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Efficacy of Probiotics on *Litopenaeus vannamei* Culture through Zero Water Exchange System

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ABSTRACT
Biosecure zero-exchange systems represent an emerging technology that provides a high degree of pathogen exclusion with minimal water exchange. An important ramification associated with reduced or zero water exchange is the increased importance of *in situ* microorganisms both in regulating biogeochemical cycles within the culture environment and in directly affecting shrimp growth and survival. The newest attempt to improve water quality and control diseases in aquaculture is the application of probiotics and/or enzymes to ponds. This concept of biological disease control, particularly using microbiological modulator for disease prevention has received widespread attention. Keeping the above points in mind, this work was performed with zero discharge using probiotics, monitoring all the physico-chemical parameters and nutrients. The microbial population of the water and sediment were analyzed throughout the culture period. Highlights of this study are, (a) In the experimental ponds 1 and 2, the shrimps had a better growth (34.5 and 32.6 g, respectively), compared to 29.8 g in the control pond and (b) There was no incidence of disease in the experimental ponds, whereas the control pond had some bacterial infections. These encouraging results may be attributed to the use of probiotics in zero water exchange system.

Key words: Shrimp culture, probiotics, zero water exchange system, physicochemical parameters, microbial load

INTRODUCTION
Aquaculture production has grown enormously in recent years and among that Penaeid shrimps are one of the most important cultured species worldwide especially in Asia due to their high economic value and export (Sekar *et al*., 2014). Approximately more than 5 million metric t of shrimps are annually produced but the current global demand for both the wild and farmed shrimp is approximately more than 6.5 million metric tons per annum (Karthik *et al*., 2014). Despite high levels of shrimp production by culture, shrimp farmers suffered significant economic losses in recent years due to disease problems that have plagued the industry. Due to the continuous outbreak of this WSSV disease in *Penaeus monodon* culture leading to loss of shrimp culture in India the farmers are seriously looking for alternative shrimp species for culture. In 2008, the Coastal Aquaculture Authority (CAA) of India introduced a new shrimp species *Litopenaeus vannamei* as an alternative *Penaeid* species in India to culture and export. The

penaeid shrimp *Litopenaeus vannamei* exhibits fast growth rate and its culture period is significantly reduces compared to *Penaeus monodon*, thus the *Litopenaeus vannamei* has been established as alternative to *Penaeus monodon* to shrimp farming in several countries such as, East, Southeast and South Asia (Karuppasamy *et al*., 2013).

Although vaccines, medicated feeds and immunostimulants are effective in combating some pathogens in other meat-producing programmes, they are either unavailable to shrimp farmers or their efficacy is unproven. Various pond management strategies like stocking of high-health seed, reducing water exchange rate and screening influent water have been employed to mitigate the risk of disease outbreak. To meet the growing demand for high-quality shrimp products, novel production systems must be designed to minimize the introduction and spread of pathogenic agents, as well as to protect coastal resources. Biosecure zero-exchange systems represent an emerging technology that provides a high degree of pathogen exclusion with minimal water exchange. An important ramification associated with reduced or zero water exchange is the increased importance of *in situ* microorganisms both in regulating biogeochemical cycles within the culture environment and in directly affecting shrimp growth and survival. The newest attempt to improve water quality in aquaculture is the application of probiotics and/or enzymes to ponds. This approach of biotechnology is known as bioremediation, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds. The concept of biological disease control, particularly using microbiological modulator for disease prevention has received widespread attention. A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains is termed as probiotics. The term 'probiotics' was firstly coined by Parker (1974) and it originated from two Greek words 'pro' and 'bios', which mean 'for life'. Probiotics generally include bacteria, cyanobacteria, fungi etc. They may be called as 'normal microbiota' or 'effective microbiota' (Rao, 2003). Thus, the present study was aimed to test the efficacy of probiotics in a zero water exchange, biosecure shrimp culture system.

MATERIALS AND METHODS

The study was carried out in a commercial shrimp farm situated on the Northern banks of Vellar estuary (Lat. 11°29' N; Long. 79°46' E). This shrimp farm with three ponds and a reservoir has a total water spread area of 2.9 ha (Pond 1-0.6 ha; Pond 2-0.6 ha, pond 3-0.7 ha and Reservoir pond -1 ha). Ponds 1 and 2 were used as experimental ponds and pond 3 was used as the control.

Pond preparation

Soil and water culture: Initially the pH of the soil was checked and was found to be between 5.9 and 6.3. Lime was applied at the rate of 500, 500 and 600 kg in ponds 1, 2 and 3 respectively and the pH was increased to 7.2. The bottom was tilled and dried. After a week of soil preparation, water was pumped in with a help of a 10 HP (Kirloskar) pump. Water was pumped from Vellar estuary into the reservoir and the pumped in water was disinfected with bleaching powder at the rate of 60 ppm ha^{-1}. The water was left undisturbed for 10 days to remove the residual chlorine. Later the water was pumped to the culture ponds.

Fertilization and stocking: For ponds 1 and 2, an organic mixture of rice bran, cow dung, yeast and a blend of probiotic bacteria were inoculated, for plankton production. In pond No. 3, initial fertilization to develop the plankton bloom was done with inorganic fertilizers in the ratio of 10:2 (N: P). Healthy and WSSV negative *Litopenaeus vannamei* seeds were purchased from a reputed hatchery at Marakkanam, Tamil Nadu. The seeds were stocked at a density of 10 m^{-2}. Before stocking, the seeds were acclimatized to the pond environment.

Feeding: Feeding was done using CP feed (Charoen Pokhpand aquaculture India Pvt. Ltd, Chennai). The feeding schedule was based on the feed chart provided by the manufacturing company. Blind feeding was done for the first 30 days. Later the feeding was adjusted based in the check tray observation and sampling. Four check trays were installed per pond. The feed ration was divided for 4 times in a day (25, 20, 30 and 25% for morning (6:00 AM), noon (12:00 PM), evening (6:00 PM) and night (1:00 AM) feeding, respectively. The feed was broadcast from the dyke during the initial phase and boat feeding followed during the later stages.

Sampling: Sampling was done in all ponds every fortnight during early hours of the day with a cast net. Five hauls were made in each pond. The shrimps caught per haul and their individual weights were recorded. Healthiness, survival rate, Average Body Weight (ABW) and Average Daily Growth (ADG) of the animals was estimated through the samples. The diameter of the cast net used for sampling was 3.3 m. The area of the net was calculated with 60% efficiency of coverage at the bottom.

Water exchange: Exchange of water was not carried out throughout the culture period, but topping up of water from the reservoir compensated the water loss due to evaporation, percolation and seepage.

Probiotics and activation: Commercially available probiotic super NB (CP aquaculture India Pvt. (Ltd) was used. Two hundred milliliter of the probiotic with rice bran, tapioca flour, sugar and yeast were added to 200 L of freshwater and left overnight with vigorous aeration. After fermentation, the slurry was applied evenly in the ponds. The dosage of the probiotic was increased as the culture days increased.

Water quality assessment: Water quality analysis was done in all the ponds following standard methods. The pH cone was used to find out the soil pH. The pH pen (Scan-2-Eutech cybernetics PTE Ltd, Singapore) was used to measure the water pH and handy refractometer (Atago, Japan) for estimating salinity. Dissolved oxygen and temperature together were measured with the help of handy D.O meter (YSI 55 model). Ammonia was determined using the sea water method as described by Solorzano (1969) and Koroleft (1969) and recorded as parts per million (ppm). Nitrate, nitrite, total phosphate and silicate were estimated following the methods described by Strickland and Parsons (1972). A secchi disc was used to measure the transparency. The total heterotrophic bacterial population was estimated following the standard procedures.

Total Heterotrophic Bacteria (THB) population: To estimate the total heterotrophic bacterial population in the experimental ponds and control pond, the water and sediment samples were collected to find out the differences in the THB population. For this study, dehydrated bacteriological medium, Zobell's 2216 (Himedia Laboratories Private Limited, Mumbai, India) was dissolved in 50% sea water and sterilized by autoclaving at 15 lb. pressure for 15 minutes. The glass wares such as petriplates and conical flasks were sterilized in a hot-air oven at 165°C for 2 h. One milliliter of sample was mixed in 9 mL of sterilized water and from this tube 1 mL was transferred to the next dilution blank. Likewise appropriate dilutions were made. From the above sample, 1 mL of aliquot was transferred to the sterile petriplates, to which 15-20 mL of melted and cooled Zobell's marine agar medium was poured and mixed with the sample thoroughly. For sediments 99 mL was used. The following procedures were similar to those done to water samples. Then the inoculated plates were incubated in an inverted position. After 48 h they were counted and expressed as Colony Forming Units (CFU).

Biometric studies: In the present study, characters of *Penaeus monodon* of cultured and wild males and females were studied separately by all possible combinations using the linear regression techniques and correlation coefficient.

RESULTS AND DISCUSSION

In normal, diseases in aquaculture practices are mostly caused by luminous bacteria *Vibrio harveyi* and it has been referred as the largest economic loss in the shrimp aquaculture due to mass mortalities (Sivakumar *et al*., 2012). To control the pathogens, the use of probiotics in aquaculture is increasing demand for its more environment friendly aquaculture practices (Kumar *et al*., 2013). Now a days, the use of probiotics in aquaculture might represent a valuable mechanism to increase shrimp growth and survival rate. In general, the Gastro Intestinal Tract (GIT) of the aquatic animal is mainly composed of gram negative bacteria (Vine *et al*., 2006). So, the incorporation of beneficial gram positive (probiotic) bacteria in feed can modify its gastro intestinal tract (Vieira *et al*., 2007).

Water quality parameters: The technique of water quality management in shrimp ponds is less understood than other aspects of shrimp farming. If water quality is not maintained properly shrimps will not feed and become more susceptible to disease, which leads to poor survival. The water quality parameters that affect the shrimp productions in ponds are salinity, temperature, pH, dissolved oxygen, transparency, toxic metabolites (ammonia, nitrite) and nutrients (nitrate, total phosphate and silicate). Addition of probiotics, as water cleaner was found to be highly beneficial in the water quality management. This fact is established clearly in the present study. Salinity and temperature are mainly dependent on climatic factors and others are altered by liming, fertilization, stocking and feeding. Boyd (1989, 1990) reported that the shrimps will respond to changes in each water quality parameter.

Temperature: Temperature plays a vital role in metabolism of shrimps. In culture pond the optimum temperature range is 25-30°C and temperature beyond this range is lethal (Boyd and Fast, 1992) to shrimps. During the present study, the temperature was between 26.7 and 34.3°C. There was no difference between the experimental and control ponds. Being a tropical country, temperature is known to be high in Tamil Nadu, especially during summer and the recorded temperature did not affect the shrimps. The variations in the temperature (Fig. 1b, c, 2a and 4) ranged from 27.8-32.8°C in the control pond, 26.9-34.3°C and 26.7-32.9°C in experimental ponds 1 and 2, respectively (Fig. 1a-c).

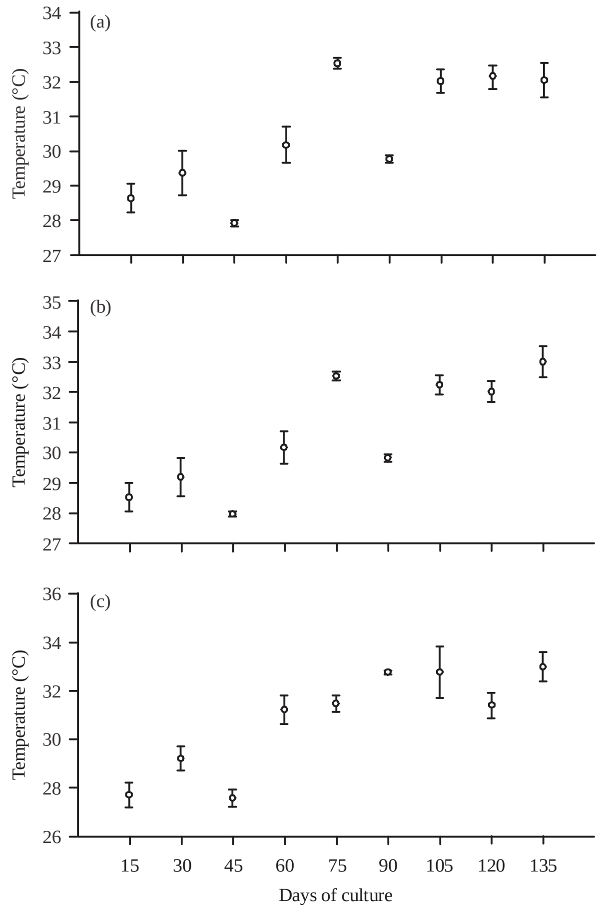

Fig. 1(a-c): Variations in temperature in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

Salinity: The normal growth of shrimp can be achieved between 15 and 20 ppt (Chen, 1976, 1985; Motoh, 1981; De Jesus *et al*., 1985; Cheng and Liao, 1986). Boyd (1989) also stated that the ideal salinity for shrimps from 15-25 ppt and high or low salinity affects the moulting frequency. In this study, salinity was varying vastly from 29-40 ppt in all the ponds. However, a normal growth occurred, in contrast to the above mentioned works. Other environmental factors would have more favourably protected the well being of the shrimps. The salinity levels varied between 29-40 ppt. There was not much difference between control and experimental ponds (Fig. 2a-c).

pH: The pH of brackish water is not a direct threat to shrimps' health because brackish water is well buffered against pH change and pH will mostly remain within the range of 6.5-9.5. The pH of the culture medium is directly related with metabolism and other physiological process of shrimps. Low pH increases the toxicity of nitrite to cultured organism (Wedemeyer and Yasulake, 1978) and the toxic form of sulfide (Chien, 1992) and high pH increases the unionized ammonia (Clot and Armstrong, 1981). It also reduces the natural pond production presumably by reducing the availability of nutrients (Alabaster and Lloyd, 1980) including phosphorus (Boyd, 1982). During the present study the water pH ranged from 6.1-8.3 in the control pond. In the experimental ponds the

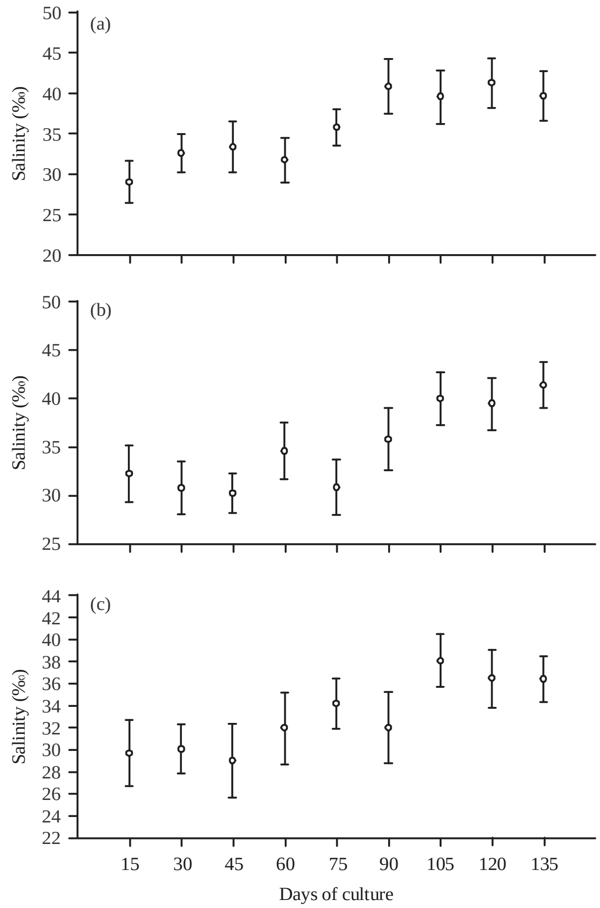

Fig. 2(a-c): Variations in salinity in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

pH was found to be maintained in the optimum ranges of 7.2-8.3 during the culture period. From the results of the present study it is evident that the probiotics are helpful in maintaining the pH at optimum levels, avoiding the otherwise possible fluctuations. The pH values in the control pond ranged from 6.1-8.3. In the experimental ponds the values ranged between 7.2 and 8.5. Fluctuation was higher in the control pond when compared to the experimental ponds (Fig. 3a-c).

Dissolved oxygen: The major factors that affect the solubility of dissolved oxygen in water are temperature, salinity, pressure and biological process. Generally the concentration of dissolved oxygen is high in the afternoon due to photosynthetic activity of phytoplankton and low in the early morning due to only respiration and no photosynthesis in the night. Boyd *et al.* (1978) and Madenjian *et al.* (1988) concluded that emergency measures must be taken if the dissolved oxygen concentration falls below 3 ppm. Law (1988) suggested that the dissolved oxygen levels should be kept above 2 ppm at all times. Liao and Murai (1986) reported that the rate of respiration in *Penaeus monodon* remained constant at dissolved oxygen concentration level of 3-4 ppm in water. Low dissolved oxygen level occurs in shrimp ponds due to phytoplankton die off and decomposition of the same (Chang and Ouyang, 1988) and can cause stress or even mortality of shrimps in ponds (Shigueno, 1975; Wickins, 1976; Madenjian *et al.*, 1987). Low dissolved oxygen increases the

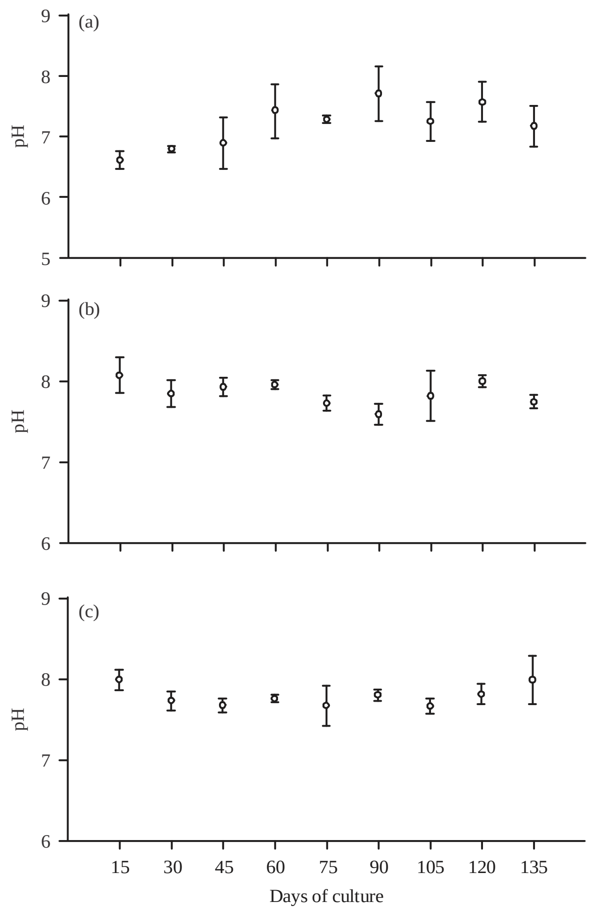

Fig. 3(a-c): Variations in pH in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

ammonia concentration and decreases the pH levels (Boyd, 1982). In the present study, in the control pond the dissolved oxygen levels ranged between 3.6-5.2 ppm. The dissolved oxygen level was always above 4 ppm in the experimental ponds, favourable for the health of shrimps. This may be correlated with the stable bloom throughout the culture period. The dissolved oxygen concentration varied from 3.6-5.2 mg L^{-1} in the control pond and 4.1-6.8 in the experimental ponds (Fig. 4a-c).

Transparency: Phytoplankton plays a significant role in the pond ecosystem and minimizes the water quality fluctuations. A stable phytoplankton population enriches the culture medium and competes with other pathogenic bacterial population for nutrients and suppresses the bacterial growth. Generally phytoplankton density is monitored by secchi disc. Water colour is also a good indicator. Dull green or yellowish or green brownish green colours are associated with green algae and diatoms. The visibility of secchi disc increases with decreasing phytoplankton abundance and decreases with increasing phytoplankton population. The optimum level of transparency is from 25-40 cm (Clifford, 1992). According to Boyd and Fast (1992) secchi disc readings of 25-35 cm are considered desirable by most shrimp farmers and the measurements should be made 800 and 1000 h or between 1400 and 1600 h (Almazan and Boyd, 1978). In the present study, the

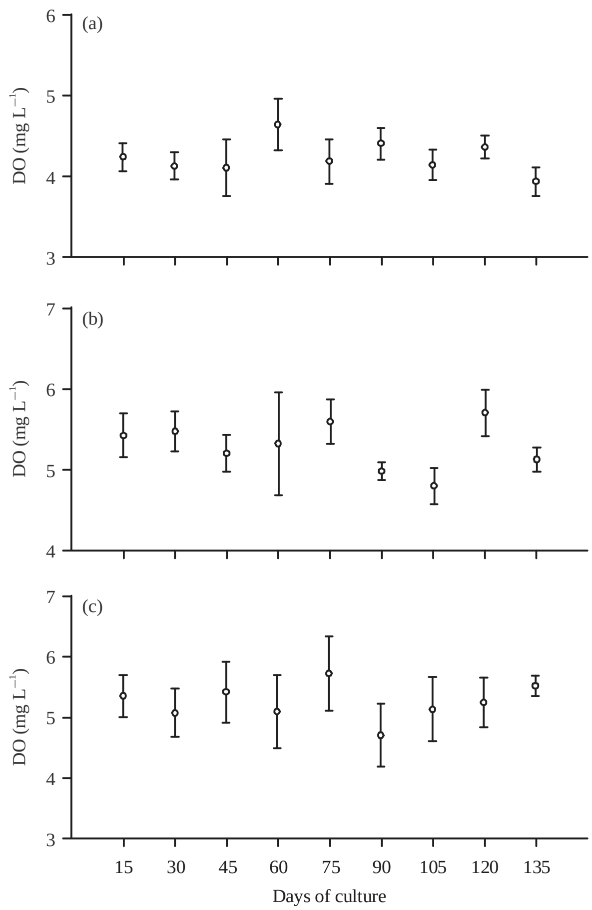

Fig. 4(a-c): Variations in DO in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

transparency levels in the control pond ranged from 90-75 cm. This was due to unstable bloom in the control pond. In the experimental ponds, the levels decreased gradually from 85-55 cm. From the results of the present study it is quite evident that probiotics are helpful in the maintenance of phytoplankton bloom and hence the recorded transparency in the experimental ponds. The transparency of the water in the control pond decreased from 90-70 cm and in the experimental ponds the transparency gradually decreased from 85-55 cm after the application of probiotics (Fig. 5a-c).

Toxic metabolites

Ammonia: Ammonia is the end product of protein catabolism in crustaceans and can account for 40-90% of nitrogen excretion (Parry, 1960) and nitrite is an intermediate product of nitrification. However, ammonia is more toxic than nitrite. Generally, ammonia exists in water both in ionized and unionized forms. Among these two, ionized ammonia is more toxic than unionized form. Ammonia concentration depends on pH, temperature and to lesser extent salinity. Chen and Sheu (1990) observed that the safe level of total ammonia for adolescent of *Penaeus monodon* was 4.3 ppm. Previously, Boyd (1982) stated that pond seldom contains more than 2 or 3 ppm of total ammonia nitrogen. The safe level of nitrite was 1.2 ppm for *P. monodon* (Law, 1988). In the present

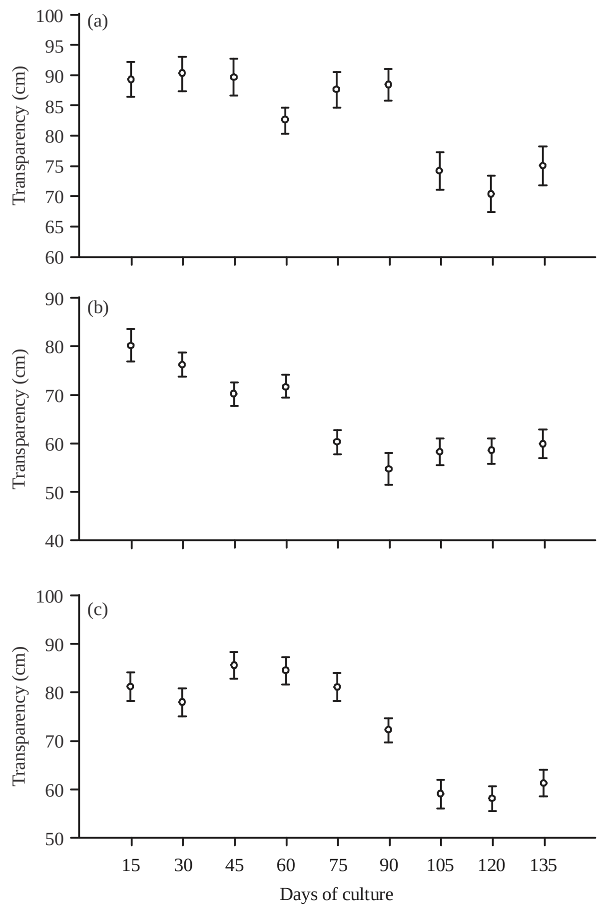

Fig. 5(a-c): Variations in transparency in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

study, values of total ammonia were found well within the safe levels, varying from 0.38-0.93 in the control pond and 0.31-0.68 in the experimental ponds. The nitrite levels ranged from 0.0016-0.0105 ppm in the control pond. In the experimental ponds, the values fluctuated between 0.0014 and 0.0077 ppm. The controlled levels of ammonia and nitrite in the experimental ponds may be attributed to the addition of probiotics. The ammonia concentration was from 0.31-0.68 ppm in the experimental ponds and in the control pond it ranged between 0.38 and 0.93 ppm (Fig. 6a-c).

Nitrite: Nitrate and phosphate are the major nutrients, which commonly determine the phytoplankton production and abundance. There is no need to apply these nutrients as fertilizers in the later stages of culture. Sometimes, nutrients do not significantly increase in the water column due to rapid uptake by phytoplankton. Nitrate is an end product of nitrification and phosphate in pond water is dependant on the addition of fertilizers and feed. Silicate is required in small quantities for diatom growth as well as for the formation of skeletal structure of the diatoms (Prabhu *et al*., 1999). The optimum levels of the nutrients for the establishment of phytoplankton are unknown (Boyd and Fast, 1992). During the present study, the concentrations of the nutrients in the experimental ponds were higher, than in the control pond. This can be

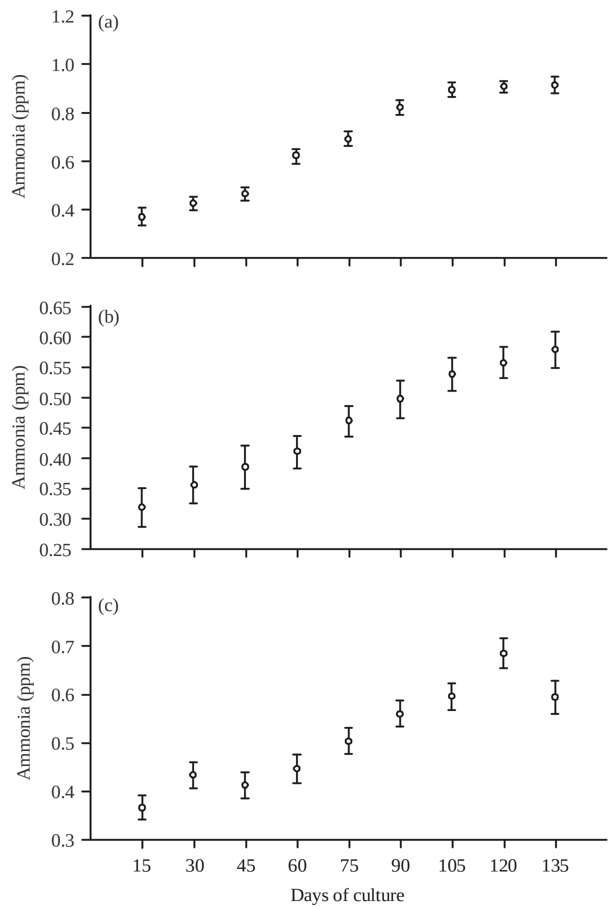

Fig. 6(a-c): Variations in ammonia in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

attributed to mineralization of organic matter by the beneficial microbes. Nitrite concentration ranged from 0.0014-0.0077 ppm in the experimental ponds. In the control pond the values of nitrite varied from 0.0016-0.0105 ppm (Fig. 7a-c).

Nutrients

Nitrate: Nitrate concentration varied from 0.0037-0.0169 ppm in the experimental ponds. In the control pond, nitrate levels ranged between 0.0029 and 0.0141 ppm (Fig. 8a-c). However, Cohen and Neori (1991), studying a zero water exchange system, observed an exponential increase in NO_2-N levels during the growth period, causing shrimp mortality. A zero water exchange system can have sudden changes of TAN and NO_2-N and accumulate NO_3-N, due to variations in the microbial biomass during the culture period, even with a higher C:N ratio (15-20:1) (Gao *et al*., 2012). Khatoon *et al*. (2009) observed higher TAN and NO_2-N concentrations in the control than in the groups treated with the addition of diatoms during the culture of *Penaeus monodon*. Sanchez *et al*. (2014) observed significant differences in concentrations of NO_2-N in tanks with and without the addition of diatoms in cultivation of *L. vannamei*.

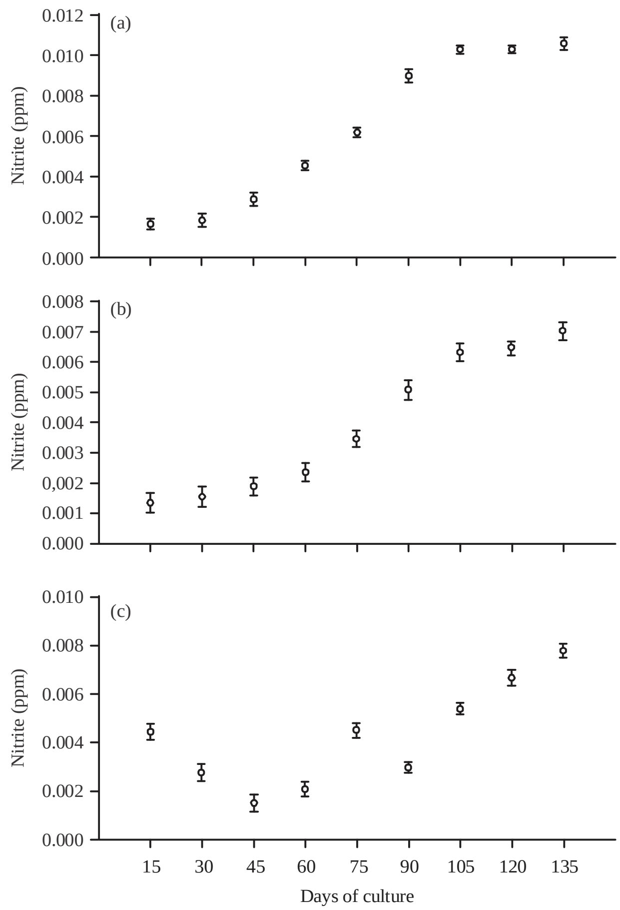

Fig. 7(a-c): Variations in nitrite in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

Total phosphate: In the experiment ponds the total phosphate levels varied from 0.0034-0.0136 ppm. The concentration in the control pond ranged between 0.0023 and 0.0097 ppm (Fig. 9a-c).

Silicate: The silicate concentration in the experimental ponds was from 0.0058-0.0131 ppm. The levels of silicate in the control pond declined. However there was an increase in the levels after 90 DOC. The values ranged from 0.0053-0.0118 ppm (Fig. 10a-c).

Total Heterotrophic Bacterial (THB) population: In general, bacterial productivity was higher in the sediment than in water. Moriarty (1998) experienced a rapid degradation of pellet feeds by bacteria due to high temperature and he concluded that the pellet feed was primarily the base for a microbial food chain. Allan *et al.* (1995) reported that the bacterial population depends upon the presence of organic load in the sediment. Putro *et al.* (1990) and Peranginangin *et al.* (1992) reported that pond waters of South East Asian countries showed relatively higher bacterial load. In the present study, the total heterotrophic bacteria were found higher in the experimental ponds than the control pond. This indicates that the higher values occurred due to the addition of

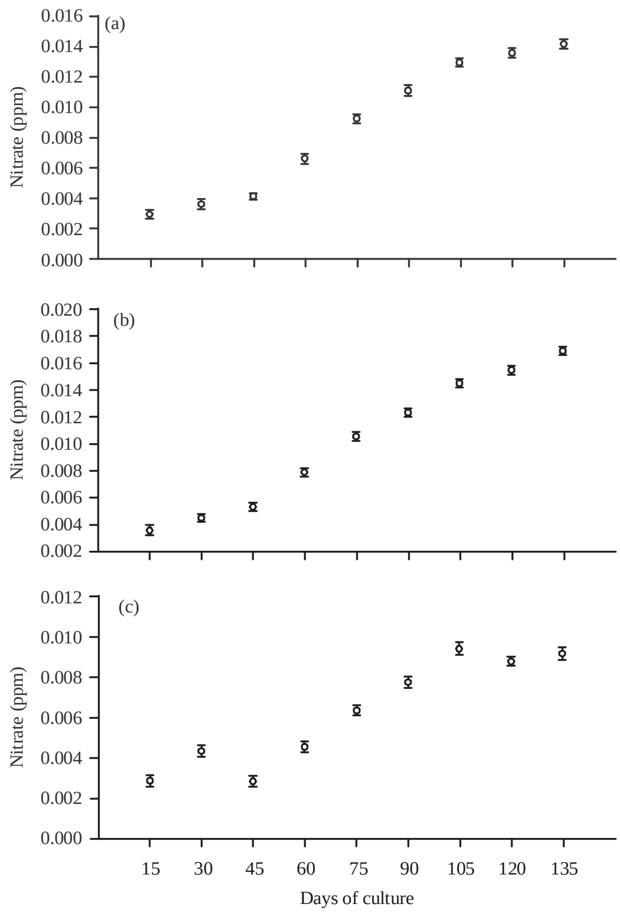

Fig. 8(a-c): Variations of nitrate in (a) Control pond, (b)Experimental pond 1 and (c) Experimental pond 2

probiotics in the experimental ponds. In the water samples, the maximum value of THB population ranged from 2×10^3-10.3×10^8 and from 2×10^3-9.9×10^8 in the experimental ponds 1 and 2, respectively. In the control pond, the values were between 1.9×10^3 and 9×10^8. In the sediment samples, the THB population was from 2.8×10^3-12.1×10^8 in the experimental pond 1 and 3×10^3-11.6×10^8 in the experimental pond 2. In the control pond the value increased from 2.5×10^2-10.8×10^7. Becerra-Dorame *et al*. (2011) (76%) and Kim *et al*. (2014) (91.5%) found higher survival rates in heterotrophic microbial-based-systems.

Growth and survival: The daily growth rate of cultured shrimps was higher in the experimental ponds (0.25 and 0.24 g), when compared to the control pond (0.22 g). The Average Body Weight (ABW) at each sampling was found to be higher in the experimental ponds. The percentage of survival was higher in the experimental ponds (81.5 and 77.5% in 1 and 2, respectively) when compared to the control pond (69.7%). Santiago (1977) reported that *Penaeus monodon* gained an average body weight of 16 g after 4 months of culture in ponds. Rajyalakshmi *et al.* (1982) reported that the daily growth rate of prawn ranged from 0.6 mm/0.039 g to 1.2 mm/0.18 g. Allan (1989) recorded the maximum growth rate of 0.17 g per day for *P. monodon* in a culture pond. Koshio (1985) reported that higher growth rate was related to higher moult frequency and higher

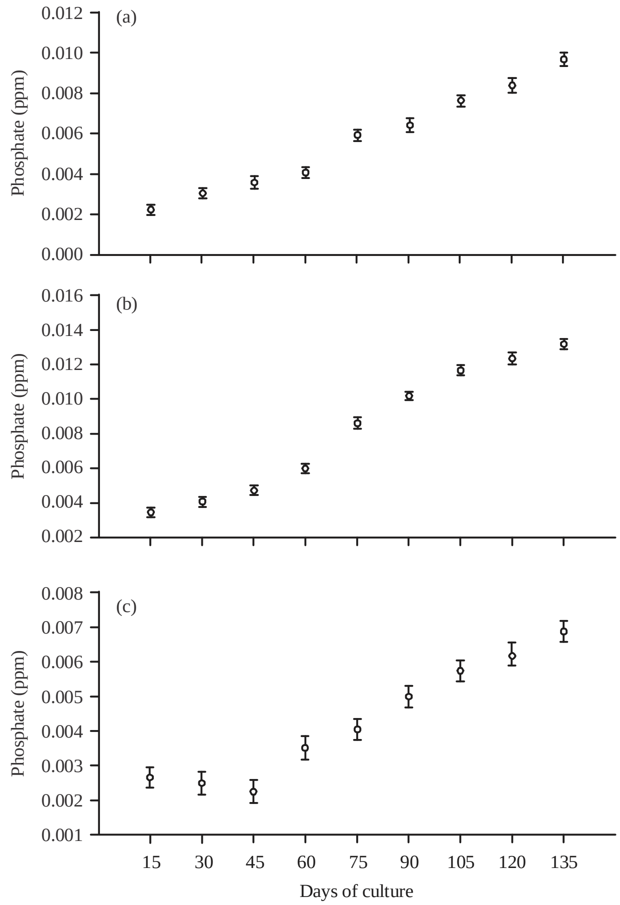

Fig. 9(a-c): Variations of total phosphate in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

weight gain. Mayavu (2002) recorded weight of 37.15 g (133 DOC) and 33.2 g (121 DOC) in semi-intensive culture system using probiotics and extensive culture ponds respectively. In the present study, the animals reached a weight of 34.5 and 32.6 g in the experimental ponds 1 and 2, respectively and 29.8 g in the control pond. This is similar to that observed by Banerjee (2010), who found a significantly higher SGR (~15% day-1) for shrimp *P. monodon* (postlarvae) reared with additional *Bacillus pumilus* and periphytic microalgae.

Food Conversion Ratio (FCR): Management and quality of feed play a major role in FCR (Feed Conversion Ratio) and production. Over feeding leads to pond bottom deterioration. Sedgwick (1979), Dall *et al.* (1990) and Lovett and Felder (1990) proved that multiple feeding will improve growth rate, better FCR and minimize the accumulation of uneaten feed as the juvenile and adult penaeid shrimps ingest what they can effectively assimilate at one time and stop feeding once their cardiac chamber has been filled. Wyban and Sweeney (1991) and Robertson *et al.* (1992) found that the growth rate of *Penaeus vannamei* significantly improved with increase in feeding frequency from one to four times per day. The same schedule of 4 times feeding per day was followed in this experiment also. Clifford (1992) postulated that maximum growth was sustained by adjustment

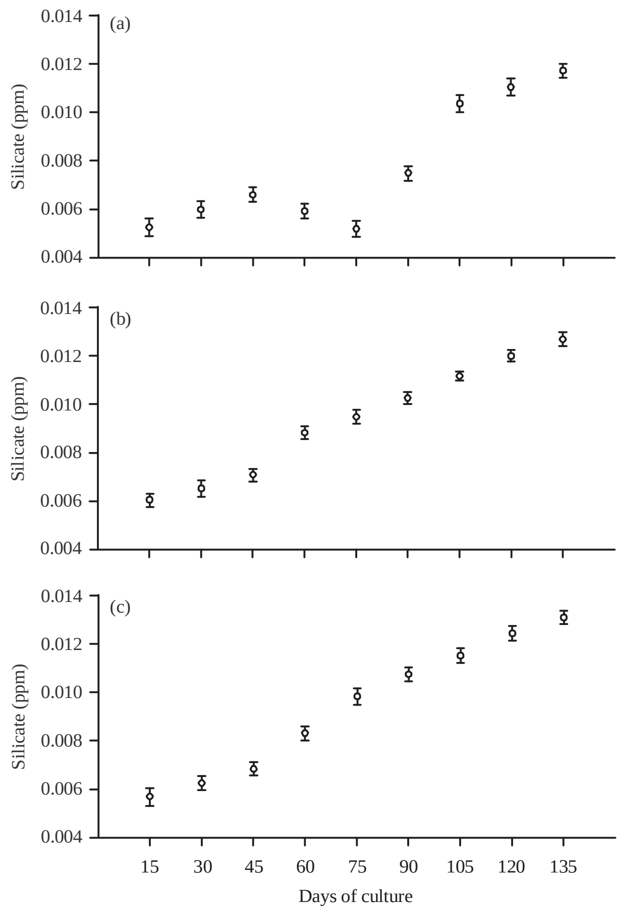

Fig. 10(a-c): Variations of silicate in (a) Control pond, (b) Experimental pond 1 and (c) Experimental pond 2

of feeding rate in such a way that the shrimps are slightly under fed. In the present study, the feed ration was adjusted based on the monitoring of feeding trays. The FCR in the experimental ponds (1.3 and 1.2 in ponds 1 and 2, respectively) were relatively lower than in the control pond (1.6). Sanchez *et al*. (2012) reported that microalgae present in the culture system significantly improved weight gain and FCR of shrimp, thus potentially reducing the feed cost associated with shrimp production. Lower FCR in a zero water exchange system was also observed by Silva *et al*. (2009) (0.8-1.2), Becerra-Dorame *et al*. (2011) (0.65-0.69) and Becerra-Dorame *et al*. (2012) (0.54-0.61).

Water exchange: The present study was carried out to understand the merits of 'zero water exchange' system of farming and the results obtained were satisfactory. This study has brought out the fact that producing shrimp in 'zero water exchange' system has beneficial effects on survival and mean final weight or FCR. Further, it has good water quality for culture during the entire duration of the grow out period. These results obtained are supported by the works carried out by McIntosh *et al*. (2000), Boyd *et al*. (1984), Tucker and Lloyd (1985), Queiroz and Boyd (1998) and Samocha *et al*. (1998). They also stated that, when carefully managed, the water quality with no water exchange could support the growth of fish and shellfish. Use of probiotics, improving water quality, increased shrimp survival and enhanced production with advantageous FCR. Based on the earlier reports and the present findings, shrimp farmers may be recommended to revise their system of farming for safety of the shrimp stock in ponds and their productivity.

Disease: According to Otoshi *et al.* (2011) and Kent *et al.* (2011), *L. vannamei* has a good ability to utilize the microbial community present in aquaculture systems as a food source. Also, in the present study, there was no incidence of disease in the experimental ponds, whereas in the control pond, there were some problems related to bacterial infection. The healthiness of shrimps in the experimental ponds may be attributed to the use of probiotics. The application of probiotics have improved the water quality and also increased the disease resistance capacity in shrimps as mentioned by Pollmann *et al.* (1980), Perdigon *et al.* (1986), Tihole (1988) and Raa *et al.* (1992). The probiotic organisms produce specific components like bacteriocins, which inhibit major pathogens (Lewus *et al.*, 1991). Though the pathogens cannot be eliminated in total from the culture system, their growth can be suppressed and kept under control by the beneficial bacteria, as established by this study. Recently, Ferreira-Marinho *et al.* (2014) also reported that, the zero water exchange treatment showed better production parameters, indicating the benefits of the addition of *Navicula* sp. as a natural food source for *L. vannamei* postlarvae in zero water exchange systems.

CONCLUSION

This study was performed with zero discharge using probiotics, monitoring all the physico-chemical parameters and nutrients. The microbial population of the water and sediment were analyzed throughout the culture period. Highlights of this study are, (a) in the experimental ponds 1 and 2, the shrimps had a better growth compared to 29.8 g in the control pond, (b) there was no incidence of disease in the experimental ponds, whereas the control pond had some bacterial infections. These encouraging results may be attributed to the use of probiotics in zero water exchange system.

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