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Fish production, water quality and bacteriological parameters: A study of saran district

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Abstract
In this paper, the effectiveness of introducing live zooplankton against direct manuring in ornamental fish ponds upon their survival and production, larvae of koi carp, *Cyprinus carpio* L., were cultured for 17 weeks in earthen ponds maintained according to three management regimes: (1) live zooplankton fed to carp larvae (LF); (2) direct fertilization with poultry manure (PM); (3) direct fertilization with cow dung (CD); and (4) a control treatment (C). There were three replicates for each treatment. The growth of heterotrophic bacteria and pathogenic microorganisms like Aeromonas sp. and Pseudomonas sp. were also examined in response to pond management.

Keywords: Koi carp ponds, *Cyprinus carpio* L. management, growth, water quality, bacteriology

Introduction
The purpose of pond fertilization is to augment fish production through autotrophic and heterotrophic pathways. Organic manures, being less expensive compared to chemical fertilizers, contain almost all the essential nutrient elements (Jana *et al*, 2001), and are traditionally applied to fish ponds to release inorganic nutrients which stimulate the growth of plankton (Wurts, 2000; Ansa & Jiya, 2002; Kadri & Emmanuel, 2003) [2]. The available organic pooi in manured ponds is usually duplicated everyday via bacterial production (Schroeder, 1987). Heterotrophic microorganisms, 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 & De, 1990) [3]. However, the role of heterotrophic bacteria in the food web and its effect on fish yield are poorly documented (Moriarty, 1987). According to our knowledge, there have been no studies on the abundance of heterotrophic bacteria in ornamental fish ponds in India.
Another aspect of pond management that has increasingly gained importance in the past decade is water quality problems in ornamental fish ponds. Pond fertilization using high amounts of animal wastes are known to have caused noticeable harm to the environment (Quines, 1988), by proliferating the growth of pathogenic bacteria like Aeromonas sp. and Pseudomonas sp. in the water-body (Hojovec, 1977; Sugita *et al*, 1985a: Jinyi *et al*, 1987). Freshwater fish in Indian ponds commonly suffer from bacterial diseases such as various kinds of skin ulcerations, albinoderma, erythroderma, furunculosis, and Verticle-scale disease, primarily caused by Aeromonas sp. and Pseudomonas sp. (Das, 2004).
Organic manuring also leads to severe depletion of dissolved oxygen, high biological and chemical oxygen demand, and high ammonia levels (Boyd, 1982), leading to stress in cultured fish (Parker, 1986). Since ornamental fish ponds in India are much smaller compared to other aquaculture ponds (measuring about 7 m×20m, with an average depth of 0.6-1.0m), there are more opportunities to control environmental conditions in ornamental fish ponds by employing proper management techniques. Introduction of live zooplankton has been investigated as a practicable alternate to pond fertilization for increasing ornamental fish yields while avoiding water quality deterioration (Jha & Barat, 2005a; Jha *et al*, 2006; Jha *et al*, 2007) [7]. However, there is a paucity of documentation pertaining to bacteriological parameters of ornamental fish ponds, particularly those under live-food and manure based management regimes.
The objective of the present study was to investigate the growth responses of heterotrophic bacteria, along with the development of Aeromonas sp. and Pseudomonas sp. in ornamental fish ponds maintained under live-food and manure based management regimes, for the culture of koi carp, *Cyprinus carpio* L.

Materials and Methods

In this study the offspring of 40 pairs of Asagi, Bekko, Kohaku and Showa koi types were obtained from a local fish farm (Saran Division, Bihar). The fish were acclimatized for seven days before the experiment. The study was conducted in twelve earthen ponds (capacity: 59600 L each) in Saran, Bihar (Saran, Gopalganj, Siwan Distt.). About two week old koi carp larvae (0.12±0.007g) were stocked in the experimental ponds and maintained at a density of 0.3 fish/L, as optimized in an earlier experiment (Jha & Barat, 2005b) [8]. Fish were cultured for 11 weeks (02 June to 18 August, 2004) according to one of the four management regimes: (1) live zooplankton was introduced into the ponds by transferring about 1000 L of plankton water everyday form a series of ponds maintained separately for culturing live plankton (LF); (2) direct fertilization with poultry manure at 0.26 kg/m^3, every 10 days (PM); (3) direct fertilization with cow dung at $0.26 kg/m^3$, every 10 days (CD); and (4) a control treatment (C), where a commercial pellet diet was used as feed.

Water samples were collected weekly at a fixed hour of the day (9.00 hour) as described earlier (Jha *et al*, 2004) and routine water quality parameters (dissolved oxygen or DO, biological oxygen demand or BOD, free CO_2, alkalinity, PO_4-P, NH_4-N, NO_2-N, NO_3-N, and specific conductivity) were estimated according to methods as described by APHA (1998). Temperature was recorded using a mercury thermometer. The pH was measured in situ using a portable pH meter (Hanna Instruments). Sediment samples were collected weekly and the amounts of total nitrogen and organic carbon in the sediment and the manures used in the experiment were estimated according to Micro-Kjeldahl's method (Anderson & Ingram, 1993) [1] and Wet Oxidation method (Walkley & Black, 1934), respectively. Samples of plankton were collected with a plankton net made of standard bolting silk cloth (No. 21 with 77 $mesh/cm^2$), twice a week. Collected plankton samples were concentrated to 20 mL and preserved in 4% formalin. Enumeration of 1mL of concentrated plankton was performed under a stereoscopic microscope using Sedgwick Rafter Counting Cell.

For bacteriological analysis, water samples were collected weekly in pre-sterilized glass bottles (125mL), and processed within 6hrs of collection. Weekly sediment samples were collected by hand and stored in pre-sterilized plastic containers. The suspension of sediment was prepared by mixing of wet sediment in 99mL of sterile distilled water. The aerobic heterotrophic bacteria were enumerated in nutrient agar by serial dilution of the sample, followed by the conventional spread plate method (Chen & Kueh, 1976; Cappuccino & Sherman, 1992). Aeromonas sp. and Pseudomonas sp. were similarly enumerated on Aeromonas Isolation Medium Base and Pseudomonas Isolation Agar, respectively. All the bacteriological media were obtained from Himedia Laboratories Ltd., Mumbai, India. After inoculation, the Petri dishes containing the culture media were incubated at 37,0 for 48 hrs. The populations of bacteria were expressed in terms of cfus./mL (colony forming units) in water, and cfus./g for the sediments. Arithmetical means from three Petri dishes for each dilution were used in the study.

Results

The amount of total nitrogen in cow and poultry manures was 2.12% and 2.59%, respectively, and the amount of organic carbon was 22.06% and 28.52%, respectively. Water temperature was between 22 ^{0}C and 38 ^{0}C during the 11 weeks. However, there was no difference in water temperature from one management regime to another on any particular sampling date. The water pH in all the treatments was neutral to acidic (Tab.-1). The values of free CO_2 and total alkalinity were significantly higher in PM ($P< 0.05$), compared to the other treatments. Total alkalinity here refers to bicarbonate alkalinity, as carbonate was not present in the water of any management regime during the entire study period. Average PO_4-P, NO_3-N, NO_2-N, NH_4-N, specific conductivity, and BOD were significantly higher ($P< 0.05$) in PM and CD, compared to the LF and control treatments (Tab.-1). However, the values of dissolved oxygen were significantly higher ($P< 0.05$) in the LF treatment, than other treatments (Tab.-1). The range of recorded pH values was highest in the LF treatment (Tab.-1). Like water, the sediment pH was also highest ($P< 0.05$) in the LF treatment (Tab.-1). The percentage of organic carbon and total nitrogen in the pond sediments were highest in the PM treatment $P< 0.05$), followed by the CD, C, and LF treatments, although the values in the latter two treatments were not significantly different ($P>0.05$) from one another (Tab.-1).

Examination of plankton showed considerable differences in species diversity and abundance between different treatments. The cladocerans formed the most abundant group in LF, whereas copepods were more dominant in all the other treatments (Tab.-2). On average, total plankton volume (no./L) was highest in LF, followed by PM, CD, and C treatments in decreasing order $P< 0.05$). Plankton population in all the treatments was dominated by zooplankton. Average zooplankton abundance (no./L) also followed the same trend as the total plankton abundance and recorded highest in LF ($P< 0.05$) (Tab.-2). In contrast, average phytoplankton abundance (no./L) was significantly higher ($P< 0.05$) in the manure based treatments (PM and CD), compared to LF and C (Tab.-2).

Results of enumeration of heterotrophic bacterial populations showed a highly variable result among the four treatments. The average counts of heterotrophic bacteria in PM (123.58×10^3 cfus./mL) and CD (95.75×10 cfusi mL) was significantly higher ($P< 0.05$) than the LF and C treatments (Tab.-3). A marked difference in the mean counts of Aeromonas sp. and Pseudomonas sp. was also observed among the treatments (Tab.- 3). Highest counts for both genera were observed in the PM treatment, followed in decreasing order by the CD and C treatments ($P< 0.05$). However, Aeromonas sp. and Pseudomonas sp. were absent from the water of LF ponds. In the pond sediments, there were no significant differences in the total aerobic heterotrophic counts between different treatments (P>0.05) (Tab. 3). However, the Aeromonas and Pseudomonas bacterial counts of the pond sediments followed similar trends, as the pond water and the highest counts for both these genera were encountered in PM, followed in decreasing abundance by the CD, C, and LF treatments ($P< 0.05$).

The final body weight of the koi carps ranged from 3.14g to 9.64g in the different treatments (Tab. -4). At harvest, maximum weight gain was achieved in the LF treatment, followed in decreasing order by PM, CD, and C treatments ($P<$ 0.05). The specific growth rate (SGR) was quite high (>4.0) in all the treatments, though the differences among the various treatments were significant ($P< 0.05$). There was a significant difference ($P< 0.05$) in the survival of koi carp among the treatments ranging from 67.21% (C) to 90.11% (LF).

Table 1: Mean±SE of major physico-chemical parameters analyzed for water and bottom sediments of the four treatments. Each mean value represents the weekly data collected during the 11-week growth period. Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented

Parameters	**Treatments**			
	LF	**PM**	**CD**	**C**
	Water			
pH	6.3–7.7	5.3–6.6	5.6–6.8	6.4–7.5
Dissolved Oxygen (mg/L)	3.63 ± 0.28^{b}	5.45 ± 0.25^{b}	5.79 ± 0.23^{ab}	6.35 ± 0.30^{ab}
BOD (mg/L)	1.65 ± 0.09^{b}	4.22 ± 0.47^{a}	3.30 ± 0.31^{a}	1.85 ± 0.15^{b}
Free CO_2 (mg/L)	2.08 ± 0.10^{c}	4.88 ± 0.26^{a}	3.57 ± 0.23^{b}	2.37 ± 0.10^{c}
Total Alkalinity (mg/L)	24.67 ± 0.85^{c}	72.22 ± 4.27^{c}	58.17 ± 3.13^{b}	29.90 ± 0.97^{c}
PO_4–P (mg/L)	0.16 ± 0.011^{b}	0.69 ± 0.083^{c}	0.51 ± 0.059^{a}	0.21 ± 0.017^{b}
NH_4–N (mg/L)	0.161 ± 0.011^{b}	0.569 ± 0.064^{a}	0.514 ± 0.048^{a}	0.203 ± 0.014^{b}
NO_2–N (mg/L)	0.006 ± 0.001^{b}	0.047 ± 0.006^{a}	0.038 ± 0.046^{a}	0.007 ± 0.001^{b}
NO_3–N(mg/L)	0.121 ± 0.007^{b}	0.462 ± 0.053^{a}	0.606 ± 0.070^{a}	$0.148 \pm 0.0.25^{b}$
Specific conductivity mmhors/cm	0.305 ± 0.012^{b}	0.711 ± 0.078^{a}		
	Sediment			
pH	0.0–6.8	0.06–5.9	4.5–55.8	5.0–6.8
Organic C (%)	1.27^{c}	2.94^{a}	2.02^{b}	1.32^{c}
Total N (%)	0.093^{c}	0.260^{a}	0.215^{b}	0.116^{c}

Table 2: Species composition, abundance (no./L) and relative abundance (% of total numbers) of plankton in experimental ponds maintained under different management regimes. Each mean value represents data from 22 samples collected twice a week during the 11-week growth period

Species	**LF**		**PM**		**CD**		**C**	
	(no./L/)	**(%)**	**(no./L/)**	**(%)**	**(no./L/)**	**(%)**	**(no./L/)**	**(%)**
Chlorella sp.	56.91	3.51	48.32	3.39	46.19	3.75	17.23	6.25
Navicula sp.	58.13	3.58	72.38	5.08	61.95	5.03	20.69	7.51
Spirogyra sp.	3.71	0.23	38.15	2.68	42.21	3.43	5.61	2.03
Scendesmus sp.	1.04	0.06	18.24	1.28	16.18	1.31	3.02	1.09
Phacus sp.	21.20	1.33	29.16	2.05	3920	3.18	3.15	1.15
Syndrad sp.	1.13	0.17	25.86	1.81	14.12	1.15	2.29	1.93
Phytoplakton	142.12	8.75	232.11	16.29	219.85	17.85	55.00	19.96
Daphnia sp.	274.38	16.90	168.13	11.80	122.64	9.96	5.26	1.91
Moina sp.	306.34	18.87	198.70	13.95	133.06	10.81	15.11	5.48
Bosmina sp.	108.26	6.67	81.43	5.72	64.19	5.21	3.20	1.16
Cladocera	688.98	42.44	448.26	31.47	319.89	25.98	23.57	8.55
Cyclops sp.	281.65	17.35	261.91	18.39	254.20	20.64	72.77	26.41
Diaptomus sp.	262.80	16.19	235.28	16.52	208.05	16.89	59.10	21.45
Nauplii	86.14	5.30	105.38	7.40	95.49	7.75	42.34	15.36
Copepoda	630.59	38.84	602.57	42.31	557.74	45.29	174.21	63.22
Brachionus sp.	55.14	3.39	58.90	4.13	62.34	5.06	10.29	3.73
Keratella sp.	106.62	6.57	82.42	5.79	71.53	5.81	12.48	4.53
Rotifera	161.76	9.96	141.32	9.92	133.86	10.87	22.77	8.26
Zooplankton	1481.33	19.25	1192.15	93.71	10.11.49	82.15	220.55	80.04
Total Plankton	1623.45	–	1425.26	–	1231.34	–	275.55	–

Table 3: Abundance of total heterotrophic bacteria, Aeromonas sp. and Pseudamonas sp. in the water and bottom sediment analyzed for the four treatments. Each mean value represents weekly data collected during the 11-week growth period. Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$

Parameters	**Treatments**			
	LF	**PM**	**CD**	**C**
	Water			
Total heterotrophic bacteria (cfu×10^3/mL)	17.08^{b}	123.58^{a}	95.75^{a}	28.17^{b}
Aeromonas sp. (cfus/ml)	–	3.63^{a}	2.01^{b}	1.08^{c}
Pseudomonas sp (cfus/ml)	–	2.10^{a}	1.29^{b}	+
	Sediment			
Total heterotrophic bacteria (cfu×10^3/mL)	32.42a	13.12^{a}	36.28^{a}	38.10^{a}
Aeromonas sp. (cfus/ml)	+	13.88^{a}	1.21^{b}	4.28^{c}
Pseudomonas sp (cfus/ml)	+	9.04^{a}	7.19^{b}	3.76^{c}

Table 4: Mean±SE of growth parameters recorded for koi carp reared in earthen ponds (2 June-18 August, 2004) under different management regimes. Different superscripts in the same row indicate statistically significant differences between means at $P<0.05$

Parameters	Treatments			
	LF	PM	CD	C
	Water			
Harvest weight (g)	9.64 ± 0.28^a	6.83 ± 0.21^b	4.17 ± 0.15^c	314 ± 0.18^d
Weight gain (g)	9.51 ± 0.28^a	6.70 ± 0.21^b	4.04 ± 0.15^c	3.01 ± 0.18^d
SGR (@/day)	5.58 ± 0.14^a	5.14 ± 0.09^b	4.15 ± 0.10^c	4.13 ± 0.04^d
Survival rate (%)	90.11 ± 0.61^a	84.50 ± 0.19^a	78.18 ± 0.38^b	67.21 ± 0.45^c

Discussion

The microbiological status of the water in which fish culture takes place depends on a wide variety of factors influencing the environment, the most important being the organic matter content (Rheinheimer, 1980; Sugita *et al*, 1985b; Zmyslowska *et al*, 2003). Variations in the abundance of heterotrophic bacteria in the water samples of the four treatments were the result of differences in management practices resulting in different organic loads in the pond system. Thus the management regimes receiving organic manures (PM and CD) recorded significantly higher populations of total heterotrophic bacteria ($P<$ 0.05), compared to other treatments (Tab. 3). The highly productive nature of the manured ponds was also supported by the greater abundance (no.IL) of total plankton, compared to the control treatment (Tab. 2).

The abundance of heterotrophic bacteria in the pond sediments did not differ from one system to another (Tab. 3). This implies that the sediment in all fish ponds in our experiment, regardless of the farming system, contained the optimal amount of essential nutrients necessary for rapid growth of heterotrophic bacteria. Jana & De (1990) [3] obtained similar results in the sediment of traditional and manure treated ponds. According to Jinyi *et al*. (1988), because of the sedimentation of applied manure and pond mud in both manure-applied and controlled ponds, the amount of bacteria in the water column decreases between the bottom of the pond and the surface layer of water with the continuous release of microorganism from the sediments. Similar results were obtained in our study (Tab. 3).

All aquaculture production systems must provide a suitable environment to promote the growth of aquatic crops. Although application of organic manure does not directly cause bacterial diseases in fish, the significantly greater abundance of pathogenic bacteria (Aeromonas sp. and Pseudomonas sp.) in the water and sediments of the manured treatments (PM and CD) could lead to diseases. Should fish resistance to disease be low, the possibility of occurrence of bacterial disease is higher in these treatments. Therefore, proper pond management should be observed to prevent any chance of bacterial disease.

Conclusion

Though it has been established that high fish yield in culture systems can be achieved by higher abundance of plankton through organic manuring, practical alternatives to pond manuring are needed because manuring may reduce water quality. Intensive stocking of ornamental fish ponds in India requires a standard water quality to be maintained throughout, so that fish growth is not adversely affected. In view of the financial constraints of marginal farmers who cannot afford modem aeration or waste-treatment equipments, raising of ornamental carp larvae in ponds fed exogenously with zooplankton is of considerable significance because not only would such feeding support high rates of survival and production, it would also maintain greater abundance of zooplankton in the system and better water quality with lower concentrations of Aeromonas sp. and Pseudomonas sp. in the system.

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