J. Mycopathol. Res. 31(2) : 75 81 (1993) Printed in India

Environmental factors affecting changes of heterotrophic bacterial population in fish ponds of West Bengal

U. K. DE
Department of Zoology, D. B. Mahavidyalaya,
Bangaon 743235, West Bengal

Density of heterotrophic bacterial population was studied bimonthly from six ponds farmed for polyculture, monoculture and traditional systems during a period of twenty-eight months. The maximum bacterial population was found in monoculture system and minimum was found in traditional system of fish farming. The highest peak was observed in post monsoon months (July to September) in all the ponds investigated. Development of larger abundance of heterotrophic bacteria in water of polyculture and monoculture ponds were perhaps due to prevalence of specific nutrient condition. The seasonal variation of heterotrophic bacterial population in these ponds attributed to the variations in the concentrations of different forms of nitrogen, phosphate, bicarbonate and dissolve organic carbon.

Key words : Seasonal variation, Heterotrophic bacteria, Fish culture ponds, Pisciculture technique

INTRODUCTION

The seasonal distribution of heterotrophic bacterial population has been studied little in tropical water ecosystem. Heterotrophic bacteria plays important roles in the nutrient condition of the fish pond. Bacterial population have no direct relationship to the fish growth except although that they are used as food source by some fishes. Primarily, heterotrophic bacteria help to determine the actual trophic level of the ponds. The purpose of this study was to provide data on physico-chemical factors and population size of heterotrophic bacteria in six fish ponds, differing in farm management, for twentyeight months.

MATERIALS AND METHODS

Six fish ponds, farmed under polyculture, monoculture and traditional systems, were selected for the present investigation. These ponds were distributed in

Kalyani and Naihati fish farm. Two fish farms were separated by a distance of 12 km. The water area and mean depth of these ponds ranged from 0.04 ha to 6.4 ha and from 1.0 to 3.6 m respectively.

The polycultural ponds were stocked with three species of Indian major carps (*Labeo rohito*, *Catla catla* and *Cirrhinus mrigala*) and three exotic carps (*Hypophthalmicthys molitrix*, *Ctenopharyngodon idella* and *Coprinus carpio*) on a ratio of 6 : 2 : 3 : 4 : 2 : 3 with a total stocking density of 6000/ha. In monoculture system fingerlings of *Cirrhinus mrigala* were introduced following the same stocking rate as in the polyculture ones. The traditional fish ponds did not follow the modern picicullure teahnique. The conventional system of fish cultivation is generally practised in majority of rural areas. The traditional fish ponds were stocked largely with three species of Indian major carps without maintaining any specific ratio. A number of stray fishes such as, *Puntius ticto*, *Heteroneoustes fossilis*, *Clarias batrachus*, *Channa marulius* etc. were also present in these ponds. All the fish ponds with the exception of those managed in the traditional way, were fertilised at monthly intervals with 50 kg ammonium sulphate or 10.5 kg N/ha or 8 kg P/ha, and manured with 2000/ha cattle dung (wet).

For microbiological and physicochemical analyses water samples were collected regularly from surface of the pond twice a month for a period of 28 months (from July 1978 to October 1980) and the monthly mean values are given in Table 1. The collection procedures were follcwed according to methods describeed by Rodina (1972) and Antipchuk (1979).

Samples of surface water from each of six ponds were collected in sterilized glass bottles (125 ml capacity) for enumeration of heterotrophic bacterial population while 500 ml plastic sampling bottles were used for examination of physico-chemical factors of water. Density of bacteria in the water samples was estimated by spread plate technique in triplicate, using nutrient agar (Difco, Bacto) medium following Antipchuk and Jana (1978a, 1978b).

RESULTS

Heterotrophic bacterial population

Determination of seasonal variation of heterotrophic bacterial community of water in these six fish ponds were studied. Viable heterotrophic bacterial populations in these ponds ranged from 0.1×10^5 cells ml^{-1} to 90×10^5 cells ml^{-1}. Density of heterotrophic bacteria differed markedly between two farm sites. The sum total for three cultural ponds of Naihati (705.27×10^5 cells ml^{-1}) was about 40.5% higher than the sum total for Kalyani farm (419.4×10^5

cells ml^{-1}). The numbers observed in polyculture system (8.79×10^5 cells ml^{-1}) was about two times higher than the counts obtained in traditional type (4.78×10^5 cells ml^{-1}). Density found in monoculture ponds remained (6.49×10^5 cells ml^{-1}) in between the two extremes.

The seasonal periodicity of heterotrophic bacteria was quite evident from these fish ponds, but the number of peaks differed considerably in these six ponds. There were three annual peaks in the mono- and polyculture ponds of Naihati as well as in the monoculture and traditional ponds of Kalyani while four and two peaks were exhibited in the polyculture-1 (Kalyani) and Traditional-2 (Naihati) respectively.

Physico-chemical character of water

Temperature of water in these ponds ranged from 18°C in winter to 34°C in summer. Water of these ponds remained always within alkaline range and pH varied between 7.3 and 8.6 (Table 1).

Table 1. Monthly mean values of physico-chemical parameters of water in various fish farming ponds (Each mean values represents the monthly data for 28 months)

Parameters	Kalyani Farm			Naihati Farm		
	Polycul-ture-1	Monocul-ture 1	Tradi-tional-1	Polycul-ture-2	Monocul-ture-2	Tradi-tional-2
Temperature (°C)	28.3	28.6	28.3	28.8	28.6	28.7
pH	8.1	8.3	8.3	8.2	8.1	8 2
Free CO_2 (mg l^{-1})	5.7	10.2	5.5	10.6	14.5	13.6
HCO^-_3 (mg l^{-1})	98.0	90.0	62.0	167.0	184.0	211.0
Total hardness (mg l^{-1})	95.0	107.0	63.0	182.2	179.0	200.0
Dissolved organic carbon (mg l^{-1})	8.1	6.8	5.9	8.1	8.2	4.6
Dissolved organic matter (mg l^{-1})	11.2	10.4	11.8	8.1	6.9	7.3
Dissolved O_2 (mg l^{-1})	11.1	10.0	10.4	8.3	8.3	5 5
PO_4P (mg l^{-1})	0.15	0.09	0.06	0 15	0.03	0.05
NH_4—N (mg l^{-1})	0.97	1.41	0 97	1.11	1.6	0.61
NO_2—N (mg l^{-1})	1.67	2.64	0.80	1.56	2 50	0.76
NO_3^-—N (mg l^{-1})	1.66	2.02	1.40	1.57	1.95	1.19
SO_4 (mg l^{-1})	30.7	28.0	24.8	26·1	27.3	25.3

Not well marked seasonal changes of pH was found in these ponds. The values of CO_2 were relatively higher (14.6 mg l^{-1}) in the ponds of Naihati (14.6 mg l^{-1}) as compared to the ponds of Kalyani (7.4 mg l^{-1}). Monoculture ponds (12.36 mg l^{-1}) showed relatively higher values of CO_2 as compared to either polyculture (8.1 mg l^{-1}) or traditional ponds (9.5 mg l^{-1}). Concentration of bicarbonate alkalinity ranged between 24 mg l^{-1} and 285 mg l^{-1}. The values of bicarbonate remained higher during the period of July through January but markedly declined during rest of the year.

The total hardness of water ranged from 34 to 266 mg l^{-1} in these ponds. The highest concentration of total hardness (266 mg l^{-1}) was recorded in summer and lower values found in monsoon (July to August). Concentration of dissolved oxygen in these ponds, ranged rrom 1.0 mg l^{-1} to 19.8 mg l^{-1}. On the cultural basis, the polyculture ponds showed higher concentration of oxygen (9.7 mg l^{-1}) than the traditional (8.0 mg l^{-1}) and monoculture (9.1 mg l^{-1}) ponds. A definite seasonal trend in the values of dissolved oxygen of water in all these fish ponds was observed.

Concentration of ammonia-nitrogen showed maximum mean values (1.50 mg l^{-1} in monoculture ponds, followed by polyculture system (1.25 mg l^{-1}) and traditional (0.54 mg l^{-1}) ones. The values of ammonia N showed a peak in winter months and then a steady decline was noticed. The nitrite-N concentration of water ranged from 0.19 to 3.80 mg l^{-1} in these ponds. The highest value of nitrite-N was found in monoculture (2.50 mg l^{-1}) and the lowest one in traditional (0.77 mg l^{-1}) ponds. The seasonal trend of nitrite level of nitrogen started a rising in July, reached the peak in winter months (Nov. and Dec.) followed by a decline to the lowest values in summer.

A marked difference in concentration of nitrate-N was observed. The values of nitrate-N in monoculture pond (1.98 mg l^{-1}) was 1.2 and 1.5 times higher than the values found in polyculture (1.61 mg l^{-1}) and traditional ponds (1.3 mg l^{-1}).

Monthly mean values of phosphate concentration obtained in polyculture ponds (0.15 mg l^{-1}) was more than two times higher than that in monoculture ponds (0.085 mg l^{-1}). The maximum concentration of phosphate was found in winter months and the lowest in April or May.

Amount of sulphate varied between 12 and 49.7 mg l^{-1} in different ponds. Sulphate concentration of polyculture ponds (28.4 mg l^{-1}) showed the highest values as compared to monoculture and traditional type of fish ponds. The highest and the lowest values of sulphate were observed during winter and summer months respectively.

Concentration of dissolved organic matter of water ranged from 1.0 to 30 mg l^{-1} in these ponds. Among the three types of fish farming ponds the highest concentration of organic matter was observed in polyculture pond and the lowest in traditional type. Values of dissolved organic matter attained the maximum in April and minimum in winter.

Organic carbon content of water ranged from 1.6 to 13.5 mg l^{-1} in these experimental fish ponds. The highest organic carbon concentration was found in polyculture (8.15 mg l^{-1}, followed in decreasing order by the monoculture (7.5 mg l^{-1}) and the traditional (5.25 mg l^{-1}) ponds. The highest peak of organic carbon content was observed in July and lowest in winter months.

Regression analysis

With a view to identifying the principal environmental factors responsible for seasonal changes of heterotrophic bacteria in the fish ponds regardless of the type of culture, a stepwise multiple regression analysis (STEP R multivariate statistics package) was run in the computer (Burroughs B 6700) using the linear concept between the dependent (Y) and independent variables (X). The analysis was performed using a reduced set of independent variables ; the logarithmic transformations (log 10) of monthly mean counts of bacteria occurring in these six ponds, regardless of the type of culture, were used as

Table 2. Stepwise multiple regression analysis of heterotrophic bacteria in water and environmental covariates. Total sum of sqauares based on 166 degrees of freedom

Step	Variable	Multiple R	R^2	$\triangle R^2$	Overall F
1	Dissolved organic matter (DOM)	0.245	0.060	0.060	10.57
2	Specific conductivity (CON)	0.297	0.088	0.028	5.12
3	HCO_3	0.404	0.162	0.134	14.40
4	PO_4—P	0.459	0.210	0.076	9.89
5	Temperature	0.488	0.267	0.191	5.93
6	NO_3—N	0.517	0.238	0.047	6.28
7	NO_2—N	0.535	0.286	0.239	4.28

Equation : Log Heterotrophic bacteria (Water = 0.063 (DOM) + 2.04 (CON) − 0.005(HCO_3) + 1.575 (PO_4−P) − 0.543 (NO —N) −0.079 (Temp.) + 0.202 (NO_2—N) + 2.204

dependent variables (Y) and 14 possible predictor variables of pond environment (physico-chemical factors) as independent parameters (X). Variables were entered into or removed from the regression equation by computing a partial

F-statistics for each variable at each step and comparing it to a preselected F-value based on the number of observations. The results of the multiple regression analysis (Table 2) of heterotrophic bacteria in water was highly significant ($P < 0.001$).

DISCUSSION

Development of microbial population in fish farming pond depends upon many factors such as organic loading, geochemical properties of the pond as well as density of the stocking fish. In the present study, farming management, through manuring schedule and fish stocking ratio, differed considerably among the monoculture, polyculture and traditional systems of fish farming. Appearence of larger abundance of heterotrophic bacteria, in the waters of polyculture and monoculture ponds, were perhaps due to prevalence of specific nutrient conditions of these ponds. Results corroborated earlier works of Antipchuk and Jana, (1978a, 1978b), Patel (1982), Roy (1984) and De (1990) and that the density difference of heterotrophic bacteria among the water bodies of three cultural types could be due to the effect of qualitative and quantitative differences of the organic loading resulted from the differences in farming management. Rheinheimer (1980) also concluded that the composition of organic material had a strong influence on the development of a particular microbial population. Tiwari and Mishra (1982) however, observed that the coefficient of heterotrophic activity was more closely related to the concentration of substrates than the population density of heterotrophic bacteria.

The statistical procedures for factor analysis showed that different forms of nitrogen (NO_2—N, NO_3—N, NH_4—N) had strong influence on the population growth of heterotrophic bacteria. Involvement of bicarbonate and dissolved organic carbon, on the seasonal change of heterotrophic bacteria, was perhaps due to their action as source of carbon.

The development of highest and lowest density of heterotrophic bacteria in polyculture and traditional ponds were associated with the amount of organic carbon. It is, therefore, concluded that the dissolved organic carbon not only affected seasonal growth of the heterotrophic bacteria but also determined the relative growth pattern in different fish ponds. Concentration of phosphate also affected directly both seasonal and spatial distribution pattern of heterotrophic bacteria in these ponds. A good degree of correlation between the density of heterotrophic bacteria and amount of oxygen was found to exist in these fish ponds. This shows that these aerobic microorganisms, require oxygen for their growth. It has been observed, in this study, the population growth of heterotrophic bacteria to be inversely correlated with diurnal changes of water temperature.

ACKNOWLEDGEMENT

The author is thankful to Dr. B. B. Jana, Department of Zoology, K. U. for his guidance and also to Prof. A. C. Guha, Principal of D. B. Mahavidyalaya, Bongaon, for his kind cooperation during the period of study.

REFERENCES

Antipchuk, A. F. (1979). Microbiological control in the fish farming ponds (in Russian). Pshevaya Promishlenostn, Moscow, 145 pp.

Antipchuk, A. F., and Jana, B. B. (1978a). Microbiological indices in the fish ponds with mono and polyculture (in Russian). Fisheries Science (Kiev), **26** : 20-25.

Antipchuk, A. F., and Jana, B. B. (1978b). Bacteriological biocoenosis in the fish ponds with mono and polyculture (in Russian). Fisheries Science (Kiev), **27** : 58-62.

De, U. K. (1990). Spatial and seasonal distribution of heterotrophic bacteria in pond water and sediments under different management practices. *Int. Revue ges. Hydrobiol.* 75(5) : 639-648.

Patel, G. N. (1982). Some microbiological indices in the trophic environment of the fish ponds in a polyculture system (Ph. D. thesis). Kalyani University, West Bengal.

Rheinheimer, G. (1980). Aquatic microbiology. John Wiley and Sons. Ltd., New York, 235 pp.

Rodina, A. G. (1972). Methods in Aquatic Microbiology, Baltimori *Univ. Press.* 461 pp.

Roy, S. K. (1984). Spatial and seasonal distribution of microbial populations involved in N-cycle and their activity in fish farming ponds under traditional, monoculture and polyculture systems. (Ph. D. thesis) Kalyani University, West Bengal.

(Accepted for publication 30 June 1993)