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# Nursery Rearing of *Penaeus vannamei* in Biofloc Systems with Different Salinities and Organic Carbon Sources

T. Anand, A. Srinivasan, P. Padmavathy, P. Jawahar, J. Stephen Sampathkumar

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## ABSTRACT

**Background:** Nursery rearing of *Penaeus vannamei* became inevitable in the Indian shrimp farming industry, since intensification of culture practices in grow-out systems caused nitrogenous wastes accumulation, diseases, mortality and premature harvests resulted in high food conversion ratio, lower production and profits. The nursery rearing in traditional water exchange systems often getting failure because of nitrogenous waste accumulation. Hence, the present experiment was planned to rear *P. vannamei* in bioflocs systems (BFS) with different salinities and carbon sources.

**Methods:** The experiment was conducted in 0.22 $m^3$ tanks with three different salinity groups *viz*., 35 ppt, 20 ppt and 5 ppt and in each salinity sugar, molasses used as carbon sources to maintain an estimated C/N ratio of 15:1 and controls without carbon sources. Experimental tanks were stocked @6 post larvae/l, with pre-salinity acclimatized *P.vannamei* seeds having 0.0029±0.0003g size and nursed for a period of 5 weeks.

**Result:** The nitrogenous waste accumulation was reduced significantly ($p<0.05$), also average body weight and survival rate of the seeds showed significant difference ($p<0.001$) between treatments and controls and within treatments ($p<0.05$). Salinity, carbon sources and their interaction influenced the growth characteristics significantly ($p<0.01$). The present experiment manifested promising results of bioflocs nurseries in rearing of *P. vannamei* seeds at different salinities.

**Key words:** Biofloc systems, Heterotrophic biomass, Nitrogenous waste, Nursery rearing, *Penaeus vannamei*.

## INTRODUCTION

After the introduction of *P.vannamei* during the year 2010 in India, the shrimp production has increased, over the years the shrimp farmers were also facing many problems in culturing this species *viz.*, unstable water quality due to intensification, size variation, slow growth, running mortality syndrome and disease incidences caused by microsporidian parasite *Enterocytozoon Hepatopenaei* (EHP), resulted in white feces diseases. Due to this, the size at harvest was reduced over the years and the farmers harvested the crop prematurely (Basavaraja, 2013; Rathipriya *et al*., 2019). Nursery systems were introduced to overcome the above issues in *P. vannamei* culture practices. But the traditional water exchange systems in nursery rearing often getting failure due to accumulation of nitrogenous wastes, hence, nursery farming of *P. vannamei* showed positive results especially with bioflocs systems (BFS) (Khanjani *et al*., 2016). The nitrogenous wastes from the nursery systems would be converted into useful heterotrophic bacterial biomass, by continuous addition of carbon sources to alter the C:N of the systems in such a way to aid the bioflocs formation under continuous aeration (Avnimelech, 1999; Menaga *et al*., 2020; Yuvarajan *et al*., 2021). The use of bioflocs technology would improve the survival, growth and final biomass of cultured animals (Khanjani *et al*., 2016). There were many studies with different stocking densities using different carbon sources in *P. vannamei* biofloc nursery systems (BNS) but dearth of knowledge in the nursery rearing of *P. vannamei* at different salinities. Hence, the present experiment was carried out for the nursery rearing of *P. vannamei* in BNS with three different salinities (35, 20 and 5 ppt) and two different carbon sources (molasses and sugar) for a period of 5 weeks with an estimated C:N of 15:1. The growth characteristics of *P.vannamei* seeds were taken into consideration to assess the system efficiency.

Department of Fisheries Science, College of Fisheries Engineering, Dr J. Jayalalithaa Fisheries University, Nagapattinam-611 002, Tamil Nadu, India.

**Corresponding Author:** T. Anand, Department of Fisheries Science, TNJFU Referral Lab, College of Fisheries Engineering, Tamil Nadu Dr J. Jayalalithaa Fisheries University, Nagapattinam-611 002, Tamil Nadu, India. Email: tanand@tnjfu.ac.in

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## MATERIALS AND METHODS

The experimental design is given in Table 1. The experiment was conducted as a part of dissertation work during the year 2020 in the Wet Laboratory of College of Fisheries Engineering, Nagapattinam. The experimental tanks were cylindrical in shape with a usable volume of 0.22 $m^3$. The experimental tanks were provided with uninterrupted turbulent aeration from the bottom of tanks through air stones connected with four blowers (each 120 $m^3$ / hour cap.). The seawater with 35 ppt was pumped into reservoir tanks

through filtration (150-250 µm mesh size) (International Office of Epizootics, 2009) from nearby Vettar estuary and it was diluted with 1ppt bore water to attain 20 and 5 ppt (Laramore *et al*., 2001). The tanks were disinfected with calcium hypochlorite (35% concentration of chlorine) to attain a minimum residual chlorine concentration of 10 ppm and which was maintained for 48 hours to kill the disease carriers (International Office of Epizootics, 2009) and after a week residual chlorine level checked and added sodium thiosulphate (7 mg/l for each 1 mg/l of residual chlorine) (Van Wyk and Scarpa, 1999). The water was pumped into experimental tanks and fertilized with dolomite at 10 g/m$^3$, superphosphate 15 g/m$^3$ and urea 15 g/m$^3$ for both treatment and control tanks for 2 to 3 days. Fifty liters of appropriate saline water were autoclaved, to that feed, respective carbon sources were added then aerated for a day and applied uniformly to only the treatment tanks for five days for biofloc development (Fig 1a) (Panigrahi *et al*., 2019). To compensate the evaporation loss, once in a week appropriate saline water was added into experimental tanks. A week after stocking the seeds, the control tanks were exchanged 10% of total volume once a week initially and once in 3 days after 20 days.

The C:N of the feed used was 7:1 and to bring the estimated C:N to 15:1, for 1g of feed added into the tanks 0.347g of sugar (96.8% free carbohydrates) and 0.368g of molasses (91.4% free carbohydrates) were applied in the treatment tanks every day between 8 to 9 am. The total ammoniacal nitrogen (TAN) level in treatment tanks also taken into consideration for the carbon dosage calculation (Avnimelech, 1999; Panigrahi *et al*., 2019).

At the start of 2$^{nd}$ week, once the total suspended solids (TSS) reached 100 mg/l, (Hostins *et al*., 2019) experimental tanks were stocked @ 6 post larvae (PL) per litre with specific-pathogen-free, pre-salinity acclimatized (Criales *et al*., 2011) *P. vannamei* post-larvae (PL) that weighed 0.0029± 0.0003 g. The PL were fed with commercial *P. vannamei* nursery feed with a size range from 0.5 mm to 1.2 mm. Crude protein, ether extract, crude fiber, total ash, nitrogen-free extract and moisture content of the feed used were 42%, 6%, 3%, 15%, 20.5% and 10.5% respectively. The animals were fed from 35 to 7% of average body weight (ABW) (Van Wyk, 1999) and fed six times a day *viz*., 6 am, 10 am, 2 pm, 6 pm, 10 pm and 2 pm. The experiments were conducted for 5 weeks from the date, seeds were released.

Water samples were collected between 8 to 9 am at weekly intervals for 8 weeks. Temperature, pH, salinity and dissolved oxygen (DO) were checked every day at 6am and 6pm (HANNA HI 9829 Multiparameter) in the experimental unit. The other water quality parameters like Total alkalinity (TA), TSS, TAN, nitrite nitrogen ($NO_2$-N), nitrate-nitrogen ($NO_3$-N), phosphate phosphorus ($PO_4$-P) and total heterotrophic counts (THC) were measured once in a week using standard methods (American Public Health Association, 2012). The biofloc volume (BFV) was measured using the Imhoff cone (Fig 2).

The ABW, average growth rate, specific growth rate and survival rate of *P. vannamei* seeds were calculated once in a week by using the formula given below. At the end, shrimps were harvested, the survival rate {Total no of animals harvested divided by the total number of animals stocked multiplied by hundred (%)} was calculated (Khanjani *et al*., 2016) (Fig 1b and c). All statistical analysis was performed using PAST 4.02 software for Windows (Hammer *et al*., 2001).

$$\text{Average body weight (g)} = \frac{\text{Total weight of shrimps collected (g)}}{\text{Number of shrimps}}$$

$$\text{Average growth rate (g)} = \frac{\text{Final weight of shrimps - Initial weight (g)}}{\text{Number of days cultured}}$$

$$\text{Specific growth rate} = \frac{\text{Log [Final weight of shrimps - Initial weight (g)]}}{\text{Number of days}} \times 100$$

## RESULTS AND DISCUSSION

### Water quality parameters

The water quality management in the nursery and hatchery systems are bit critical than the grow out ponds since, the larval forms are more susceptible to toxic metabolites. Though there was no significant difference ($p>0.05$) in the temperature and DO levels of treatments and control they were within the optimum for growing *P.vannamei* (Emerenciano *et al*., 2013). DO and salinity is inversely proportional hence, DO levels were slightly higher at 5ppt treatments (Boyd *et al*., 2018). The pH, TA and TSS values showed significant difference ($p<0.05$) between treatments, controls and within treatments. BFV showed significant difference ($p<0.01$) between and within treatments. The pH, TA and TSS levels were within the optimum range for growing *P.vannamei* (Van Wyk and Scarpa, 1999; De Morais *et al*., 2020). Regular addition of carbon sources in the BFS with

**Table 1:** The experiment design of *P. vannamei* bioflocs nursery rearing systems.

| Particulars | 35 ppt group | | | 20 ppt group | | | 5 ppt group | | |
|---|---|---|---|---|---|---|---|---|---|
| Carbon source | Sugar | Molasses | Control (No carbon added) | Sugar | Molasses | Control (No carbon added) | Sugar | Molasses | Control (No carbon added) |
| Experimental tanks marked as | T35S | T35M | CL35 | T20S | T20M | CL20 | T5S | T5M | CL5 |

higher DO levels resulted in lower pH due to degradation of organic matter and formation of heterotrophic biomass (Emerenciano *et al*., 2013). TA was lower than the controls since the assimilation of nitrogenous matter consumed alkalinity in the treatments (Furtado *et al*., 2014). In the present experiment a reasonable level of TSS content was maintained and PL of *P.vannamei* might have consumed the bioflocs (Schveitzer *et al*., 2013; Khanjani *et al*., 2020).

**Nutrient dynamics in bioflocs nursery rearing systems**

The TAN, $NO_2$-N, $NO_3$-N and $PO_4$-P values showed significant difference ($p<0.01$) between treatments and controls, whereas significant difference was not observed within treatments (Fig 3, 4 and 5a to c). It was observed that TAN values started increasing slowly till 5$^{th}$ week, after that it showed downtrend, while $NO_2$-N, $NO_3$-N started increasing. But in the controls TAN, $NO_2$-N and $NO_3$-N was gradually increasing from the first week and attained the peak at the 7$^{th}$ week. So, after the bioflocs were established in the treatments, the assimilation was dominated whereas, nitrification was solely responsible for TAN assimilation in the control tanks. This indicated the fact that the heterotrophic bacterial biomass (HBB) in BNS outwitted the nitrifiers in the nitrogenous nutrient assimilation which was an impediment for the development of the nitrifiers in BFS (Cortes-Lorenzo *et al*., 2015). In BFS insignificant levels of $NO_2$-N and $NO_3$-N were observed in treatments, which used to happen due to assimilation of nitrogenous compounds. Though there was an increase in the TAN, $NO_2$-N and $NO_3$-N concentrations in the treatment groups during the first 2 to 3 weeks, it was within optimal level for rearing and growing PL of *P.vannamei* (Lin and Chen, 2001; Lin and Chen, 2003). The distinction in the levels of TAN, $NO_2$-N and $NO_3$-N values between treatment and controls served as an evidence that HBB assimilated the TAN in BNS (Avnimelech, 1999). A significant difference in the THC between treatments and controls also expounded the fact very well (Khanjani *et al*., 2020). The results exhibited that nitrification was the major source of nitrogenous matter oxidation in controls whereas in BNS, assimilation as well as nitrification also served as the major nitrogenous matter oxidation process (Emereniano *et al*., 2013).

The $PO_4$-P values were higher than the controls and similar observations were made in the earlier studies also (Fig 6a to c). Unlike the plankton based systems the microbial flocs in the BNS often unable to assimilate the accumulated phosphorus arising out of non-ingested feed, fecal matter and decomposed organic matter hence, periodical removal of excess phosphorus is necessary in such systems (Panigrahi *et al*., 2019).

**Growth characteristics**

Salinity, carbon sources and their interaction showed significant influence ($p<0.05$) between different treatments in the ABW and survival rate of *P. vannamei* BNS (Table 2). A significant difference ($p<0.01$) was found in the ABW,

**Table 2:** Growth characteristics of *P. vannamei* seeds in the nursery biofloc systems.

| Growth characteristics | T35S | T35M | CL35 | T20S | T20M | CL20 | T5S | T5M | CL5 | P<** |
|---|---|---|---|---|---|---|---|---|---|---|
| Final ABW (g) | 2.233± 0.11$^{a}$ | 1.937± 0.06$^{b}$ | 1.543± 0.11$^{c}$ | 2.517± 0.13$^{ad}$ | 2.013± 0.25$^{abe}$ | 1.573± 0.14$^{cf}$ | 2.873± 0.12$^{*}$ | 2.197± 0.05$^{abeg}$ | 1.700± 0.05$^{bcfh}$ | 0.001 |
| Growth rate (g/day) | 0.0638± 0.003$^{a}$ | 0.0553± 0.002$^{b}$ | 0.044± 0.003$^{c}$ | 0.0719± 0.004$^{ad}$ | 0.0575± 0.007$^{abe}$ | 0.0449± 0.004$^{cf}$ | 0.0821± 0.003$^{h}$ | 0.0628± 0.001$^{abei}$ | 0.0486± 0.001$^{bcfj}$ | 0.001 |
| Specific growth rate | 17.50± 0.05$^{a}$ | 17.35± 0.03$^{ab}$ | 17.12± 0.07$^{c}$ | 17.62± 0.05$^{ad}$ | 17.29± 0.13$^{be}$ | 17.04± 0.09$^{cf}$ | 17.56± 0.04$^{adg}$ | 17.38± 0.02$^{abeh}$ | 17.03± 0.03$^{cfi}$ | 0.001 |
| Survival rate (%) | 95.8± 1.6$^{a}$ | 90.0± 1.5$^{b}$ | 80.5± 1.5$^{c}$ | 96.3± 0.7$^{ad}$ | 87.3± 1.5$^{be}$ | 78.3± 1.2$^{cf}$ | 88.3± 1.5$^{beg}$ | 79.8± 1.2$^{cfh}$ | 63.7± 2.5$^{*}$ | 0.001 |

Note: Initial ABW was 0.0029±0.0003 g.

** Repeated measures ANOVA significance level.

Note:In Table 2 * superscript showed significant difference with all the treatment in that row.

Mean values within a row with same superscripts are not significantly different and Different superscripts like $^{abc}$ are depicted for significant difference ($p<0.05$) based on Tukeys' pairwise significance tests.

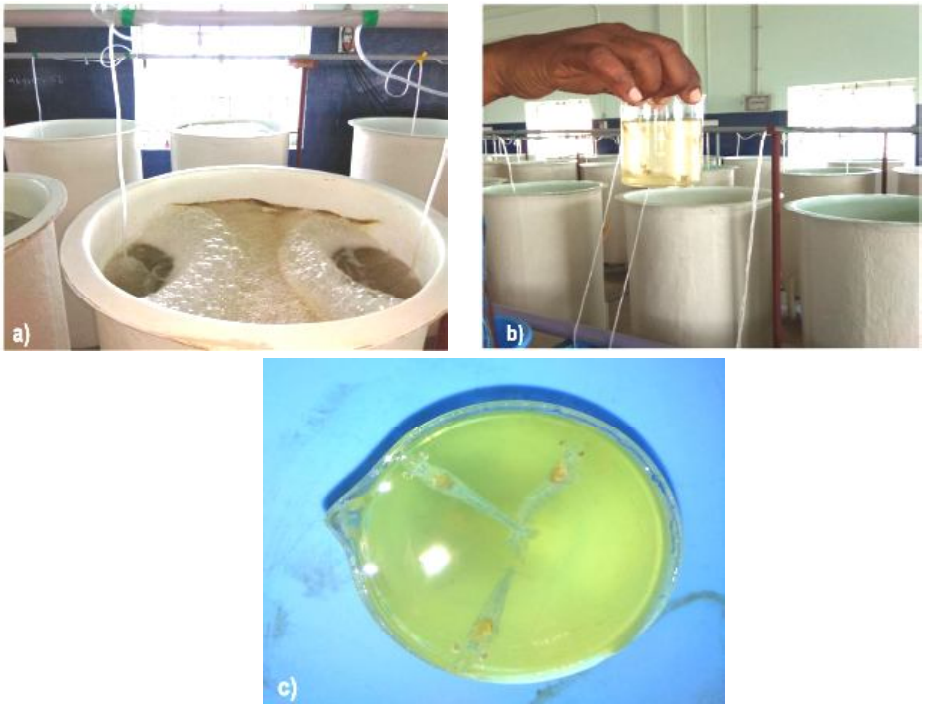


**Fig 1:** a). Bioflocs developed experimental tank b) and c) Sampling of *P. vannamei* seeds reared in bioflocs systems.

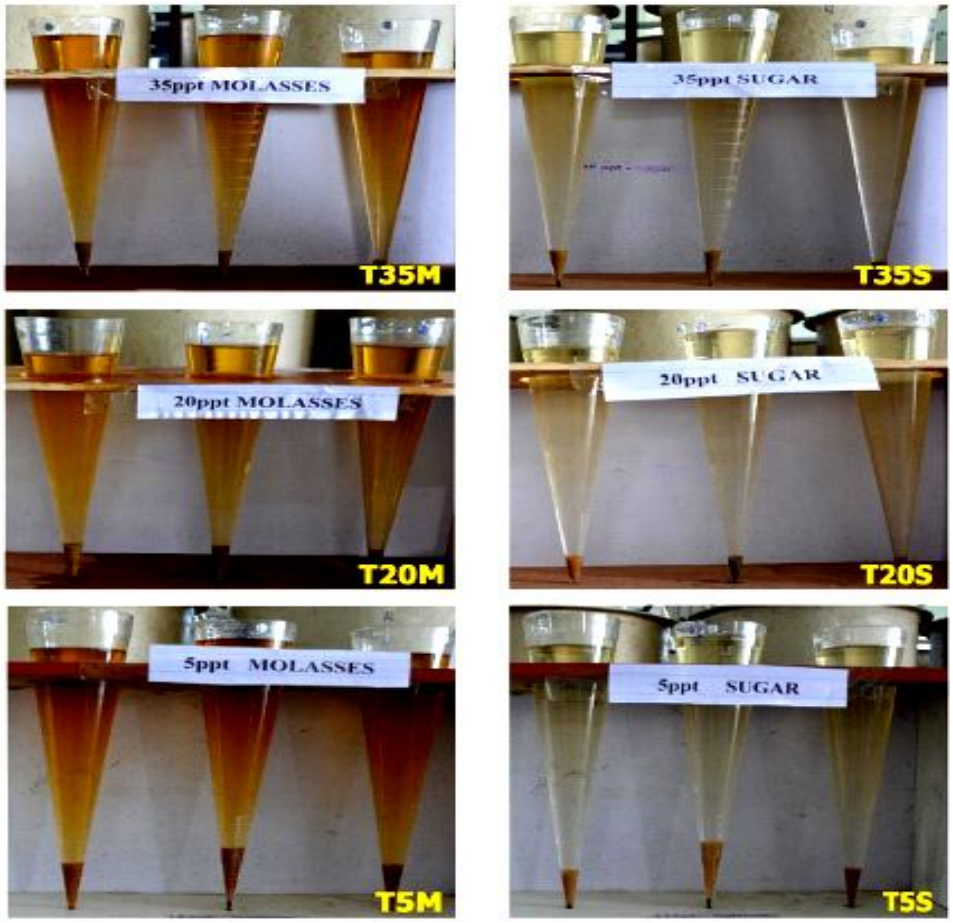


**Fig 2:** Measuring the biofloc volume (BFV) using Imhoff cone at 4th week of experiment.

between treatments and controls and within the treatments ($p<0.05$). Sugar treatments recorded significantly higher growth rate than molasses treatments irrespective of salinity. ABW was 2.23 to 2.87 g in sugar treated tanks and 1.93 to 2.19 g in molasses treated tanks and showed an increasing trend with decrease in salinity. Growth rate was higher at 5 ppt than 35 or 20 ppt. Earlier studies reported that use of BFS as nurseries for Penaeid shrimps yielded better growth of cultured organisms with higher ABW and survival rate if optimal water quality is maintained. In the present experiment irrespective of carbon sources 5 ppt showed higher growth rate than 20 and 35 ppt (Bray *et al*., 1994).

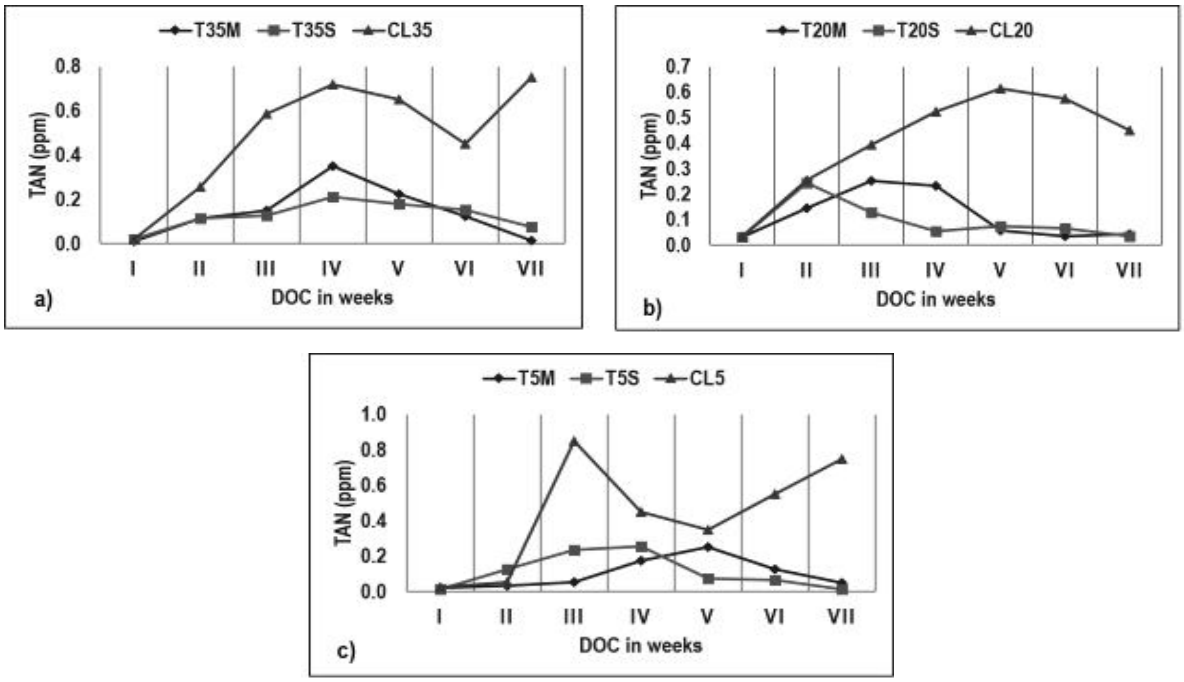


**Fig 3:** Total ammoniacal level (TAN) in *P.vannamei* biofloc nursery systems a) at 35 ppt b) at 20 ppt c) at 5 ppt.

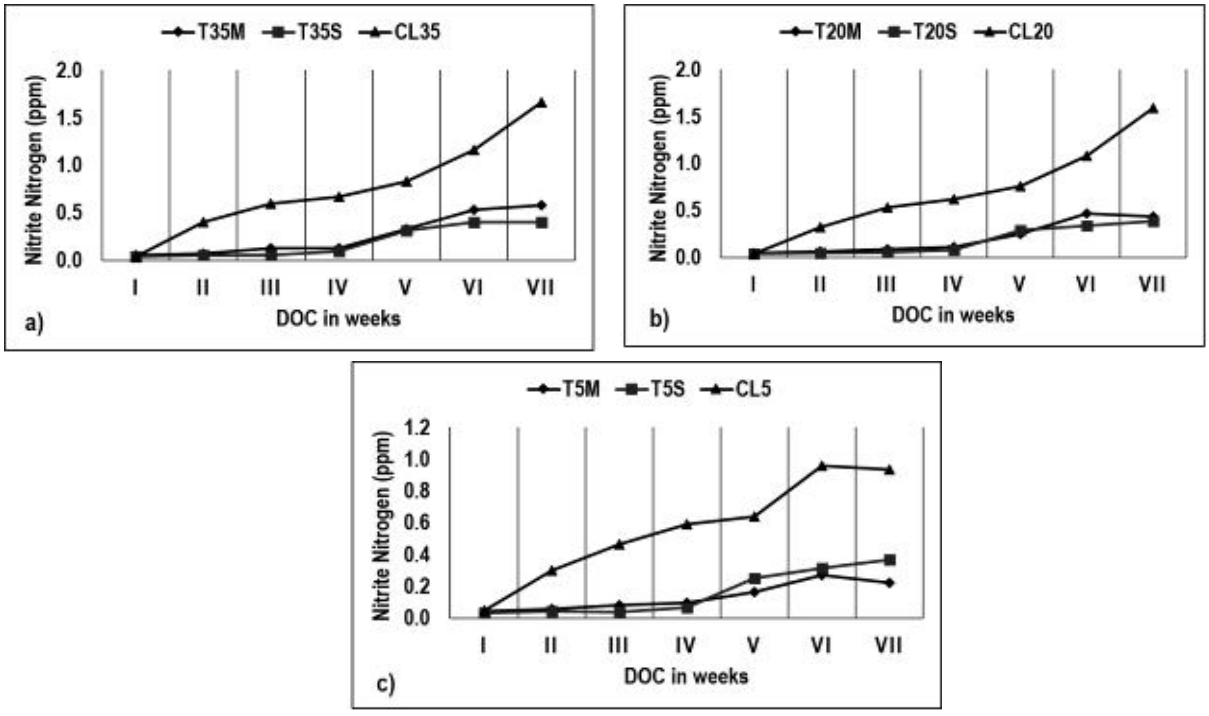


**Fig 4:** Nitrite nitrogen level ($NO_2$-N) in *P. vannamei* biofloc nursery systems a) at 35 ppt b) at 20 ppt c) at 5 ppt.

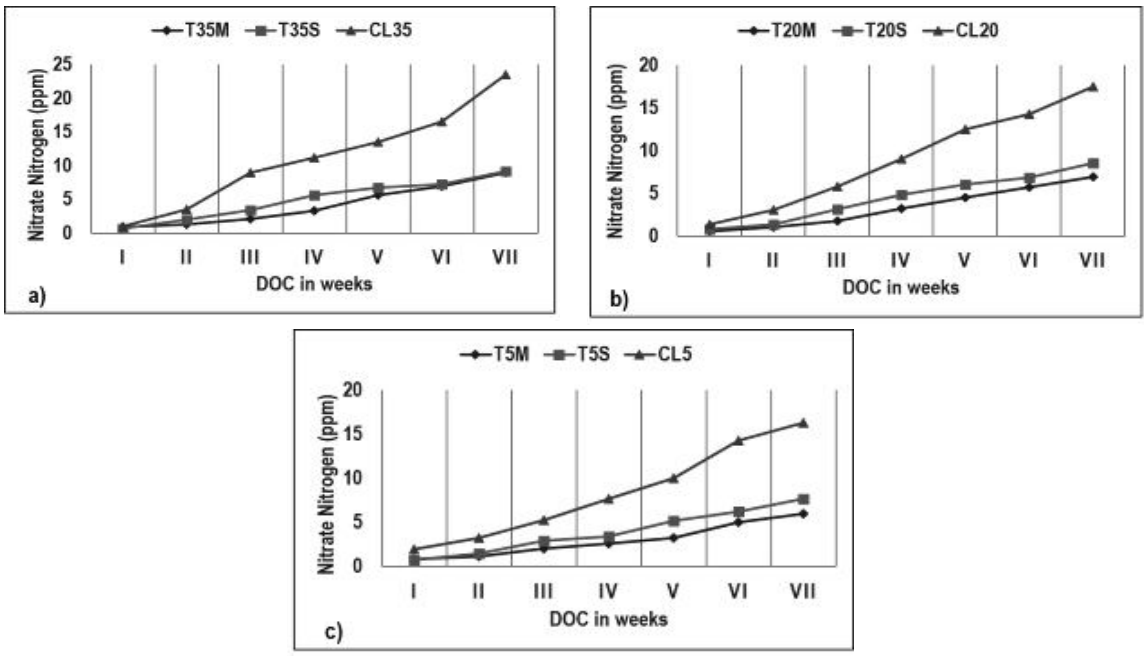


**Fig 5:** Nitrate nitrogen level ($NO_3$-N) in *P.vannamei* biofloc nursery systems a) at 35ppt b) at 20ppt c) at 5ppt.

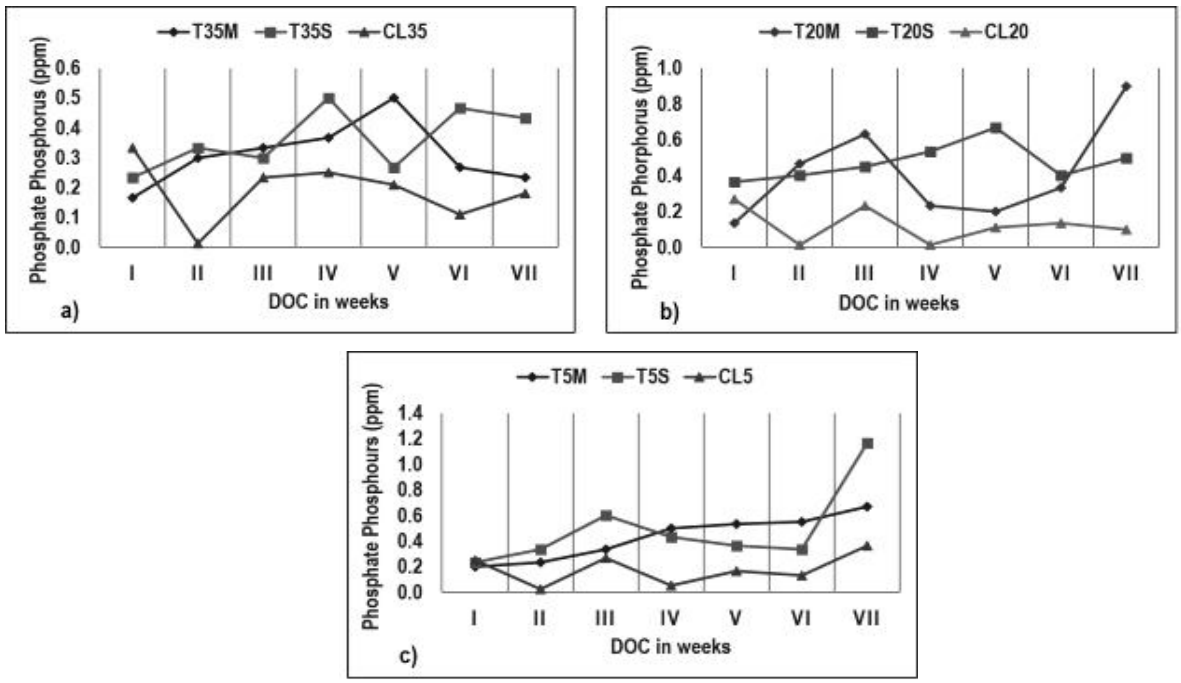


**Fig 6:** Phosphate phosphorus level ($PO_4$-P) in *P.vannamei* biofloc nursery systems a) at 35ppt b) at 20ppt c) at 5ppt

Among various carbon sources applied in the present experiment sugar showed higher ABW irrespective of salinities, this is in accordance with the earlier studies conducted by Xu *et al*. (2012).

A significant difference ($p<0.01$) in the survival rate was observed between the control, treatments and within treatments ($p<0.05$) except between 35 and 20 ppt. The survival rate was increasing with increase in salinity, similar results were observed with previous studies conducted with low salinities (Li *et al*., 2007). But *P. vannamei* can be cultured from 1 to 45 ppt if it is acclimatized for the salinity during the post larval development stage which would modify the ensuing salinity tolerance limits of juveniles (Criales *et al*., 2011). Among the carbon sources sugar showed higher survival rate ranging from 88.3 to 96.3% followed by molasses with 79.8% to 90% (Table 2).

## CONCLUSION

The nursery rearing of *P.vannamei* in BFS showed beneficial effects in a wide range of salinity from 5 to 35 ppt than the conventional water exchange systems. The average body weight decreasing and survival rate increasing with increase in salinity. Though the ABW was higher at 5ppt and survival rate was higher at 35 ppt, both ABW and survival rate was better at 20 ppt salinity and among the carbon sources sugar showed promising results in nursery rearing of *P. vannamei* seeds.

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