International Journal of Fisheries and Aquatic Studies 2015; 3(2): 124-131

ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(2): 124-131

www.fisheriesjournal.com
Received: 12-08-2015
Accepted: 13-09-2015

DV Prasuna Devi
Department of Zoology,
Sri Venkateswara University,
Tirupati- 517502, India

K Hareesh
Department of Zoology,
V.S.U.P.G. Centre,
Kavali - 524 201, India.

M Srinivasulu Reddy
Department of Zoology,
Sri Venkateswara University,
Tirupati- 517502, India.

Correspondence
M Srinivasulu Reddy
Department of Zoology,
Sri Venkateswara University,
Tirupati- 517502, India.

Enhancement of growth potentials in freshwater prawn *Macrobrachium rosenbergii* through supplementation of probiotic diets of *Bacillus subtilis* and *Lactobacillus rhamnosus*

DV Prasuna Devi, K Hareesh, M Srinivasulu Reddy

Abstract
In the present investigation Wild prawns and Hatchery PL raised prawns were fed with two different probiotic diets. The probiotic diets include *Bacillus subtilis* and *Lactobacillus rhamnosus*. Feeding trail experiments were conducted for 120 days and growth parameters were monitored through Weight gain, Specific growth rates, Food conversion ratio, Feed efficiency ratio, Percent survival, Muscle somatic index, Protein efficiency ratio, Normalised biomass index, Microbial analysis and activities of Antioxidant enzymes including Superoxide dismutase and Catalase were monitored and recorded. Regular water quality analysis was conducted for several water parameters including temperature, Transparency, Dissolved oxygen content, pH, Conductivity, Turbidity, Total Alkalinity, Nitrite (NO_2), Nitrate (NO_3),Ammonia (NH_3), Total Nitrogen, Total Phosphorous, Chlorophyll-a and Chemical oxygen demand (COD) were monitored and recorded. Feed trail experimental results obtained clearly demonstrate that Probiotic diet (PB-I) containing *Lactobacillus rhamnosus* Supplementation induces highest growth potentials in terms of weight gain compared to Probiotic diet II (PB-II) containing *B.subtilis* supplemented and Control group fed with Commercial diet. The FCR values also recorded to be better with PB-I diet compared to PB-II and Control diet, demonstrates the efficient utilization of feed by the prawn *M. rosenbergii*. The water quality parameters are seems to be more congenial for inducing better growth potentials when PB-I and PB-II are being made available for feeding in the feeding trail experiments. The Antioxidant enzymes represented by Superoxide dismutase and Catalase demonstrates the reduction of ROS in the body of Prawns there by inducing highest and best growth potentials in Freshwater Prawn *M. rosenbergii.* All the changes are more pronounced in prawns collected from wild source compared to Hatchery raised prawns indicating that the wild source of prawns are showing the better growth rates and efficient mechanisms of detoxification, there by the growth potentials recorded are to be superior to the Hatchery raised prawns. Both the Probiotics selected *B. subtilis* and *L. rhamnosus* are considered to be more efficient inducing best and highest rate of growth potentials in Freshwater prawn *M. rosenbergii.*

Keywords: freshwater prawn, *Macrobrachium rosenbergii, Lactobacillus rhamnosus*

1. Introduction
Aquaculture has emerged as one of the most promising and fastest growing food producing sector around the globe, which provides avenues for high quality protein, income generation, employment and foreign exchange earnings. Recent developments in the Aquaculture sector and increasing demand for fish production resulted in intensification of the Aquaculture practices. Globally, Prawn or shrimp farming has been a significant Agro based economic activity since early 1970's. Introduction of Intensive culture systems create highly stressful environment for the candidate species of culture that further suppress the immune response, leading to the outbreak of infectious diseases. In recent times, shrimp culture all over the world has been frequently affected by viral and bacterial diseases inflicting huge loss [23, 9]. Pathogenic Microorganisms implicated in these outbreaks were Viruses, Bacteria, Fungi, and certain Protozoon parasites. For Prevention and control of the diseases, Antibiotic, Pesticides, and other toxic chemicals were used possibly creating Antibiotic resistance Bacteria, persistence of Pesticides and other toxic chemicals in Aquatic environment and creating human health hazards. Probiotics are beneficial Microorganisms that protect the host from diseases. Fuller [11] defined Probiotics as "Live Microbial Feed supplements which are

beneficially affect the host by improving its intestinal Microbial balance". Microbes play very important and crucial role in Aquaculture systems, both at Hatchery and Grow-out levels, as water quality as well as disease incidences are directly affected by Microbial activity [27]. The range of Probiotics examined for use in Aquaculture has encompassed both Gram-negative as well as Gram-positive Bacteria, Yeast, Unicellular algae etc. In particular, the probiotics have been reported to be successful with a wide range of Invertebrates [35, 29, 52, 7, 6, 26, 34]. In, Aquaculture, most probiotics are supplied as live supplements in diets, which have the ability to survive passage through the intestinal tract [11]. A wide variety of mechanisms have been suggested, contributing to the beneficial effects of probiotics, including the removal of pathogens by the beneficial population, which is often considered as the most important mechanism [12, 14, 16]. Some studies have attributed to the enhancement of a normal growth to the nutritional benefits of probiotic bacteria, such as vitamin production, availability of minerals as well as trace elements and production of important digestive enzyme [19]. Thus, the use of probiotics in the culture of Aquatic organisms is increasing with the demand for more environmental-friendly aquaculture practices. An effective method to overcome the pathogens problems is to administer the probiotic into the rearing Water system or through food [25, 42, 50]. Many different genera, including photosynthetic Bacteria, Yeast, *Bacillus* and *Lactobacillus* have been evaluated as probiotics in Fish and Shell Fish [44, 30, 27, 28]. The use of Lactic Acid Bacteria (LAB) as Probiotics and non-specific immunostimulants [47, 48]. Skjermo & Vadstein [41] has been proposed, in addition to their effects on improved water quality and nutrition [36] as a means to increase larval survival and Aquaculture output. There has been great interest in the use of LAB as probiotic in Aquaculture industry [13, 28, 43]. Through the probiotics have been shown to be effective in a wide range of species for growth promotion, enhanced nutrition, immunity and survival rate, very few attempts have been made to investigate the effect of probiotics i.e the effect of administration of *Bacillus subtilis* and *Lactobacillus rhamnosus*-suplimented diets on the growth pattern of fresh water prawn *Macrobrachium rosenbergii.* Attempts were also made into study the activity levels of Antioxidant system of Prawn during Probiotic treatments with the increase in the Farm areas, there has been a concomitant increase in demand of the seed of prawn in the coastal areas. Availability of seeds poses to be an obstacle in the development of the commercial culture of candidate species. So long the demand of seed was used to be met from the natural collection, but of late, the natural sources are drastically reduced and now hatchery-produced seed is meeting a significant part of the demand. So by keeping the above back ground, the objective of this present study was to know the production performance of prawn farming using wild and hatchery sourced young freshwater prawn *M. rosenbergii.*

2. Materials and Methods

Freshwater Prawn young individuals both wild and Hatchery raised ones are stocked in six different ponds with uniform stocking density of 2000 Nos/ Acre. Wild individuals were collected from adjacent areas of Buckingham Canal near Ramayapatnam Seacoast (Latitude 15^0 02^1 55^{11} N, Longitude 80^0 02^1 50^{11} E) and Hatchery raised once are collected from local Aquaculture Ponds of almost equal size located at Ramayapatnam, 16 kms away from Kavali, Nellore Dist. Andhra Pradesh. In the present investigation Three each ponds were selected for stocking of Wild and Hatchery raised young ones are selected and stocked and the experiments were conducted for 120 days. First pond was treated as Control pond and the prawns were fed with commercial diet. Second pond was treated as Probiotic treated pond (PB-I), and the prawns were fed with *Lactobacillus rhamnosus* supplemented diet. Third Pond was considered as Probiotic treated pond (PB-II), and the animals were fed with *Bacillus subtilis* supplemented diet. The Lactic Acid Bacterium, *Lactobacillus rhamnosus* and *Bacillus subtilis* were collected from IMTECH, Chandigarh. The Probiotic Bacteria *L. rhamnosus* was cultured in Man-Rogora Sharpe broth (MRS, Himedia), and *B. Subtilis* was cultured in Bacillus broth (Himedia) and incubated under continuous agitation of 180 rpm at 37 ℃ for 24 hrs. The Bacterial culture was centrifuged at 4000 rpm for 15 min at 4℃ and harvested. The collected Bacteria were suspended in normal saline solution to 5×10^{13} CFU/ml of *Bacillus Subtilis* and 3×10^5 CFU/ml of *L. rhamnosus*. The Probiotic Experimental diets were oven dried at 35℃ per 1-2 hrs. The Control commercial feed was sprayed with sterile culture medium. The feeding trails were conducted for 120 days. At the Regular intervals Growth parameters, Microbial analysis and selected Antioxidant enzyme assays were performed to monitor the effect of Probiotic treatments on the culture activity of prawn *M. rossenbergii*. For conduction of Microbial analysis 6 prawns were selected randomly from each pond and the digestive tract was selected. Prawn digestive tract were removed with Tweezers and scalpel and homogenized with sterile saline solution. Dilution was spread on the following culture media. MRS Agar (Lactic Acid Bacteria selective) and Bacillus Agar (Selective for *Bacillus* bacteria) 30℃ for 24 hrs. Gram staining was performed with colonies grown in MRS and *Bacillus* selective agar [40]. The colonies were identified based on Morphological and Biochemical characteristics [24]. Antioxidant enzyme assays were also performed in the hemolymph of prawns. Hemolymph was withdrawn from the base of the Third walking leg of the shrimp using a syringe containing 1.5 ml of anticoagulant, Sodium citrate. Superoxide dismutase (SOD) activity was determined by the method of Kakkar *et al.* [22]. The assay mixture contained 0.1 ml of sample, 1.2 ml of Sodium Pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of Phenazine methosulphate (186 µm), 0.3 ml of Nitro Blue Tetrazolium (300 µm), 0.2 ml of NADH (750 µm). Reaction was initiated by the addition of NADH. After incubation at 30 ℃ for 90 Sec, the reaction mixture was stirred vigorously with 4.0 ml of n-Butanol. The mixture was allowed to strand for 10 min, Centrifuged and Butanol layer was separated. The colour intensity of the chromogen in Butanol layer was measured at 560 nm against n-Butanol and concentration of SOD was expressed as units/ml of hemolymph. Absorbance values were compare with a standard curve generated from known SOD. Catalase was assayed according to the method of Aebi [1]. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The reaction mixture contained 0.01M Phosphate Buffer (pH 7.0), 2 mM H_2O_2 and 0.2 ml of enzyme extract. The specific activity of catalase was expressed in terms of Units/ml of hemolymph. Absorbance values were compare with a standard curve generated from know CAT. At the end of 120 days feeding trail experiment, Growth Parameters, Food conversion ratio (FCR), Specific growth rates (SGR), Percent survival, Normalised biomass index (NBI), Food efficiency ratio (FER) (1/FCR), Protein efficiency Ratio (PER) and Muscle somatic index (MSI) were

also determined.

NBI: (Final weight ✕ Prawn Number –Initial Weight ✕ Prawn number) ×100

$$\text{SGR: } \frac{\text{Log Final weight} - \text{Log Initial Weight}}{\text{Days of Experiment}} \times 100$$

FCR: Feed Consumed (g) × Number of prawn/ Weight gain (g)

$$\text{Weight gain (\%): } \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

FER: 1/FCR

PER: Weight gain/ Protein intake

$$\text{MSI: } \frac{\text{Muscle weight}}{\text{Weight of the Animal}} \times 100$$

Determination Physico Chemical Properties of water

Regular analysis of collected water from Culture Ponds was analysed for several Parameters. Some parameters were measured in the field such as Dissolved Oxygen (DO), pH; Temperature (℃), Transparency (SD), and Conductivity etc. DO, Temperature and pH of water were measured with Elico Field water kit. SD was measured by Secchi disk. Total Nitrogen (TN) and Total Phosphate (TP) were detected with the National Standards by for monitoring of water quality analysis. TN was determined by the Alkaline Potassium Persulfate digestion Ultraviolet Spectrophotometric method. TP was determined by using Ammonium Phosphomolybdate Colorimetric method. Nitrate Nitrogen (NO_3^-) Using the phenol disulfonic acid spectral light degree method. Nitrite Nitrogen (NO_2^-) Using naphthalene ethylene-diamine Spectrophotometry. Ammoia Nitrogen (NH_3-N) was measured by Nesslers reagent colorimetric method. Chemical oxygen demand (CODMn) was specified by acidic Potassium Permanganate method of permanganate index. Chlorophyll-a was estimated according AOAC Methods. All the Water quality parameters were conducted by following the procedures of APHA [4] and AOAC [3].

3. Results and Discussion

After feeding trail experiments at end of 120 days of experimentation with freshwater Prawn *M. rosenbergii* both Wild and Hatchery raised prawns, after fed with Control group fed with commercial diet Growth parameters and Probiotic treated group (PB-I) fed with *Lactobacillus rhamnosus*-supplemented diet and other Probiotic treated Group-II (PB-II) fed with *Bacillus subtilis*-supplemented diet were monitored and recorded. The parameters including Growth potentials, Population counts of Bacteria, Percent survival, Food conservation ratio, Specific Growth rates, Normalized Biomass index, Histosomatic index were recorded at the start of the Experiment and at the completion of 120 days of feeding trail experiment. The Growth parameters obtained for freshwater prawn *M. rosenbergii* after fed with commercial fed and Probiotic supplemented diets were presented in Table.1. The trends obtained for growth potentials in the case of both the wild and Hatchery raised ones are almost followed the same trend, but are more pronounced in wild young prawn culture activity. In all the culture ponds, the stocking density was 2000 Nos/Acre. The survival rates recorded minimum with 88% with Hatchery raised young prawn culture i.e., PB-I group fed with *B. Subtilis*-supplemented feed and maximum survival of 97% recorded with wild young prawn culture fed with commercial diet. The Prawns selected for stocking were weighing around 3.50 g. The final weights recorded for wild young prawn culture were for control 43.48 g compared to 68.33 g for PB-I group fed with *B. subtilis* supplemented feed and for 81.45 g for PB-II group *L. rhamnosus* supplemented feed. The final weights recorded for Hatchery raised young one prawns were 36.78 g, 52.39 g and 66.73 g for control, PB-I and PB-II, respectively. Similarly weight gain was observed to be 40.03 g, 64.79 g and 77.86 g for control, PB-I and PB-II, respectively for wild group prawn culture compared to 33.29 g, 48.94 g and 63.15 g for control, PB-I and PB-II, respectively for Hatchery raised prawn culture. Weight gain (%) recorded to be highest g 2196%, 1830% and 1160% for PB-II, PB-I and controls of wild prawn culture, whereas 1764%, 1419% and 954% recorded with PB-II, PB-I and controls of Hatchery raised young prawn culture. The Specific growth rates (SGR) were1.114, 1.071 and 0.917, for PB-II, PB-I and controls respectively for wild young prawns compared to 1.059, 0.993 and 0.816 for PB-II, PB-I and control, respectively for Hatchery raised prawns. The Normalised Biomass Index values were recorded to be maximum with PB-II, followed by PB-I and control of both the cases of culture operation. The Feed conversion ratio (FCR), appears to be best for PB-II followed by PB-I and controls of both wild and Hatchery raised prawn culture operations. The Food Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) values were also recorded to b maximum with PB-II group compared to PB-I and control groups in both the types of culture operation. The Muscle somatic Index (MSI) and Muscle Weight (MW) were recorded to be maximum with PB-II, followed by PB-I and controls of both the types of culture operation. Water quality analysis was conducted at field level for every 20-30 days depending on the convenience throughout the 120 days of experimental trails. Water quality parameters like, Water Temperature, Transparency, Dissolved oxygen, pH, Conductivity, Turbidity, Total Alkalinity, Nitrites (NO_2), Nitrate (NO_3), Ammonia, Total Nitrogen, Total Phosphorus, Chlorophyll-a and Chemical Oxygen Demand (COD) were monitored and presented in Table -2. Length-weight relationship pattern in freshwater prawn *M. rosenbergii* in both the types of culture systems was monitored for every 30 days for 120 days of experimentation. The trends obtained for both the types of culture operation is appears to be same. The percent deviation of control (PDC) values over Initial weights of their respective values appears to show a upward trend in gaining weight and reaching maximum weights at the end of the experiment. Similarly values also recorded to be percent deviation experimental (PDE), calculated against its respective control group values with PB-I and PB-II treated groups. Similarly Length values of prawns in both the types of cultures were monitored and presented in Table. 3. The Length-Weight relationship clearly reveals that with the advancement of culture activity, the Weight and Length of prawns also shown a parallel progress. The bacterial population content studies, hemolymph activity levels of antioxidant activity levels of enzymes such as Superoxide dismutase (SOD), and catalase of prawns *M. rosenbergii* in two different types of culture operation were estimated or assayed and presented in Table. 4.

The values recorded to be relatively higher with Wild Prawn culture compared to Hatchery raised prawn culture. In both the types of culture operations, there was a significant increase in the Bacterial population densities and antioxidant activity levels in PB-I and PB-II fed groups over their respective control groups.

Table 1: Growth Parameters in Freshwater Prawn after fed with Probiotic diets.

Parameter	Wild PL's Culture			Hatchery PL's Culture		
	Control	PB-I	PB-II	Control	PB-I	PB-II
Stocking density	2000	2000	2000	2000	2000	2000
Survivability (%)	97	92	93	92	88	89
Initial weight (g)	3.45±0.28	3.54±0.27	3.59±0.25	3.49±0.24	3.45±0.26	3.58±0.27
Final weight (g)	43.48±0.59	68.33±0.72	81.45±0.92	36.78±0.36	52.39±0.65	66.73±0.68
Weight gain (g)	40.03±0.54	64.79±0.83	77.86±0.88	33.29±0.41	48.94±0.48	63.15±0.59
Weight gain (%)	1160	1830	2169	954	1419	1764
Specific Growth Rate (SGR)	0.917	1.071	1.114	0.816	0.993	1.059
Normalizes Biomass Index (NBI)	38.83	59.61	72.41	30.63	43.06	56.2
Food Conversion Ratio (FCR)	2.59	2.03	1.82	2.78	2.34	2.08
Food Efficiency Ratio (FER)	0.386	0.493	0.549	0.36	0.427	0.481
Protein Efficiency Ratio (PER)	8.22	8.74	9.13	7.41	8.05	8.41
Muscle weight	26.31±1.75	43.04±1.89	52.13±2.05	21.33±1.68	32.08±2.14	41.49±2.13
Muscle Somatic Index (MSI)	60.51±2.13	62.99±2.19	64.00±2.72	57.99±1.88	61.23±1.94	62.22±2.12

All values are Mean ± SD of six individual observations.

Table 3: Length – Weight Relationship in Freshwater Prawn *M. rosenbergii.*

DOC	Parameter	Wild PL's culture			Hatchery PL's culture		
	Weight(g)/Length(mm)	Control	PB-I	PB-II	Control	PB-I	PB-II
0	Initial weight	3.45±0.28	3.54±0.27	3.59±0.25	3.49±0.24	3.45±0.26	3.58±0.27
0	Initial Length	50-54	50-54	51-55	50-54	50-54	50-54
30	Weight	10.75±0.23	14.39±0.26	16.97±0.29	9.25±0.24	13.05±0.23	15.12±0.25
	PDC	+212	+306	+373	+165	+278	+322
		PDE	+34	+58	PDE	+41	+63
30	Length	71-75	76-80	80-84	65-69	73-77	77-81
60	Weight	15.18±0.34	27.36±0.32	32.19±0.34	13.15±0.34	23.42±0.35	28.41±0.32
	PDC	+340	+673	+797	+277	+579	+694
		PDE	+80	+112	PDE	+78	+116
60	Length	80-84	95-99	102-106	75-79	90-94	96-100
90	Weight	29.93±0.38	44.71±0.32	53.76±0.49	21.74±0.35	38.21±0.38	42.39±0.34
	PDC	+768	+1162	+1397	+523	+1008	+1084
		PDE	+49	+80	PDE	+76	+95
90	Length	100-104	110-114	120-124	90-94	108-112	109-113
120	Weight	43.48±0.59	68.33±0.72	81.45±0.92	36.78±0.36	52.39±0.65	66.73±0.68
	PDC	+1160	+1830	+2169	+954	+1419	+1764
		PDE	+57	+87	PDE	+42	+81
	Length	110-115	130-134	137-141	105-109	118-122	128-132

All values are Mean ± SD of Six individual observations.
DOC: Days of Culture.
PDC: Percent deviation over Zero day Control.
PDE: Percent deviation of Respective Experimental value.
All values are Statically significant at P <0.001.

Table.4: Bacterial Population densities, Activity levels of Superoxide dismutase and Catalase activities in the Hemolymph of Wild and Hatchery cultured *M. rosenbergii***.**

Parameter	Wild PL's Culture			Hatchery PL's Culture		
	Control	PB-I	PB-II	Control	PB-I	PB-II
Bacterial Population[a]	3.18×104	7.08×104	8.93×104	3.04×104	6.34×104	7.31×104
	PDC	+123	+181	PDC	+108	+140
Superoxide dismutase[b]	3.15±0.34	6.33±0.39	8.16±0.42	2.84±0.32	5.75±0.39	7.12±0.45
	PDC	+101	+159	PDC	+102	+151
Catalase[b]	3.49±0.35	5.13±0.42	7.12±0.52	3.12±0.36	4.72±0.46	6.11±0.42
	PDC	+47	+104	PDC	+51	+96

All values are Mean ± SD of six individual observations.
Values are expressed as a: CFU/g; b: Units/ml of hemolymph.
All values are Statically significant at P <0.001.
PDC: Percent deviation over Control

The present study was undertaken to ascertain the efficiency of Probiotics on the growth rates, survival and Antioxidant enzymes of the most important cultivable Prawn species *M. rosenbergii*. To date, probiotics can be considered a valid

alternative to the use of Antibiotics in Aquaculture and in particular in prawn culture activity, to prevent high mortality and to improve welfare and promote growth and survival. In the last two decades, many studies were conducted and several authors reported promising results using a single beneficial Bacterial strain on the culture of Finfish species [5]. The present investigation clearly demonstrate that the growth patterns of fresh water Prawn *M. rosenbergii* were shown to increase when fed with *L. rhamosus* and *B. subtilis* supplemented diets, when compared with commercial diet. Probiotics that have been examined for use in Crustacean Aquaculture particularly for prawn culture operation includes Bacteria, Yeast, and Microalgae [28, 38, 14, 44, 46, 47, 50]. Lactic Acid Bacteria, *Bacillus* species were recently employed to improve the aquatic environment in Aquaculture [10, 48]. *Lactobacillus* species have yielded strong Antimicrobial activity against the pathogenic microorganisms [37]. Numerous other Researchers have reported encountering results in the application of probiotics in Aquaculture [31, 45]. The major source of prawn fry is from nature. Coastal tidal waters, estuaries and adjoin areas of Bay of Bengal were the main sources of prawn seed from natural sources. Farmer's believed that seeds collected from nature have much better performance in terms of growth and survivability than the hatchery produced seeds. Therefore, they like to stock seed from reverine source, through its price is much higher than the hatchery seed. Traditionally, farmers prefer to stock wild post larvae rather than hatchery-Produced fry because, until recently, the production of the hatchery post larvae has been limited and farmers consider item to be as lower quality. The survival as wild post larvae is reported to be much higher than that of Hatchery-Produced fly. Most of the farmers directly stock post larvae without rearing in nursery systems. But in recent times, few farmers have started to use hapa i.e., net enclosure or separate small ponds for nursing the post larvae to improve survival rates. Several authors reported that the rate of survival is very poor in Hatchery PL than that of wild PL. In the present investigation, the results obtained, clearly demonstrated that the survivability of Prawns obtained from wild PL's is better that of prawns obtained from Hatchery PL's. This might be explained to their natural origin, greater capability to adapt new environmental condition and to use natural feed property. Besides this, wild PL derived prawns were habituated with adverse environmental condition, so they become stronger that prawns of Hatchery derived PL's. More over the survival rates of prawns and PL's depends on large number of factors, of which water quality parameters are considered to be important and basic factors for the survivability of prawns.

The water quality parameter of all the selected Aquaculture ponds were analysed and presented (Table.2). The water quality parameters includes Water Temperature, Total Phosphorus, Total Nitrogen, Chlorophyll-a content, Ammonia and other shown to have interaction among themselves and its out play will influence the culture operation of prawns. When prawns are culture intensively and fed protein-rich feeds, they can produce high concentrations of ammonia and may be discharged into the surrounding water. If feed is uneaten, then more ammonia is present than it is consumed by prawns. It has been reported that for every kilogram of feed, about 30 grams of ammonia will be excreted by prawns into the pond. Un-ionized ammonia is very toxic to prawn and causes gill damage and reduced growth at low concentrations [2]. In ponds, the equilibrium between NH_3 and NH_4^+ is affected by temperature and pH. At any given pH, more toxic ammonia is present in warmer water than in cooler water; un-ionised ammonia is the toxic from and predominates when pH is high [21, 51]. The uneaten feed materials are the main sources of organic nitrogen, will gradually accumulated in the water following time and not immediately decomposed by microbial decomposition; when accumulated in large amounts, heterotrophic bacteria multiply in the water will also increase about amount for decomposition, thus ammonia- nitrogen content in water. In this present study, ammonia nitrogen content concentrated relatively high in water compared to water quality requirements for freshwater prawn grow-out facilities, but it is not influencing the mortality of prawns, which may directly influence the productivity rates. Phosphorus is a limiting nutrient needed for the growth of all plants-aquatic plants and algae. In the freshwater prawn Aquaculture ponds, phosphorus is found in the form of inorganic and organic phosphates (PO_4).

Table 2: Water quality analysis of Culture Ponds.

Parameter	Wild PL's Culture			Hatchery PL's Culture		
	Control	PB-I (L.R)	PB-II (B.S)	Control	PB-I (L.R)	PB-II (B.S)
Temperature (˚C)	27.2±2.3	26.9±2.1	27.1±1.9	27.3±2.4	27.1±2.2	27.3±2.3
Transparency (cm)	58.8±1.5	56.7±1.6	59.2±1.3	58.4±1.7	59.2±1.6	59.1±1.5
Dissolved Oxygen (DO mg/L)	6.99±1.58	7.78±1.63	8.84±1.58	7.05±1.63	7.82±1.38	8.49±1.34
pH	7.32±0.21	7.42±0.24	7.59±0.25	7.74±0.23	7.48±0.22	7.39±0.24
Conductivity (mg/cm)	0.345±0.078	0.413±0.089	0.494±0.085	0.445±0.445	0.504±0.083	0.515±0.085
Turbidity (NTU)	29±3	28±2	30±3	31±30	30±3	2930±3
Total Alkalinity (mg/L) $CaCo_3$)	33.2±1.6	34.3±1.5	33.7±1.8	34.3±1.7	33.8±1.8	34.1±1.9
Nitrite (NO_2) (mg/L)	0.21±0.03	0.28±0.04	0.32±0.05	0.25±0.04	0.28±0.05	0.31±0.05
Nitrate (NO_3) (mg/L)	3.58±0.24	4.39±0.29	5.04±0.38	3.74±0.35	5.18±0.33	5.79±±0.35
Ammonia (NH_3) (mg/L)	0.534±0.028	0.635±0.032	0.674±0.042	0.658±0.039	0.795±0.042	0.785±0.049
Total Nitrogen (mg/L)	7.494±0.155	6.745±0.154	6.454±0.195	6.959±0.175	7.124±0.185	7.049±0.195
Total Phosphorous (mg/L)	0.715±0.074	0.834±0.079	0.855±0.082	0.819±0.075	0.855±0.079	0.899±0.073
Chlorophyll 'A'	103.18±10.42	115.75±11.34	143.64±13.75	114.15±10.74	135.49±10.45	155.74±10.79
COD Mn (mg/L)	9.75±0.25	10.12±0.34	10.38±0.32	10.04±0.29	11.14±0.32	11.75±0.38

All values are Mean ± SD of six individual observations.

Nitrogen is major factor that affects to growth of algae in water body and it also plays an important role for freshwater prawn growth. Several authors reported that freshwater prawn culture, in intensive model that nutrients needed for the prawn growth in almost whole depending on artificial compound feed feeding, natural environment provides nutrients portions of the very few [32, 15, 20]. Generally the demand of nitrogen in nutrients portions for freshwater culture will be decreased gradually following period culture time. The amount of DO in water is largely dependent upon the water temperature; colder water can carry more dissolved oxygen that warmer water. The pH of a solution is the concentration of hydrogen ions, expressed as negative logarithm; lower pH values indicate increasing acidity, while pH levels higher than 7 indicate increasingly alkaline solution. Chlorophyll-a concentration and DO content are closely related Haiying *et al.* [17] illustrated that chlorophyll-a in summer was positively correlated with Total phosphorous and Dissolved oxygen, whereas it was negative correlation with Total nitrogen and Nitrate nitrogen [17].

Superoxide dismutase (SOD) is one of the main antioxidant defence enzymes generated in response to oxidative stress. Several authors reported the activity of SOD was significantly lowered in WSSV-infected *Fenneropenaeus indicus* [33, 38]. Chang *et al.* [8] observed that the shrimp fed with β-glucan (BG) diets showed significantly higher level of Oxygen concentrations than BG free group as observed in shrimp treated with *C. dactylon* plant extract. Holmblad and Soderhall [18] observed that SOD is related to immunity in crustaceans. The high level of oxygen content in freshwater prawns fed with *B. Subtilis* and *L. rhamnosus* incorporated diets indicates that the probiotics selected in the present investigation are of potential immunostimulant in nature. Hydrogen peroxide is toxic to cells and Catalase is a major primary antioxidant defence component that catalyses the decomposition of H_2O_2 which is produced by the action of Superoxide dismutase to H_2O. The present study revealed that the Catalase assay of hemolymph of freshwater prawns fed with two different probiotics incorporated diets showed increased levels of Catalase when compared to control group of prawns in the two different types culture operations.

The present investigation may be conducted that

A) Prawns selected from wild sources are showing better survival and growth rates compare to Hatchery PL derived prawns.
B) Probiotics, *Bacillus subtilis* and *Lactobacillus rhamnosus* selected in the present investigation were effectively inducing best growth potential and other related parameters, demonstrating the potential nature of growth modulators or immunostimulants.
C) The growth potentials inducing weight gain specific growth rates, Food conversion ratio, Food efficiency ratio and Antioxidant enzyme activity level were significantly increased after fed with probiotic incorporated diets forms a basis to understand the efficiency of selected probiotics in the present investigation.
D) The selected probiotics selected in the present investigation also seems to maintain a congenial water condition, which in turn induces highest and best growth potentials.
E) The scientific community is interesting to find safer antioxidants to be extracted from natural sources to prevent oxidative deterioration of food and to minimise oxidative damage to living cells. In the case of crustaceans a well-defined acquired immunity system is lacking and hence the dietary supplementation of natural oxidants possessing antimicrobial properties may be preferred as effective alternatives to promote growth potentials in Freshwater *M. rosenbergii* culture operation.

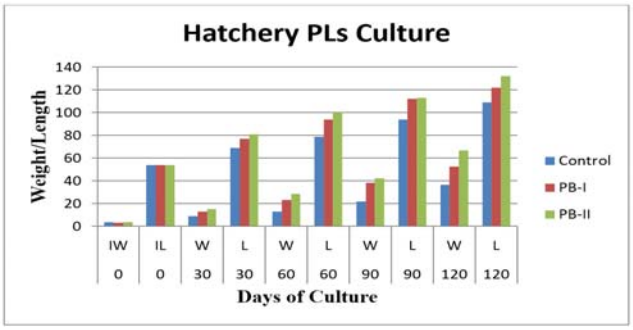

Fig 1: Relationship between Length-Weight and Days of Culture in Hatchery raised Prawns.

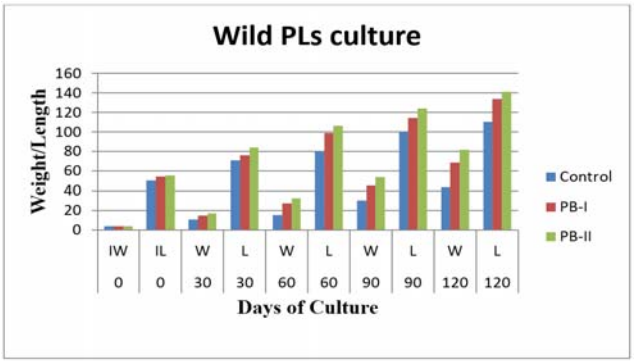

Fig 2: Relationship between Length-Weight and Days of Culture in Prawns collected from Natural environment.

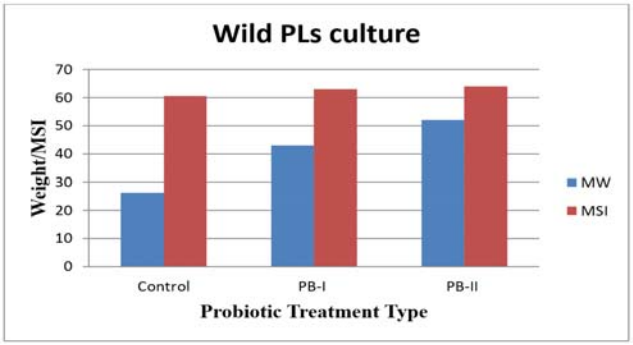

Fig 3: Relationship between Muscle Weight and Muscle Somatic Index in Prawns collected from Natural environment.

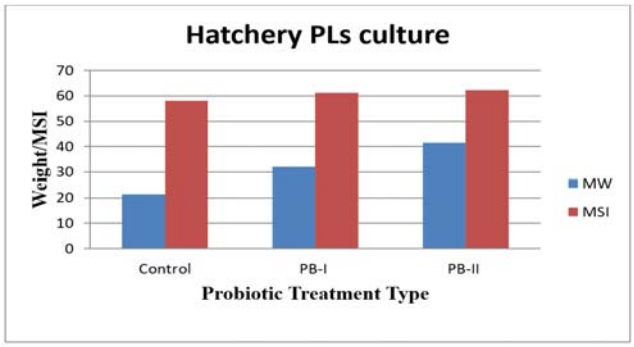

Fig 4: Relationship between Muscle Weight and Muscle Somatic Index in Prawns collected from Hatchery.

4. Acknowledgements
Prof. MSR thank UGC, New Delhi for financial assistance and Dr. V. Sreekanth, Hyderabad for Technical help and Prof. KVS. Sarma for his help in Statistical Analysis.

5. References

1. Aebi H. Catalase. In: Methods in Enzymatic Analysis. Bergmeyar (Ed). Academic Press. New York, 1974, 674-684.
2. Andrew L. Grow out Pond and Water Quality Management. University of Maryland, 2007, 6-17.
3. AOAC. *Official Methods of analysis*. 17th Edn. Association of Official Analytical Chemists, Washington, DC, 2000.
4. APHA. Standard Methods for the Examination of Water and Wastewater, 19th edn. American Public Health Association, New York, 2005.
5. Avella M, Gioacchini G, Decamp O, Makridis P, Bvacciatelli C, Carnevalli O. Application of multi-species of *Bacillus* in sea bream larviculture. Aquaculture 2010; 305:12-19.
6. Bhavani MY, Suneetha CKS, Naidu M, Srinivasulu Reddy. Monitoring the effect of water and feed probiotics on the growth potentials in the freshwater prawn *Macrobrachium rasenbergii***.** Int J Fish Aqua. Sci. 2015; 5:59-70.
7. Bhavani M. Studies on the Determination of Nutritional requirements for the Freshwater prawn *Macrobrachium rosenbergii* (de Man). Ph.D Thesis, S.V. University, Tirupati, 2015.
8. Chang CF, Chen HY, Su MS, Liao IC. Immunomodulation by dietary β-1, 3-glucan in the brooders of the black tiger shrimp *Penaeus monodon*. Fish Shellfish Immunol 2000; 10:505-514.
9. FAO. Yearbook of fishery statistics, Food and Agriculture Organization of the United Nations, Rome, Italy 2005; 96:2.
10. Farzanfar A. The use of Probotics in shrimp Aquaculture. FEMS. Imm. A Med. Microb 2006; 48:149-158.
11. Fuller R. *Probiotics: The scientific basis*. Chapman & Hall, London, UK, 1992.
12. Gatesoupe FJ. The use of probiotics in aquaculture. *Aquaculture* 1999; 180:147-165.
13. Gatesoupe FJ. Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollac, *Pollachius pollachius*. *Aquaculture* 2002; 212:327-360.
14. Gomez-Gill B, Roque A, Turnbull JF. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* 2000; 199:259-270.
15. Gopa M, Mukhopadhyay PK, Chattopadhyay DN. Nutrition and feeding in freshwater prawn *Macrobrachium rosenbergii* farming. Aqua Feeds Formulation & Beyond 2005; 2:17-19.
16. Gullian M, Thomposon F, Rodriguez J. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei***.** *Aquaculture* 2004; 233:1-14.
17. Haiying L, Keming QU, Shaosai MA. Survey of both the variation and the absorption and consumption budget of dissolved oxygen in culture ponds. Marine Fisheries Research. 2005; 26(2):80-84.
18. Holmblad T, Soderhall K. Cell adhesion molecules and antioxidative enzymes in a crustacean: Possible role in immunity. Aquaculture 1999; 172:111-123.
19. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huisint Veld J. Overview of gut flora and probiotics. Internat. J Food Microbiol. 1998; 41:85-101.
20. Jinliang L, Xuefen C, Qiuming L, Chunyu L, Jinling C, Shuye S. Study on nitrogen and phosphorus budgets and production performance in higher place pond of Litopenaeus vannamei. South China Fisheries Science. 2010; 6(5):13-20.
21. John A, Hargreaves, Craig ST. Managing Ammonia in Fish Ponds. SRAC, 2004, 4603.
22. Kakkar P, Das B, Viswanathan PN. A modified Spectrophotometric assay of Superoxide dismutase. Ind. J Biochem. Biophys. 1984; 21:131-132.
23. Karunasagar I, Pai R, Malathi GR, Karunasagar I. Mass mortalities of *Penaeus monodon* larvae due to antibiotic resistant Vibrio varveyi infection. Aquaculture 1994; 128:203-209.
24. Krieg NR, JG Holt. Bergeg's Manual of Systematic Bacteriology. Williams & Wilkins co. Baltimore 1984; 1:161-172.
25. Krishnaprakash R, Saravanan R, Murugesan P, Rajagopal S. Usefulness of Probiotics in the Production of High quality shrimp *Penaeus monodon* seeds in Hatcheries. World Journal of Zoology. 2009; 4(2):144-147.
26. Keysami M, Mohammadpour CR. Saad, Probiotic activity of *Bacillus subtilis* in juvenile fresh water prawn, *Macrobrachium rosenbergii* (de Man) at different methods of administration to the feed, Journal of Aquaculture International. 2012; 20(3):499-511.
27. Kumar R, Mukherjee SC, Prasad KP, Pal AK. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp Labeo rohita (Ham). Aquaculture Research. 2006; 37:1215-1221.
28. Lara-Flores M, Olvera-Novoa MA, Guzman-Mendez BE, Lopez-Madrid W. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia Oreochromis niloticus. Aquaculture 2003; 216:193-201.
29. Lin HZ, Guo Z, Yang Y, Zheng W, Li ZJ. Effect of diatary probiotic on apparent digestibility coefficients of nutrients of white shrimp *Litopenaeus vannamei* Boone. Aquacult. Res 2004; 35:1441-1447.
30. Mcintosh D, Samocha TM, Jones ER, Lawrence AL, Mckee DA, Horowitz S. The effect of a commercial bacteria supplement on the high-density culturing of *Litopenaeus vannamei* with a low protein diet in an outdoor tank system and no water exchange. Aquacult. Engg 2000; 21:215-227.
31. Merrifield DL, Bradley G, Baker RTM, Daveis SJ. Probiotic applications for rainbow trout *Oncorhynchus mykiss*. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria post antibiotic treatment. Aqua. Nut 2010; 16:496-503.
32. Michael BN. The diet of prawns. FAO/UNDP/THA/75/008; 1980; THA: 75:008/80/WP/12.
33. Mohankumar K, Ramasamy P. White Spot Syndrome virus infection decreases the activity of antioxidant enzymes in Fenneropenaeus indicus. Virus. Res 2007; 115:69-75.
34. Naresh SY, Suneetha M, Srinivasulu Reddy. Effect of *Lactobacillus rhamnosus* and *Bacillus subtilis* Supplemented Probiotic diets on the growth patterns and antioxidant enzyme activity in Penaeus monodon and Penaeus indicus. Ame. Int. J Res. For. App. Nat. Sci.

2014; 14:76-80.
35. Requelme C, Araya R, Escribano R. Selective incoporation of bacteria by *Argopecten purpuratus* larvae: implications for the use of probiotics in culturing systems of the Chilean scallop. Aquaculture 2000; 181:25-36.
36. Ronnestad J, Thorsen A, Finn RN. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 1999; 177:201-216.
37. Rossland EGIA, Borge T, Langsrud, T Sorhang. Inhibition of *Bacillus cereus* by Strains of *Lactobacillus* and *Lactococcus* in milk. Int. J Food. Microb. 2003; 89:205-212.
38. Saad AS, Habashy MM, Sharshar KM. Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (de Man), to diets having different levels of Biogen®. World Appl. Sci. J. 2009; 6:550-556.
39. Sarathi M, Ahmed I, Venkatesan C, Balasubramaniyan G, Prabavathy J, Hameed ASS. Comparative study on immune response of *Fenneropenaeus indicus* to *Vibro alginolyticus* and white spot syndrome virus. Aquaculture 2007; 271:8-20.
40. Shariff M, Yusoff FM, Devaraja TN, Srinivasa Rao SP. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp *Penaeus monodon* (Fabricius), ponds. Aquacult. Res 2001; 32:181-187.
41. Skjermo J, Vadstein O. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 1999; 177:333-343.
42. Soundarapandian P, Sankar S. Effect of Probiotics on the survival and Production of Black tiger shrimp Penaeus monodon. Int. J Zool. Res. 2008; 4(1):35-41.
43. Seenivasan Bhavan CPS, Radhakrishnan S, Shanthi R. Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for enhancing the Survival, Growth and Levels of Biochemical Constituients in the Post Larvae of the Freshwater prawn Macrobrachium rosenbergii. Tur. J Fish. Aquatic Sci. 2012; 12:23-31.
44. Suralikar V, Sahu NP. Effect of feeding probiotic *Lactobacillus cremoris* on growth and survival of *Macrobrachium rosenbergii* post larvae. J Appl. Ani. Res. 2001; 20:117-124.
45. Swain SM, Singh C, Arul V. Inhibitory activity of Probiotics *Streptococcus phocae* P180 and *Enterococcus faecium* MC13 against Vibriosis in shrimp Penaeus monodon. World. J Microb. Biot. 2009; 25:697-703.
46. Uma A, Abraham TJ, Jeyaseelan MJP, Sundararaj V. Effect of probiotic feed supplement on performance and disease resistance of Indian white shrimp, Penaeus indicus. J Aquacult. Trop. 1999; 14:159-164.
47. Venkat HK, Sahu NP, Kamal KJ. Effect of feeding *Lactobacillus*- based probiotics on the gut microflora, growth and survival of post larvae of *Macrobrachium rosenbergii* (de Man). Aquacult. Res 2004; 35:501-507.
48. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbial Mol. Biol. Rev 2000; 64:655-671.
49. Wang X, Li H, Zhang X, Li Y, Ji W, Xu H. Microbial flora in the digestive tract of adult penaeid shrimp Penaeus chinensis, J Ocean Univ. Qingdao. 2000; 30:493-498.
50. Wang YB. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 2007; 269:259-264.
51. Yunfa Z, Ming L, Wenzhong D, Jianbo C, Xiaoze G, Yong T. Analysis of Influencing Factors on Water Quality Determination of Total Nitrogen by GB 11894-89. Hubei Agricultural Sciences 2009; 48(5):1232-1234.
52. Ziaei-Nejad S, Rezaei MH, Takami GA, Mirvaghefi GL. Shakouri M. The effect of *Bacillus* spp. Bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp, Fenneropenaeus indicus. Aquacluture 2006; 252:516-524.