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Assessment of bacterial loads and water quality parameters of three different types of fish pond

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Abstract. The present study was conducted to determine bacterial loads in the water columns and mud of three different pond systems,includingan overwintering fish fry pond,a brood fish rearing pond, anda recreational fish pond (L-shaped fish pond) situated at the Field Laboratory Complex of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.Three samplings were done atevery 15-day interval. Bacterial loads in water columns and mud were determined using the drop count method. Water quality parameters, including temperature, dissolved oxygen, pH, and ammonia, were also determined. Significantly ($p<.001$),the highest bacterial loads in three different water columns were observed in the overwintering fish fry pond, and the lowest bacterial loadswere found in the L-shaped fish pondat all sampling points. Bacterial loads in the bottom water were also significantly higher than those in the surface and middle layers of the water in all ponds. Pond mud was significantly ($p<.001$) richer in bacterial loads than pond water. Bacterial load was strongly correlated with temperature, and the correlation faded with the increase in depth of the water column.
Keywords: Bacterial load, Sediment, Water quality, Pond

Introduction

Bacteriology is one of the most important areas for determining the pond dynamics and the health and hygiene of the fish farming system. The bacteriological quality of water plays a vital role in the diseases spreading in farmed fish. In aquaculture, bacterial pathogens represent an important cause of fish infections and mortalities (Arifin*et al.* 2013). Bacteria are responsible for many of the dominant chemical processes that occur in fish ponds. They also help to maintain the delicate balance between the host and the environment. However, bacteria can also be primary or secondary pathogens and cause many infectious aquatic diseases (Lightner 1993). Bacteria in aquatic systems have been employed as an index of the abundance of the microbial community (Uddin and Al-Harbi 2004). Studies on bacterial populations in aquaculture are important in predicting the possibility of disease outbreaks and providing information for developing an optimal strategy for effective management to improve production and environmental conditions. Detailed information about the bacterial load in fish pond water is essential torecognizing and correcting the abnormal conditions of fish, which can be a prelude to the appearance of disease epizootics (Uddin and Al-Harbi 2004).

Water is the prime abiotic factor that supports life in this world. The physicochemical properties of water monitor the lives of aquatic organisms living in it. Any change in the water quality has a direct influence on biotic communities, where different species of flora and fauna exhibit great variations in their responses to the altered environment. Water quality is a dynamic web of physical, biological, and chemical factors that constitute the water environment and influence the production of fish and other aquatic environments (Boyd 1978, Piper *et al*. 1982).

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There is growing awareness of the influence of bacterial composition on the health and growth of fish (Uddin and Al-Harbi 2012,Razavilar*et al*. 2013). Recent interest in the microbial study of aquaculture products also increases the importance of knowledge of the microflora associated with fish and the water in which they live (Reilly and Kaferstein 1997). Bacterial load and bacterial type in fish ponds have received the attention of researchers (Otta*et al*. 1999,Naim*et al*. 2007). The objective of the study is to find out the bacterial loads and water quality parameters in three different types of pond water and sediment.

Materials and Methods

Three different pond systems,including an overwintering fish fry pond, a brood fish pond, and a recreational fish pond (L-shaped fish pond) situated at the Field Laboratory Complex of the Faculty of Fisheries, Bangladesh Agricultural University, were selected for the present study. Water samples were collected on day 1, day 15, and day 30 from the upper, mid, and bottom layers of the ponds. About 200 mL of water was collected in a sterile plastic bottle, transported to the Fish Disease Laboratory, BAU, Mymensingh, serially diluted using 0.85% physiological saline, and plated on Tryptone Soya Agar (TSA). The plates were incubated at 25°Cfor 48 h. The colonies were counted and expressed in colonyformingunit per mL (cfu/ml). Mud samples were also collected from the bottom of the ponds, and bacterial loads were determined as cfu/g. During every sampling point,water quality parameters, e.g., dissolved oxygen (DO) (mg/l), pH, and ammonia contents were also determined using commercial kits. Water temperature was measured by hand thermometer and denoted as °C.

Results

Bacterial load in fish pond water:Among the three ponds, the highest and lowest bacterial loads were observed in the overwintering fry pond and L-shaped fish pond, respectively, for all three sampling days (Table I). However, the bacterial load in the brood fish pond was significantly higher than in the L-shaped fish pond and lower than in the overwintering fry pond for all three sampling days.

A significant difference was found among the three ponds for all three columns. On all sampling days, it was observed that bacterial loads in the bottom water were significantly higher than those in the surface and middle layers of the water for all ponds (Table I). At day 30, the highest level of bacterial load $(52.6\pm3.9)\times10^5$cfu/ml was observed in the bottom water of the overwintering fish fry pond, while the lowest $(2.4\pm0.9)\times10^5$cfu/mlwas found in the surface water of the L-shaped fish pond. Throughout the study period, the highest andlowest bacterial loads for bottom water were $(52.6\pm3.9)\times10^5$cfu/mland $(11.9\pm1.6)\times10^5$cfu/ml, respectively; for the middle water column, they were $(33.9\pm2.9)\times10^5$cfu/mland $(3.9\pm0.9)\times10^5$cfu/ml, respectively; while for surface water,they were $(15.3\pm2.9)\times10^5$cfu/mland $(1.2\pm0.6)\times10^5$cfu/ml, respectively (Fig. 1).

Table I. Bacterial load (cfu/ml) in different water columns of different ponds

Experi-mental pond	Bacterial load (cfu/ml) x 10^5								
	Day 1			Day 15			Day 30		
	Surface water	Middle water	Bottom water	Surface water	Middle water	Bottom water	Surface water	Middle water	Bottom water
Over-wintering fish fry pond	(11.9± 1.24)	(16.57± 1.23)	(38.5± 2.24)	(12.6± 2.21)	(22.23± 1.98)	(43.9± 3.21)	(15.3± 2.9)	(32.9± 2.9)	(52.6± 3.9)
Brood fish pond	(6.3± 1.03)	(11.65± 1.98)	(28.3± 2.03)	(7.5± 2.02)	(15.8± 2.3)	(32.9± 3.02)	(9.2± 1.8)	(22.3± 2.6)	(34.1± 2.8)
L-shaped fish pond	(1.2± 0.6)	(3.9± 0.9)	(11.9± 1.6)	(2.3± 0.9)	(6.3± 1.07)	(12.3± 1.9)	(2.4± 0.9)	(7.8± 1.3)	(12.6± 1.9)
p value	.000	.021	.0361	.003	.005	.000	.000	.000	.000

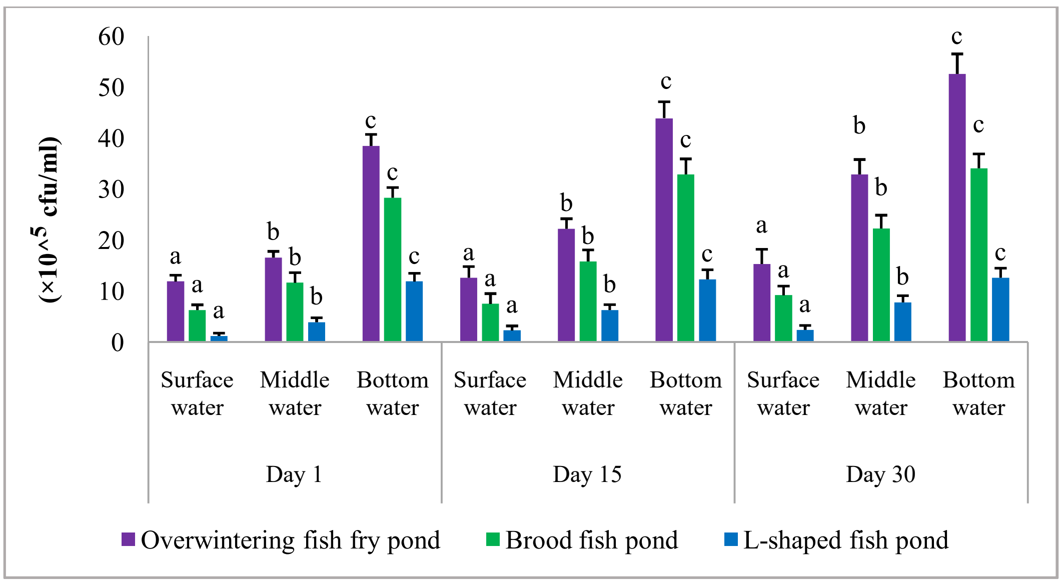

Fig. 1. Bacterial load (cfu/ml) of different water columns of different ponds.

Bacterial load (cfu/g) in pond mud:The highest and lowest bacterial loads in pond mud were (32.6±4.36)×10^6cfu/gin the overwintering fish fry pond on day 30 and (21.9±3.79)×10^6 cfu/gin the L-shaped fish pond on day 1, respectively (Table II). Though there was a significant difference between the overwintering fish fry pond and the brood fish pond, and the overwintering fish fry pond and the L-shaped fish pond, there was no significant difference between the last two ponds (Fig. 2).

Table II. Bacterial load (cfu/g) in fish pond mud

Experimental Pond	Bacterial load (cfu/g) x10^6		
	Day 1	Day 15	Day 30
Overwintering fish fry pond	$(26.5\pm4.56)\times10^6$	$(27.2\pm4.23)\times10^6$	$(32.6\pm4.36)\times10^6$
Brood fish pond	$(22.3\pm3.52)\times10^6$	$(22.9\pm2.53)\times10^6$	$(23.1\pm3.89)\times10^6$
L-shaped fish pond	$(21.9\pm3.79)\times10^6$	$(22.3\pm2.98)\times10^6$	$(22.6\pm2.72)\times10^6$
p value	0.363	.0279	<.001

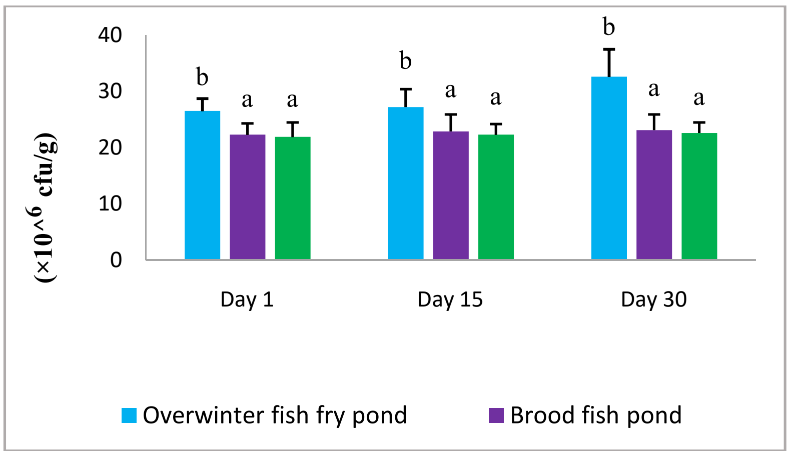

Fig. 2. Bacterial load (cfu/g) of pond mud.

Water quality parameters of different fish ponds:On day 30, the L-shaped fish pond showed the highest level of dissolved oxygen content (6.3±0.89 ppm) and temperature (24.9±1.89 ºC) than the other ponds, and the lowest level of pH (7.0±0.42) and ammonia content (0.02±.01ppm) was found here (Table III). The overwintering fish fry pond displayed the highest level of ammonia content (0.5±.01 ppm), but the lowest level of dissolved oxygen content (4.0±0.23 ppm). Besides, the brood fish pond showed the highest level of pH (8.1±0.78) and the lowest level of temperature (24.3±1.53 ºC). However, there was no significant difference in pH or temperature among the ponds.

Table III.Water qualityparameters of different fish ponds

Experimental pond	Days	Dissolved Oxygen (ppm)	pH	Ammonia (ppm)	Temperature (ºC)
Overwintering fish fry pond	1	4.5±0.56	7.8±0.98	0.3±.009	19.3±1.56
	15	4.4±0.29	7.7±0.75	0.33±.01	22.3±1.86
	30	4.0±0.23	7.8±0.46	0.5±.01	24.7±1.20
Brood fish pond	1	5.9±0.71	7.8±0.82	0.20±.006	19.2±2.01
	15	5.3±0.57	7.4±0.43	0.22±.008	22.6±1.75
	30	5.9±0.36	8.1±0.78	0.26±.01	24.3±1.53

L-shaped fish pond	1	6.3±0.89	7.6±0.73	0.01±.005	19.8±1.46
	15	6.7±0.52	7.6±0.62	0.02±.01	22.4±0.98
	30	6.8±0.56	7.0±0.42	0.02±.01	24.9±1.89

Influence of temperature on bacterial loads: It was found that temperature had a significant influence on the bacterial composition of surface water but not on pond mud. As depth increased, the impact of temperature decreased (r = 0.025). In the case of surface water, temperature posed a strong and significant positive correlation with the bacterial load (Table IV). A moderate but strong relationship was observed for the middle layer, while a very poor impact was observed for the bottom water and mud. This indicates that, with increasing depth, temperature could do very little for microbial growth in water.

Table IV. Correlation between temperature and bacterial load of pond water and mud

Bacterial load						
		Temp-erature	Surface water	Middle water	Bottom water	Mud
Temperature	Pearson Correlation	1				
	Sig. (2-tailed)					
Bacterial load in surface water	Pearson Correlation	0.61	1			
	Sig. (2-tailed)	0.04				
Bacterial load in middle water	Pearson Correlation	0.628	.939**	1		
	Sig. (2-tailed)	0.250	0.000			
Bacterial load in bottom water	Pearson Correlation	0.52	.988**	.946**	1	
	Sig. (2-tailed)	0.696	0.000	0.000		
Bacterial load in mud	Pearson Correlation	0.285	.860**	.851**	.832**	1
	Sig. (2-tailed)	0.457	0.003	0.004	0.005	
** Correlation is significant at the 0.01 level (2-tailed)						

Discussion

Bacterial load is an important indicator of aquatic health, disease management, and understanding the microbial dynamics of aquatic environments, particularly aquaculture ponds. In this study, bacterial loads were determined in the water and sediment of anoverwintering fish fry pond, a brood fish pond, and a recreational L-shaped fish pond used for fish angling. The water taken from the pond bottom was highly abundant with bacterial populations. However, regardless of pond type and culture system, pond sediments had significantly higher bacterial loads when compared to water columns. In the mud, bacterial load was found in a range between $(21.9\pm3.79)\times10^6$ cfu/g and $(32.6\pm4.36)\times10^6$ cfu/g, while $(52.6\pm3.9)\times10^5$cfu/ml was the highest bacterial load observed in water. Similarly, Trakroo and Agarwal (2011)found total viable counts of bacteria were $(4.90\pm0.06)\times10^3$cfu/ml in pond water and $(3.50\pm0.07)\times10^4$ cfu/g in sediment during a study on the bacterial flora of farm-raised common carp in India.Bisht*et al.*(2014) studied bacterial populations associated with farmed common carp fingerlings during the winter and summer seasons and found that the bacterial count in the pond sediment ($6.40x10^4$)cfu/g was about 10 times higher in comparison tothe pond water ($6.93x10^3$)cfu/g.The sediment was a more appropriate medium to grow and thrive for bacteria than the water column because it was less influenced by perturbations caused by rainfall and transient movements of waterborne substances. In sediments, organic enrichment and the consequent modification of the characteristics of the benthic environment determined an increase in the bacterial population (Sultana *et al*. 2021). As a result, higher organic matter production in a highstocking-density commercial culture system influenced the size and composition of the microbial population more than in the other two pond systems. With the increasing days of culture, the bacterial load in sediment also increased but was not significantly related to the influence of rising temperatures.

Bacterial load was found by Faruk*et al.* (2018) in a range between 2.36×10^6 cfu/ml and 4.61×10^6cfu/ml in the three tilapia ponds during a production cycle in a commercial farm.In the present study, the bacterial load was found to be in a range between $(1.2\pm0.6)\times10^5$cfu/ml and $(52.6\pm3.9)\times10^5$ cfu/ml in the water of the three ponds. The highest bacterial load was observed in the overwintering fish fry pond for all sampling days, ranging from $(11.9\pm1.24)\times10^5$cfu/ml to $(52.6\pm3.9)\times10^5$cfu/ml in water, which was also evident in studies carried out by Gołaś*et al*. (2002) and David *et al*. (2015). However, these bacterial loads were higher than David *et al*. (2015) but lower than Gołaś*et al*. (2002). In the case of the three ponds, the bacterial load was lowest in the L-shaped fish pond, where fish culture was done on an extensive basis. L-shaped fish ponds acted as a natural ecosystem that proliferated the growth of bacteria in a nature-based system. As a result, the bacterial count remained in a suitable condition, as stated by Thornber*et al*. (2020).

With the increasing time of sampling, the bacterial load increased in every pond, as there was a strong correlation between water and water quality parameters. In the present study, recorded DO levels were found within the optimum range (4-7 ppm), which supports the findings observed by Abedin*et al*. (2017) and Roy andBarat (2011). The optimum DO level is important for the success of intensive aquaculture. However, the lowest level of DO was observed in overwintering fry pond, indicating the highest level of biological oxygen demand in these ponds. In ponds where the DO level was lowest, the bacterial load was highest throughout the study period, which indicates a strong negative correlation between DO and bacterial loads. However, there was no strong relationship between the bacterial load and the pH of the fish pond. In the commercial pond, the ammonia level remained high, though the bacterial load was higher, which was hoped to degrade ammonia into nitrate or nitrite.

Temperature also followed an increasing trend, which stimulated higher microbial growth in pond water and sediment but was in the optimum range for fish production. There are reports that temperatureincreases the bacterial load in aquatic ecosystems. Among many studies, Al-Harbi and Uddin (2006) reported the same trend of bacterial floral count with increasing temperature. From the correlation study, it was seen that bacterial load was strongly correlated with temperature, and the correlationfaded with the increasing depth of the water column.

The result of this study indicated that aquaculture operations facilitate more microbial growth in aquatic environments, possibly from feed and feces waste. As a result, overwinteringfish fry pondswere found to harbor a higher bacterial load in the water and soil than the brood fish ponds and L-shaped fish ponds, where the semi-intensive form of aquaculture was practiced. However, considering the depth, it was observed that the bottom water contained a higher bacterial load than the surface water, as most of the bacterial community preferred to stay either in the soil or near the soil column. Moreover, with increasing days of the sampling period, the bacterial loads in the water and pond mudgrew faster because more decomposed materials settled in the bottom and were suspended on the water's surface. The L-shaped fish pond with its outstanding water quality showed the highest DO content, but ammonia was significantly poorer than in the other two ponds. However, with an increasing timeframe, ammonia in all ponds showed an increasing trend, while others showed cluttered trends. Surface bacterial load was influenced to some extent by the temperature, but with increasing depth, the correlation became weaker, and for the bottom, there was a very poor relationship between the temperature and bacterial load. Much progress has been made through this experiment, and hopefully, it would make sense to widen the gateway to future investigations. It still possessed some limitations, based on which this research suggested further studies to delve into insights from molecular and physiological perspectives.

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