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Research Paper

Influence of Probiotics on Water Quality and Fish Yield in Fish Ponds

K. Sunitha[1,*] and P. Padmavathi[1]

[1] Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjunanagar – 522 510, A.P., India

* Corresponding author, e-mail: (ksunitha.chinni@gmail.com)

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Abstract: *Increased production of fish through intense culture practices often leads to not only stress and disease problems but also alteration in water quality. The organic wastes and bad water quality influence the growth of pathogenic microorganisms. Bioremediation using pond probiotics is considered a promising way to circumvent these problems. For this study, three earthen fish ponds stocked with Pangasius sutchi, Catla catla and Labeo rohita were selected and studied for a culture period. Two ponds were treated with probiotics having Nitrosomonas and Nitrobacter species and one pond was used as control. In these ponds, physico-chemical parameters of water, bacterial loads of total heterotrophic bacteria (THB), beneficial bacteria (Nitrosomonas and Nitrobacter species) and pathogenic bacteria (Pseudomonas), zooplankton and fish yields were studied. The results indicate that the concentrations of ammonia, nitrite and orthophosphates were higher in control ponds than in the treated ponds. In treated ponds, zooplankton, THB and beneficial bacterial loads were observed to be increased whereas pathogenic Pseudomonas loads decreased. Fish yields were relatively higher in probiotic treated ponds (37.35 and 37.00 t/ha/year) than in control pond (32.47t/ha/year). The factors responsible for the improved water quality and high fish yields under the influence of probiotics are analyzed and discussed.*

Keywords: Fish, pond probiotics, physico-chemical parameters, bacterial loads, zooplankton.

Introduction:

Aquaculture is one of the fastest growing food sectors of the world. However, poor water quality and disease out breaks are the main constraints to aquaculture production thereby affecting both economic development and socio-economic status of local people in many countries. The recent attempt being made to improve water quality in aquaculture is the application of probiotics and/ or enzymes to the ponds. It involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and to get rid of undesirable waste compounds. In recent years, research on probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture practices (Maeda et al., 1997; Moriarty, 1997 and 1998; Gatesoupe, 1999; Prabhu *et al*., 1999; Gomez- Gil *et al*., 2000; Verschuere *et al*., 2000; Rao, 2001; Shariff *et al*., 2001; Irianto and Austin, 2002; Balcázar, 2003; Ali, 2006; Lakshmanan and Sounderpandian, 2008; Sreedevi and Ramasubramanian, 2010; Dimitroglou *et al*., 2011; Iribarren *et al*., 2012). Of late, in the coastal districts of Andhra Pradesh, India, cat fish such as *Pangasius sutchi* is cultured along with major carps in order to get higher production and more profits. However, fish production in some ponds decreased drastically due to poor water quality and diseases. Freshwater fish in Indian ponds commonly suffer from bacterial diseases such as various kinds of skin ulcerations including the most dreaded Epizootic ulcerative syndrome (EUS), albinoderma, erythroderma, tail and fin rot and hemorrhagic septicemia, primarily caused by *Aeromonas* and *Pseudomonas* species (Das, 2004). Keeping in view of the beneficial effects of probiotics as bioremediation and biocontrol agents, some enthusiastic farmers are recently using commercially available probiotics in their fish ponds. However, no attempt has been made so far to study the efficacy of probiotics in improving water quality and fish production of carp-catfish culture ponds of this region. Hence, the present study was aimed at investigating the influence of probiotics on water quality and fish yield in ponds treated with probiotics and compare the results with those of the untreated pond in a fish farm in Krishna district, Andhra Pradesh, India.

2. Material and Methods:

2.1. Fish Ponds

Three fish ponds located in a farm at Balliparru near Machilipatnam (latitude 16º 09' N, longitude 81° 12' E) in Krishna district, Andhra Pradesh, India were chosen for the present study. The earthen ponds are designated as Pond A, B and C and were stocked with catfish (*Pangasius sutchi)* and Indian major carps, catla (*Catla catla)* and rohu (*Labeo rohita*) (Table 4). The fish were fed with supplementary feed mostly with deoiled rice bran, groundnut oil cake and slaughter waste at the rate of 2% body weight of fish per day. Prior to and after the release of fish, all the ponds were treated with organic manures such as poultry manure, compost and cattle dung, and inorganic fertilizers such as superphosphate. The particulars of fertilizers and lime used in different months in three ponds are given in Table1. Pond A and B were treated with probiotics whereas Pond C was kept as control pond i.e. without probiotics treatment.

The study was carried out for a culture period of 332 days from August 25, 2009 to July 23, 2010. The extent (water spread area) of Pond A, B and C is 3.22 ha, 3.22 ha and 3 ha respectively. The depth of the ponds ranged between 1.2 and 1.5 m. In the present study, physico-chemical parameters of water, bacterial loads, qualitative and quantitative analysis of zooplankton, and fish growth and yield were studied.

2.2. Pond Probiotics

In this study, commercially available probiotics with *Nitrosomonas* species and *Nitrobacter* species manufactured by KCP Sugar and Industries Corporation Limited, Vuyyuru, Krishna district, Andhra Pradesh, India were used. Probiotics were used in Pond A and B at monthly intervals with *Nitrosomonas* probiotics @ 1.62 kg/ha and *Nitrobacter* probiotics @ 0.82 kg/ha.

Table 1: Particulars of inputs used in Pond A, B and C

Month & Year	Organic Fertilizers				Inorganic Fertilizers				Lime		
	Quality	Quantity Kg/ha			Quality	Quantity Kg/ha			Quantity Kg/ha		
		Pond				Pond			Pond		
		A	B	C		A	B	C	A	B	C
Aug 2009	Poultry manure	1750	2000	2000	Super phosphate	4.5	4.5	2.5	--	--	75
Sep 2009	Compost	1675	1800	1800	Super phosphate	6.5	6.5	3.5	--	--	--
Oct 2009	Poultry manure	200	200	745	--	--	--	--	--	--	--
Nov 2009	--	--	--	--	--	--	--	--	--	--	75
Dec 2009	--	--	--	--	--	--	--	--	--	--	--
Jan 2010	Poultry manure	975	975	820	--	--	--	--	--	--	75
Feb 2010	Cattle dung	1175	1175	1175	--	--	--	--	--	--	25
Mar 2010	--	--	--	--	--	--	--	--	--	--	--
Apr 2010	Poultry manure	--	--	250	--	--	--	--	--	--	--
May 2010	Poultry manure	--	1975	1825	--	--	--	--	--	--	--
Jun 2010	Poultry manure	1975	--	--	--	--	--	--	--	--	--

2.3. Water Samples

In the present study, physico-chemical parameters of water and bacterial loads were studied at fortnight intervals by collecting water samples in between 8 and 10 A.M. The physico-chemical parameters such as temperature, transparency, dissolved oxygen, pH, total alkalinity, total hardness, total dissolved solids, nitrite, nitrate, ammonia, phosphorus and iron of water were estimated by following the methods suggested in Golterman and Clymo (1969), Wetzel and Likens (1979) and APHA (1999). Primary productivity was determined using the light and dark bottle method described by Vollenweider (1969). To study the correlations among physico-chemical parameters of water studied, simple correlation coefficients were calculated and correlation matrix has been developed using SPSS 17.0 software.

2.4. Bacteriological Analysis

Bacteriological analysis was carried out for the isolation and enumeration of Total heterotrophic bacteria (THB), *Nitrosomonas* species, *Nitrobacter* species and *Pseudomonas* species in three ponds. Samples for bacterial analysis were collected in well cleaned, dried and sterile bottles which were sterilized at 121^0 C under pressure of 15 lbs for 15 minutes. Column water samples were collected for the analysis of THB and *Pseudomonas* whereas bottom water samples for *Nitrosomonas* and *Nitrobacter*. After collection, 1ml of the sample was transferred to sterile conical flask (150 ml) containing 99ml of sterile distilled water and serial dilution was performed to get 10^{-1},10^{-2},10^{-3}, 10^{-4} and 10^{-5} samples. THB was enumerated by adopting the spread plate method (Chen and Kueh, 1976; Cappuccino and Sherman, 1992). From the diluents, 0.1 ml was spread in the plates and incubated in an inverted position at 37^0 C for 20-24 h. *Nitrosomonas* species was cultured by using Winogradsky phase-I medium whereas *Nitrobacter* species by Winogradsky phase-II medium. From the diluents, 0.1 ml was inoculated into the medium and plates were incubated at 28 ± 2^0 C for 48 h. *Pseudomonas* species was cultured by using *Pseudomonas* base medium (Hi-medium Mumbai). From the diluents, 0.1 ml of the sample was inoculated into the medium and incubated at 37^0 C for 24-48 h. All the determinations were carried out in triplicates. Following incubation, plates containing viable colonies were used to calculate bacterial population results. The colonies were counted and expressed as cfu/ml.

2.5. Zooplankton Analysis

Qualitative samples of plankton were collected by towing the plankton net made of silk bolting cloth No.25 (mesh size 50µ) in the surface and subsurface layers of pond water for about 15 min. Quantitative samples were collected by filtering 100 L of pond water through the plankton net. The plankton obtained was fixed in 5% formalin. Sample volumes were adjusted to 10 ml and two sub-samples of 1.0 ml capacity were removed and counted in a Sedgwick-rafter cell (Edmondson, 1959). The average of three counts was converted to number of individuals per liter (ind. /l) of pond water sampled.

2.6. Fish Yield

Growth studies of fish were made from the sample netted from the pond at regular monthly intervals. The mean weight with the standard deviation for the number of fish sampled for each species was taken. The growth increments in every month were also calculated. The details of the harvesting data such as the number of fish harvested, the mean weight and the total biomass obtained for each species with gross and net yields are given in Table 4.

3. Results:

3.1. Physico-Chemical Parameters of Water

The parameters of water studied and their values of Mean ± S.D (n=20) and ranges during the culture period in three ponds are shown in Table 2.

Table 2: Physico-chemical parameters of water in Pond-A, B and C

Physico-chemical parameters	Pond-A		Pond-B		Pond-C	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Air temperature(ºC)	27.02±3.09	20.50-30.20	26.96 ±3.03	20.90- 31.10	29.06±1.99	25.50-33.00
Water temperature(ºC)	29.17±2.32	25.30-31.40	29.66±2.11	25.20-32.70	29.93±1.35	27.60-32.50
Secchi disc transparency(cm)	29.19±1.48	26.10-32.20	28.36±2.15	24.50-31.20	29.37±1.97	26.50-34.20
Dissolved oxygen(mg/l)	4.80±0.81	3.20-6.20	4.09±1.30	2.40-6.40	3.50±0.88	2.00-5.20
pH	7.87±0.43	7.29-8.88	7.92± 0.35	7.50-8.56	7.92±0.40	7.44-8.65
Total alkalinity(mg/l as $CaCo_3$)	148.70±24.68	110-192	150.7±18.13	120-180	134.50±24.50	100-184
Total hardness(mg/l as $CaCo_3$)	153.05±30.79	100-220	164.2±18.51	130-195	147.75±21.97	105-190
Conductivity(mS)	14.55± 6.45	6.72-26.10	13.76±5.78	7.06-26.60	8.53±3.58	3.51-15.80
Total dissolved solids (ppt)	12.48±6.47	4.28-23.60	12.05±6.39	5.24-24.90	7.64±4.37	2.41-16.70
Ammonia-N(mg/l)	0.41±0.07	0.28-0.53	0.38±0.06	0.27-0.50	0.51±0.08	0.38-0.73
Nitrite-N(mg/l)	0.04±0.05	0.08-0.25	0.03±0.01	0.09-0.07	0.07±0.02	0.02-0.11
Nitrate-N(mg/l)	0.26±0.09	0.10-0.45	0.26±0.09	0.12-0.46	0.21±0.07	0.09-0.41
Orthophosphates(mg/l)	0.41±0.09	0.25-0.59	0.45±0.06	0.32-0.58	0.56±0.09	0.42-0.75
Iron(mg/l)	0.28±0.06	0.18-0.41	0.27±0.05	0.19-0.36	0.39±0.11	0.10-0.59
Gross primary productivity ($gc/m^3/hr$)	0.19±0.19	0.07-0.87	0.14±0.11	0.05-0.40	0.09±0.15	0.02-0.75
Net primary productivity ($gc/m^3/hr$)	0.09±0.05	0.02-0.24	0.06±0.10	0.02-0.37	0.09±0.07	0.02-0.26
Community respiration ($gc/m^3/hr$)	0.08±0.13	0.02-0.62	0.07±0.08	0.02-0.38	0.05±0.03	0.02-0.16

3.2. Bacterial Analysis

The relative bacterial loads of Total Heterotrophic Bacteria (THB), *Nitrosomonas*, *Nitrobacter* and *Pseudomonas* in three ponds are shown in Figure 1(a-d). THB were observed to be high in Pond A and B and low in Pond C. The loads recorded were $5.25x10^5$ to $7.80x10^5$ cfu/ml in Pond A, $5.42x10^5$ to $7.90x10^5$ cfu/ml in Pond B and $2.05x10^5$ to $4.72x10^5$ cfu/ml in Pond C. *Nitrosomonas* and *Nitrobacter* loads are relatively higher in Pond A and B than in control pond whereas *Pseudomonas* were observed to be higher in control pond than in treated ponds A and B. *Nitrosomonas* loads ranged from 2.01×10^3 to 4.80×10^3 cfu/ml in Pond A, 2.25×10^3 to 4.90×10^3 cfu/ml in Pond B and 1.24×10^3 to 2.00×10^3 cfu/ml in control pond and *Nitrobacter* loads in Pond A, B and C ranged from 2.20×10^3 to 4.95×10^3, 2.10×10^3 to 4.15×10^3 and 1.10×10^3 to 2.15×10^3 respectively. *Pseudomonas* loads in Pond A and B ranged from 1.00×10^5 to 2.96×10^5 cfu/ml and 1.02×10^5 to 2.9×10^5 cfu/ml and in control pond from 2.36×10^5 to 7.25×10^5 cfu/ml.

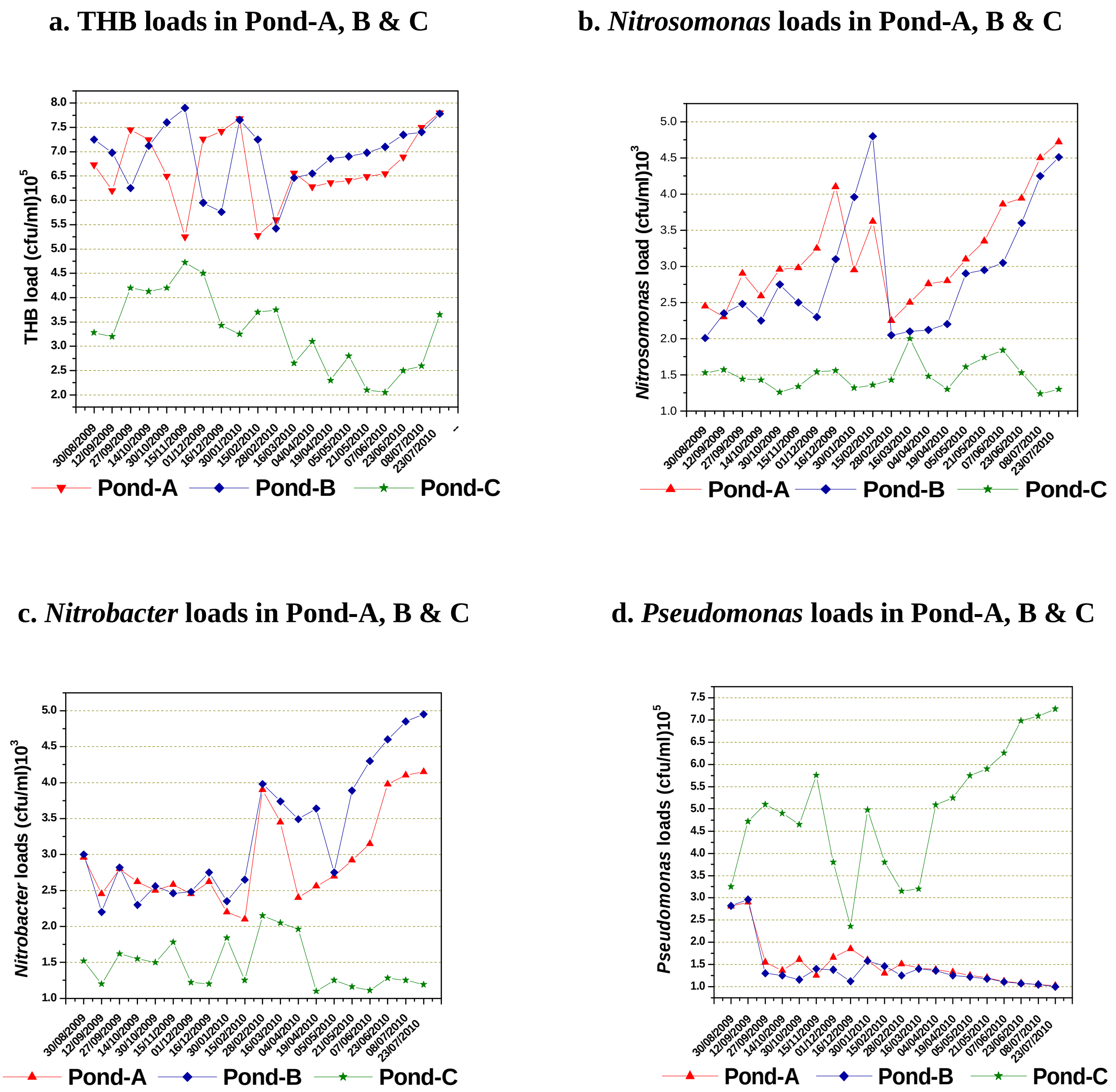

Figure 1: Relative abundance of bacterial loads in the three ponds

3.3. Zooplankton

Zooplankton was mainly represented by Rotifera, Copepoda and Cladocera in the order of dominance whereas the other zooplanktonic forms were almost negligible. Zooplankton was identified up to species level. The total number of species recorded in pond A, B and C were 17 (10, 3 and 4), 16 (9, 3 and 4) and 16 (9, 3 and 4) respectively. The numbers in parentheses represent the number of species of rotifers, cladocerans and copepods respectively. The levels of zooplankton with Mean ± S.D values (n=20), their ranges and the dominant species during the culture period are shown in Table 3.

Table 3: Zooplankton abundance (Mean± SD) in Pond A, B and C

POND	Rotifers (ind./l)	Cladocerans (ind./l)	Copepods (ind./l)	Total Zooplankton (ind./l)
Pond-A	185.45±64.82 (112-349) ab	29.526±14.80 (4-52) eg	108.2±70.81 (18-260) hj	338.65±105.69 (180-586)
Pond-B	161.15±46.10 (109-252) bc	49.625±24.79 (16-95) fg	142.05±49.60 (101-253) ij	347.95±70.91 (252-482)
Pond-C	111.0±33.37 (59-177) ad	25.157±17.93 (8-91) Gf	57.0±32.22 (11-99) hk	192.3±58.48 (114-325)

*The values in parantheses are the ranges "a to k" indicates the dominant species and two letters indicates the order of dominance

a= *Brachionus calyciflorus* b=*Brachionus caudatus*
c= *Keratella tropica* d= *Polyarthra remata*
e= *Diaphanosoma excisum* f= *Moina micrura*
g= *Ceriodaphnia cornuta* h= *Mesocyclops thermocyclopoides*
i= *Microcyclops varicans* j= *Heliodiaptomus cinctus*
k=*Rhinediaptomus indicus*

3.4. Fish

The number of fish stocked and harvested with their mean weights and gross and net yields are shown in Table 4. The gross yields obtained in Pond A,B and C are 33.97, 33.66 and 29.53 t/ha/332 days. The corresponding net yields are 32.29, 32.04 and 28.09 t/ha/332 days.

Table 4: Fish yield data in three ponds

Data	Pond - A		Pond - B		Pond – C	
A. Fish stocked (no./ha)	20,785		21,049		22,016	
		Mean wt.(g)		Mean wt.(g)		Mean wt.(g)
Catfish	12,880	(75.3)	13,354	(74.3)	15,183	(64.0)
Catla	3,220	(124.4)	3,059	(77.7)	3,333	(72.8)
Rohu	4,685	(65.4)	4,636	(84.1)	3,500	(63.5)
B. Fish harvested (no./ha)	20,106		20,478		20,990	
		Mean wt.(kg)		Mean wt.(kg)		Mean wt.(kg)
Catfish	12,556	(1.542)	13,144	(1.574)	14,841	(1.345)
Catla	3,026	(1.909)	2,894	(2.049)	3,066	(1.658)

Rohu	4,524 (1.953)	4,440 (1.586)	3,083 (1.456)
C. Total Biomass of fish Harvested (kg)	33,973	33,661	29,533
Catfish	19,361	20,689	19,961
Catla	5,777	5,930	5,083
Rohu	8,835	7,042	4,489
D. Gross Yield (t/ha/332 d)	33.97	33.66	29.53
(t/ha/year)	37.35	37.00	32.47
E. Net Yield (t/ha/332 d)	32.29	32.04	28.09
(t/ha/year)	35.50	35.22	30.88

4. Discussion:

In the present study, water quality of the ponds treated with probiotics was observed to be suitable for survival and growth of cultured fish. During the period of observation, the amplitude of variation in water temperature was very narrow (Table 2) and is considered as a characteristic of tropical waters (Dewan *et al.*, 1991; Ahmed *et al*., 2000). The water temperature in general follows with the pattern of fluctuation of air temperature and showed a strong positive correlation in Pond A (r = 0.616; P < 0.01), Pond B (r = 0.857; P<0.01) and Pond C (r = 0.639; P<0.01).

Transparency of water showed an inverse correlation with temperature in Pond A (r = -0.090; P>0.05); Pond B (r = -0.509; P<0.05) and Pond C (r = -0.443; P>0.05). Transparency of water also correlated negatively with dissolved oxygen (r = -0.457; P<0.05) in Pond A and C (r = -0.326; P>0.05). These relations are interdependent and could be explained by the fact that the high temperatures are conducive to the development of phytoplankton. With the increase in the densities of phytoplankters, a corresponding decrease in the secchi disc transparency and increase in dissolved oxygen content was observed. Hence, a negative correlation between dissolved oxygen and transparency of water was discernible. Optimum levels of dissolved oxygen maintained in Pond A and B might be due to the beneficial effect of probiotics which favored mineralization of organic matter.

pH of water has been found to be positively correlated with dissolved oxygen in Pond A (r = 0.591; P<0.05), Pond B (r = 0.485 P<0.05) and Pond C (r = 0.466 P<0.05). Due to intense photosynthetic activity, the free carbon dioxide (if present) or the carbon dioxide of bicarbonate is drawn out by the algae; this results in the decrease of free CO_2 and bicarbonate and increase of carbonate. It is well established that the carbonates increase the pH of water on hydrolysis. Simultaneously, the photosynthetic release of oxygen increase the dissolved oxygen content in the water. The relationship of pH, free carbon dioxide, carbonates and bicarbonates has been discussed by many workers (Seenayya, 1971 and Rao, 1972). It was generally noticed that the higher pH values coincided with the period of greater photosynthetic activity of abundant phytoplankton which received support from the relation between primary productivity and pH (r = 0.600 P<0.01). Total hardness showed a positive correlation with total alkalinity in Pond A (r = 0.853; P<0.01) and Pond B (r = 0.644; P<0.01). According to Boyd (1982), the total hardness is usually related to total alkalinity as the cations of hardness and anions of alkalinity are normally derived from the solution of carbonate minerals. Arce and Boyd (1980) also observed a high positive correlation between total alkalinity and total hardness in pond waters.

Conductivity and Total dissolved solids (TDS) represent mineral content of the water, they exhibit significant positive relationship in Pond A (r=0.933; P<0.01), Pond B (r = 0.958; P<0.01) and Pond C (r = 0.900; P<0.01). Higher values of conductivity were noticed in the month corresponding to the application of organic manure in the ponds. This could be explained by the fact that poultry manure has high soluble inorganic salts and is responsible for the increase of conductivity in the water (Ray and David, 1969).

The nutrients, nitrate-N, nitrite-N, and ammonia-N in the pond water showed varied distribution which might be due to biological or chemical reactions or combination of these two. In fish ponds, mineralization of fertilizers, feed wastes and excreta often increases the ammonia concentration, which is harmful to fish above 0.1 mg/l. Hence it is a critical water quality parameter to be maintained at optimal level in fish ponds. The nitrogen cycle involves the oxidation of ammonia to nitrite by bacteria of the genus *Nitrosomonas* and the subsequent oxidation of the nitrite to nitrate by *Nitrobacter*. Inputs of ammonia cannot be eliminated from the water body. However, it can be converted to non-toxic nitrate by nitrifying bacteria which can be accomplished by means of probiotic treatment. The levels of ammonia and nitrites were relatively low in Pond A and B than in Pond C. This might be because of the use of nitrifying bacteria in the form of probiotics. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite observed in Pond A and B compared to Pond C can be supported. A significant positive correlation could be discernible between nitrate-N and dissolved oxygen in Pond A (r=0.0.462; P<0.05) and Pond B (r = -0.633 P <0.05). The oxidation of various forms of inorganic nitrogen in the well oxygenated surface water might have resulted in the increased concentration of nitrates. A negative correlation has been observed between ammonia and temperature in Pond A (r = -0.448; P<0.01), Pond B (r = -0.373; P>0.05) and Pond C (r = -0.102; P>0.05). This indirect correlation may be explained by the fact that during high temperatures, the intense photosynthetic activity of phytoplankters release oxygen into the pond waters and as such a considerable amount of ammonia might have been converted to oxidized forms in addition to the activity of nitrifying bacteria.

Though phosphorus is considered as the most important critical factor in the maintenance of pond fertility (Boyd, 1982), high levels lead to eutrophication and water deterioration. It was observed that orthophosphate concentrations were maintained at relatively low levels in probiotic treated ponds than in control ponds. Rao (2001) reported that the probiotic bacteria utilize phosphate for their body metabolic activities and thus diminish this nutrient in pond waters. Orthophosphates showed a significant positive relationship with dissolved iron in Pond A (r = 0.452; P<0.05), Pond B (r = 0.482; P<0.05) and Pond C (r = 0.485; P<0.05). Seenayya (1971) also observed that phosphate and iron were in some way related to each other. Primary production or the organic carbon fixed through photosynthetic activity by phytoplankton helps in understanding the productive function of aquatic system (Odum, 1971). The well maintained nutrient levels in probiotic treated ponds A and B support the abundant primary producers and then the next trophic levels like zooplankton and fish. Primary productivity showed positive relationship with dissolved oxygen and pH in the ponds studied.

Probiotic bacteria are known to improve water quality in many ways. Heterotrophic bacteria necessitating some organic sources of carbon in addition to inorganic forms for growth have a significant role in the decomposition of organic matter and production of particulate food materials from dissolved organics (Jana and De, 1990). There are many studies on the relationship between heterotrophic bacteria and water quality (Guo *et al*., 1988; Fang *et al*., 1989; Liu *et al*., 1992). Total heterotrophic bacteria (THB) were observed to be high in Pond A and B and low in Pond C. The loads recorded were 5.25×10^5 to 7.80×10^5 cfu/ml in Pond A, 5.42×10^5 to 7.9×10^5 cfu/ml in Pond B and 2.05×10^5 to 4.72×10^5 cfu/ml in Pond C. The high levels of heterotrophic bacteria in Pond A and B might be due to the consumption of more organic matter as their sources of carbon and improve water quality. Heterotrophic bacteria are known to utilize nitrogen rich substances and release ammonia or ammonium salts (Jana and Barat, 1983). However, as Pond A and B are treated with nitrifying bacterial probiotics, which convert ammonia to nitrates, relatively low levels of ammonia compared to control pond were observed.

Nitrosomonas loads in Pond A, B and control ponds ranged from 2.01×10^3 to 4.80×10^3 cfu/ml, 2.25×10^3 to 4.9×10^3 cfu/ml and 1.24×10^3 to 2.00×10^3 cfu/ml, and *Nitrobacter* loads from 2.20×10^3 to 4.95×10^3, 2.10×10^3 to 4.15×10^3 and 1.10×10^3 to 2.15×10^3 respectively. As the Ponds A and B are treated with probiotics having *Nitrosomonas* and *Nitrobacter* species, their abundance in these ponds can be explained. These bacterial loads were also observed to be gradually increasing by the end of the culture period (Figure 1b and 1c). As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite observed (Table 2) in Pond A and Pond B compared to Pond C can be supported.

Several species of *Pseudomonas* and *Aeromonas* are pathogenic and reported to cause various kinds of skin ulcerations including the most dreaded Epizootic ulcerative syndrome (EUS), albinoderma, erythroderma, tail and fin rot and hemorrhagic septicemia (Das, 2004). In the present study, *Pseudomonas* loads in Pond A and B ranged from 1.00×10^5 to 2.96×10^5 cfu/ml and 1.02×10^5 to 2.90×10^5 cfu/ml and in control Pond from 2.36×10^5 to 7.25×10^5 cfu/ml (Figure 1d). It was observed that *Pseudomonas* loads showed changing patterns from sampling to sampling with decreasing trend to the end of the culture period. Customarily, the indigenous bad bacteria can cause diseases but when the probiotics are introduced into the ponds, the new comers eliminate the pre-existing bacteria out of the nutrients queue. Thus, the old/bad bacteria, never having had to compete for food, cannot keep pace with the aggressive probiotics. Further, probiotic bacterial excretions make the pond medium less inhabitable for bad bacteria. Not only do probiotic bacteria have terrific appetites; they excrete exo-enzymes as a natural byproduct of their metabolic activity. These enzyme excretions infuse and spread throughout the pond medium, changing its chemistry and destroying bacteria. Thus the low levels of *Pseudomonas* in probiotic treated Pond A and B can be explained. The general conclusion obtained from the present study is that the probiotics played a major role in maintaining optimum water quality parameters especially dissolved oxygen, ammonia, nitrite, nitrate and phosphates throughout the culture period. It is clear from the bacterial load data that the *Nitrosomonas* and *Nitrobacter* species were dominated and suppressed the *Pseudomonas* species in the probiotic treated ponds when compared to the control pond. Hence by using probiotics, it can be possible to improve water quality and prevent the occurrence of bacterial diseases in fish ponds.

Zooplankton forms the natural source of food for carps (Khan and Siddique, 1973). Zooplankton was abundant in Pond A and B compared to control pond. This might be because of the fact that probiotics favor the growth and development of zooplankton. Ludwig (1999) also stated that probiotics maximize zooplankton, as they form another nutrient for existing zooplankton in the pond medium and thus strengthen up the food supply to culture organisms. However, the relative abundance of different groups of zooplankton in aquatic ecosystems varies not only with the variations in environmental conditions but also on predatory pressure. In fish ponds, predation exerts perhaps the major influence on zooplankton abundance. In the present study, among zooplankters, rotifers were dominant followed by copepods and cladocerens throughout the culture period in three ponds (Table 3). The low number of cladocerans and copepods might be due to the preferential predation by carp. In natural ponds, where the intensity of predation is less, the zooplankton was dominated by copepods followed by cladocerans and rotifers (Durga Prasad, 1981). Geiger (1983) states that the predation exerts perhaps the largest single influence on pond zooplankton communities. When fish predation is low, the number of smaller sized plankton is greatly reduced and the large cladocerans and copepods prevail. However, when the fish predation is high, the large cladocerans and copepods are greatly reduced in numbers and the small rotifers, small cladocerans and copepod nauplii become prevalent. Though this trend is common in three ponds, total number of the zooplankton was found to be abundant in Pond A and B than in Pond C.

The weight attained by the fish at the time of harvesting in the three ponds indicates that the fish grow well in probiotic treated Ponds A and B than in Control pond (Table 4). The gross and net yields obtained respectively were 33.97 and 32.29 t/ha/332days in Pond A; 33.66 and 32.04 t/ha/332d in Pond B; 29.53 and 28.09 t/ha/332d in Pond C. The high yields obtained in these ponds might be due to high stocking densities of catfish and carps and maintaining good water quality by the use of probiotics.

5. Conclusion

Hence, from the present study, it can be concluded that probiotics played a major role in maintaining optimum water quality parameters (especially dissolved oxygen, ammonia, nitrite, nitrate and phosphates, bacterial loads and zooplankton) throughout the culture period which resulted in better growth, survival and disease resistance in the culture fish. Hence higher yields with better growth and survival can be achieved by using probiotics in aquaculture ponds.

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