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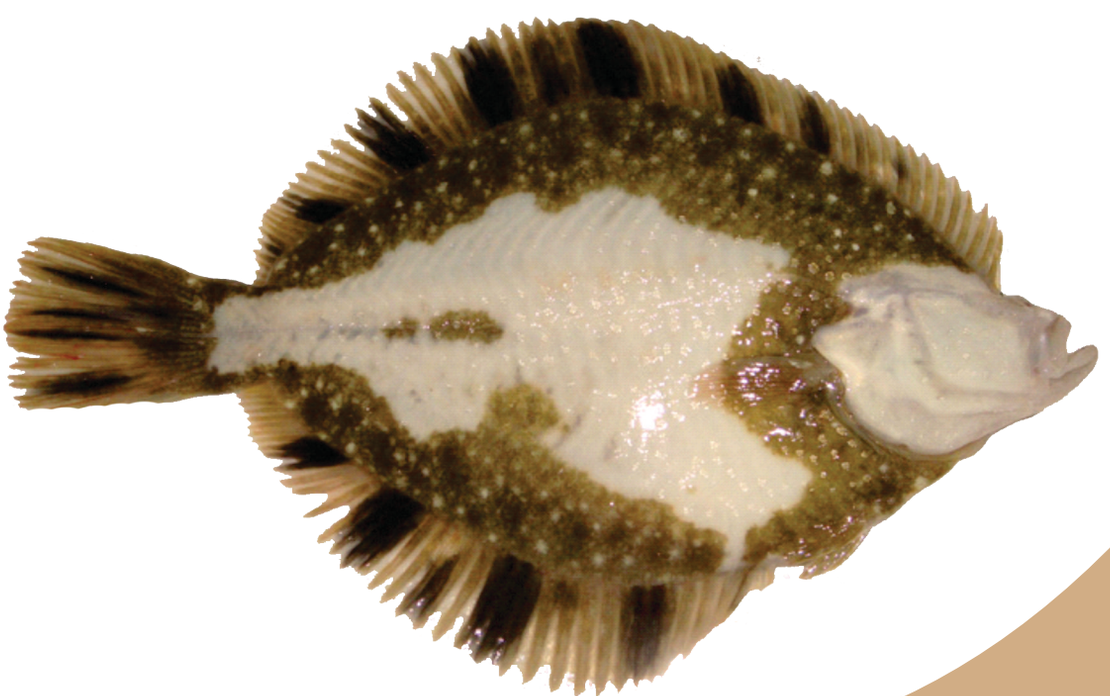

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Effect of culture intensity and probiotics application on microbiological and environmental parameters in *Litopenaeus vannamei* culture ponds

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Abstract

The present study examined the complex interaction among stocking density and extent of probiotic use with production and environmental parameters in *Litopenaeus vannamei* culture ponds to suggest suitable management strategies. The study was conducted in *L. vannamei* culture ponds with stocking density of 35 nos sq m^{-1} (Group I) and 56 nos sq m^{-1} (Group II) and probiotic application @16.5 kg ha^{-1} and 157 kg ha^{-1}, respectively. There was no significant difference noted between the two groups of ponds in respect to ammonia oxidizing bacteria (AOB) in sediment and nitrite oxidizing bacteria (NOB) in water samples, whereas significantly higher levels of AOB in water samples of high intensity culture ponds (Group II) and NOB in sediment samples of Group I were observed. The levels of sulphur oxidizing bacteria (SOB) and sulphur reducing bacteria (SRB) in Group I pond water and in Group II sediment were significantly higher than their corresponding levels in the other group. In both the groups, ammonia, nitrite and sulphide concentrations were below toxic limits prescribed for shrimp farming. Comparing the production parameters at harvest revealed that low intensity culture ponds (Group I) had higher growth rate, average body weight and significantly lower FCR and higher survival rate than high intensity culture ponds (Group II). The results indicated that application of microbial products in higher quantities did not benefit significantly, and there is a need to regulate quantum and schedule of biological product usage for economically sustainable shrimp culture.

Key words

Culture intensity, *Litopenaeus vannamei,* Microbial dynamics, Probiotics

Introduction

Shrimp culture worldwide is dominated by Pacific white leg shrimp, *Litopenaeus vannamei*, owing to the development of Specific Pathogen Free (SPF) stocks and genetically improved strains (Briggs *et al*., 2004). Many countries in South East Asia have initiated scientific culture of this shrimp since 2000. In India, *L. vannamei* has been introduced in the year 2009 with strict guidelines from Coastal Aquaculture Authority (CAA), Government of India (CAA, 2014). In the last four years, *L. vannamei* has not only drastically replaced *Penaeus monodon* culture areas (MPEDA, 2014) but also revived aquaculture in previously abandoned shrimp farming areas. Unlike *P. monodon*, white leg shrimp is being cultured at high rate and this intensification without proper management practices may impose stress on the pond environment with adverse effects on ecosystem, health and increased susceptibility of shrimp to diseases.

Studies on the nutrient budgeting in shrimp culture systems have reported that only 30% nitrogen provided through feed is assimilated into shrimp biomass (Kutako *et al*., 2009) and accumulation of unutilized nutrients in pond bottom leads to reduced dissolved oxygen and increased level of ammonia and hydrogen sulphide metabolites that are toxic to shrimps (Mevel and Chamroux, 1981). Environmental stress arising from unfavourable or adverse changes in pH

(Lin *et al*., 2010), salinity (Wang and Chin, 2005), temperature (Yeh *et al*., 2010), ammonia (Liu and Chen, 2004) and nitrite (Liao *et al*., 2012) in culture ponds suppresses the immune competence of penaeidshrimp in addition to decrease in survival, feeding, moulting and growth.Therefore, maintaining healthy pond environment is key to successful shrimp production. Traditionally, regular water exchange is practised to maintain the quality pond environment, but due to deteriorating quality of available water (Kautsky *et al*., 2000) and threat of diseases (Chamberlain, 2001) zero water exchange system is being followed in recent times. These closed systems, if not properly managed suffer from severe deterioration of environmental parameters, compromising of shrimp growth and survival (Thakur and Lin, 2003; Tacon*et al.*, 2002).

A stocking density of 60 nos sqm^{-1} is permitted by CAA for *L. vannamei* farming (CAA, 2014). However, optimal stocking density depends on the farming practices being followed by the farmers (Sanchez-Zazueta *et al*., 2013). As stocking density is inversely proportional to growth, it is essential to understand impact of stocking density on the health of pond environment and production. A realistic approach for profitable shrimp culture is to find a balance between the culture intensity, environment and economic sustainability. To ameliorate the shortcomings of intensive aquaculture, particularly the pond environment quality, chemical, herbal and other biological products are widely used during the culture. However, indiscriminate use of such products may lead to adverse impact on the environment and increase in cost of production.

Microbes grossly influence aquaculture pond environment through decomposition of organic matter and various elemental transformation processes. Nitrogen recycling bacteria (ammonia and nitrite oxidizing bacteria) along with other bacterial communities carry out nitrogen fixation, ammonification, nitrification or denitrification in aquaculture pond. Ammonia oxidizing bacteria mostly *Nitrosomonas* spp. oxidize toxic ammonia into nitrite while nitrite-oxidizing bacteria mostly *Nitrobacter* spp oxidize nitrite to nitrate. This accumulated nitrate is utilized by phytoplanktons in the aquaculture pond and improves the natural productivity. Similarly, sulphur recycling bacteria (sulphur reducing and oxidizing bacteria) are predominately involved in reduction and oxidation of sulphates and hydrogen sulphide in pond bottom (Avnimelech *et al.*, 1995) which indirectly helps in augmenting organic matter degradation.

Microbial products like, probiotics are being extensively applied in aquaculture to maintain the pond environment and improving the shrimp health (Boyd, 1995). Commercial products containing *Bacillus pumilus, B. megaterium, Bacillus subtilis, B. polymyxa, B. licheniformis, B. subtilis, Lactobacillus acidophilus, Saccharomyces cerevisiae, Pseudomonas aeruginosa, Nitrosomonas europaea*, *Nitrobacter winogradskyi, Rhodobacter* spp. and *Rhodococcus* spp. are being used extensively in India for shrimp cultures. Scientific reports on the beneficial effects in shrimp culture are not available for some of the probiotics bacteria. The beneficial effects of probiotics have been attributed to production of antibiosis compounds, competition for pathogen adhesion sites, modulation of host immune responses, competition with pathogens for nutrients, interference with quorum sensing, antibacterial, antiviral and antifungal activitiesand stress management in addition to improvement in water quality (Farzanfar, 2006; Mahapathra *et al*., 2013). Though several commercial probiotic products are applied in shrimp culture, their efficacy in demonstrating the intended benefit has not been proven unequivocally (Shariff *et al*., 2001; Devaraja *et al*., 2002; Hena Abu *et al*., 2008). Several reports are available on the beneficial effects of probiotics application on shrimp health, growth and production under laboratory and yard experiments; however similar studies are limited under field conditions (Devaraja *et al*., 2002). Further to obtain the intended beneficial effects of probiotics under field condition, type of culture system, quality and quantity of feed, stocking density and other environmental conditions are critical (Hena Abu *et al*., 2008). Levels of probiotic application and their effect on the environmental parameters and microbial dynamics in *L. vannamei* grow-out culture have not been reported. The objective of the present study was to examine the impact of stocking density and probiotic application level on pond health and shrimp yield to suggest optimal *L. vannamei* culture management practice under tropical Indian conditions.

Materials and Methods

Shrimp ponds and culture practice: The study was conducted in six shrimp ponds at Surat area off the coast of Arabian Sea, Gujarat, India during April to September 2012. The dry ponds were ploughed and subsequently filled with tidal water filtered through a five stage filtration unit (20,20,40, 60 and 80 mm mesh size)and treated with 40 ppm chlorine(calcium hypochlorite containing 30% active chlorine). The ponds were then fertilized with farm made fermented rice juice (10 kg Jaggery+ 10 kg Rice bran+ 100 g yeast fermented for two days) on every 3rd day till sufficient bloom developed.Shrimps, up to 30 days of culture (DOC), were fed with commercial feed @ 20 kg $million^{-1}$ post larvae (PL) with a daily increment of 4 kg. After 30 DOC, shrimp were fed @ 8 % body weight which was gradually reduced to 2% towards the end of culture period. Six ponds were grouped into two, low intensity (Group I, 0.8 ha each) and high intensity culture ponds (Group II, 1.0 ha each) with 3

ponds in each group.

Low intensity culture management (Group I): Ponds under low intensity culture (Group I) were stocked with postlarvae of *L. vannamei* (PL_{14}) @ average stocking density of 35 nos. sqm^{-1} following standard procedures. Aeration was provided round the clock @ 8 HP ha^{-1} from 7 to 85 DOC and thereafter @12 $HPha^{-1}$ till the harvest. Commercial probiotic product 1 (*Rhodobacter* spp.) was applied @ 6 lit ha^{-1} at 27 and 52 DOC and probiotic product 2 (*Bacillus licheniformis* Strain 1 and 2; *Bacillus megaterium*, *B. pumilus*, *B. subtilis* fortified with digestive enzymes) was applied @ 1kg ha^{-1} at 59 and 69 DOC followed by one dose of probiotics product 3 (*B. subtilis,B. licheniformes*) @ 2.5 kg ha^{-1} on 83 DOC.

The fermented rice juice was applied @ 15$kgha^{-1}$ at 26 and 28 DOC andmineral supplements ($MgCl_2$ @ 25kg ha^{-1} and KCl @ 4$kgha^{-1}$) were applied on 22 and 135 DOC and multi-mineral mixture @ 10kg ha^{-1} was applied once in every 30 days). Liming was done with commercial products, agricultural lime and dolomite @120$kgha^{-1}$ at regular intervals.

High intensity culture management (Group II): Ponds under high intensive culture were stocked with postlarvae of *L.vannamei* (PL_{14}) with average stocking density of 56 nos sq m^{-1} following standard procedures. Aeration was provided @ 10 $HPha^{-1}$ for the first 30 DOC and thereafter @14 $HPha^{-1}$ till final harvest. Commercial probiotic product1 (*Thiobacillus denitrificans*, *Bacillus pumilus*, *B.megaterium*) was applied @ 4.0 $kgha^{-1}$ on weekly intervals till 43 DOC, and thereafter @ 5.0 kg ha^{-1} once on every fourth day till harvest. Probiotic product 2 (*B. subtilis, B. licheniformes*) was applied @ 400gha^{-1} at weekly interval starting from 12 DOC till 53 DOC, and thereafter @ 100 to 150gha^{-1} daily till harvest.

Molasses was applied @ 10kg ha^{-1} at three days intervals till 20 DOC and once a week, thereafter till 68 DOC. Mineral supplement ($MgCl_2$ @ 25$kgha^{-1}$ and KCl @ 4$kgha^{-1}$ till 50 DOC was applied at weekly intervals and multi-mineral mixture was used from 50 DOC @ 10kg ha^{-1} till 98 DOC. Liming was done with Dolomite/agrilime@25$kgha^{-1}$ in the first half and @50$kgha^{-1}$ in the second half of culture period at regular intervals. Throughout the culture period zero water exchange system was followed, and the water was topped up as and when required to compensate seepage and evaporation.

Sample collection and laboratory analysis: Water (one feet below the surface) and sediment (at soil-water interface) samples were collected from four corners and centre of each ponds on DOC 5, 35, 59, 92 and 122 (referred Phase I, II, III, IV and V respectively) in sterile bottles and plastic bags, respectively, and transported on ice to laboratory. Samples were processed within 4 hrs of collection for bacteriology and stored for further analysis under refrigeration.

Physico-chemical parameters of water *viz.,* pH, salinity, calcium, magnesium, total hardness, carbonate, bicarbonate, total alkalinity, nitrite nitrogen and total ammonia nitrogen (TAN) were examined following the standard procedures of (APHA 2005) and sediment samples were analysed for pH, electrical conductivity (EC), organic carbon, available nitrogen and phosphorus (Jackson, 1973).

Total plate count (TPC) and presumptive vibrio count (PVC) of water and sediment samples were examined by counting the number of colony forming units (cfu) on Zobell marine agar and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Gilliland *et al.*, 1976; Austin, 1988). For TPC, samples were diluted tenfold (10^{-1} to 10^{-10}) and 100 µl of contents from each dilution were plated in triplicate using spread plate method. Similarly for PVC, the samples were diluted tenfold (10^{-1} to 10^{-5}) and 100 µl of contents from each dilution were plated in triplicate using spread plate method. Ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) were enumerated by most probable number (MPN) method following 7-10 days incubation at 30ºC using modified and original Winogradsky media respectively. Since AOB oxidize NH_3 to NO_2, presence of AOB was confirmed by detection of NO_2 in tubes. Presence of NOB was confirmed by detecting NO_3 in tubes (Rodina, 1972). Similarly, sulphur oxidizing bacteria (SOB) and sulphur reducing bacteria (SRB) were enumerated by MPN technique using specific medium (Letain*et al.*, 2007) and M559 HiMedia, Mumbai, India, respectively overlaid with liquid paraffin. Lowering of pH in the tubes following 4-6 days incubation at 30°Cindicated presence of SOB while blackening of medium due to ferrous sulphide formation indicated presence of SRB (Rodina, 1972).

Growth and production: Shrimp growth and production was monitored throughout the culture period for all the ponds under study by measuring average body weight (ABW) and average daily gain (ADG) at regular intervals. At the end of the culture period production parameters viz., survival (%), feed conversion ratio (FCR), total biomass (kg) and production ($kgha^{-1}$) were recorded. Group I ponds had partial harvesting at 100 DOC and high intensity culture ponds (Group II) at 103 and 125 DOC. Final harvesting was done on 165 and 166 DOC in Group I and Group II, respectively.

Statistical analysis: Data on physico-chemical characters of soil and water, bacterial population, growth and production between the groups was analysed by t-test to determine significant association at $P<0.05$. Pearson Correlation Coefficient test was performed to examine relation between probiotic application and physico-chemical parameters and bacterial populations.

Table 1 : Levels (range) and average of physico-chemical parameters of water and sediment samples of low intensity (Group I) and high intensity (Group II) *L. vannamei* culture ponds.

Parameters	Group I		Group II	
	Range	Mean±SD	Range	Mean±SD
Water				
pH	7.52-7.95	7.68^{a}±0.13	7.20-7.98	7.52^{b}±0.25
Salinity(gl^{-1})	32-42	37.07^{a} ±2.96	32-39	36.80^{a}±1.82
Calcium (mgl^{-1})	172- 348	262.13^{a}±58.98	194- 314	264.133^{a}±40.17
Magnesium (mgl^{-1})	895-1777	1380.47^{a}±361.18	769-1895	1181.73^{a}±305.64
Total hardness(mgl^{-1}as $CaCO_3$)	1535-8308	1625.93^{a}±428.19	3975-8562	1445.87^{a} ±298.80
NO_2^-N(mgl^{-1})	0.005-0.907	0.246^{a}±0.307	0.005-0.825	0.213^{a}±0.290
TAN (mgl^{-1})	0.115-0.533	0.34^{a}±0.13	0.121- 0.802	0.27^{a}±0.17
CO_3^{-2} (mgl^{-1})	0.000-0.41	0.169^{a}±0.249	0.000-0.12	0.077^{a}±0.076
HCO_3^-(mgl^{-1})	227.000-403	216.33^{a}±35.01	124.44-185.44	129.07^{b}±15.28
Total alkalinity(mgl^{-1}as $CaCO_3$)	176.000-330	215.6^{a}±35.84	102 – 194	129.07^{b}±15.85
Sediment				
Electric conductivity (dSm^{-1})	3.10 – 12.41	6.95^{a}±2.58	4.97-11.8	6.94^{a}±1.77
pH	7.6 – 8.63	8.15^{a}±0.28	7.4-8.4	7.69^{b}±0.28
Organic carbon (%)	0.33 –0.85	0.634^{a}±0.155	0.58-0.92	0.751^{b}±0.097
Available Nitrogen (kg ha^{-1})	106.26 – 267.26	184.57^{a}±47.14	103.04-222.18	149.4^{b}±31.63
Available Phosphorus (kg ha^{-1})	107.23 – 213.43	162.88^{a}±30.48	126.09-292.82	206.01^{b}±44.22
Available potassium (kg ha^{-1})	2695 – 7355.04	5185.2^{a}±1463.17	4747.68-578.88	5796.6^{a}±471.99

Note: Values are Mean±SD

Results and Discussion

In low intensity culture ponds (Group I), water pH, bicarbonate and total alkalinity were significantly ($p<0.05$) different from that of high intensity culture ponds, whereas other parameters viz., salinity, calcium, magnesium, total hardness, nitrite-N, total ammonia-N and carbonate did not show significant difference between the groups (Table 1).

There was a slight decrease in salinity after mid culture in both the groups due to heavy rainfall during that period. Though a definite trend was not maintained, calcium and magnesium level was maintained more or less same in the pond water of both the groups. Average total ammonia nitrogen (TAN) values were within the optimum range and did not exceed 0.5 mgl^{-1} in both the groups. Similarly, nitrite N values were also within the optimum range. In both the groups, TAN and NO_2-N values decreased towards the end of culture. Carbonate ion concentration was nil to traces in pond water both groups, and total alkalinity levels were mainly due to bicarbonate ion concentrations.

In Group I pond, sediment pH (8.15±0.28), organic carbon (0.634±0.15%), available nitrogen (184.57±47.14 mg kg^{-1} soil) and available phosphorus (162.88±30.48 mg kg^{-1} soil) were significantly ($p<0.05$) different from that of high intensity culture ponds (pH 7.69±0.28;OC - 0.751±0.097%; available N 149.4 ±31.63; available P 206.01±44.22), whereas other parameters like electrical conductivity (EC) and available potassium did not show significant ($p<0.05$) difference (Table 1).

Soil salinity and conductivity in both the groups followed the trend of water salinity. High pH values were observed in low intensity culture ponds (7.92 to 8.41) as compared to high intensity culture ponds (7.50 to 7.92). Soil in low intensity culture ponds had low organic carbon content (0.42 to 0.71%) as compared to high intensity culture ponds (0.67 to 0.82%), and the values towards the end of culture were low as compared to initial periods.

Physico-chemical parameters showed significant positive correlation between salinity, Mg ion concentration and total hardness (TH). Mg^{2+} ion was highly correlated with TH than Ca^{2+} ion in both groups. Similarly, HCO_3^- concentration had high significant positive correlation with total alkalinity. Nitrite-N had significant negative correlation with Ca ($r=0.673$) and positive correlation with TAN ($r=0.527$) in low intensity culture ponds (Group I).

TPC of bacteria, indicative of heterotrophic bacterial populations, were low in water as compared to that in sediments in both the groups. Total bacterial counts in water inlow intensity culture ponds (Group I) showed a typical bell shaped

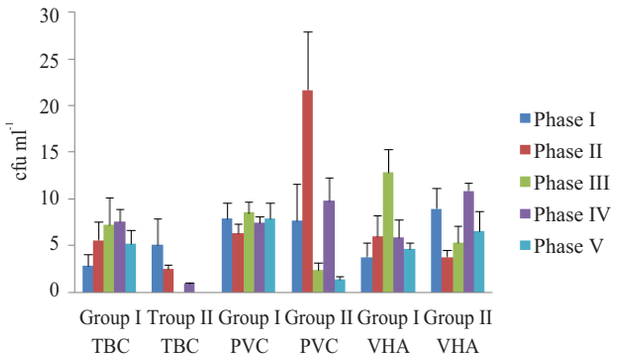

Fig. 1 : Total bacterial (x10^6), Presumptive *Vibrio* (x10^2) and *V. harveyi* (x10) counts in Group I and Group II *L. vannamei* culture pond water

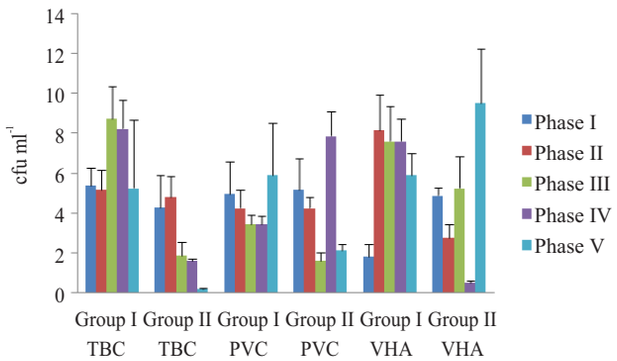

Fig. 2 : Total bacterial (x10^6), Presumptive *Vibrio* (x10^2) and *V. harveyi* (x10) counts in Group I and Group II *L. vannamei* culture pond sediment

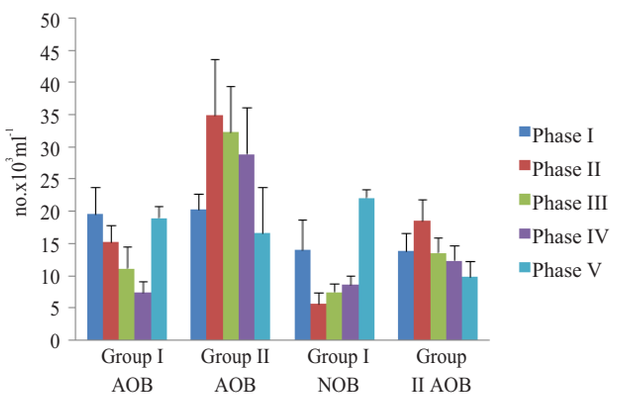

Fig. 3.: Ammonia oxidizing bacteria (AOB) and Nitrite oxidizing bacteria (NOB) population (x10^3)in Group I and Group II *L. vannamei* culture pond water

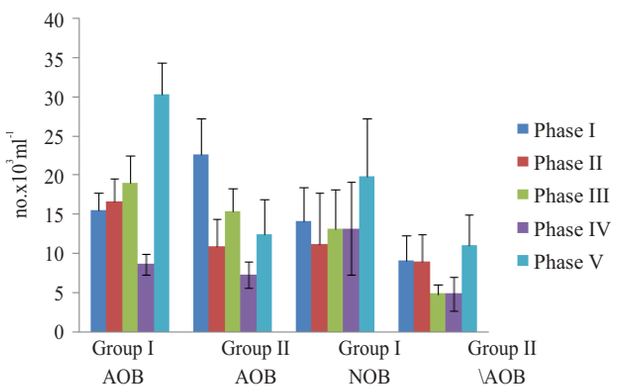

Fig. 4.: Ammonia oxidizing bacteria (AOB) and Nitrite oxidizing bacteria (NOB) population (x10^3) in Group I and Group II *L. vannamei* culture pond sediment

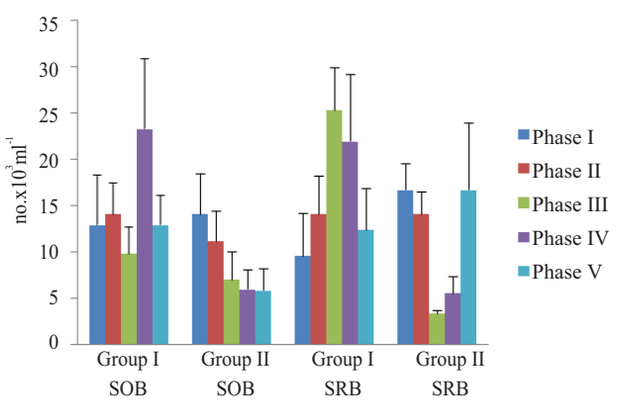

Fig. 4.: Ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) population (x10^3) in Group I and Group II *L. vannamei* culture pond sediment

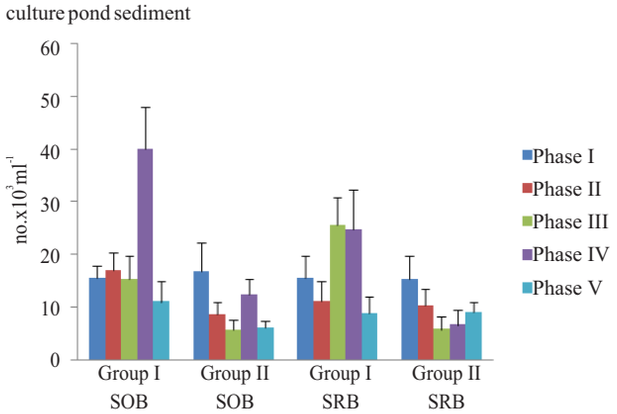

Fig. 6.:.Sulphur oxidizing bacteria (SOB) and Sulphur reducing bacteria (SRB) population (x10^3) in Group I and Group II *L. vannamei* culture pond sediment

curve with peak (7.60±1.34x10^{6}cfuml^{-1}) in mid cycle (Phase III), while a gradual decrease was observed in high intensity culture ponds (Group II) till the harvest of the crop (Fig. 1).

Presumptive *Vibrio* counts measured by number of bacterial colonies on TCBS plate were highly fluctuated in high intensity culture ponds (Group II), while a steady and higher count was found in low intensity culture ponds (Group I) (Fig. 1).*V. harveyi* level measured in VHA media, showed a trend similar to TBC in low intensity culture ponds (Group I) and PVC in high intensity culture ponds (Group II) (Fig. 1). In sediment, there were wider fluctuations in these bacterial

Table 2 : Average levels of environmental and pathogenic bacterial in low intensity (Group I) and high intensity (Group II) *L. vannamei* culture ponds (Mean±SD)

TBC ($x10^6$)		PVC ($x10^2$)	VHA (x10)	AOB ($x10^3$)	NOB ($x10^3$)	SOB ($x10^3$)	SRB ($x10^3$)
Water							
Group I	5.72±2.34[a]	7.65±1.31[a]	6.66±3.67[a]	14.54±5.99[a]	11.66±7.09[a]	14.67±6.25[a]	16.7±7.55[a]
Group II	1.75±2.27[b]	8.62±8.07[a]	7.14±8.81[a]	26.67±8.97[b]	13.71±3.67[a]	8.87±4.29[b]	15.32±6.67[b]
Sediment							
Group I	6.54±2.31[a]	4.38±1.58[a]	6.21±2.67[a]	18.03±7.71[a]	14.31±5.87[a]	19.79±11.41[a]	17.17±8.29[a]
Group II	2.53±1.96[b]	4.19±2.47[a]	4.56±3.35[a]	13.76±6.15[a]	7.75±3.69[b]	9.01±5.10[b]	9.45±4.35[b]

Values are mean ±SD; Means in the same column within water and sediment category with different superscript are significantly different (P< 0.05)

Table 3 : Production details of low intensity (Group I) and high intensity (Group II) cultures of *L.vannamei*

Particulars	Group I	Group II
Stocking (nos m^{-2})	35	56
Partial harvesting 1		
DOC	100	103
Biomass (kg ha^{-1})	1270.16±380.77	2069.06±220.53
ABW (g)	19.66[a]±3.00	16.25[a]±0.49
ADG (g)	0.19[a]±0.033	0.16[a]±0.003
Partial harvesting 2		
DOC	-	125
Biomass (kg ha^{-1})	-	2011.09±789.86
ABW(g)	-	21.94±0.70
ADG(g)	-	0.18±0.005
Final harvest		
DOC	165	166
Biomass (kg ha^{-1})	5856.31±256.45	4074.45±849.01
ABW(g)	28.80±3.21	32±1.35
ADG(g)	0.18±0.02	0.19±0.003
Overall Total		
Production (kg ha^{-1})	7126.47[a]±366.90	8154.60[a]±1175.52
ADG	0.19±0.02[a]	0.176±00[a]
ABW	24.23±2.32[a]	23.39±3.01[a]
Survival	84.84±7.34[a]	61.53±5.94[b]
FCR	1.43±0.07[a]	1.92±0.25[b]
Total cost of biological materials (Rs.)	13302.70	71726.00
Cost of biological kg^{-1} shrimp (Rs.)	2.33	9.03

Means in the same row with different superscript are significantly different (P< 0.05).

counts in both the groups (Fig. 2).

AOB in low intensity culture ponds (Group I), pond waters showed a steady decreasing trend till Phase IV (19.67±4.16 $x10^3ml^{-1}$ in 5 DOC to 7.47±1.79 $x10^3ml^{-1}$at 92 DOC), which again increased almost to original level towards the end of culture (19.06±7.12$x10^3ml^{-1}$), whereas in high intensity culture (Group II) pond water AOB levels increased during the first half (35±8.72 $x10^3ml^{-1}$) followed by a gradual decrease till harvest (Fig. 3). NOB population in low intensity culture pond (Group I) water was consistently low in the middle phases (35 DOC 5.77±1.62 x 10^3ml^{-1}, 59 DOC 7.57±1.36 x 10^3ml^{-1}, 92 DOC 8.63±1.35 x 10^3ml^{-1}) but peaked in the last phase of culture (22.20±7.60$x10^3ml^{-1}$). In high intensity culture ponds (Group II) pond waters, NOB levels reached peak at Phase II (18.67±3.21$x10^3ml^{-1}$) and gradually declined till the end of the culture (Fig. 3). In sediment, there were wider fluctuations in AOB and NOB populations in both the groups. AOB population was highest towards the end of culture period (30.33±4.04$x10^3g^{-1}$) in Group I ponds, but high counts were observed during initial periods in high intensity culture ponds (Group II) (22.67±4.62$x10^3g^{-1}$). Though the NOB population differed widely in two groups, highest density was found in last phase of culture (19.87±7.38$x10^3g^{-1}$ in Group Iand 11.07±3.90$x10^3g^{-1}$ in Group II) (Fig. 4).

Among the sulphur recycling bacterial populations, SOB density were highly fluctuating in pond waters of low intensity culture ponds (Group I), while a steady decreasing trend was observed in high intensity culture ponds (Group II). In case of SRB, two groups of ponds showed opposite trend with highest bacterial load (25.33±4.62$x10^3ml^{-1}$) in Group I ponds and lowest load (3.40±0.35$x10^3$/ml) in Group II ponds during the mid-culture period (Fig. 5). Population of sulphur bacteria (SOB and SRB) in pond sediments showed more or less similar trend as that of water samples in both the groups. High intensity culture ponds (Group II) had lower SOB and SRB levels in sediment as compared to those in low intensity culture ponds (Group I) throughout the culture period (Fig. 6).

Though no correlation was observed between levels of nitrogenous toxicants and bacteria involved in nitrogen cycle, overall heterotrophic bacterial populations in low intensity culture (Group I) pond water was significantly ($p<0.05$) higher (5.72±2.34 $x10^6ml^{-1}$) than in high intensity culture ponds (Group II) (1.75±2.27 $x10^6ml^{-1}$). AOB was significantly ($p<0.05$) higher in high intensity culture ponds (26.67±8.0 $x10^3ml^{-1}$) as compared to that in low intensity culture ponds (14.54±5.99 $x10^3ml^{-1}$). However for sulphur recycling bacteria, both SOB (14.67±6.25 $x10^3ml^{-1}$) and SRB (16.7±7.55 $x10^3ml^{-1}$) levels were significantly ($p<0.05$)

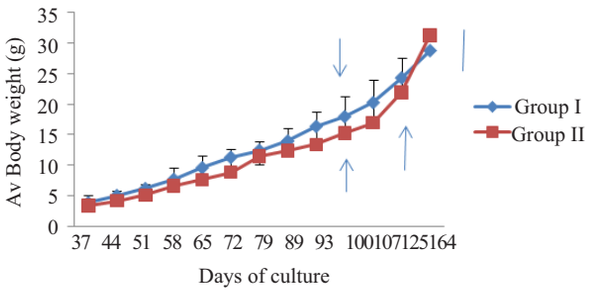

Fig. 7 : Average body weight (g) of *L. vannamei* in Group I (♦) and Group II (■) culture ponds

higher in low intensity culture ponds as compared to those in high intensity culture ponds (SOB 8.87±4.295 x10^3 ml^{-1} and SRB 15.32±6.67 x10^3 ml^{-1}). Like water, low intensity culture ponds (Group I) had significantly ($p<0.05$) higher TBC (6.54±2.31x10^6 g^{-1}), NOB (14.31±5.87x10^3 g^{-1}), SOB (19.79±11.41x10^3 g^{-1}) and SRB (17.17±8.29x10^3 g^{-1}) as compared to high intensity culture ponds (Group II) sediments(TBC-2.53±1.96x10^6 g^{-1}, NOB-7.75± 3.69x10^3 g^{-1}, SOB-9.01±5.10x10^3 g^{-1}, SRB-9.45±4.35x10^3 g^{-1}), while no significant ($p<0.05$) difference was observed in the levels of PVC, VHA and AOB between them.

First partial harvesting recorded a production of 1270±381 kg ha^{-1} with ABW of 19.66 g and ADG of 0.19 g in low intensity culture ponds (Group I) and 2069±221 kg ha^{-1} with ABW of 16.25±0.49 and ADG 0.16 g in high intensity culture ponds (Group II). Second partial harvesting in high intensity culture ponds (Group II)ponds registered a production of 2011.09 ±789.86 $kgha^{-1}$ with ABW of 21.94±0.70 and ADG of 0.18 g. Comparison of production parameters at harvest revealed that low intensity culture ponds (Group I) had higher growth rate (0.19±0.02$gday^{-1}$) and average body weight (24.23±2.32 g) as compared to high intensity culture ponds (Group II) (growth rate-0.18 $gday^{-1}$ and ABW-23.39±3.01g) (Fig. 7). Low intensity culture ponds (Group I) had significantly ($p<0.05$) lower FCR (1.43±0.07) and higher survival rate (84.84±7.34 %) as compared to high intensity culture ponds (Group II) (FCR-1.92±0.25, survival-61.53±5.94%). Though high intensity culture ponds (Group II)yielded total higher production (8154.60±1175.52 $kgha^{-1}$) than low intensity culture ponds (Group I) (7126.47±366.90 $kgha^{-1}$), it was not statistically significant ($p<0.05$). With respect to the value of biological products per kg shrimp produce,low intensity culture (Group I) system accounted for 25.80 % less as compared to high intensity culture (Group II) (Table 3).

In modern intensive shrimp culture system, increasing emphasis is focused on microbes for mitigating the deteriorating pond environment. Nitrogen and sulphur cycling are critically important for pond environment and accumulation their intermediate metabolites at higher levels in the system is toxic (Burford and Lorenzen, 2004). In the present study, both the groups showed optimal environmental parameters for shrimp growth. Stocking density, level of probiotic use, aeration and fertilization were different in two groups. High intensity culture ponds (Group II) showed improved environmental parameters despite higher stocking density, suggesting the beneficial role of higher use of probiotics. However, for economics and environmental sustenance the level of probiotics use need to be rationalized.

Heterotrophic bacteria influences the level of vital environmental parameters like, DO, pH and ammonia in addition to primary production through nutrients recycling, especially nitrogen and phosphorus (Lemonnier *et al*., 2010). Hence, higher concentrations of these bacteria in low intensity culture ponds (Group I) might be responsible for better water quality and production parameters. Level of TBC was higher in the first half of the culture period in high intensity culture ponds (Group II) and might be due to regular addition of molasses till DOC 68. Reduction in TBC numbers towards the end of the culture in Group II might be due to regular application of probiotics and partial harvesting.

Uniformity in PVC levels in low intensity culture ponds (Group I) and high fluctuations in high intensity culture ponds (Group II), indicated stability and volatility, in the pond dynamics. Further, no significant difference in VHA concentration was observed between the groups. Bacterial species present in probiotic product used in both the groups were known to enhance shrimp immunity and inhibit growth of pathogenic Vibrios (Nakayama *et al*., 2009; Zokaeifar *et al*., 2012). Composition of microbes in probiotic products and their frequency of application might be responsible for the limiting the levels of PVC and *V. harveyi* despite the higher stocking density in high intensity culture ponds (Group II) compared to low intensity culture ponds (Group I).

A specific pattern was observed in nitrogen recycling bacterial populations in pond environment, indicating the dynamic mechanism in build-up and removal of nitrogenous toxicants as culture progress. It was interesting to note that a proportional increase in TAN level was also observed throughout the study, suggesting the role of AOBs in reducing the nitrogenous toxicants. Generally, role of bacteria in controlling the build-up of toxicants like ammonia and nitrite has been well documented (Dang *et al*., 2011; Fernandes *et al*., 2010). Significantly higher AOB numbers in high intensity culture ponds (Group II) may be attributed to frequent application of product with *Thiobacillus dentrificans* which is known to have unusual and

environmentally relevant metabolic repertoire of coupling of denitrification with sulphur oxidation (Letain *et al.,*2007). The level ofthese nitrifying bacteria were similar to the earlier reports of Cheng and Liu (2001).Though TAN level also increased proportionately, it was not statistically significant as compared to Group I.

Accumulation of unutilized feed, creating an anaerobic environment, is responsible for blackening the bottom of pond which adversely affected the shrimp health and growth. Bacteria involved in sulphur recycling are crucial for converting sulphur and sulphur related compounds accumulated during the course of culture. Similar to previous observations, higher population of SRBs were detected both in water and sediment (Srinivasa Rao and Karunasagar, 2000). However, the level recorded in both the groups were much lower than the previous reports (Rao *et al*., 2000) in *P. monodon*. The observed higher level of sulphur oxidizing bacteria in water samples contrary to previous observations (Rao *et al*., 2000) might be attributed to higher aeration followed in the study ponds. Level of sulphate reducers and sulphide oxidizers reportedly suggest efficient sulphur cycling in an environment (Madrid *et al.*, 2001). The observations of the present study is similar to the previous report of Devaraja *et al.* (2002) indicating that level of sulphur recycling bacteria maintain naturally depending on the conditions of the pond environment, and their numbers may not differ significantly by extraneous administration of soil probiotics.

Understanding the complex inter-relationship between microbial dynamics and environment quality with respect to species and intensity of culture systems help to develop suitable intervention strategies. In the present study, the level of microbial populations and physico-chemical parameters suggested the role of bacterial products in maintaining healthy pond environment. The study also suggests ample scope for reducing expenditure on usage of probiotics without compromising the production.

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