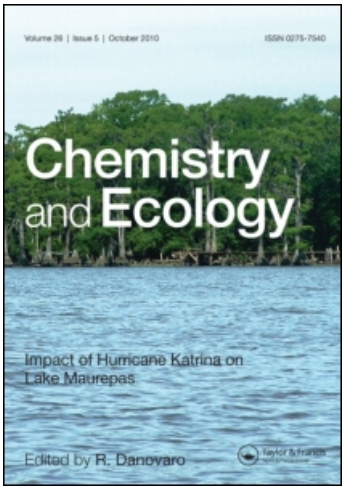

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Dose Dependent Interactions Between Ammonification Potential and Bacteria in Three Tropical Pond Ecosystems

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DOSE DEPENDENT INTERACTIONS BETWEEN AMMONIFICATION POTENTIAL AND BACTERIA IN THREE TROPICAL POND ECOSYSTEMS

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Using six different doses of peptone (0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 gl^{-1}) and nine different periods of incubation (1, 2, 3, 4, 6, 8, 10, 12 and 14 days), the rates of ammonification potential (AP) were monitored in three tropical ponds of different ecological status, during winter and summer periods. During the winter, the AP (ammonification potential) coupled with counts of HB (heterotrophic bacteria) and AB (ammonifying bacteria) exhibited peaks on day 4 of incubation in all the test doses of peptone in all the three ponds, except in the lowest dose in eutrophic and chemically polluted pond where the peaks preceded by a day. The responses of AP to the lowest doses of substrate did not differ between summer and winter, while the AP-peaks at higher doses were delayed by 2 days in summer. The concentrations of nitrate in *vitro* were the inverse and direct functions of the AP and dissolved oxygen of water. At moderate dose (0.75 $g\,l^{-1}$) of peptone, both AP and dissolved oxygen were at their optimal since the nitrate synthesis was limited by dissolved oxygen at higher doses, and by AP at the lowest dose of peptone.

Keywords: Ammonification potential; peptone doses; ammonifying bacteria; tropical aquaponds

INTRODUCTION

High concentrations of ammonia are a major problem in fish culture ponds especially in an intensive culture system. Ammonia occurs in water primarily as NH_4^+ and as undissociated NH_4OH, the latter being highly toxic to many organisms specially to fish (Lloyd, 1961).

Exposure of ammonia has a major effect on ionic regulation in juvenile fish (Paley *et al.*, 1993). Unionised ammonia accounts for the major component of metabolic ammonia excretion by diffusion down its concentration gradient (Wright and Wood, 1985). A major part of the dietary nitrogen is excreted by fish as ammonia (Guerin-Ancy, 1976). Decomposition of organic forms of nitrogen found in excretion is also a source of ammonia in fish culture ponds. Excretion of ammonia and amino acids of zooplankton (Brezonik, 1972), as well as the release of those compounds through direct autolysis after cell death (Krause, 1964), are a greater important source of ammonia in fish ponds.

Ammonia is typically associated with a waste excretion in microbial metabolism. The process of ammonification in the aquatic system has been recently reviewed by Jana (1994). Some of the ammonifying microorganisms are substrate specific, using only peptone rather than simple amino acids, or using urea but not uric acid. In contrast, other species are able to use a wide variety of organic nitrogen sources (Kormondy, 1986). While examining the diversity of 68 isolated strains of ammonifying bacteria, Sepers (1981) showed utilization of 41 organic compounds as the sole carbon and energy source, suggesting that microorganisms are capable of utilizing a wide variety of substrates.

It is probable that the functional responses of ammonifying bacteria are influenced by the amount of substrate and some environmental factors of a particular habitat. Since there are different sources of ammonia, and ammonia is often considered as an index of ecological health, it is of considerable interest to monitor ammonification rates under different pond conditions. Tropical fish ponds offer an excellent opportunity for measurement of ammonifications because of high temperatures and organic enrichment. Little information about the incubation time and dose of substrate required for ammonification is available for tropical ponds though a relatively old method is available for temperate fish ponds (Rodina, 1972). The purpose of the investigation was, therefore, to provide some basic data in the optimal incubation time and substrate dose for determining ammonification in tropical ponds which differ in their productivity and state of ecological health.

METHODS

Three ponds differing in qualitative and quantitative aspects of biological production and state of ecological health were selected for this investigation. Pond-A (area, 1.2 ha; depth, 1.2 ± 0.3 m) is a mesotrophic water body used for fish culture. Pond-B (area, 0.02 ha; depth, 1.3 ± 0.4 m) is an organic enriched eutrophic system, whereas Pond-C (area, 1.96 ha; depth, 1.15 ± 0.5 m) is a shallow pond receiving industrial effluents.

Surface pond water was collected from several sites within each pond and then pooled into a single composite sample. The required amount of peptone was mixed with filtered (cotton wool) pond water and then poured into 125 ml sterilized glass bottles which were glass capped; these were incubated *in situ* at the temperature of the pond water for periods up to 4 days during the winter and up to 14 days during the summer experiments. According to Rodina (1972), ammonification potential was determined using 1 g peptone (A.R.) as an energetic substrate per litre of water. In this study, six peptone doses (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 g l^{-1}) were selected during winter and three (0.25 g l^{-1}; 0.75 g l^{-1} and 1.5 g l^{-1}) during summer. Each treatment had four replicas. The peptone used in this study was found to contain 16% N. After the required incubation periods, bottles were removed from the pond and were analysed for NH_4-N and NO_3-N concentrations and other physico-chemical parameters, which were determined according to the standard methods described in APHA (1984). Ammonical nitrogen was measured by phenol-hypochlorite method using nitroprusside as a catalyst (Wetzel, 1991).

For enumeration of bacteria, aliquotes of ten-fold dilution of water samples were prepared in sterile distilled water from 10^{-1} to 10^{-5}. The conventional nutrient agar spread plate technique (Chen and Kueh, 1976) in an aerobic condition was used to enumerate viable counts of heterotrophic bacteria (HB). Ammonifying bacteria (AB) were enumerated using the plate count method with Remy's medium (Allen, 1957). As water temperature of these tropical fish ponds remained fairly high (28–33°C) for most of the year, culture plates were incubated at 37 ± 1°C considering the mesophilic adaptations of bacteria and incubations were made for 36 hours in the case of AB and 48 hours in the case of HB. The arithmetic mean calculated from four parallel determinations was used.

The data were statistically evaluated using Kruskal-Wallis one-way analysis of variance by ranks to find significant differences among treatments. Also, the treatment means were compared using one way analysis of variance and Duncan's multiple range test (Montgomery, 1984) was used to find the significant difference between treatment means. Statistical significance was accepted at the $P < 0.05$ level.

RESULTS

Natural Ammonification (NA)

The NA ranged from 7.2 to 14.6 mg l^{-1} in four ponds investigated (Table I). In general, NA was relatively higher in rates during winter than in summer. Of all the ponds, the activity was highest in Pond-C (14.6 mg $l^{-1} \pm 0.64$) followed by Pond-B (10.8 mg $l^{-1} \pm 0.5$) and Pond-A (7.6 mg $l^{-1} \pm 0.69$) during the period of investigation. The winter summer ratio, estimated up to 4 days, was higher in Pond-C (1.31) compared to Pond-B (1.28) or to Pond-A (1.04). In both winter and summer, NA tended to rise gradually with increase in length of incubation till day 4; a downward trend was observed beyond 4 days incubation during summer.

Ammonification Potential (AP)

Responses of AP to different doses were variable in the three ponds and were highly dependent upon days of incubation (Table II). Treatment

TABLE I Natural ammonification (NH_4-N mg l^{-1} $time^{-1}$) measured for different days of incubation (1–14) in three ponds during winter and summer ($\pm$SE)

Days of incubation	*Pond-A*		*Pond-B*		*Pond-C*	
	Winter	*Summer*	*Winter*	*Summer*	*Winter*	*Summer*
1	7.2 ± 0.12	6.98 ± 0.15	7.2 ± 0.16	6.90 ± 0.08	10.8 ± 0.24	9.20 ± 0.09
2	7.4 ± 0.13	6.99 ± 0.10	9.5 ± 0.12	7.00 ± 0.04	12.3 ± 0.12	9.64 ± 0.01
3	7.4 ± 0.12	7.03 ± 0.16	10.1 ± 0.21	7.34 ± 0.13	13.4 ± 0.14	9.78 ± 0.23
4	7.6 ± 0.08	7.20 ± 0.16	10.8 ± 0.12	7.70 ± 0.09	14.6 ± 0.24	10.31 ± 0.14
6		7.00 ± 0.13		7.41 ± 0.08		10.00 ± 0.01
8		7.00 ± 0.07		7.08 ± 0.01		9.83 ± 0.25
10		6.82 ± 0.04		6.79 ± 0.23		9.11 ± 0.08
12		6.41 ± 0.09		6.32 ± 0.13		8.62 ± 0.25
14		6.34 ± 0.10		6.27 ± 0.02		8.12 ± 0.07

differences were found not significant ($P > 0.05$) in the treatments with low doses (0.75 g l^{-1}) of peptone. The AP at 1.5 g peptone was significantly higher ($P < 0.05$) than that of 1.25 g peptone in most of the cases. AP showed a gradual rise as days of incubation increased showing higher values on day 4 for all the test doses in Pond-A and for all but the lowest dose (0.25 g l^{-1}) in both Pond-B and Pond-C. In the lowest dose, the AP peaked one day earlier (day 3) than with higher dose.

The responses of AP to different peptone doses remained the same in both summer and winter. Increase in days beyond 4 of incubation resulted in gradual decline of AP along with decreasing dose of peptone, showing the lowest value in lowest dose of peptone (Table III), implying that AP was substrate limited.

AP/NA Ratio

The ratio between AP and NA in different doses of peptone showed the similar variations as observed in AP, suggesting less variability of NA in the three ponds investigated (Figure 1).

Enumeration of Bacteria *in vitro*

Initially, the counts of both heterotrophic bacteria (HB) and ammonifying bacteria (AB) in pond waters were largest in Pond-C ($25–36 \times 10^3$) followed by Pond-B ($13–21 \times 10^3$) and Pond-A ($12–16 \times 10^3$) (Figure 2). Enumeration of HB and AB in the peptone enriched water samples after 4 days of incubation showed their rise in numbers as the amount of peptone dose increased. However, the bacterial density was not found to exhibit a clear cut relationship ($r < 0.66$; $P > 0.05$) with the ammonia content of peptone treated samples.

Among the three ponds, both HB and AB were highest in Pond-B (ANOVA; DMR test) at all but the highest dose. At the highest dose, in Pond-C HB and AB were maximum in winter and minimum in summer.

Changes of Nitrate *in vitro*

Increase in days of incubation from day 1 to 3 resulted in sharp decline in nitrate concentration in all the doses of peptone, regardless

TABLE II Measurement of ammonification potential (NH_4-N mg l^{-1} $time^{-1}$) in different doses of peptone and in different days of incubation in three ponds during winter (±SE). Same subscripts among peptone doses revealed lack of significant differences (DMR test, $P < 0.001$)

Substrate Dose	*Pond-A* *Days of incubation*				*Pond-B* *Days of incubation*				*Pond-C* *Days of incubation*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
0.25 g l^{-1}	17.60^{a} ±0.79	34.56^{a} ±0.943	51.84^{a} ±0.199	69.12^{a} ±0.947	60.48^{ab} ±0.35	77.76^{ab} ±0.671	95.04^{abc} ±0.889	60.48^{a} ±0.685	43.20^{abc} ±0.377	51.88^{ab} ±0.508	115.20^{abcd} ±1.108	81.84^{ab} ±0.478
0.50 g l^{-1}	16.10^{a} ±0.14	34.56^{a} ±0.427	42.12^{a} ±0.172	56.68^{a} ±0.247	50.01^{ab} ±0.117	54.74^{ab} ±0.444	65.60^{abc} ±0.142	72.94^{cb} ±0.247	29.64^{a} ±0.584	39.64^{a} ±0.193	62.92^{abc} ±0.427	65.54^{a} ±0.646
0.75 g l^{-1}	19.15^{a} ±0.219	28.72^{a} ±0.184	40.21^{a} ±0.212	51.71^{a} ±0.150	40.21^{a} ±0.248	48.83^{a} ±0.334	54.58^{a} ±0.121	64.58^{a} ±0.307	22.98^{a} ±0.103	37.34^{a} ±0.086	52.98^{a} ±0.156	60.32^{a} ±0.092
1.00 g l^{-1}	17.22^{a} ±0.182	37.29^{b} ±0.222	63.43^{cb} ±0.112	69.71^{ab} ±0.130	39.28^{a} ±0.091	49.52^{a} ±0.201	58.77^{b} ±0.315	72.11^{c} ±0.219	33.78^{b} ±0.230	41.33^{b} ±0.053	62.09^{bc} ±0.130	78.04^{b} ±0.457
1.25 g l^{-1}	17.02^{a} ±0.377	39.09^{c} ±0.187	59.84^{c} ±0.071	78.28^{c} ±0.275	41.41^{a} ±0.194	52.06^{b} ±0.067	58.63^{b} ±0.177	82.46^{d} ±0.071	41.61^{c} ±0.261	54.85^{c} ±0.140	62.62^{c} ±0.275	86.92^{c} ±0.362
1.50 g l^{-1}	37.06^{b} ±0.099	45.61^{d} ±0.078	57.02^{b} ±0.190	95.35^{d} ±0.088	48.47^{b} ±0.037	51.32^{b} ±0.027	61.30^{c} ±0.065	97.60^{e} ±0.124	57.02^{d} ±0.174	59.87^{d} ±0.136	65.57^{d} ±0.134	100.20^{d} ±0.121

TABLE III Measurement of AP (NH_4-N mg l^{-1} $time^{-1}$) during days of incubation in three ponds during summer (± SE). Same subscripts among peptone doses revealed lack of significant differences (DMR test, $P < 0.001$).

Days of incubation	*Pond-A* *Substrate dose (g l^{-1})*			*Pond-B* *Substrate dose (g l^{-1})*			*Pond-C* *Substrate dose (g l^{-1})*		
	0.25	0.75	1.50	0.25	0.75	1.50	0.25	0.75	1.50
1	13.52^{a} ± 0.172	15.18^{b} ± 0.218	28.53^{c} ± 0.132	46.56^{ab} ± 0.692	30.96^{a} ± 0.190	37.32^{b} ± 0.091	33.28^{a} ± 0.566	17.70^{a} ± 0.477	44.21^{b} ± 0.293
2	26.60^{a} ± 0.923	22.11^{a} ± 0.357	35.13^{b} ± 0.059	59.88^{ab} ± 0.637	37.59^{a} ± 0.196	39.52^{b} ± 0.125	39.96^{a} ± 0.273	28.75^{a} ± 0.741	46.10^{b} ± 0.085
3	39.92^{a} ± 0.409	30.96^{a} ± 0.163	43.96^{b} ± 0.123	73.20^{ab} ± 0.688	42.04^{a} ± 0.279	49.18^{b} ± 0.130	88.72^{ab} ± 0.612	40.80^{a} ± 0.069	52.52^{b} ± 0.740
4	53.24^{a} ± 0.321	39.79^{a} ± 0.400	73.42^{b} ± 0.170	60.12^{a} ± 0.320	46.56^{a} ± 0.138	73.42^{b} ± 0.176	79.92^{ab} ± 0.198	46.45^{a} ± 0.327	73.42^{b} ± 0.521
8	43.48^{a} ± 0.659	90.97^{b} ± 0.166	104.54^{c} ± 0.311	43.20^{a} ± 0.372	76.60^{b} ± 0.263	85.53^{c} ± 0.124	72.00^{a} ± 0.266	79.90^{b} ± 0.414	104.54^{c} ± 0.167
10	39.20^{a} ± 0.405	86.18^{b} ± 0.306	86.72^{b} ± 0.272	41.00^{a} ± 0.116	70.60^{b} ± 0.479	75.24^{c} ± 0.135	70.40^{a} ± 0.728	76.07^{b} ± 0.612	80.78^{c} ± 0.382
12	32.00^{a} ± 0.135	83.79^{b} ± 0.166	85.53^{b} ± 0.107	39.06^{a} ± 0.238	62.24^{b} ± 0.152	73.65^{c} ± 0.204	57.60^{a} ± 0.553	73.41^{b} ± 0.071	78.40^{c} ± 0.229
14	32.00^{a} ± 0.419	82.72^{b} ± 0.222	78.40^{b} ± 0.164	37.20^{a} ± 0.773	58.53^{b} ± 0.582	70.56^{c} ± 0.060	57.60^{a} ± 0.264	62.64^{b} ± 0.731	72.03^{c} ± 0.376

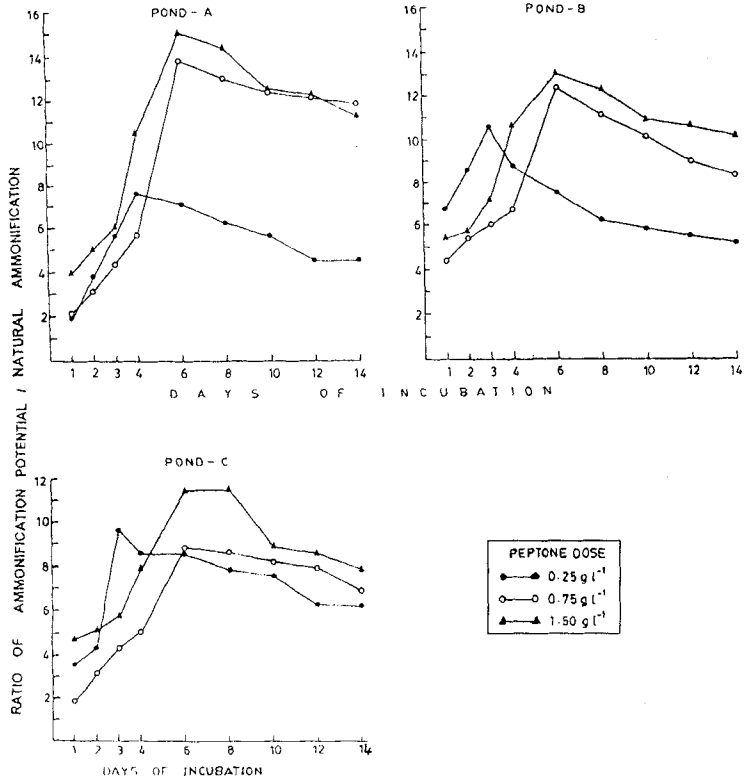

FIGURE 1 Ratios between the ammonification potential in three doses of peptone and natural ammonification measured in three ponds during the summer.

of season. However, there was an increase in nitrate concentration of water when the days of incubation increased from 3 to 4 during winter and from day 3 to 14 during summer (Figure 3). Among three doses of peptone, the concentration of nitrate was highest at 0.75 g l^{-1} as compared to either 0.25 g l^{-1} or 1.50 g l^{-1}.

Water quality *in vitro*

In *in vitro* examination of water quality, revealed marked decline of pH, the rate of decline was more pronounced at high dose than at low dose. The responses of chemical oxygen demand, orthophosphate and specific conductivity of water to different doses of peptone were opposite

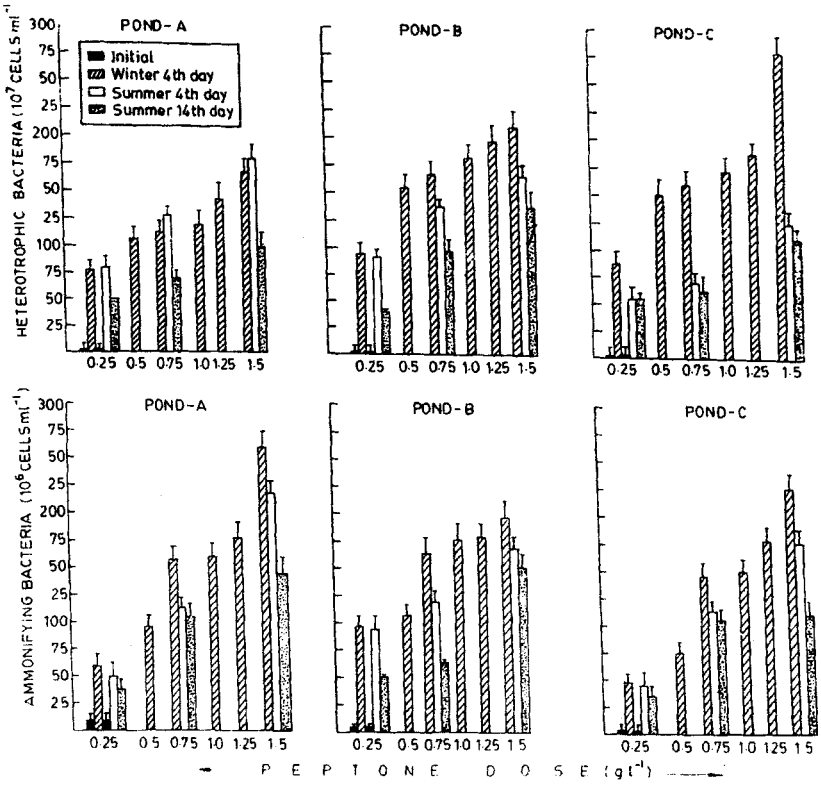

FIGURE 2 Responses of heterotrophic and ammonifying bacteria to six different doses of peptone in three ponds during winter and summer (±SE).

to that of pH (Table IV). Dissolved oxygen disappeared completely from the water within 24 hours after peptone application and this anaerobic condition, reexamined to day 4 of incubation in lower doses (0.25 g l^{-1} and 0.75 g l^{-1}). However, prolonged absence of DO occurred in high doses of peptone. The rate of recovery was faster in 0.25 g l^{-1} than in 0.75 g l^{-1}. This was found to be true for all the ponds (Table IV).

Water quality *in vivo*

The values of most of the water quality parameters tended to increase on day 4 as compared to initial values. However, there was no marked differences in temperature, pH, specific conductivity, total alkalinity and

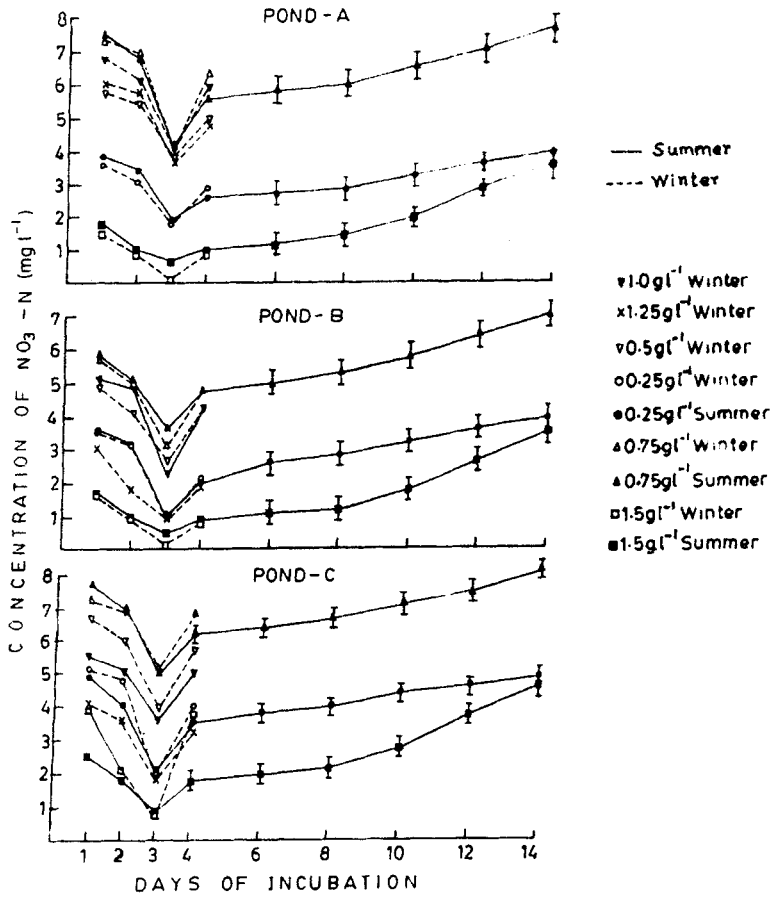

FIGURE 3 Changes of nitrate concentrations of water in different doses of peptone in three ponds during winter and summer (± SE).

orthophosphate of pond water between initial and that after 4 days. Differences were greater in the values of oxygen (up to 15%) and chemical oxygen demand (up to 26%) rather than other parameters analysed (Table V).

DISCUSSION

The ammonification potential was found to be a function of the abundance of ammonifying and heterotrophic bacteria of the pond

TABLE IV *In vitro* analysis of water quality parameters used for measurement of NA and AP on day 4 of the incubation

Parameters	*Season*	*Natural ammonification*			*Ammonification potential*								
					Substrate (0.25 g l^{-1})			*Substrate (0.75 g l^{-1})*			*Substrate (1.50 g l^{-1})*		
		Pond-A	*Pond-B*	*Pond-C*	*Pond-A*	*Pond-B*	*Pond-C*	*Pond-A*	*Pond-B*	*Pond-C*	*Pond-A*	*Pond-B*	*Pond-C*
pH	Winter	8.0	8.60	8.1	7.5	8.0	7.7	7.2	7.3	7.20	6.60	6.90	6.90
	Summer	8.2	9.6	8.97	7.61	8.42	8.22	7.72	7.98	6.93	6.91	7.40	7.03
Ortho-phosphate	Winter	0.052 ±0.17	0.047 ±0.14	0.056 ±0.06	0.061 ±0.12	0.048 ±0.22	0.099 ±0.12	0.116 ±0.09	0.082 ±0.07	0.103 ±0.09	0.092 ±0.24	0.061 ±0.09	0.52 ±0.21
(mg l^{-1})	Summer	0.030 ±0.03	0.038 ±0.08	0.062 ±0.07	0.058 ±0.05	0.043 ±0.09	0.097 ±0.07	0.107 ±0.07	0.080 ±0.06	0.108 ±0.12	0.075 ±0.07	0.073 ±0.08	0.41 ±0.09
Chemical oxygen demand	Winter	60 ±0.65	88 ±0.59	80 ±1.11	90 ±0.87	102 ±0.72	135 ±2.06	145 ±1.07	220 ±1.13	280 ±0.95	380 ±0.96	366 ±2.02	400 ±0.68
(mg l^{-1})	Summer	72 ±0.81	76 ±0.78	118 ±0.86	98 ±0.88	105 ±1.83	196 ±3.01	180 ±0.95	270 ±2.12	368 ±1.22	586 ±1.09	420 ±1.89	623 ±2.83
Specific conductivity (10^{-2} mMHO)	Winter	0.46	0.59	0.73	0.63	0.65	0.89	0.95	0.92	1.27	1.47	1.47	1.79
	Summer	0.48	0.62	0.78	0.69	0.69	0.92	0.99	1.05	1.34	1.45	1.58	1.81
Dissolved oxygen	Winter	7.2	7.62	3.4	0.2	0	0	0	0	0	0	0	0
(mg l^{-1})	Summer	7.2	6.8	3.7	0	0	0	0	0	0	0	0	0

TABLE V Concurrent changes of water quality in three ponds during the measurement of ammonification potential (± SE)

Parameters	*Pond-A*				*Pond-B*				*Pond-C*			
	Winter		*Summer*		*Winter*		*Summer*		*Winter*		*Summer*	
	Initial	*Day 4*	*Initial*	*Day 4*	*Initial*	*Day 4*	*Initial*	*Day 4*	*Initial*	*Day 4*	*Initial*	*Day 4*
Temperature (°C)	21.0	23.3	28.0	32.0	21.9	23.6	30.0	33.0	21.5	23.9	31.0	33.0
pH	7.8	7.9	8.05	8.0	8.1	8.2	9.42	9.2	7.7	7.6	9.0	9.1
Specific conductivity (10^{-2}m MHO)	0.46	0.47	0.43	0.45	0.59	0.61	0.45	0.47	0.73	0.73	1.19	1.21
Total alkalinity ($mg\ l^{-1}$)	130.6 ±1.2	130.0 ±1.3	213.32 ±0.96	212.6 ±1.43	180.0 ±1.11	183.8 ±1.61	235.33 ±1.66	230.6 ±1.73	150.0 ±0.97	142.8 ±1.32	220.14 ±1.44	227.3 ±1.65
Dissolved oxygen ($mg\ l^{-1}$)	7.32 ±0.12	8.1 ±0.14	8.0 ±0.06	9.2 ±0.10	7.7 ±0.09	8.0 ±0.07	9.2 ±0.08	9.4 ±0.08	4.0 ±0.14	5.0 ±0.13	4.0 ±0.06	4.2 ±0.08
Orthophosphate $mg\ l^{-1}$	0.059 ±0.001	0.061 ±0.004	0.028 ±0.002	0.033 ±0.002	0.233 ±0.006	0.240 ±0.003	0.216 ±0.011	0.225 ±0.02	0.109 ±0.001	0.113 ±0.002	0.095 ±0.004	0.091 ±0.008
COD ($mg\ l^{-1}$)	80 ±0.216	85 ±0.081	53.4 ±0.188	66.6 ±0.574	84 ±0.309	90 ±0.17	120 ±0.419	166.6 ±0.287	100 ±0.492	100 ±0.34	173.4 ±0.349	180.2 ±0.188

($r > 0.75$; $P > 0.001$). The phenomenon was also found to be substrate limited because of the fact that the AP peaked earlier with low dose and later in the high dose substrates. Despite relatively low initial counts of both groups of bacteria, the AP-peak in the highest dose of peptone in Pond-A was the result of fast multiplication, induced by the nutrient enrichment of the pond in the absence of environmental stress. The environmental stress caused by the discharge of diverse toxic agents from several chemical factories in Pond-C was primarily responsible for the sharp reduction of generation time of bacteria even with their highest initial counts among all the ponds. Eventually, this resulted in relatively smaller population size and consequently less AP. This suggests that causative bacteria were *r*-selected in Pond-A and K-selected in Pond-C.

A reduction of 7–11°C water temperature during the winter (20.5–21.5°C) was found to be responsible for AP induction by 23% over the rates observed during the summer (28–33°C). The ammonification was stated to be favoured within the temperature range of 30°C and 35°C (Rheinheimer 1980, Jana and Roy 1985, Jana 1994).

The concentration of ammonia is a factor which influences the activity of nitrifiers. Wada and Hattori (1971) reported that below a concentration of 5 g atom l^{-1} of NH_4-N, there was no nitrification in the ocean. The direct relationship between the AP-rates and nitrate concentration, at least among the lower doses of substrate in the

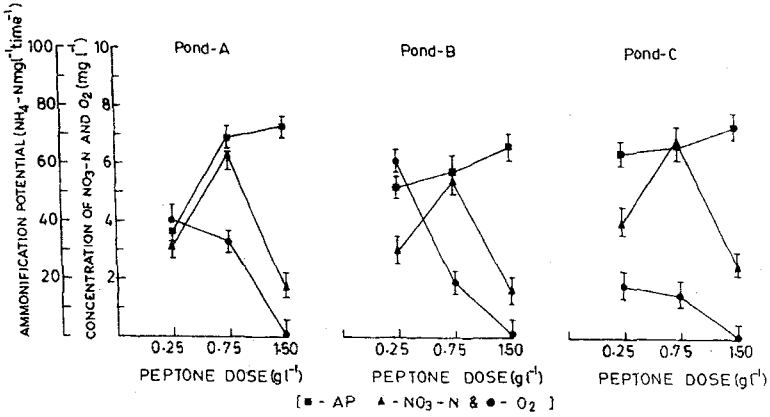

FIGURE 4 Responses of AP, NO_3-N and O_2 to different doses of peptone in three ponds investigated.

present study, suggests that a certain amount of ammonia is a prerequisite for nitrification to occur. Since nitrate synthesis was distinctly higher at 0.75 g l^{-1} peptone as compared to either 0.25 g l^{-1} or 1.50 g l^{-1} of peptone, it appears that both AP and DO perhaps remained favourable at their optimal for nitrification at moderate dose of peptone. At low peptone dose, nitrate synthesis was limited by AP because DO was present in adequate amount, whereas with a high dose, DO limited the nitrification. This suggests that both factors were involved in the nitrification process.

A strong inverse correlation between the AP/NO_3 ratio and the dissolved oxygen of water suggests that production of nitrate per unit of AP was faster at high dissolved oxygen level than at a reduced level of dissolved oxygen. According to Gunderson *et al.* (1966), even though the presence of oxygen is a necessary condition for nitrification, very low levels of dissolved oxygen are sufficient since nitrification is known to occur down to 0.3 mg l^{-1} of dissolved oxygen. It appears that dissolved oxygen is not only essential for nitrification, but also a certain amount of dissolved oxygen is necessary for the AP because of a sharp decline of AP-rates at zero concentration of dissolved oxygen, though the AP maintained an inverse relationship with dissolved oxygen within the range of 0.4 to 6.4 mg l^{-1} (Figure 5). Jana and Barat (1984) observed higher ammonification due to greater abundance of AB in the water with high oxygen tension.

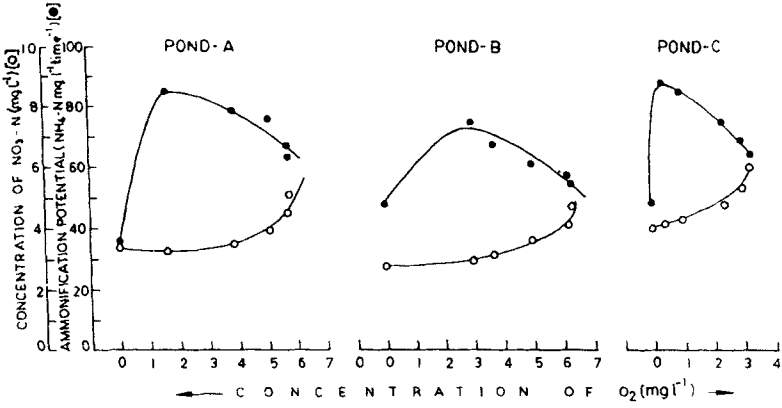

FIGURE 5 Responses of AP and NO_3-N to different concentrations of D.O. in three ponds (overall mean values of doses and days of incubation were considered).

A recommended method for the measurement of ammonification potential in temperate fish ponds is the use of 300 g peptone per 300 ml of water with an incubation period for 3 days (Rodina, 1972). This study clearly shows that incubation time for AP was variable depending upon the substrate dose as well as on the ecological health of the pond in question.

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