1000 and 2000 m^{-3} (Arnold et al., 2006), *L. vannamei* at densities up to 1556 m^{-2} (Bratvold and Browdy, 2001; Moss and Moss, 2004) and in giant fresh water prawn (Tidwell et al., 1999). Added surface area created by the substrates enhances the natural food supplement for the shrimp and provides refuge for shrimp from negative behavioral interactions at higher densities (Arnold et al., 2006). In the present Exp 1, even at higher density, no significant differences in FCR or PER noticed among the treatments indicate shrimp gained considerable nutritional value by grazing the periphyton developed over the substrate which resulted significant reductions in FCR. Apart from nutritional benefit, incorporation of periphyton as a dietary ingredient was also reported to act as immunostimulant and enhance digestive enzyme activities in shrimp (Anand et al., 2013b; Kumar et al., 2015; Anand et al., 2015a). Thus, in the era of antibiotics, this ecosystem based models can impart immunity and reduces disease outbreak in the long run. In Exp 2, no significant difference in final growth of tiger shrimp at in presence or absence of aeration indicates the comparatively low aeration requirement when shrimps reared at lower density. Better FCR or PER indicates that even in the absence of aeration, shrimp can perform better when submerged substrates are provided for growth of periphytic community. Significantly higher survival obtained in both the trails when submerged substrates are provided further reassures the fact that additional area generated by substrate installation provides more area for living (Arnold et al., 2009; Anand et al., 2015b), and thereby reduces crowding stress or increases survival (Bratvold and Browdy, 2001; Audelo-Naranjo et al., 2011). This further confirms that this system has the potential to adopt by small scale shrimp farmers with less investment for a sustainable farm outcome.

5. Conclusion

Provision of submerged substrates in shrimp culture improves the growth performance of shrimps and help to maintain better water quality parameters. The present study reveals that the scope for development of shrimps in low density traditional ponds with minimum aeration which can reduce the energy cost. Similarly, substrate addition enhanced the survival of shrimp through provision of additional living surface area which in turn can lead to increased final biomass even in a low density system. Also, study reveals that optimization of stocking density of cultured animal in substrate based system is highly imperative for maximum utilization of developed periphyton in the system. Selective algal feeding preference by the shrimps and composition shift in periphyton community with respect to aeration or in biofloc based systems are subjects of further research.

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