

## Response of Differential Application Frequency of Poultry Litter on Mineralization Potential in Fish Culture Tanks with Special Reference to the Abundance of Ammonia Oxidizing Bacteria and Fish Growth

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### ABSTRACT

The primary concern of the fish culture is nutrient budget of the particular ecosystem. Nitrogen is the chief macronutrient of aquatic system and biological nitrogen transformation in the aquaculture pond is dominated by ammonifying, nitrifying and denitrifying bacteria. The first step of nitrification is mediated by Ammonia Oxidizing Bacteria (AOB) and the functional response of AOB is influenced by substrate availability. Livestock manures are enriched with essential nutrient elements having pronounced effect in increasing organic content of the soil sediment in aquaculture pond. So, input of organic manure such as poultry litter provides major source of macronutrients and also produces more substrate for AOB by enhanced degradation of organic matter. Although excess input of organic matter may produce deleterious effect due to accumulation of toxic nitrogen species such as ammonia and nitrite. Greater abundance of AOB is beneficial for subsequent transformation of ammonia into valuable by-products. So, the present study was conducted to establish a cost effective scientific protocol for administration of poultry litter in fish pond with special reference to the abundance of AOB. Weekly application of poultry litter at a dose of 50 Kg ha<sup>-1</sup> day<sup>-1</sup> with bimonthly lime application at a dose of 37.5 Kg ha<sup>-1</sup> month<sup>-1</sup> was found to be beneficial for fish ponds. The study also explained the impact of the abundance of AOB on absolute growth rate of fish.

**KEY WORDS:** AMMONIA OXIDIZING BACTERIA, ABSOLUTE GROWTH RATE, POULTRY LITTER AND FISH GROWTH.

### ARTICLE INFORMATION

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## INTRODUCTION

Nitrogen is the prime regulating nutrient of aquaculture pond which greatly influences the productivity of aquatic ecosystem. Availability of nitrogen in aquatic system depends upon the nitrogen mineralization. Nitrification is the crucial step of nitrogen mineralization for nitrogen cycling in aquatic environment (Lu et al., 2015). Bacterial dynamics regulate the chemical transformation of organic nitrogen into simpler one through ammonification, nitrification and denitrification. In the first step of nitrification, ammonia-oxidizing bacteria (AOB) plays the pivotal role for oxidation of ammonia to nitrite which is subsequently, oxidized to nitrate by nitrite-oxidising bacteria (NOB) (Kim et al., 2005; Abeliovich, 2006; Kumari et al., 2011; Daims et al., 2016, Ghosh et al 2019).

Ammonia accumulation beyond the threshold level is detrimental to aquatic life depending on the pH of the water body (Hargreaves, 1998). In the aquatic environment, ammonia can be utilized by phytoplankton directly or it can be oxidized by ammonia-oxidizing microorganisms (Ebeling et al., 2006) to be transformed into valuable by-product. Very low proportion of nitrifiers (AOB and NOB) leads to nitrogen toxicity in the system (Jiao et al., 2009). Aquaculture ponds in which inorganic fertilisers are applied for rapid solubilisation have inadequacy in essential nutrients and may exerts untoward effects on soil structure, composition, micro-flora, macro-flora and many other characteristics of the pond ecosystems (Jana et al., 2001b).

However, organic manures are rich in both macro and micro nutrients that enrich the organic matter content of pond soil (Jana et al., 2001a) and incorporation of animal wastes as a source of organic matters in the aquatic system increase trophic status of ponds there by enhance fish production (Orhibhabor and Ansa, 2006). A minor portion of livestock manure applied to the aquaculture pond can be directly consumed by fish, and the remaining portion release nutrients to enhance the growth of photosynthetic organisms (Little and Edwards, 1999) as the excreta is enriched with nutrient of recovered 72-79% of Nitrogen (N), 82-92% of Potassium (K) and 61-87% of Phosphorus (P) in feed given to animals (Singh et al., 2014 Ghosh et al 2019).

The litter is very useful fertilizer for the fish farming ponds as it is loaded with crucial nutrients such as nitrogen, phosphorus, potassium (Gupta et al., 2012) and different trace elements, such as Cu, Zn and As (Bolan, 2010). Digestive tracts of poultry birds are short and therefore 80% of chicken manure may remain as undigested feed-stuff (Chen, 1981) which may contain about 20-30% total protein (Pudadera et al., 1986). Intensification of the macronutrients may lead to stressed condition to aquatic lives with deterioration of water and sediment qualities. Hence, organic manure should be applied in fish ponds at proper doses to promote fish production (Jhingran, 1995) because application of excess nitrogen in the form of fertilizer may have

adverse effects on water quality by the accumulation of toxic nitrogenous compounds such as ammonia and nitrite. That can be controlled by maintaining the optimal environment for the proliferation of nitrifying bacteria which will cause removal of the ammonium nitrogen (Shan and Obbard, 2003). Although fish cultures under the practice of integrated poultry-fish farming system are recommended to be safe for human consumption (Orhibhabor and Ansa, 2006). Poultry litter input at a dose of 50 kg ha<sup>-1</sup> day<sup>-1</sup> keeps the system conducive for fish farming (Tamuli, 2006). Magnitude of the abundance of bacteria highly depends on the time taken for mineralization of the applied organic matter. Taking this into consideration, the present study was carried out to find out a rational and scientific protocol for application of poultry litter in fish ponds.

The present investigation was carried out on a freshwater minor carp, bata (*Labeo bata*). It was selected for the present study because of its wide acceptability in India due to its good taste, omnivorous feeding habit, very well growth rate in shallow waters (Ghosh et al., 2019) and availability of artificially propagated seed. The experiment was conducted to ascertain the optimal mode of application of poultry litter (daily, weekly and monthly) with a dose of 50 kg ha<sup>-1</sup> day<sup>-1</sup> to obtain maximum abundance of AOB thereby enhancing the nitrogen cycling and growth rate of *Labeo bata* for sustained biological productivity of fish ponds.

## MATERIAL AND METHODS

A 120-day experiment was conducted with 3 different application frequencies (monthly, weekly and daily) in twelve tanks to find out the most effective mode of application of poultry litter with special reference to the AOB abundance during January 02 – April 30, 2017 in Gayeshpur, Nadia West Bengal (22°58'7.788' N, 88°29'44.556"E). Twelve circular tanks with water holding capacity of 300L were selected for the experiment (three replication for each) as control (C) with no organic input, treatment 1 (T<sub>1</sub>) with monthly input of poultry litter, treatment 2 (T<sub>2</sub>) with weekly input of poultry litter and treatment 3 (T<sub>3</sub>) with daily input of poultry litter. Each of the tanks were provided with virgin alluvial soil base of 15 cm and filled with ground water, 40 days prior to initiation of the experiment. After initial liming to maintain the water pH, poultry litter was added to each tank for manuring and 15 well acclimatized fish fingerlings (*Labeo bata*) were stocked in each tank. Previously selected dose of poultry litter (50 Kg ha<sup>-1</sup> day<sup>-1</sup>) through a pilot study was preferred as the optimal dose of application for the present experiment with bimonthly lime application of 37.5 Kg ha<sup>-1</sup> month<sup>-1</sup> (Table:1).

On day 0 of the experiment, treatment specific poultry litter was applied for the 1st time and samples were collected after manuring. Then the treatment specific poultry litter application frequency (monthly, weekly and daily) was followed all throughout. Samples were collected prior to poultry litter application on the day of sampling during remaining period of the experiment.

The samples were collected at fixed hour (8.00-9.00a.m) of the day during the study period from 3 places of the tank to create a homogeneous mixture of sample of water as well as of sediment.

For microbial study aliquots were prepared with serial dilution of  $10^{-2}$ - $10^{-4}$  for sediment samples. For sediment sample a homogeneous sediment suspension was prepared by mixing 1 g of the wet soil with 99 mL sterile distilled water. Then the aliquot dilutions were subjected to pour plate technique for microbial analysis. Selective medium was prepared for the isolation of AOB from sediment samples following standard methodology (Drews, 1974). Composition of the medium was as follows ( $\text{g L}^{-1}$ ): ammonium sulphate, 1.0; potassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ ), 0.5; sodium chloride, 2.0; magnesium sulphate, 0.2; ferrous sulphate, 0.05; calcium carbonate, 6.0; cresol red (0.5%), 0.1; agar, 15 and a volume of 1L medium was prepared with adding distilled water. Mostly abundant bacterial species were subjected to SEM (Scanning Electron Microscopy) analysis to produce a magnified image of those particular organisms for further study (Photograph: 1 & 2). Physico-chemical parameters

(viz. pH, alkalinity, free  $\text{CO}_2$ ,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$ ) of the surface water samples were monitored following standard methods (APHA, 1995).

Dissolved oxygen (DO) and chemical oxygen demand (COD) were determined by standard methodology of Wetzel & Likens (1991) and Golterman et al. (1978), respectively. Primary productivity was monitored following dark and light bottle method (Vollenweider, 1974). All the fish from each tank were harvested at the end of 120 days and length and weight of the 10 fish from each treatment were recorded. Absolute growth rate and specific growth rate of *Labeo bata* were measured using the formula of:  $\Delta W = (W_t - W_i) / t$  and  $\text{SGR} = (\log W_t - \log W_i) \times 100 / t$  respectively, where  $W_i$  is the initial weight,  $W_t$  is the final weight and  $t$  is days on trial (Lugert et al., 2014). Observed data were statistically evaluated (SPSS, 20). To determine the treatment difference minutely, one way analysis of variance (ANOVA) was performed among treatments and days of sampling. The level of significance was accepted at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ .

Table1. Experimental protocol

a. Treatment variation					
Input	Experimental input	Control (C)	Treatment 1 (T <sub>1</sub> )	Treatment 2 (T <sub>2</sub> )	Treatment 3 (T <sub>3</sub> )
Poultry litter	Frequency of application	0	Monthly	Weekly	Daily
	Dose of application	0	45g	11.25g	1.5g
b. Common input					
Input	Common input	Control (C)	Treatment 1 (T <sub>1</sub> )	Treatment 2 (T <sub>2</sub> )	Treatment 3 (T <sub>3</sub> )
Lime	Frequency of application	Bimonthly after sampling in all the experimental tanks			
	Dose of application	37.5 Kg ha <sup>-1</sup> month <sup>-1</sup> in all the experimental tanks			

## RESULTS AND DISCUSSION

Interactions between water quality parameters and AOB and that in turn with the biological productivity of the aquatic system were established by the experiment. In the present investigation water temperature and dissolved oxygen (DO) were higher in control tanks than the treatment tanks. On the other hand free  $\text{CO}_2$ , carbonate alkalinity, bicarbonate alkalinity and chemical oxygen demand in all the treatments were greater than control (Table:2). Ammonia and nitrite accumulation beyond the assimilatory capacity of the particular system (Paul et al., 2020) is detrimental for the system where as nitrate is the mostly preferred form. Ammonia can be utilized by phytoplankton as the preferred N substrate, only when it is present within the desirable limit (Hargreaves, 1998). The unionized form of ammonia ( $\text{NH}_3$ ) is much more toxic than ionized ammonia ( $\text{NH}_4^+$ )

because of its capability to diffuse across cell membranes (Fernandes et al., 2010).

Transition between the two forms of ammonia greatly depends on pH, at pH 7.3 about 1% get unionized; at pH 8.3 about 10% of ammonia get unionized and at pH 9.3 about 50% of ammonia get unionized (Hargreaves, 1998). In the present experiment pH of all the tanks ranged between 7.59-8.54, thereby maintaining ammonia concentration supportive for fish growth. Both carbonate ( $\text{CO}_3^{2-}$ ) and bicarbonate ( $\text{HCO}_3^-$ ) alkalinity were observed in the treatment tanks whereas in case of control carbonate alkalinity was absent throughout the experiment. The net primary production in all treatments gradually increased with time in response to poultry litter load and was highest in T<sub>3</sub> followed by T<sub>2</sub>, T<sub>1</sub> and control.

Table 2. Mean±SE values of water quality parameters excepting pH (range values for pH) of control and treatments tanks throughout the period of investigation

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Temperature (°C)	22.84±5.35	21.69±6.17	22.14±6.26	21.73±6.37
pH of water	7.63-8.14	7.73-8.42	7.59-8.51	7.65-8.54
Free CO <sub>2</sub> (mgL <sup>-1</sup> )	16.56±6.99	28.74±4.78	29.78±5.26	30.81±5.89
Carbonate Alkalinity (mgL <sup>-1</sup> )	0	12.33±2.13	18.33±4.81	15.5±4.84
Bicarbonate Alkalinity (mgL <sup>-1</sup> )	242.21±14.83	273.38±21.02	279.74±32.94	280.82±34.75
*DO(mgL <sup>-1</sup> )	10.67±0.68	10.08±0.71	10.11±0.91	10.13±1.09
*COD(mgL <sup>-1</sup> )	31.21±4.50	45.12±9.94	47.74±14.73	47.92±16.08
Ammonium- Nitrogen (mgL <sup>-1</sup> )	0.203±0.0390	0.2707±0.0726	0.2741±0.0889	0.2711±0.0976
Nitrite- Nitrogen (mgL <sup>-1</sup> )	0.011±0.0074	0.0194±0.0104	0.0193±0.0123	0.0194±0.0128
Nitrate- Nitrogen (mgL <sup>-1</sup> )	0.344±0.0187	0.4339±0.0596	0.4431±0.0729	0.4483±0.0741
Ortho- phosphate (mgL <sup>-1</sup> )	0.038±0.0109	0.0594±0.022	0.0603±0.0276	0.0597±0.0293
*NPP(mgC/m <sup>3</sup> /hr)	201.25±12.65	285.12±30.28	300.25±27.28	298.56 ±35.25

\*DO=Dissolved Oxygen, COD=Chemical Oxygen Demand, NPP= Net primary productivity

### Abundance of Ammonia Oxidizing Bacteria (AOB):

Abundance of AOB in different treatments varied remarkably (Table: 3) with differential application frequencies of poultry litter @ 18,250 Kg ha<sup>-1</sup> yr<sup>-1</sup> (monthly, weekly, daily), throughout the 120 days period of experiment which were initially indifferent (P>0.05, ANOVA). From 4<sup>th</sup> to 120<sup>th</sup> day the AOB counts ranged between 9×10<sup>2</sup> cfu g<sup>-1</sup> to 42×10<sup>2</sup> cfu g<sup>-1</sup>, 24×10<sup>2</sup> cfu g<sup>-1</sup> to 103×10<sup>2</sup> cfu g<sup>-1</sup>, 18×10<sup>2</sup> cfu g<sup>-1</sup> to 138×10<sup>2</sup> cfu g<sup>-1</sup> and 17×10<sup>2</sup> cfu g<sup>-1</sup> to 144×10<sup>2</sup> cfu g<sup>-1</sup> in control (C), treatment-1 (T<sub>1</sub>), treatment-2 (T<sub>2</sub>) and treatment-3 (T<sub>3</sub>) respectively. The average count was maximum (68.79×10<sup>2</sup> ± 40.63×10<sup>2</sup> cfu g<sup>-1</sup>) in T<sub>3</sub>, followed by T<sub>2</sub> (67.12×10<sup>2</sup> ± 38.78×10<sup>2</sup> cfu g<sup>-1</sup>), T<sub>1</sub> (54.51×10<sup>2</sup> ± 21.40×10<sup>2</sup> cfu g<sup>-1</sup>) and lowest in control (20.36×10<sup>2</sup> ± 9.22×10<sup>2</sup> cfu g<sup>-1</sup>). Abundance of AOB within the range 780-2325 cells g<sup>-1</sup> in sediment was reported by Jana and Roy (1985).

Each AOB contains two or three *amoA* gene copies which code for the enzyme ammonia monooxygenase. Ammonia monooxygenase catalyses the oxygenation of ammonia to hydroxylamine. Quantitative analyses for AOB by RtPCR reported that the concentrations of the AOB *amoA* gene in the freshwater aquaculture pond sediments ranged from 4.05±3.83×10<sup>4</sup> to 3.11±1.65×10<sup>5</sup> copy g<sup>-1</sup> in China (Lu et al., 2015). In a constructed wetland *amoA* gene copy numbers of AOB in water samples during summer observed within the range of 5.3±0.6×10<sup>4</sup> copy mL<sup>-1</sup> to 8.1±0.5×10<sup>6</sup> copy mL<sup>-1</sup> (Sims et al., 2012). Although the systems as well as methodology were different but all the study concluded more or less similar range of AOB population.

Variability of AOB counts in all the treatments and control group were changed remarkably as time progressed. Temporal variation of AOB count was highest (8.01 folds) in T<sub>3</sub>, followed by T<sub>2</sub> (7.69 folds), T<sub>1</sub> (5.55 folds) and lowest (2.53 folds) in C during the period

of investigation. There was continuous increase in the population of AOB in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> from the initial day while lowest count of AOB was observed on 21<sup>st</sup> day of experiment in C. On the other hand maximum count was observed on 75<sup>th</sup> day of experiment in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and on 60<sup>th</sup> day of experiment in C.

The experiment clearly revealed that counts of AOB were increased with time due to gradual increment of organic inputs (poultry litter) in all the treatments whereas in control it was decreased until 21<sup>st</sup> day of the experiment and increased thereafter. In T<sub>1</sub>, administration of the monthly dose in a single application contributed significantly higher concentration of AOB until 15<sup>th</sup> day and then gradually declined up to 30<sup>th</sup> day and again increased subsequently as time progressed with the increment of organic input. On the other hand as split doses were applied in T<sub>2</sub> and T<sub>3</sub>, they received continuous supply of organic matter and the abundance of AOB was increased with time throughout the period of experiment. While after 90<sup>th</sup> day, as the temperature raised beyond 30°C that had a negative impact on the abundance of AOB and as a result the bacterial count declined up to 120<sup>th</sup> day in all the treatment tanks and also in control.

On 4<sup>th</sup> day there were significant differences between the C & T<sub>1</sub> (P<0.01, ANOVA), C & T<sub>2</sub> (P<0.05, ANOVA) and C & T<sub>3</sub> (P<0.05, ANOVA). During the first few days population of AOB in T<sub>2</sub> and T<sub>3</sub> were much lower than T<sub>1</sub> and the degree of differences were maximum on 11<sup>th</sup> and 15<sup>th</sup> days (P<0.01, ANOVA) while T<sub>2</sub> and T<sub>3</sub> were mostly alike (P>0.05, ANOVA). On 21<sup>st</sup> day for the 1st time after beginning, there were no significant (P>0.05, ANOVA) differences between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. On 30<sup>th</sup> day, the abundance of AOB was changed as lime was applied on day 11 which caused decrease of AOB in T<sub>1</sub> until the 2<sup>nd</sup> monthly application of poultry litter. On the other hand T<sub>2</sub> and T<sub>3</sub> received continuous supply of organic manure at weekly and daily intervals, respectively.

That is why the abundance of AOB in  $T_2$  and  $T_3$  exceeded the count of  $T_1$  on day 30. After 60<sup>th</sup> day even after manure application  $T_1$  failed to reach the AOB abundance of  $T_2$  and  $T_3$  and the magnitude of difference increased significantly ( $P < 0.01$ , ANOVA) up to 120<sup>th</sup> day of experiment. AOB population of C was much lower than  $T_1$ ,  $T_2$  and  $T_3$  and the degree of difference ( $P < 0.001$ , ANOVA) was increased day by day. The fact implies

Figure 1: Scanning electron microscopy of isolated AOB

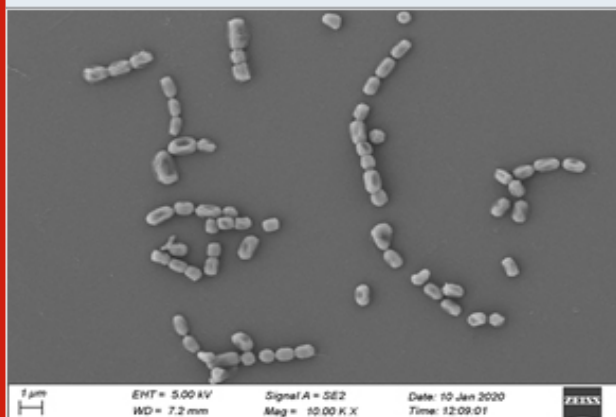
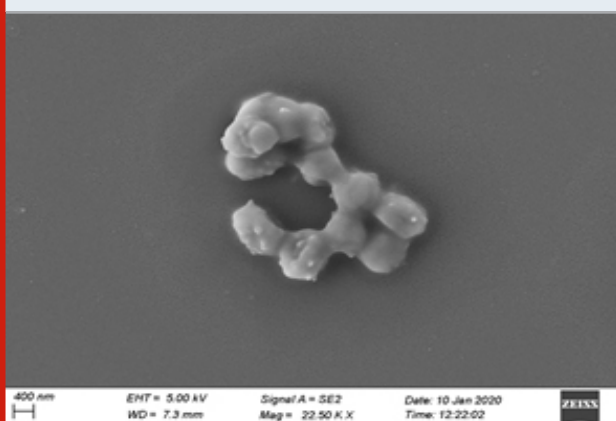


Figure 2: Scanning electron microscopy of isolated AOB



**Interaction between water quality and AOB:** As ammonia oxidizers obtain energy by oxidation of ammonia so ammonia concentration is likely to be correlated with AOB abundance (Jana and Roy, 1985). In the present study distribution pattern of AOB against treatment specific ammonia concentration was checked properly (Figures: 1, 2, 3, 4). In the present study, all the species of nitrogen (Ammonium-N, Nitrate-N, Nitrite-N) were positively correlated ( $p < 0.05$ ) with AOB abundance. Highest concentrations of ammonium-N and nitrate-N were observed in  $T_3$  followed by  $T_2$ ,  $T_1$  and control whereas highest concentration of nitrite-N was seen in  $T_2$  followed by  $T_3$ ,  $T_1$  and control. Concentrations of the various species of nitrogen were more or less similar in  $T_2$  and  $T_3$  which corroborated the similar findings regarding the abundance of AOB.

Probable reason of such distribution pattern of nitrogen is the application difference of poultry litter among the systems. Although doses were same for  $T_1$ ,  $T_2$  and

the impact of the application of poultry litter and the frequency of application in  $T_2$  and  $T_3$  were found to be beneficial over  $T_1$  ( $P < 0.05$ , ANOVA). This suggests that weekly dose of the poultry litter application ( $T_2$ ) is indifferent from AOB abundance of daily dose ( $T_3$ ) in fish tanks but weekly application is beneficial and viable as it requires less labour and time.

Table 3. Mean±SE values of AOB abundance in control and treatment tanks in different days of sampling during the period of investigation

Days	Ammonia oxidizing bacteria( $\text{cfu} \times 10^2 \text{ g}^{-1}$ )			
	Control	Treatment 1	Treatment 2	Treatment 3
0	15.67±2.05	16.67±1.25	17.33±1.25	17±0.82
4	13.00±1.63	26.67±2.05	19.67±1.25	18.67±1.25
7	13.33±2.49	34.33±2.49	24.33±2.49	23±1.63
11	12.67±1.25	43.00±3.27	28.67±1.70	26.67±2.05
15	10.67±1.25	63.33±4.9	36.67±2.05	38.33±2.62
21	10.00±0.82	52.00±3.74	45.00±1.63	48.00±2.16
30	22.67±3.86	48.33±2.49	86.67±7.76	90.33±5.73
45	35.00±2.45	78.00±5.10	97.67±5.44	110.33±2.87
60	39.67±1.70	73.67±4.99	117.33±5.31	120.33±4.99
75	28.00±2.45	92.67±8.58	133.33±4.64	136.33±5.44
90	22.67±1.25	70.67±3.68	101.33±7.36	102.00±5.35
105	21.00±2.94	64.00±6.16	84.00±4.08	83.00±3.27
120	20.67±2.49	45.33±5.44	80.67±2.87	80.33±6.02

$T_3$  but split dose of application resulted in continuous supply of the substrate in the system where as in case of monthly application substrate level started to fall after 15 to 21 day. The control system received no organic input and the only source of nitrogen was fish excreta and dead parts of plankton. So, ammonium-N, nitrate-N and nitrite-N were much less in control tanks than the treatments. In addition to that as the control systems did not have any source of phosphate so ortho-phosphate ranges of the control tanks were negligible throughout the experiment.

Figure 1: Relationship between ammonium nitrogen concentration and abundance of AOB in control tanks

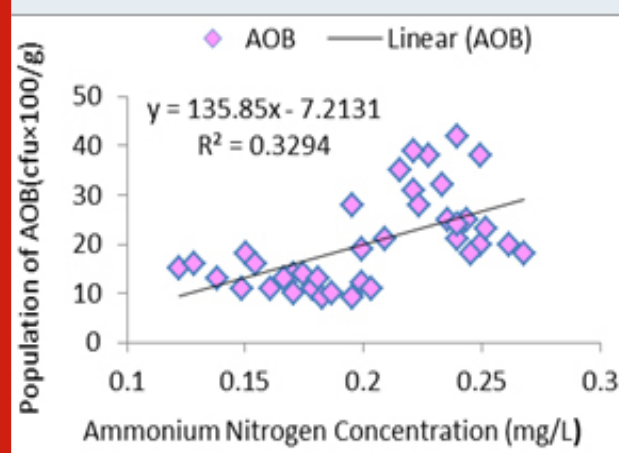


Figure 2: Relationship between ammonium nitrogen concentration and abundance of AOB in Treatment 1

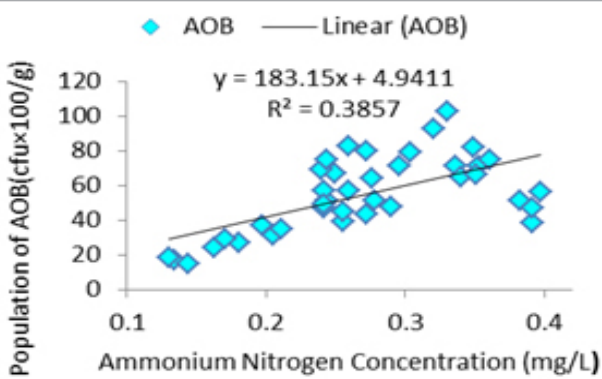


Figure 4: Relationship between ammonium nitrogen concentration and abundance of AOB in Treatment 3

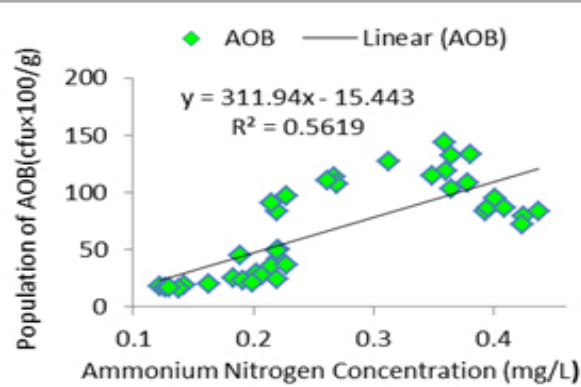
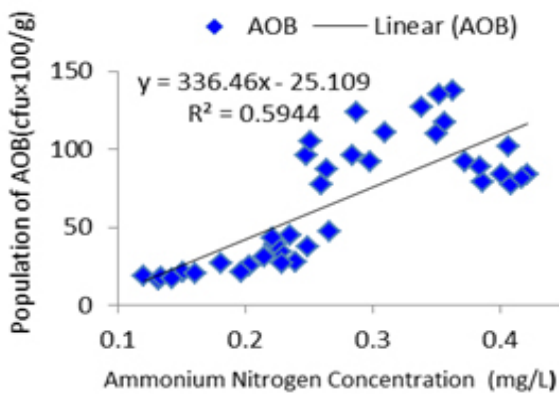


Figure 3: Relationship between ammonium nitrogen concentration and abundance of AOB in Treatment 2



formulated fish feed (Mondal et al., 2015). The present study also postulates the similar specific growth rate except the control system. Average specific growth rate of the C, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 0.75±0.08 %·d<sup>-1</sup>, 1.30±0.09 %·d<sup>-1</sup>, 1.43±0.10 %·d<sup>-1</sup> and 1.42±0.10 %·d<sup>-1</sup> respectively. The control system was devoid of nutrient supply as no organic input was there, which affected the fish growth. While T<sub>2</sub> and T<sub>3</sub> were mostly alike just like as the other parameters. Specific growth rate of *Labeo bata* was observed to be positively correlated (Figure: 7) with the abundance of AOB. That clarifies the fact of considerably higher growth rate in treatments according to their bacterial abundance and other nutrient parameters.

**Fish growth:** Absolute growth rate was significantly different (P<0.001, ANOVA) among control and treatments with highest specific growth rate in T<sub>2</sub> followed by T<sub>3</sub>, T<sub>1</sub> and lowest in control (Table: 4, Figure: 5). Specific growth rate of the fish also followed the similar pattern (Figure: 6). Specific growth rate of *Labeo bata* was observed within the range of 1.24±0.07 to 1.64±0.09 in an investigation with

Figure 5: Absolute growth rate of fish in control and treatments tanks

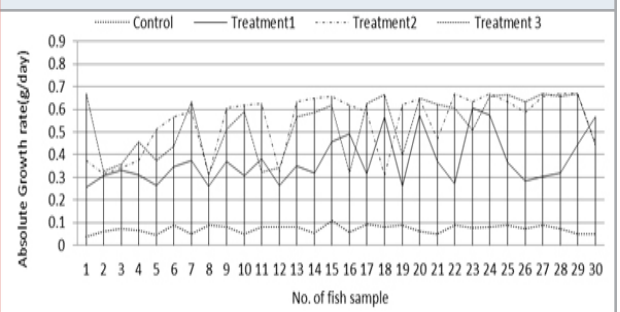


Table 4. Growth performance of *Labeo bata* in treatment and control tanks

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Mean initial weight (g)	5.23±0.18	5.23±0.18	5.23±0.18	5.23±0.18
Final weight (g)	9.87±2.09	46.11±12.92	66.79±15.35	64.84±15.59
Mean initial length (cm)	1.23±0.15	1.23±0.15	1.23±0.15	1.23±0.15
Final length (cm)	10.52±0.82	16.71±1.37	18.68±1.49	18.49±1.51
*AGR (d <sup>-1</sup> )	0.07±0.02	0.37±0.11	0.55±0.13	0.53±0.13
*SGR (%·d <sup>-1</sup> )	0.75±0.08	1.30±0.09	1.43±0.10	1.42±0.10
Survival rate (%)	82.22%	88.89%	93.33%	91.11%

\*SGR=Specific growth rate, \*AGR=Average growth rate.

Figure 6: Specific growth rate of fish in control and treatments tanks

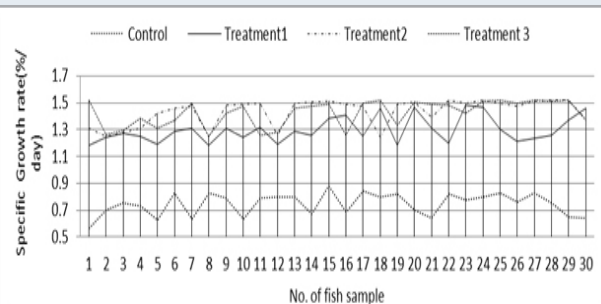
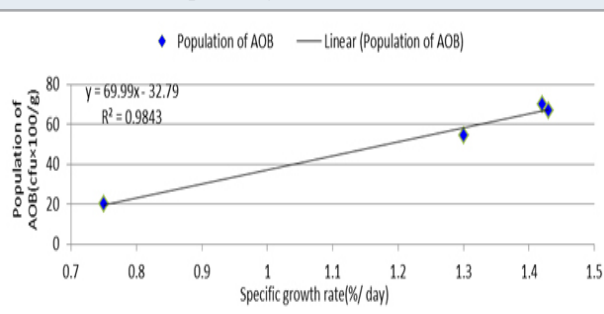


Figure 7: Relationship between treatment specific AOB abundance and Specific growth rate of *Labeo bata*



## CONCLUSION

It is evident from the investigation that AOB population developed maximally when all the Species of nitrogen (Ammonium-N, Nitrate-N and Nitrite-N) is relatively high. That is because of substrate dependency of AOB on ammonia and subsequent productions of nitrite which can be further get utilized by the nitrite oxidizing bacteria of the system. As a result increment of nitrite in the system can cause a better growth of planktons thereby enhancing fish growth. Previously it was seen that split dose of fertilizer is beneficial over a single annual dose (Jana et al., 2001a). The present study also revealed that split application of poultry litter at weekly intervals is beneficial over the application of poultry litter at monthly intervals. No remarkable difference regarding fish growth, water quality and AOB abundance were seen between daily and weekly application as both the systems had a continuous nutrient supply. In case of monthly application of poultry litter the nutrient gets exhausted after certain time, that results into a sudden drop after 21 day in each monthly cycle.

On the other hand, as AOB is highly temperature sensitive, the density of AOB drops in high temperature even after having plenty of substrate in the systems. Excess ammonia can be removed from the system by using nitrifying bacteria (Krishnani et al., 2009) thereby maintain the nitrogen budget of the system. In the present experiment positive relation between the abundance of AOB and specific growth rate of *Labeo bata* contributes to the similar finding of maintaining the nitrogen budget thereby enhancing fish growth. After all AOB needs our

special attention for further use in aquaculture to increase biological productivity. In addition to that optimum dose and mode of poultry litter application in fish farming ponds by means of integrated farming can be taken as a useful tool for sustained development and income generating practice for village people. So beside scientific findings this study also have a good social impact for betterment of the economic status of rural people.

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