

## Research Article

# Studies on Biosecured Shrimp Culture of *Penaeus monodon* (Fabricius, 1798)

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Submitted: 07 December 2016

Accepted: 28 February 2017

Published: 15 March 2017

ISSN: 2379-0881

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## Keywords

- Shrimp Culture
- *Penaeus monodon*

## Abstract

Aquaculture has been the fastest growing food production sector in the world among other sectors over the past 30 years. However, in some cases the rapid growth of the aquaculture sector has outstripped planning and regulation environmental impact and marketing have become unavoidably overriding issues in aquaculture development. At the same time demand for product quality and safety increased significantly. There is an urgent need in aquaculture to develop microbial control strategies, since outbreaks are recognized as important constraints to aquaculture production and since the development of antibiotic resistance has become a matter of growing concern. One of the alternatives to antimicrobials in disease control could be the use of probiotic bacteria as microbial control agents. This work to study the use of probiotic bacteria in the culture of larval aquatic organisms which may improve the survival rates of these larvae also stimulates their immune.

## INTRODUCTION

Shrimp aquaculture expanded significantly throughout Asia and Latin America during the 1980's and this expansion was possible by abundant wild seed resources, static production of shrimp from capture fisheries and high profits from shrimp culture (Fast and Menasveta, 2000). 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. In Asia, mortalities of cultured shrimp due to White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) have resulted in economic losses of about \$1 billion per year since 1994 (Lightner *et al.*, 1998). In Ecuador alone, Taura Syndrome Virus (TSV) has been responsible for an estimated loss of 400 million US\$ in revenue per year and this virus has had an equally devastating impact in other shrimp farming countries of the Western Hemisphere including the U.S. (Brock *et al.*, 1997). 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. Biosecured 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.

Aquaculture uses resources from and interacts with the environment. Many aquaculture operations generate metabolic waste products (e.g., feces, ammonia, uneaten food etc.) that are released into the receiving waters. In some cases, the organic particulate waste will accumulate on the seabed in the immediate vicinity of the farm, while soluble waste will eventually end up in the receiving waters. Organic enrichment of the benthic ecosystem may result in formation of anoxic conditions. Under extreme cases, reduction in macrofauna biomass, abundance and species composition may also follow [4]. In semi-intensive and intensive pond systems, sometime upto 40% of pond volume is exchanged daily. For example, old shrimp production practices in Taiwan, required upto 43 m<sup>3</sup> of water for every 1 Kg of shrimp produced [5]. Often on large farms, water exchange is based on a set schedule with occasional emergency flushes [6] rather than as an ongoing response to changing pond conditions. The concept of biological disease control, particularly using microbiological modulator for disease prevention has received widespread attention.

## MATERIALS AND METHODS

The study was carried out in a commercial shrimp farm situated at Marakkanam near Pondicherry. This shrimp farm with three ponds had a total water spread area of 2.9 ha (Pond 1 – 0.6 ha; Pond 2 – 0.7 ha and Pond 3 – 1.6 ha). Ponds 1 and 2 were used as experimental ponds and pond 3 was used as control.

## Pond preparation

**Soil 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, water was pumped in with the help of a 10 HP pump.

**Water culture:** Water was pumped from Uppanar estuary into the reservoir and the pumped in water was disinfected with bleaching powder at the rate of 60 ppm/ha. The water was left undisturbed for 10 days to remove the residual chlorine. Later the water was pumped to the culture ponds.

**Fertilizing the ponds:** In 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).

## STOCKING

Healthy and WSSV negative *Penaeus monodon* seeds were purchased from a reputed hatchery at Marakkanam, Tamil Nadu. The seeds were stocked at a density of 10/m<sup>2</sup>. Before stocking, the seeds were acclimatized to the pond environment as given below.

1. Seed bags were allowed to float in the water surface in each pond for 30 min. to adjust the temperature.
2. The bags were opened and the pond water was introduced slowly by sprinkling into the bags for 60 min. to equalize with pond water quality.
3. The bags were drawn to different parts of the pond and seeds were released slowly.

The survival rate after socking was estimated using survival cages (happa nets), laid near the outlet of each pond with 100 PLS in each pond. Based on the survival rate on the 3<sup>rd</sup> day, the feed ratio was decided.

## FEEDING

Feeding was done using CP feed (Charoen Pokhpan aquaculture India Pvt. Ltd, Thailand). 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 on the check tray observation and sampling. Four check trays were provided per pond.

The feed ration was divided into 4 times in a day (25%, 20%, 30% and 25% at morning (6.00 AM), noon (12.00 Noon), evening (6.00 PM) and night (1.00 AM) and provided respectively. The feed was broadcasted from the dyke during the initial phase and center feeding using a float 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 animal was

estimated through the samples. The diameter of the cast net used for sampling was 3.3 mts. The area of the net was calculated with 60% efficiency of coverage at the bottom.

## WATER EXCHANGES

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

## PROBIOTICS

Commercially available probiotic super NB (CP aquaculture India Pvt. (Ltd) was used.

### Activation of Probiotics

200 ml 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 method. pH cone was used to find out the soil pH. 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 [9] and recorded as parts per million (ppm). Nitrate, nitrite, total phosphate and silicate were estimated following the methods described by [10]. 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 glasswares such as petriplates and conical flasks were sterilized in a hot-air oven at 165°C for 2 hours. One ml of sample was mixed in 9 ml of sterilized water and from this tube 1ml was transferred to the next dilution blank. Likewise appropriate dilutions were made. From the above sample, 1ml 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 hours they were counted and expressed as colony forming units (CFU)

## RESULTS

### Water quality Parameters

**Temperature:** The variations in the temperature are plotted in Figures (1, 2 and 3) and it ranged from 27.8 to 32.8°C in the

control pond, 26.9 to 34.3°C and 26.7 to 32.9°C in experimental ponds 1 and 2 respectively (Figures 1, 2 and 3).

**Salinity:** The salinity levels varied between 29 to 40 ppt. There was not much difference between control and experimental ponds. The results are given in Figures (4, 5 and 6).

**pH:** The pH values in the control pond ranged from 6.1 to 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 (Figures 7, 8 and 9).

**Dissolved Oxygen:** The dissolved oxygen concentration varied from 3.6 to 5.2 mg/l in the control pond and 4.1 to 6.8 in the experimental ponds (Figures 10, 11 and 12).

## TRANSPARENCY

The transparency of the water in the control pond decreased from 90 to 70 cm and in the experimental ponds the transparency gradually decreased from 85 to 55 cm after the application of probiotics (Figures 13, 14 and 15).

## TOXIC METABOLITES

### Ammonia

The ammonia concentration was from 0.31 to 0.68 ppm in the

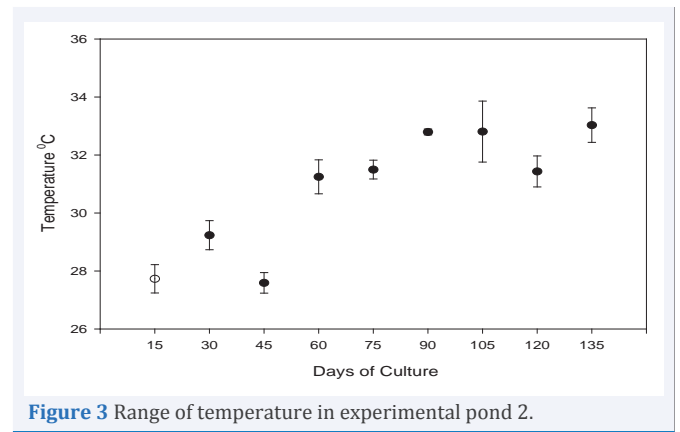


Figure 3 Range of temperature in experimental pond 2.

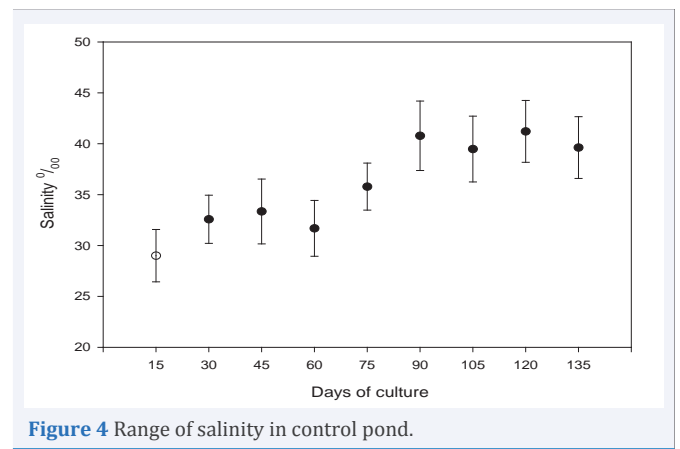


Figure 4 Range of salinity in control pond.

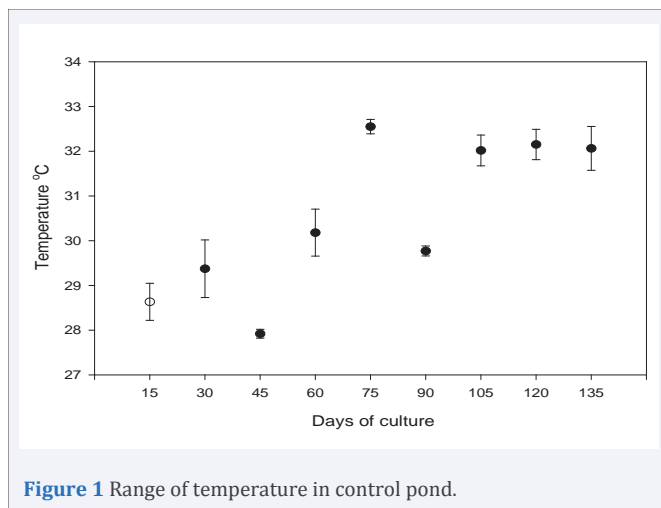


Figure 1 Range of temperature in control pond.

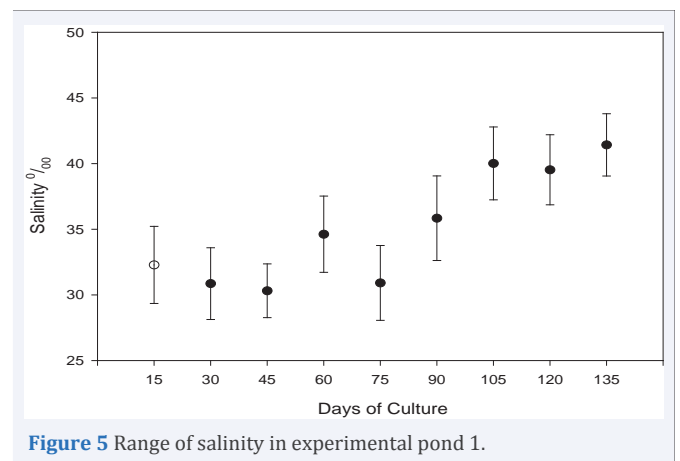


Figure 5 Range of salinity in experimental pond 1.

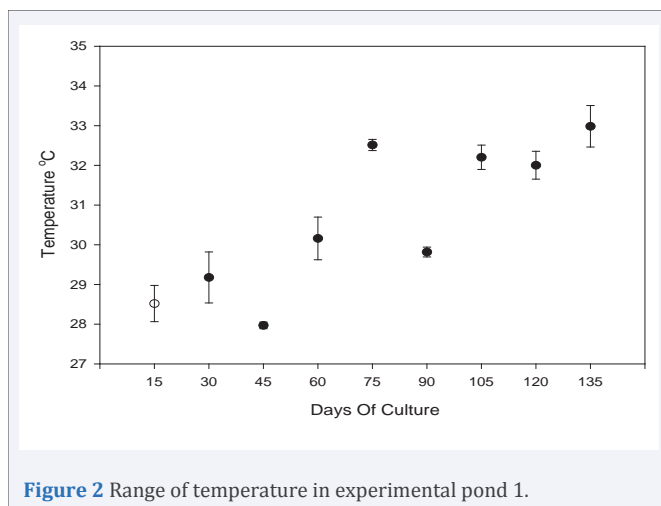


Figure 2 Range of temperature in experimental pond 1.

experimental ponds and in the control pond it ranged between 0.38 and 0.93 ppm (Figures 16, 17 and 18).

### Nitrite

Nitrite concentration ranged from 0.0014 to 0.0077 ppm in the experimental ponds. In the control pond the values of nitrite varied from 0.0016 to 0.0105 ppm (Figures 19, 20 and 21).

## NUTRIENTS

### Nitrate

Nitrate concentration varied from 0.0037 to 0.0169 ppm in the experimental ponds. In the control pond, nitrate levels ranged

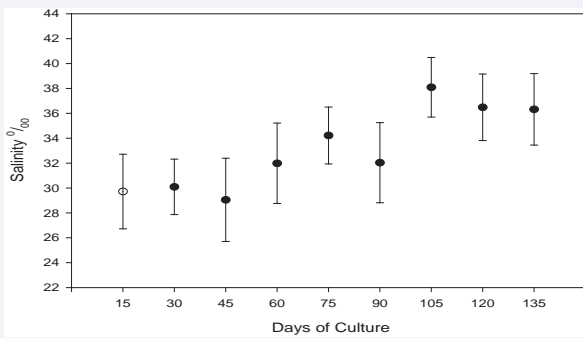


Figure 6 Range of salinity in experimental pond 2.

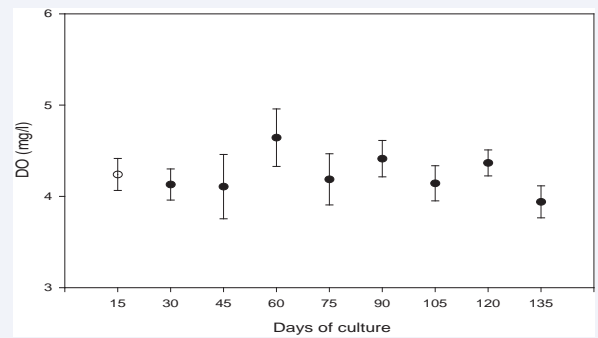


Figure 10 Range of DO in control pond.

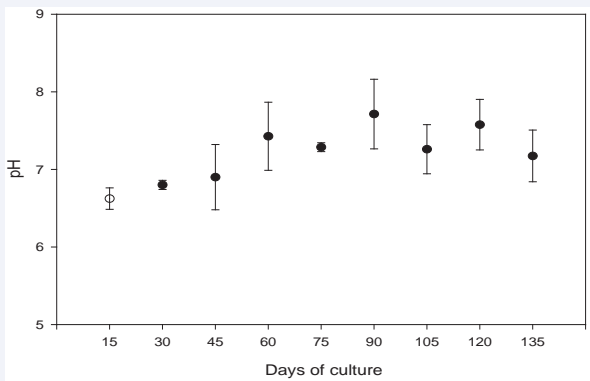


Figure 7 Range of pH in control pond.

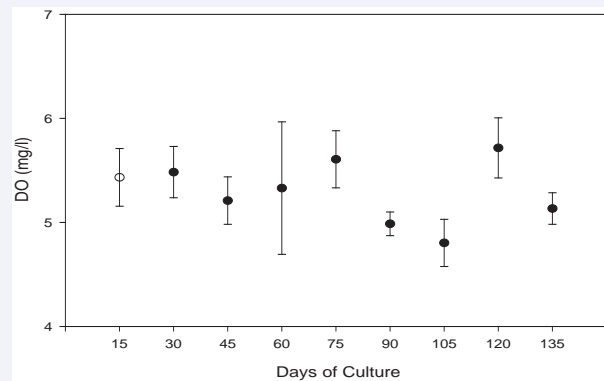


Figure 11 Range of DO in experimental pond 1.

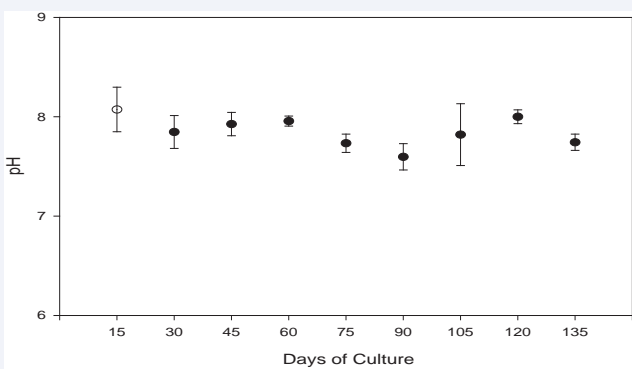


Figure 8 Range of pH in experimental pond 1.

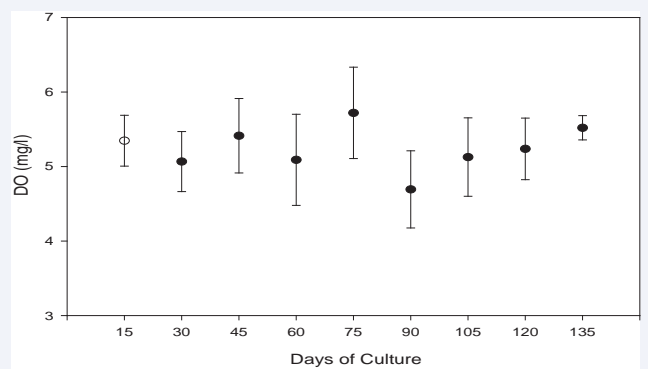


Figure 12 Range of DO in experimental pond 2.

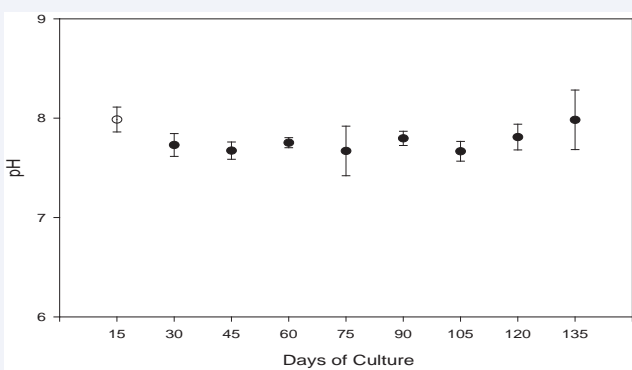


Figure 9 Range of pH in experimental pond 2.

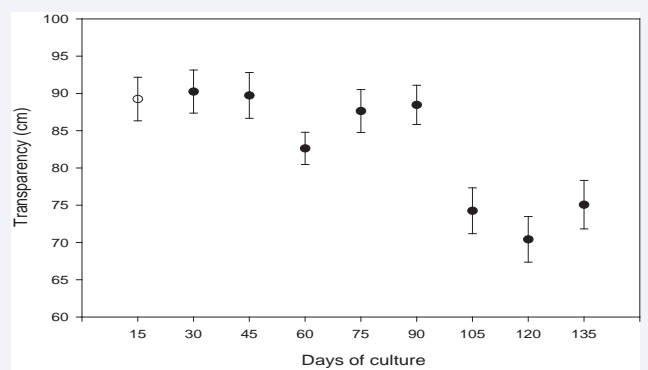


Figure 13 Range of transparency in control pond.

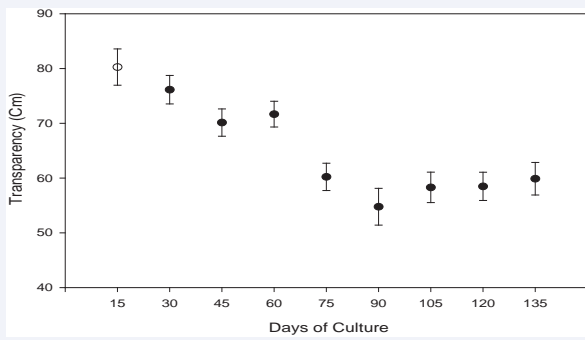


Figure 14 Range of transparency in experimental pond 1.

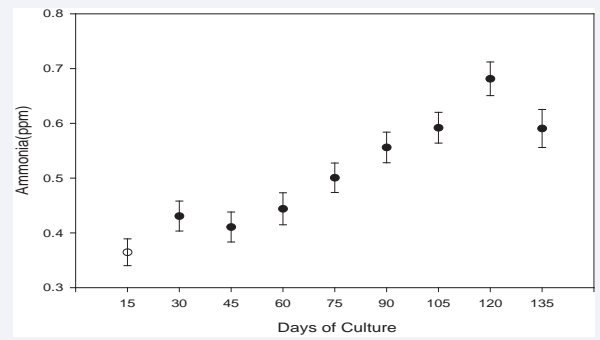


Figure 18 Range of ammonia in experimental pond 2.

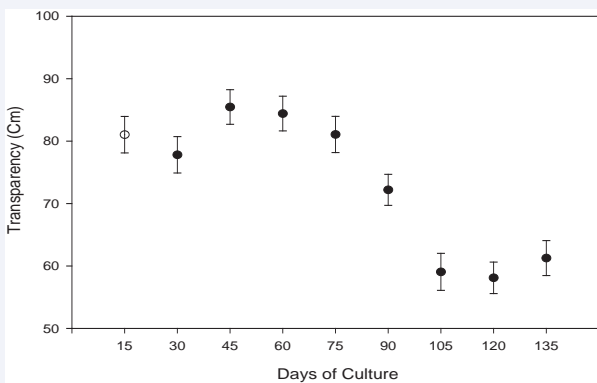


Figure 15 Range of transparency in experimental pond 2.

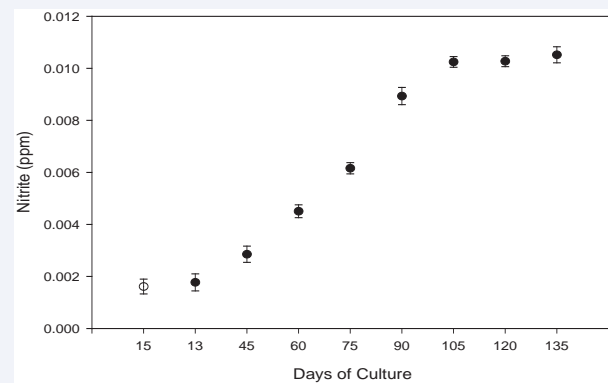


Figure 19 Range of nitrite in control pond.

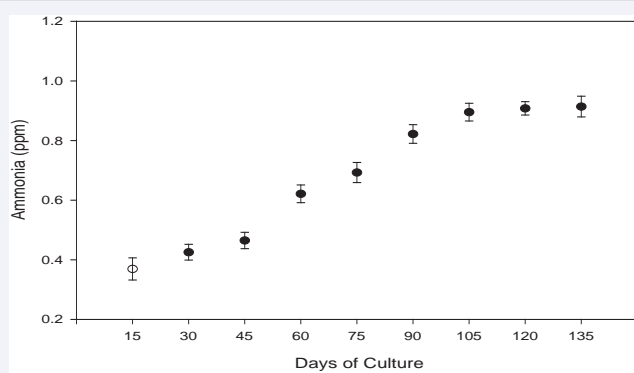


Figure 16 Range of ammonia in control pond.

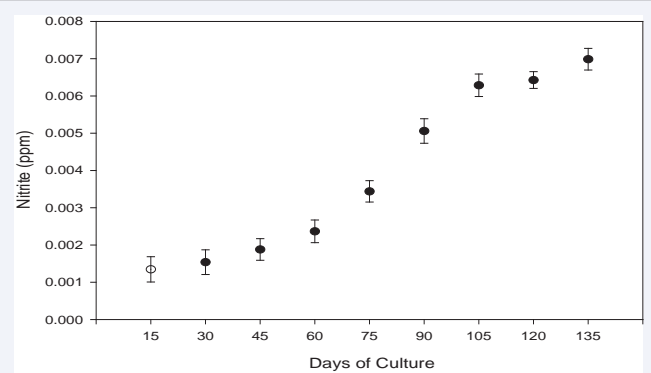


Figure 20 Range of nitrite in experimental pond 1.

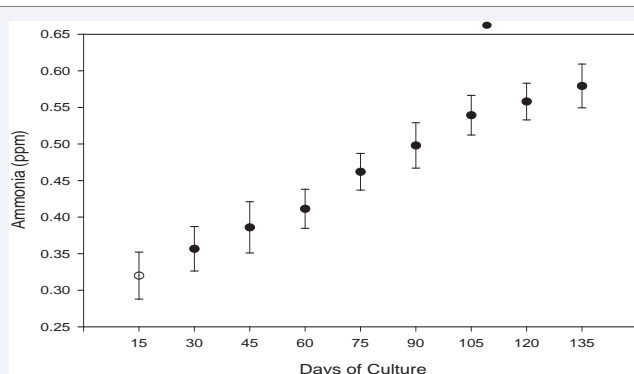


Figure 17 Range of ammonia in experimental pond 1.

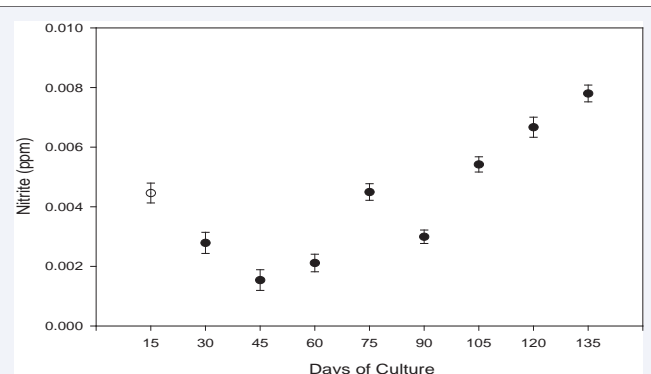


Figure 21 Range of nitrite in experimental pond 2.

between 0.0029 and 0.0141 ppm (Figures 22, 23 and 24).

### Total phosphate

In the experiment ponds the total phosphate levels varied from 0.0034 to 0.0136 ppm. The concentration in the control pond ranged between 0.0023 and 0.0097 ppm (Figures 25, 26 and 27).

### Silicate

The silicate concentration in the experimental ponds was from 0.0058 to 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 to 0.0118 ppm (Figures 28, 29 and 30).

### Total Heterotrophic Bacterial Population (THB)

In the water samples, the maximum value of THB population ranged from  $2 \times 10^3$  to  $10.3 \times 10^8$  and from  $2 \times 10^3$  to  $9.9 \times 10^8$  in the experimental ponds 1 and 2 respectively. In the control pond, the values were between  $1.9 \times 10^3$  and  $9 \times 10^8$ .

In the sediment samples, the THB population was from  $2.8 \times 10^3$  to  $12.1 \times 10^8$  in the experimental pond 1 and  $3 \times 10^3$  to  $11.6 \times 10^8$  in the experimental pond 2. In the control pond the value increased from  $2.5 \times 10^2$  to  $10.8 \times 10^7$  (Tables 1, 2 and 3).

### 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

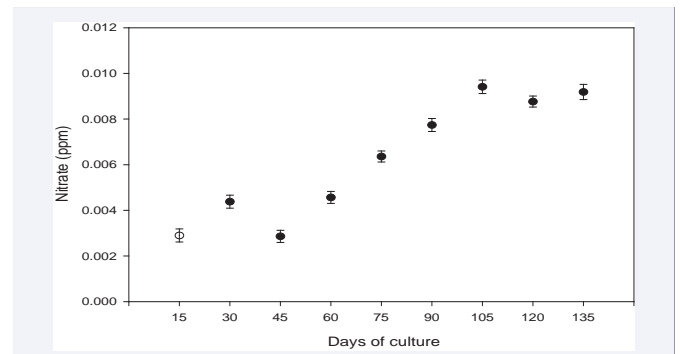


Figure 24 Range of nitrate in experimental pond 2.

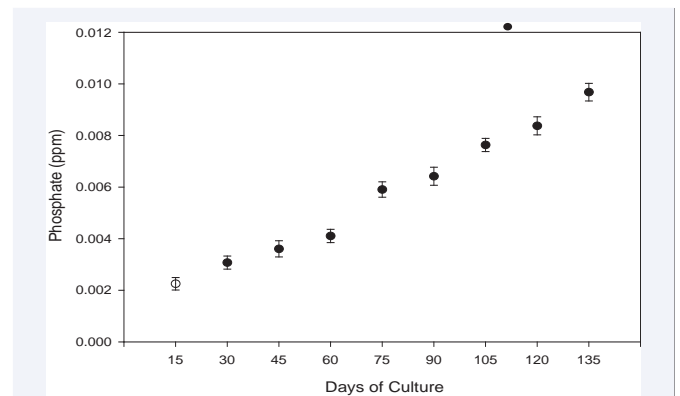


Figure 25 Range of total phosphate in control pond.

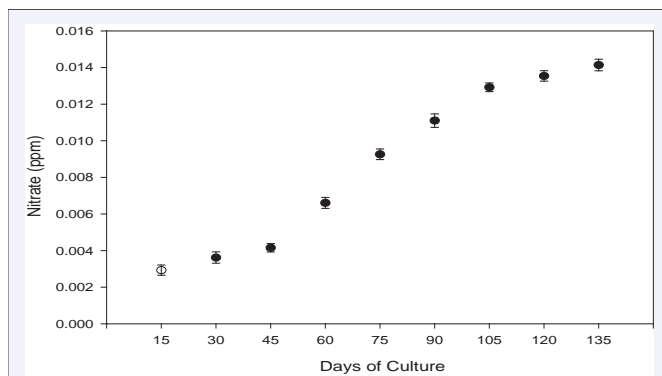


Figure 22 Range of nitrate in control pond.

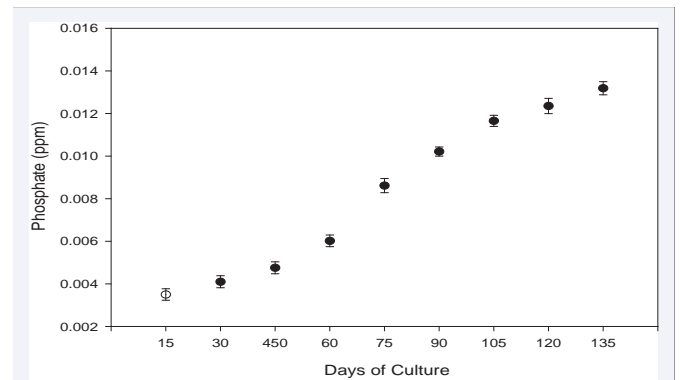


Figure 26 Range of total phosphate in experimental pond 1.

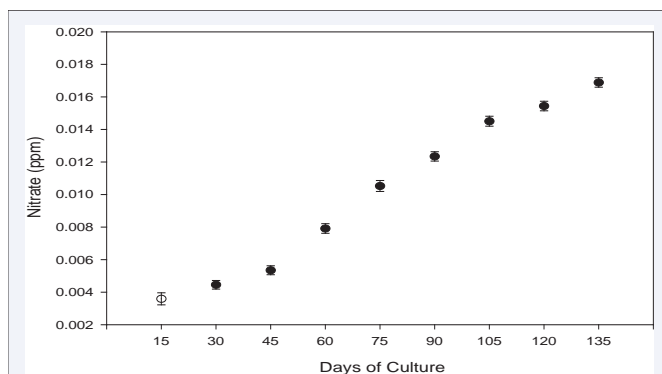


Figure 23 Range of nitrate in experimental pond 1.

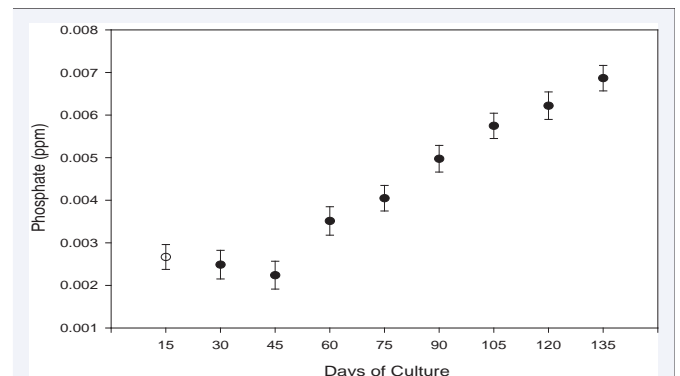


Figure 27 Range of total phosphate in experimental pond 2.

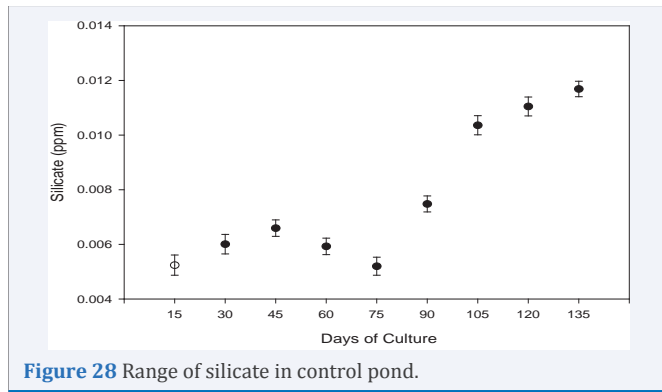


Figure 28 Range of silicate in control pond.

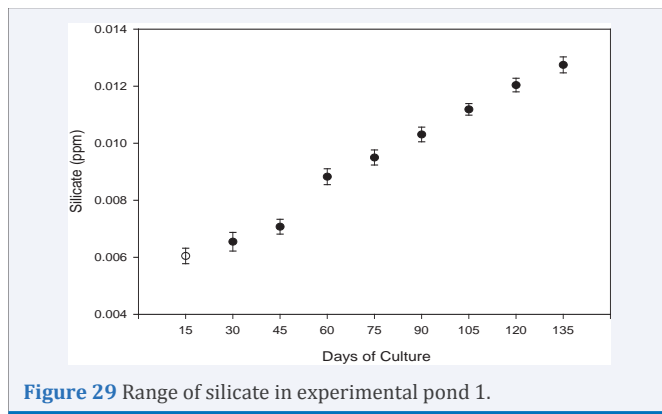


Figure 29 Range of silicate in experimental pond 1.

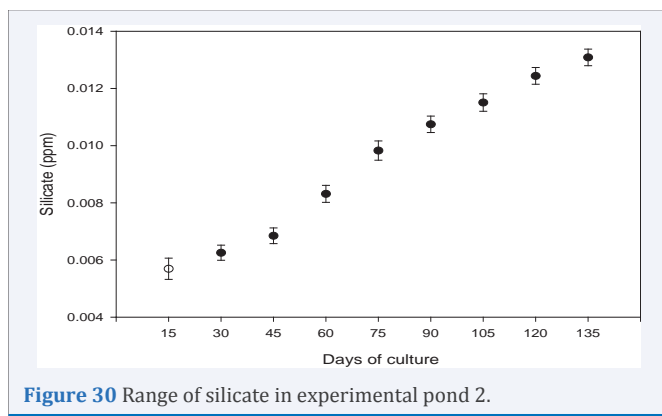


Figure 30 Range of silicate in experimental pond 2.

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%) (Table 4, 5 and 6).

### FOOD CONVERSION RATIO (FCR)

The FCR calculated in the experimental ponds were 1.3 and 1.2 in ponds 1 and 2 respectively and relatively lower than the control pond (1.6).

### DISCUSSION

#### Water quality management

The technique of water quality management in shrimp ponds is less understood than other aspects of shrimp farming. If water

Table 1: THB population in water and sediment sample (control pond).

DOC	Water (CFU/ml)	Sediment (CFU/gm)
15	1.9x 10 <sup>3</sup>	2.5x10 <sup>2</sup>
30	2.6x 10 <sup>4</sup>	3x10 <sup>3</sup>
45	1.3 x 10 <sup>4</sup>	3.5 x 10 <sup>3</sup>
60	3.7 x 10 <sup>5</sup>	4.3 x 10 <sup>7</sup>
75	4.6 x 10 <sup>7</sup>	5.8 x 10 <sup>4</sup>
90	6.1 x 10 <sup>7</sup>	8.2 x 10 <sup>6</sup>
105	8 x 10 <sup>8</sup>	9.8 x 10 <sup>7</sup>
120	8.4x10 <sup>8</sup>	10.2x10 <sup>7</sup>
135	9x10 <sup>8</sup>	10.8x10 <sup>7</sup>

Table 2: THB population in water and sediment sample (Experimental pond 1).

DOC	Water (CFU/ml)	Sediment (CFU/gm)
15	2.4x10 <sup>3</sup>	2.8x10 <sup>3</sup>
30	2.8x10 <sup>3</sup>	3.4x10 <sup>3</sup>
45	1 x 10 <sup>3</sup>	1.3 x 10 <sup>3</sup>
60	2.1 x 10 <sup>3</sup>	3.7 x 10 <sup>5</sup>
75	5.1 x 10 <sup>5</sup>	5.3 x 10 <sup>6</sup>
90	4.8 x 10 <sup>7</sup>	7.9 x 10 <sup>5</sup>
105	7.8 x 10 <sup>7</sup>	9.6 x 10 <sup>6</sup>
120	9.1x10 <sup>8</sup>	10.4x10 <sup>7</sup>
135	9.7x10 <sup>8</sup>	11.3x10 <sup>7</sup>

Table 3: THB population in water and sediment sample (Experimental pond 2).

DOC	Water (CFU/ml)	Sediment (CFU/gm)
15	2 x 10 <sup>3</sup>	3 x 10 <sup>3</sup>
30	2.6 x 10 <sup>4</sup>	3.3 x 10 <sup>3</sup>
45	1.6 x 10 <sup>4</sup>	3.0 x 10 <sup>4</sup>
60	2.0 x 10 <sup>5</sup>	4.0 x 10 <sup>4</sup>
75	3.7 x 10 <sup>5</sup>	5.3 x 10 <sup>5</sup>
90	5.5 x 10 <sup>6</sup>	7.0 x 10 <sup>6</sup>
105	6.2 x 10 <sup>6</sup>	8.3 x 10 <sup>7</sup>
120	8.8 x 10 <sup>7</sup>	10 x 10 <sup>7</sup>
135	9.3 x 10 <sup>8</sup>	10.8 x 10 <sup>8</sup>

Table 4: Growth and survival in Control pond.

Days Of Culture	Survival %	ABW (gm)	Biomass (kg)	ADG (gm)
45	90	4.5	283.5	0.1
60	75	6.0	315.0	0.1
75	73	9.5	485.4	0.23
90	70	14.5	710.5	0.33
105	64	21.0	940.8	0.43
120	60	26.5	1113.0	0.36
135	56	29.8	1168.1	0.22

**Table 5:** Growth and survival in Experimental pond 1.

Days of Culture	Survival %	ABW (gm)	Biomass (kg)	ADG (gm)
45	95	4.8	273.6	0.10
60	88	7.8	411.8	0.2
75	85	11.6	591.6	0.25
90	80	16.2	777.6	0.30
105	75	23.1	1039.5	0.46
120	74	29.3	1300.9	0.41
135	74	34.5	1531.8	0.34

**Table 6:** Growth and survival in Experimental pond 2.

Days Of Culture	Survival %	ABW (gm)	Biomass (kg)	ADG (gm)
45	90	4.6	248.4	0.10
60	83	7.1	353.5	0.16
75	80	10.6	508.8	0.23
90	76	15.8	720.4	0.34
105	74	22.6	1003.4	0.45
120	70	28.4	1192.8	0.38
135	70	32.6	1369.2	0.28

quality is not maintained properly shrimps will not feed and become more susceptible to disease, which leads to poor survival and growth [11], studied the problems in culturing black tiger shrimp (*Penaeus monodon*) – semi intensive way as an Indian experience. 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 [12], 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 to 30°C and temperature beyond this range is lethal [13] 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.

### Salinity

*Penaeus monodon* is a euryhaline species which can adapt easily to wide variation in salinity. The normal growth of *P. monodon* can be achieved between 15 and 20 ppt [14-19] also stated that the ideal salinity for *P. monodon* is from 15 to 25 ppt and high or low salinity affects the moulting frequency. In this

study, salinity was varying vastly from 29 to 40 ppt in all the ponds.

### 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 to 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 [20] and high pH increases the unionized ammonia [21]. It also reduces the natural pond production presumably by reducing the availability of nutrients [22] including phosphorus [23]. During the present study the water pH ranged from 6.1 to 8.3 in the control pond.

### 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 [24,25], concluded that emergency

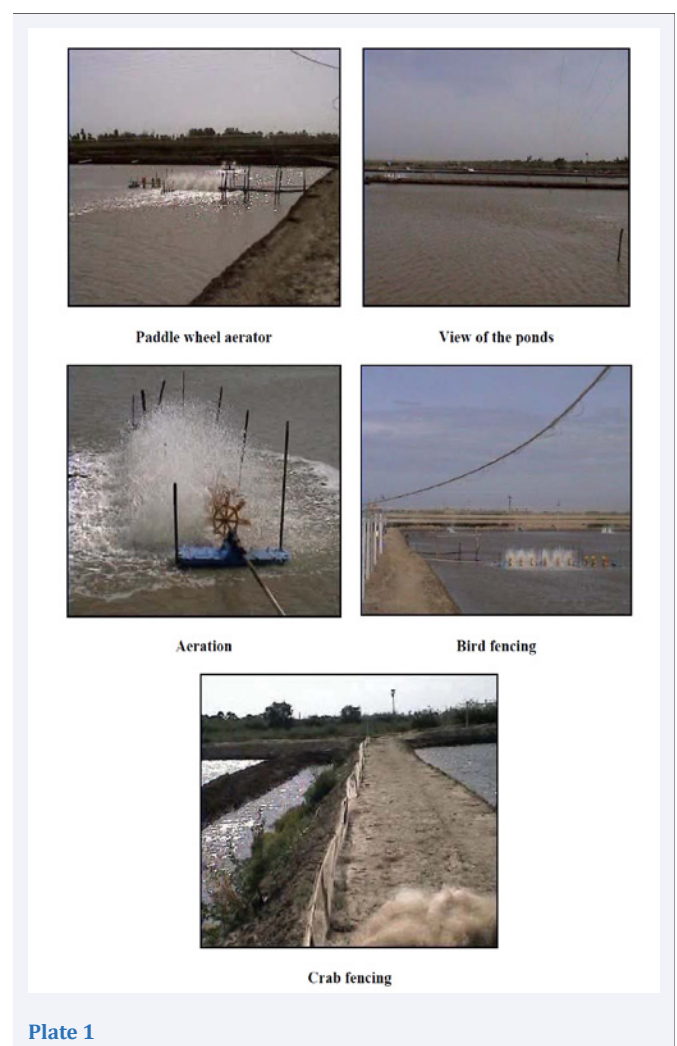


Plate 1



measures must be taken if the dissolved oxygen concentration falls below 3 ppm. [26] suggested that the dissolved oxygen levels should be kept above 2 ppm at all times, [27] reported that the rate of respiration in *Penaeus monodon* remained constant at dissolved oxygen concentration level of 3 to 4 ppm in water. Low dissolved oxygen level occurs in shrimp ponds due to phytoplankton die off and decomposition of the same [28] and can cause stress or even mortality of shrimps in ponds [29-31]. Low dissolved oxygen increases the ammonia concentration and decreases the pH levels [23]. In the present study, in the control pond the dissolved oxygen levels ranged between 3.6 to 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.

### 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 to 40 cm [32]. According to Boyd and Fast (1992) secchi disc readings of 25 to 35 cm are considered desirable by most shrimp farmers and the measurements should be made 800 and 1000 hr or between 1400 and 1600 hr. (Almazan and Boyd, 1978). In the present study, the transparency levels in the control pond ranged from 90 to 75 cm. This was due to unstable bloom in the control pond. In the experimental ponds, the levels decreased gradually from 85 to 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.

### Toxic metabolites

Ammonia is the end product of protein catabolism in crustaceans and can account for 40 to 90 % of nitrogen excretion [33], 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 [34], observed that the safe level of total ammonia for adolescent of *Penaeus monodon* was 4.3 ppm. Previously, [23] 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* [26]. In the present study, values of total ammonia were found well within the safe levels, varying from 0.38 to 0.93 in the control pond and 0.31 to 0.68 in the experimental ponds. The nitrite levels ranged from 0.0016 to 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.

### Nutrients

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 dependent on the addition of fertilizers and feed. The optimum levels of the nutrients for the establishments of phytoplankton are unknown [13]. During the present study, the concentrations of the nutrients in the experimental ponds were higher, than in the control pond. This can be attributed to mineralization of organic matter by the beneficial microbes.

### TOTAL HETEROTROPHIC BACTERIAL (THB) POPULATION IN POND WATER AND IN SEDIMENT

In general, bacterial productivity was higher in the sediment than in water [35], 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 probiotics in the experimental ponds.

### FEED MANAGEMENT

Management and quality of feed play a major role in FCR (Feed Conversion Ratio) and production. Over feeding leads to pond bottom deteriorations [36-38] 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 [39-40]. 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 [32], postulated that maximum growth was sustained by adjustment 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.

### GROWTH

[41] Reported that *Penaeus monodon* gained an average body weight of 16 g after 4 months of culture in ponds [42], reported that the daily growth rate of prawn ranged from 0.6 mm/0.039g to 1.2 mm/0.18 g. [43] 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 weight gain [45], 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 g and 32.6 g in the experimental ponds 1 and 2 respectively and 29.8 g in the control pond.

## 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 grow out period. These results obtained are supported by the works carried out by [46-50]. 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 improves 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 [50-53], studied the usefulness of applying probiotics in shrimp culture pond.

## DISEASE

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 [53-56]. The probiotics organisms produce specific components like bacteriocins, which inhibit major pathogens [57]. 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.

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Cite this article

Natarajan S, Deivasigamani B (2017) Studies on Biosecured Shrimp Culture of *Penaeus monodon* (Fabricius, 1798). *Ann Aquac Res* 4(1): 1031.