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Fish Production, Water Quality and Bacteriological Parameters of Koi Carp Ponds Under Live-food and Manure Based Management Regimes

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Abstract：To test 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 11 weeks in earthen ponds maintained according to four management regimes: (1) live zooplankton fed to carp larvae (LF); (2) direct fertilization with poultry manure (PM); (3) direct fertilization with cowdung (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. Values of dissolved oxygen were significantly higher (P<0.05) in the water of LF ponds, compared to other treatments, while the PM and CD treatments recorded were significantly higher (P<0.05) values of PO_4 – P, NH_4 – N, NO_3 – N, NO_2 – N, specific conductivity, alkalinity, and BOD, compared to the LF and C treatments. The percentages of organic carbon and total nitrogen in the bottom sediments were higher in the PM and CD treatments compared to LF (P<0.05). Average counts of heterotrophic bacteria in the water of PM and CD ponds were significantly higher than other treatments (P<0.05). The development of *Aeromonas* sp. and *Pseudomonas* sp. were significantly higher (P<0.05) in the PM and CD treatments. Weight gain of koi carp stocked in LF was significantly higher (P<0.05) than that of fish in the other treatments. There was a significant difference in the survival rate of koi carp among the treatments ranging from 67.21% in C to 90.11% in LF. The results suggest that raising koi carp larvae in ponds and feeding them exogenously with zooplankton would support high rates of survival and production through maintenance of better water quality and greater abundance of zooplankton in the system. Significantly lower abundance of *Aeromonas* sp. and *Pseudomonas* sp. in the LF treatment considerably lowered any possibility of occurrence of bacterial disease.

Key words: Koi carp ponds; *Cyprinus carpio* L.; Management; Growth; Water quality; Bacteriology

活体和施肥管理体系下锦鲤池塘产量、水质及细菌学参数

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摘要：为探讨在观赏池塘中的投放浮游动物以及直接投放动物粪便对锦鲤（*Cyprinus carpio* L.）的生长及产量的影响，在池塘中进行了为期 11 周的实验。实验按如下四种管理系统进行处理：1.给幼体锦鲤投喂浮游动物饲料(LF 组)；2.直接投放家禽粪便(PM 组)；3.直接投放牛粪(CD 组)；4.不投放任何食物，仅进行常规管理(C 组) 。每组实验重复三次。同时检测非自养细菌及致病微生物(如: *Aeromonas* sp. 和 *Pseudomonas* sp.)的生长状况，以此了解池塘的管理状况。在 LF 组中，其水体含氧量较高，与其它组相比具显著差异(P<0.05)。而 PM 、CD 组与 LF、C 组比较，在 PO_4 – P, NH_4 – N, NO_3 – N, NO_2 – N 的关系, 导电率、碱度以及生化需氧量等较高，且差异显著(P<0.05)，在池塘底部淤泥中的总氮量及有机碳百分率方面 PM、CD 与 LF 组相比，具有显著差异（P <0.05）。PM 与 CD 组与其它组相比在池塘中的非自养细菌（*Aeromonas* sp.和 *Pseudomonas* sp.）的繁殖率较高，皆具显著差异 P<0.05)。LF 组中锦鲤的体重增长率较其它组高（P<0.05)。锦鲤幼体在 C 及 LF 组中的成活率分别

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为: 67.21% 和 90.11%。结果提示：提高锦鲤幼体的存活率及其产量可通过对水质的管理(即保持优良水质)及提高池塘中浮游生物丰富度加以获得。值得注意的是：LF 组中非自养细菌（*Aeromonas* sp. 与 *Pseudomonas* sp.）比率的过低将导致细菌性疾病的发生。

关键词：锦鲤池塘；鲤鱼.；管理；生长；水质；细菌学
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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). The available organic pool 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). 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×20 m, with an average depth of 0.6-1.0 m), 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). 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.

1 Materials and methods

The koi carp used in this study were the offspring of 40 pairs of Asagi, Bekko, Kohaku and Showa koi types and were obtained from a local fish farm (Rainbow ornamentals, Jalpaiguri, India). The fish were acclimatized for seven days before the experiment. The study was conducted in twelve earthen ponds (capacity: 59600 L each) in Raninagar village, Jalpaiguri, India. About two week old koi carp larvae (0.12±0.007 g) 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). 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.

There were three replicates for each treatment. The ponds used for culturing plankton were maintained separately according to similar management condition as applied to the PM ponds. The application rate of 0.26

kg/m^3, every 10 days, for both the poultry and cow manures, were standardized in an earlier experiment (Jha et al, 2004). A single layer of nylon bird netting covered the entire experimental unit. Constant water levels were maintained in the experimental ponds by supplying ground water periodically to compensate for loss due to evaporation. However, as the experiment was conducted during the rainy season, evaporation loss was minimal and was mostly replenished naturally by rainwater. Approximately 1000 L of excess water was discharged from the live food ponds (LF), everyday during the introduction of plankton water. A plankton cloth was tied over the outflow water pipe to prevent any loss of zooplankton during the process. In the control ponds, a commercial pellet diet (Tokyo pellets, Japan) containing 32% crude protein was added, based on the amount of 5% body weight of cultured fish, daily.

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) 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 (125 mL), 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 1g 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℃ 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.

The weights of the fish were recorded at the beginning of the experiment and during harvest to the nearest 0.001 g. However, individual data could not be recorded from every harvested fish. In its place, 1000 fish were randomly selected from each pond and data was collected. For this the fish were anaesthetized with tricaine methene sulphonate (MS-222) of 0.4 g/L concentration. Dead fish were removed daily, and were not replaced during the course of the study. Differences between the number of fish stocked and the number of fish at harvest were used to calculate mortality percentage in each treatment.

The specific growth rate (SGR) was calculated as: $SGR= 100[(\ln W_t – \ln W_0)/t]$; where W_0 and W_t are the initial and final weights of live fish (g) respectively, and (t) is the culture period in days (Ricker, 1975). Fish growth and survival were assessed by one way analysis of variance (ANOVA) and where a significant difference ($P<0.05$) was detected, Tukey's HSD test (Zar, 1999) was applied to compare and rank means.

2 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℃ and 38℃ 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 (123.58×10^3cfus./mL) and CD (95.75×10^3cfus./ 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 3.14 g to 9.64 g 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).

Tab. 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	6.1 – 7.5
Dissolved oxygen (mg/ L)	6.63 ± 0.28^{a}	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 ± 0.010^{b}	0.69 ± 0.083^{a}	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 ± 0.004^{a}	0.007 ± 0.001^{b}
NO_3 – N (mg/ L)	0.121 ± 0.007^{b}	0.462 ± 0.053^{a}	0.380 ± 0.046^{a}	0.148 ± 0.013^{b}
Specific conductivity (mmhos/ cm)	0.305 ± 0.012^{b}	0.711 ± 0.078^{a}	0.606 ± 0.070^{a}	0.364 ± 0.025^{b}
		Sediment		
pH	5.2 – 6.8	4.6 – 5.9	4.5 – 5.8	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}

Tab. 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
Scenedesmus sp.	1.04	0.06	18.24	1.28	16.18	1.31	3.02	1.09
Phacus sp.	21.20	1.30	29.16	2.05	39.20	3.18	3.16	1.15
Synedra sp.	1.13	0.07	25.86	1.81	14.12	1.15	5.29	1.93
Phytoplankton	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	91.25	1192.15	83.71	1011.49	82.15	220.55	80.04
Total Plankton	1623.45	-	1424.26	-	1231.34	-	275.55	-

Tab. 3 Abundance of total heterotrophic bacteria, *Aeromonas* sp. and *Pseudomonas* 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

	Treatments			
	LF	PM	CD	C
		Water		
Total heterotrophic bacteria (cfus×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 (cfus×10^5 /g)	32.42 ^{a}	38.12 ^{a}	36.28 ^{a}	38.10 ^{a}
Aeromonas sp. (cfus./g)	+	13.88 ^{a}	10.21 ^{b}	4.28 ^{c}
Pseudomonas sp. (cfus./ g)	+	9.04 ^{a}	7.19 ^{b}	3.76 ^{c}

+ = <1.0; –= absent.

Tab. 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

	Treatment			
	LF	PM	CD	C
Harvest weight (g)	9.64 ± 0.28 ^{a}	6.83 ± 0.21 ^{b}	4.17 ± 0.15 ^{c}	3.14 ± 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}	4.51 ± 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}

3 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./L) of total plankton, compared to the control treatment (Tab. 2).

Lower counts of heterotrophic bacteria in control ponds not receiving any organic manuring have been reported earlier by many authors (Barat & Jana, 1990; Jana & De, 1990; Barik et al, 2001; Majumdar et al, 2002). As such, the control system appeared to be less productive, indicated from the significantly lower plankton abundance (P<0.05), compared to the manured treatments (Tab. 2). According to Ludwig (1999), when organic fertilizers are added to a pond, they are decomposed by bacteria and the water rapidly gains nutrients from the bottom. The released nutrients are rapidly utilized by phytoplankton and other bacteria, which are simultaneously grazed by single cell protozoan and other zooplankton. In control ponds, as also observed in our study, there are few nutrients, and hence few living organisms.

Although heterotrophic bacteria and phytoplankton are important components in the cycle of organic matter and inorganic nutrients in aquatic ecosystems, they may affect each other positively or negatively, depending on the nutrient conditions of their environment (Wang & Priscu, 1994; Kamjunke et al, 1997; Duvall et al, 2001). Because bacteria have a high surface area to volume ratio (Currie & Kalff, 1984), it has been suggested that bacteria should be superior competitors with phytoplankton for nitrogen and phosphorus (Elser et al, 1995). However, in our experiment, higher abundances of heterotrophic bacteria in manured treatments correlated with high phytoplankton abundance (in PM, r=0.625; P<0.01; in CD, r=0.588; P < 0.01).

Brett et al (1999) suggested that the underlying mechanisms behind the positive correlation between phytoplankton and bacteria are tangled in complex interactions between factors such as inorganic nutrient concentrations, organic nutrient availability, protozoan bactivory, availability of physical substrate, as well as light and temperature. Such complications could prevent augmented bacterial populations from having significant effects on phytoplankton. In experiments by Cottingham et al (1997), bacteria did not buffer phytoplankton responses to nutrient enrichment. In view of the continuous grazing pressure on bacteria and phytoplankton by zooplankton and on zooplankton by fish larvae, it is very difficult to estimate the exact population density of bacteria, phytoplankton or zooplankton in any aquatic system. However, the overall results clearly demonstrate the importance of pond management on the growth responses of heterotrophic bacteria.

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) 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).

Greater abundance of *Aeromonas* sp. and *Pseudomonas* sp. in the water and sediments of PM and CD, compared to the control treatment, indicate their sewage character. Very high counts of *Aeromonas* sp. and *Pseudomonas* sp. in ponds manured with animal excreta have been reported by many authors (Cloete et al, 1984; Jinyi et al, 1987; Jinyi et al, 1988; Hamza et al, 1998) The introduction of live plankton in the LF treatment, however, significantly reduced the population of total heterotrophic bacteria, as well as *Aeromonas* and *Pseudomonas* in both water and sediment, compared to the manured treatments (Tab. 3).

The water quality was also influenced by the management conditions. Significantly high $NH_4 – N$ in the PM and CD treatments could be related to the greater abundance of heterotrophic bacteria in these treatments, apart from ammonifying bacteria, which was not enumerated in our experiment, many heterotrophic bacteria are known to utilize nitrogen-rich substrates and release ammonia or ammonium salts (Jana & Barat, 1983). Yao & Zhaoyang (1997) reported that the contact layer between pond mud surface and water is the major source of nutrition. The organic nitrogen decomposed to $NH_4 – N$ by bacterial activity adheres to the surface of the mud before being released in the water where it continuously rises to the surface of the water and escapes into the air (Blackburn & Henriksen, 1983; Mei et al, 1995).

Depletion of dissolved oxygen after manure application often leads to heterotrophic organisms in the water utilizing $NO_3 – N$ as electron receptors instead of oxygen, thus converting it to nitrite (Boyd, 1990). Higher concentration of BOD, $NH_4 – N$, $NO_2 – N$ and other nutrients, along with the higher counts of *Aeromonas* sp. and *Pseudomonas* sp. in the manure treated culture regimes may have lowered the grazing activity by the carp, compared to the LF treatment. Neutral to acidic pH in the water of a majority of the treatments (Tab. 1) could be related to the acidic nature of water bodies in North Bengal (Nath et al, 1994; Jha & Barat, 2003; Jha et al, 2003). Lower range of pH values in the PM and CD ponds could be attributed to the animal manure applied in these treatments (Jha et al, 2004; Jha et al, 2006; Jha et al, 2007).

Zooplankton is required as a first food for most cultured fish (Ludwig, 1999). In an earlier experiment, a direct correlation (r=0.957; P<0.05) was observed between the weight gain of koi carp and the amount of zooplankton present in tanks under different doses of organic manuring (Jha et al, 2004). The maximum concentration of zooplankton in the LF treatment could be the consequence of improved water quality, expressed in terms of lower values of BOD, $NH_4 – N$ and $NO_2 – N$, and higher values of dissolved oxygen, which is conducive to fast reproduction of some of the major zooplankton constituting the main food item of carps (Jana & Chakrabarti, 1993), and also due to the regular introduction of plankton.

Higher weight gain and survival rate of koi carp in the LF treatment could be attributed to better water quality (Tab. 1) in that treatment (Jha & Barat, 2005c). Again, the differences in the weight gain of koi carp observed among the different treatments were not essentially due to changes in the water quality, since, weight gain in the C treatment was lower than PM and CD treatments P<0.05), despite having better water quality. It might well be that the weight gain was more directly related to the differences in food concentration, although the zooplankton concentration and water quality were closely related to each other.

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.

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 modern 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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