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Quantitative assessment of bacterial flora during a production cycle in commercial farm raised tilapia

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Abstract. Study of bacterial flora is one of the important aspects of assessing the pond dynamics and fish health in farming systems. The present study determined bacterial load in pond water, sediment and gill of farmed tilapia (*Oreochromis niloticus*) throughout a 60 days production cycle in a private farm of Trishal, Mymensingh. Around 30 g sized tilapia fingerlings were stocked in three ponds at a stocking density of 6,1000 fingerling/ha. Sampling for bacteriology and water quality was done every 15 days intervals. No particular trend was observed in bacterial load in either pond water, sediment or gill of fish throughout the study. Highest bacterial load in pond water was $5.83 \pm 4.48 \times 10^6$cfu/ml found at day 30. In the pond sediment, bacterial load was $3.50 \pm 0.50 \times 10^7$cfu/g observed at the end of the experiment on day 60. In fish gill, average bacterial load among the ponds ranged between $1.81 \pm 2.32 \times 10^7$ and $2.81 \pm 3.62 \times 10^7$cfu/g. Water quality parameters including temperature, NO_3, NO_2, PO_4, NH_4 and DO of pond water revealed that they were within the suitable range for the freshwater fish culture throughout the farming phase.

Key words: Bacterial flora, Tilapia, Water quality.

Introduction

Tilapia culture has expanded rapidly in a wide range of farming environments from extensive to intensive in both fresh and brackish water in Asia including Bangladesh and many other countries of the world. It is now one of the favorite food fishes in Bangladesh. There has been a tremendous progress of tilapia farming all over the country and the production increased from 2,140 mt in 1999 to about 423,000 mt in 2015. There are over 6000 medium and large scale commercial farms involved in the production of table size tilapia. Tilapia comprises about 12.5% of total aquaculture production in Bangladesh (Hussain *et al.* 2014). In intensive pond culture of tilapia, the bacterial community may contribute to food web. They may be eaten directly by tilapia. Moreover, the nitrogen and phosphorus may be recycled to stimulate primary production through the activity of decomposed microorganism (Moriarty 1997). Bacteriology is one of the most important areas for determining the pond dynamics and health and hygiene of fish farming system. Aquatic microorganisms influence the water quality and are closely associated with the fish physiology and diseases. The success of practical aquaculture depends on water quality which is greatly influenced by aquatic microorganisms. There is growing awareness of the influence of bacterial composition

of fish on the health and growth of the host (Uddinand Al-Harbi 2012; Razavilar *et al.* 2013).

Fish obtains majority of the bacteria into the gut and skin from water, sediment and feed as fish lives in water and some burrow in soil (Sugita *et al.* 1988). The bacterial contents of the water used for growing fish affects the quality of the fish and fish products produced. Frazier and Westhoff (1978) stated that the bacterial flora of living fish depends upon the microbial content of water in which they live. Some normal bacterial flora of water can be found on the body surface or in the intestines of fish and may cause disease epizootics under environmental stress. Thus, environment plays a crucial role in disrupting the balance between the host and the pathogen. Bacteria in aquatic systems, specially, freshwater systems have been employed as an index of abundance of the microbial community. The bacterial load and composition may change with age, individuals, nutritional status, environmental conditions, and the complexity of the fish digestive system (Uddin and Al-Harbi 2004).Studies on bacterial population 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 condition. Detailed information about the bacterial load in fish culture pond water is essential in order to recognize and correct the abnormal conditions of fish which can be a prelude to the appearance of disease epizootics. As, there is dearth of information on the bacterial load in farmed tilapia in Bangladesh, the objective of the present study was therefore to determine the bacterial load in pond water, sediment and gills of fish during a production cycle of tilapia.

Materials and Methods

Study sites: This experiment was conducted in a private tilapia farm named Abdul Halim Tilapia Farm in Trishal Upazilla under Mymensingh district. Three ponds of sizes 4048 m^2 (Pond 1), 2631 m^2 (Pond 2), and 1821 m^2 (Pond 3) with an average depth of 1.5 m used to grow marketable sized tilapia were selected. These ponds were completely dependent on the ground water supply throughout the production cycle.

Culture strategy: About 35 days old hatchery reared tilapia fingerling were collected from a private hatchery and these fingerling were reared in nursery ponds for 60 days. Before releasing fingerling, the ponds were prepared using 250 kg lime, 1250 kg cow dung, 25-35 kg urea and 12.5 kg TSP per ha. After 5-7 days, around 30 g body weight fingerlings were stocked in the stocking pond at the rate of 6,1000 fingerling/ha. We changed the water of the pond daily at the rate of 5% when the fish reached more than 100g body weight. We fed the fish with commercial tilapia feed at 8-10% body weight, 3-4 times a day at the beginning, 6-8% body weight 3-4 times a day when they reached 50-100g, 5-6% body weight 3 times a day when they were 100-200 g size, 1.5-4% body weight 3 times a day when they were 200gsize. Fish was harvested after two months of culture when they reached around 250-300 g size.

Sampling for bacteriological analysis: We determined bacterial load in pond water, sediment and in fish gills at every 15 days interval for 60 days. We prepared Tryptone soya agar (TSA, Hi-media) having pancreatic digest of casein 15g, enzymatic digest of soya bean 5 g, agar 15 g per 500g following standard procedure.

Determination of colony forming unit (cfu/ml or cfu/g): Bacterial load was determined using serial dilution technique, expressed as colony forming unit (cfu/ml or cfu/g) and determined using drop count method described by Miles and Misra (1938). Cfu/ml or cfu/g was counted by using following formulae:

Cfu/ml or cfu/g = Average no. of colonies × dilution factor ×50

Recording of water quality parameter: Water quality parameters such as temperature, dissolved oxygen, ammonium, nitrate and phosphate were measured by using a commercial test kit (SERA Test Kit, Sera GbaH D 52518 Heinsberg, Germany) and recorded throughout the experimental period.

Results

Bacterial load (cfu/ml) in pond water: We noticed slight variations in bacterial loads of pond water during the study period. The highest mean bacterial load of $5.8 \pm 4.48 \times 10^6$cfu/ml was found at 30th day and lowest average load of $1.93 \pm 2.65 \times 10^6$ cfu/ml (Table I) was found at 60th day of sampling. Among the three ponds the highest average bacterial loads was seen in Pond 2 (4.61×10^6cfu/ml) while the lowest load of (2.36×10^6cfu/ml) was observed in Pond 1.

Table I. Bacterial load in pond water

Days	Bacterial load (cfu/ml)			
	Pond 1	Pond 2	Pond 3	Mean ± SD
0	3.4×10^6	3.2×10^6	3.5×10^6	$3.36 \pm 1.52 \times 10^6$
15	4.5×10^6	3.5×10^5	3.2×10^6	$2.63 \pm 2.12 \times 10^6$
30	3.0×10^6	1.1×10^7	3.5×10^6	$5.83 \pm 4.48 \times 10^6$
45	4.5×10^5	3.5×10^6	5.0×10^6	$2.98 \pm 2.31 \times 10^6$
60	3.5×10^5	5.0×10^6	4.5×10^5	$1.93 \pm 2.65 \times 10^6$
Mean ± SD	$2.34 \pm 1.854 \times 10^6$	$4.61 \pm 3.588 \times 10^6$	$3.13 \pm 0.770 \times 10^6$	

Bacterial load (cfu/g) in pond sediment: The highest mean bacterial load of $3.50 \pm 0.50 \times 10^7$cfu/g was observedat 30th day and lowest average load of $1.50 \pm 1.73 \times 10^7$ cfu/g was calculated at 15th day of sampling (Table II).During sampling, Pond 3 had the highest average load (2.74×10^7cfu/g) followed by Pond 2 (1.82×10^7) and Pond 1 (1.38×10^7cfu/g).

Table II. Bacterial load in pond sediment

Days	Bacterial load (cfu/g)			
	Pond 1	Pond 2	Pond 3	Mean ± SD
0	4.5×10^6	3.0×10^6	4.4×10^7	$1.72\pm2.32\times10^7$
15	4.5×10^6	3.5×10^7	5.6×10^6	$1.50\pm1.73\times10^7$
30	1.2×10^7	9.5×10^6	8.0×10^7	$3.39\pm3.99\times10^7$
45	4.5×10^7	3.5×10^6	4.2×10^6	$1.75\pm2.37\times10^7$
60	3.0×10^6	4.0×10^7	3.5×10^6	$3.50\pm0.50\times10^7$
Mean ± SD	$1.38\pm1.779\times10^7$	$1.82\pm1.789\times10^7$	$2.74\pm3.401\times10^7$	

Bacterial load (cfu/g) in fish gill: In fish gills, the highest mean bacterial load of $2.81\pm3.62\times10^7$cfu/g was observed after 60th day and lowest average load of $1.81\pm2.32\times10^7$cfu/ml was recorded at the starting day of sampling (Table III). Among the ponds, both highest (8.5×10^6cfu/g) and lowest (4.5×10^6cfu/g) bacterial load in fish gills were noticed in Pond 3 at 60th and 15th day of sampling respectively. Average highest bacterial load was recorded in Pond 2 was 2.88×10^7 and lowest was 1.75×10^7 in Pond 3.

Table III. Bacterial load in fish gill

Days	Bacterial load (cfu/g)			
	Pond 1	Pond 2	Pond 3	Mean ± SD
0	4.5×10^7	4.5×10^6	5.0×10^6	$1.81\pm2.32\times10^7$
15	5.0×10^7	4.5×10^6	4.5×10^6	$1.97\pm2.62\times10^7$
30	5.5×10^6	5.0×10^6	6.5×10^7	$2.51\pm3.44\times10^7$
45	4.5×10^6	6.0×10^7	4.5×10^6	$2.30\pm3.20\times10^7$
60	6.0×10^7	7.0×10^7	8.5×10^6	$2.81\pm3.62\times10^7$
Mean ± SD	$3.3\pm2.6127\times10^7$	$2.88\pm3.3235\times10^7$	$1.75\pm2.6606\times10^7$	

Water quality parameters of fish ponds: The temperature range was recorded 27-30°C during the sampling period. However, the amount of DO ranged from 4-5 mg/l, Nitrite and Nitrate were calculated 0.3 mg/l and PO_4 was found around at 1 mg/l from three different ponds.

Discussion

The success of fish culture systems highly depends on various environmental factors as well as microorganisms resides in pond water and sediment. In the present study, bacterial flora was determined in water, sediment and gills of tilapia from grow out ponds. Variations in bacterial loads were observed throughout 60 days study period which did not show any particular trend. Quite a high number of bacteria were

observed here in the tilapia cultured ponds. Maximum bacterial load in pond water was $5.83 \pm 4.48 \times 10^6$cfu/ml recorded at the middle stages of culture systems. Bacterial load was found in a range between 2.36×10^6 cfu/ml and 4.61×10^6cfu/ml in the three ponds.

The success of intensive culture of tilapia depends on water quality in pond which is influenced by native micro flora. Intensive production usually leads to an increase in disease due to poor water quality and high stocking densities of species. In the present study, water quality parameters were in the suitable range throughout the culture period. Dissolved oxygen ranged from 4-5 mg/l throughout the study period. Nitrite and nitrate remained same and it was calculated 0.3 mg/l. Phosphate level was also found quite same in three different ponds. The water temperature during the sampling period ranged from 27-30°C. No particular correlation was thus found between bacterial load and water quality parameters.

Braz and Biol (2013) stated the levels of total bacteria in the water varied between 1.3×10^4 and 67.3×10^4cfu/100 ml during microbiological parameters study of reservoir water used for irrigation and culture of tilapia in floating net cages. Uddin and Al-Harbi (2004) reported that total viable count of bacteria in a tilapia pond water was ranged from $7.8 \pm 0.9 \times 10^3$ to $1.3 \pm 1.1 \times 10^4$cfu/ml in Spring, $9.2 \pm 1.7 \times 10^3$ to $6.4 \pm 1.3 \times 10^4$ cfu/ml in Summer, $5.1 \pm 1.7 \times 10^3$ to $2.2 \pm 1.0 \times 10^4$cfu/ml in Fall and $6.7 \pm 2.1 \times 10^2$ to $2.5 \pm 0.6 \times 10^3$cfu/ml in Winter. Similar observation was reported by Sharmila *et al.* (1996) where they found mean total viable bacterial counts in semi intensive pond water 1.8×10^3- 4.5×10^3cfu/ml in India. Banu *et al.* (2001) observed that the mean bacterial load in surface water varied from 1.39×10^5 to 3.11×10^7cfu/ml while that of bottom water ranged from 1.01×10^6 to 5.90×10^7 cfu/ml which was relatively similar to the present study.

The organic matter content in pond sediment may gradually increase due to the feed input and fertilizer. The uneaten feed that accumulate at the bottom of pond has a significant role in increasing bacterial load in pond sediment. Bacterial load in pond sediment has an important role in improving water quality because of decomposing organic matter and reducing harmful bacteria in sediments. In the present study, the highest bacterial count ($3.50 \pm 0.50 \times 10^7$cfu/g) in pond sediment was observed at the later stages of culture cycle. Dhanasevi (2014) reported total bacterial count in sediment ranging from 6.49×10^5 to 1.29×10^6cfu/g during a study of three intensive tilapia ponds in Hai Duong province, Vietnam. Al-Salim *et al.* (2009) estimated total viable bacterial counts ranging from $2.8 \pm 1.9 \times 10^1$ to $7.0 \pm 2.2 \times 10^3$cfu/ml in ponds water while $4.2 \pm 2.2 \times 10^4$ to $3.8 \pm 2.5 \times 10^6$cfu/g in ponds sediments. The present study showed the bacterial load in pond sediment was higher among these reported observation. However, bacteria counts observed in the present study were lower than the results reported by Al-Harbi and Uddin (2003). They reported that the quantity of bacteria in hybrid tilapia pond sediment ranged from $9.3 \pm 1.1 \times 10^6$ to $1.9 \pm 1.5 \times 10^8$cfu/g.

Another study of Al-Harbi and Uddin (2005) showed that the bacteria loads in brackish water tilapia pond sediment ranged from $1.2\pm3.1\times10^6$ to $7.3\pm1.1\times10^7$cfu/g.

Fish gill is a delicate site for bacterial colonization. Gill filaments are directly involved in respiration systems and bacterial content easily affect the circulatory systems of a fish body. Bacterial loads in fish gills were also studied and found the bacterial load in fish gill ranged from $1.81\pm2.32\times10^7$ to $2.81\pm3.62\times10^7$cfu/g. High metabolic rate and bacteria in water had a reflection on the bacterial composition of the fish gill. The influence of physiological stress and environmental factors on the variation of bacterial load in the gill filaments were also important considerations. Ingestion of free-living bacterial community under natural and stable conditions may possibly lead to the establishment of variation of bacterial load in gill filaments. Al-Harbi and Uddin (2005) reported that the presence of a high bacterial load in gill and intestine of fish might be due to high metabolic activity of fish associated with increased feeding rates at higher temperature. They also reported that pond water and sediment bacteria influenced the bacterial composition of gills and intestine of tilapia. Uddin and Al-Harbi (2012) observed that the total viable bacterial counts in gill filaments of carp and catfish ranged from $3.3\pm3.8\times10^6$ to $7.9\pm5.6\times10^6$ and $1.1\pm4.6\times10^5$to $2.3\pm5.2\times10^6$cfu/g respectively which were quite similar to the present study. The results of the present study were also supported by the findings of Al-Harbi and Uddin (2003) where total bacterial load was $3.4\pm1.8\times10^6$ to $5.8\pm0.4\times10^7$ cfu/g in the intestine and $7.1\pm0.7\times10^5$ to $8.7\pm1.1\times10^6$cfu/g in the gills. It was noticed that bacterial density was higher in pond water than in the sediment. Water quality parameters were in the suitable range throughout the culture systems. Further study should include determination of both qualitative and quantitative bacteria that would give a clear picture of bacterial flora in commercial tilapia ponds in Bangladesh.

Literature Cited

Al-Harbi, A.H. and M.N. Uddin, 2003. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquac. Res.,* 34(1): 43-48.

Al-Harbi, A.H. and M.N. Uddin, 2005. Bacterial biodiversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture,* 250: 566-572.

Al-Salim, N.K., N.R. Khamees and K.S. Al-Niaeem, 2009. Intestinal bacteria in the University of Basrah fish ponds, Iraq. *J. Al-Anbar Univ. Pure Sci.,* 3(1): 1-7.

Banu, A.N.H., M.A. Islam and M.B.R. Chowdhury, 2001. Bacterial load in pond water and different organs of a Indian major carp *Cirrhinus mrigala* Ham. *Bangladesh J. Fish. Res.,* 5(1): 53-58.

Braz, J. and S.C. Biol, 2013. The influence of fish culture in floating net cages on microbial indicators of water quality. *Braz. J. Biol.,* 73(3): 1519-6984.

Dhanasevi, K.R., 2014. Population of bacteria in tilapia (*Oreochromis niloticus*) culture pond deposit in Vietnam. *Adv. Aquac. Fish. Manage.,* 3(1): 217-222.

Frazier, W.C. and D.C. Westhoff, 1978. Food Microbiology, 3rd Edition. McGraw-Hill Book Co. 540 p.

Hussain, M.G., B. Kumar Barman, M. Karim and E.H. J. Keus, 2014. Progress-and-the-future-for-tilapia-farming-and-seed-production-in-bangladesh. www.thefishsite.com/articles/1885/

Miles, A.A. and S.S. Misra, 1938. Estimation of the bactericidal power of the blood. *J. . Hyg.*(Lond.), 38: 732–749.

Moriarty, D.J. W., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture,* 151(1-4): 333–349.

Razavilar, V., M.R. Khani and A.A. Motallebi, 2013. Bacteriological study of cultured silver carp (*Hypophthalmicthys molitrix*) in Gilan province, Iran. *Iranian J. Fish. Sci.,* 12(3): 689-701.

Sharmila, R., T.J. Abraham and V. Sundararaj, 1996. Bacterial flora of semi-intensive pond-reared *Penaeus indicus* (H. Milne Edwards) and the environment. *J. Aquac. Trop.* 11: 193-203.

Sugita, H., M. Tsunchara, T. Ohkoshi and Y. Deuchi, 1988. The establishment of intestinal microflora in developing gold fish (*Carassius auratus*). *Microbiol. Ecol,.* 15: 333-344.

Uddin, M.N. and A.H. Al-Harbi, 2012. Bacterial flora of polycultured common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*). *Int. Aquat. Res.*, 4: 1-10.

Uddin, M.N. and A.H.Al-Harbi, 2004. Seasonal variation of bacterial flora in ponds in Saudi Arabia used for tilapia aquaculture. *J. Appl. Aquac.,* 16: 53-61.

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