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Bacterial content in fish, water and sediments of different fish ponds in the Patuakhali Science and Technology University (PSTU) campus: a food safety aspect for the consumers in PSTU

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ABSTRACT

The present study was to investigate the bacterial content in water, sediments and fish samples and to determine the water quality parameters (water temperature, dissolve oxygen, pH and transparency) in different ponds (Lalkomol, Nilkomol, Salsabil, Jalpoddo, Tarangatonu and Nildighi) in Patuakhali Science and Technology University (PSTU) campus, in the consecutive three season (rainy, winter and summer). Water quality parameters were determined using HANNA test kit and bacteriological study was conducted following standard plate count (SPC) method. Different water quality parameters in different seasons among the different ponds in PSTU campus were significantly correlated with each other's. Air temperature showed strongly positive relationship with water temperature and water temperature showed positive relationship with transparency, pH and DO. Transparency showed positive relationship with pH and negative relationship with DO. On the other hand pH showed positive relationship with DO. Among the three samples and three seasons for the study, in fish sample and summer season had higher bacterial load than other sample and seasons. All fish, water and sediment sample in different rainy, winter and summer seasons, the total viable count of bacteria in fish were higher than the recommended limit for fish. Improve water quality, proper hygiene and sanitation steps should be taken into consideration to reduce bacteria thus for public health safety especially for the employee and people adjacent to PSTU.

Keywords: Bacterial load, water, fish, sediment, water quality.

INTRODUCTION

Fish and fisheries have been playing an important role in addressing nutritional and livelihood security, especially for the poor in the developing countries. Globally, over 3.1 billion people get at least 20% of their animal protein intake from fish (FAO, 2016). In some Asian countries, it could be much higher 58, 63 and 75 in Indonesia, Bangladesh and Cambodia, respectively (Delgado et al., 2003). In addition, contributing to nutritional security, aquaculture has been providing livelihood improvement millions of people across the world (Siddiq et al., 2013). Fisheries sector is also important in agro-based economy of Bangladesh, contributing 60% of total animal protein intake, 3.69% to the national GDP and 23.12% to the agricultural GDP, providing full time employment to 1.28 million and part time work to 12 million people and contributing 1.92% to the total export earnings (DoF, 2016). The total fish and fisheries production in 2014–2015 was 3.6 million metric tonnes, of which 47.71% was from inland culture, 17.84% was from marine capture and 34.45% was from inland capture fisheries (DoF, 2016).

The southern districts of Bangladesh specifically Patuakhali district is rich in both inland open and closed water fisheries resources. Due to geographical location, the water bodies of this region are highly productive and aquaculture is an important commercially viable activity. The main rivers namely Paira, Bishkhali, Lohalia, Andarmanik, Agunmukha and some other canals are available in this region, which are important freshwater fisheries resources for inland capture fishery production of Patuakhali district (Ali et al., 2014). Besides freshwater resources, Patuakhali region also has vast estuarine and marine water from the Bay of Bengal. Moreover, to further increase the culture based fisheries production in this southern region water quality parameter and bacterial content considered as an important factor because the bacterial contents affects the quality of the fish and fish products produced (Gray, 2005).

Patuakhali Science and Technology University (PSTU) campus is situated at Dumki upazilla in Patuakhali district. There are 6 (six) ponds situated at PSTU campus under the faculty of fisheries. The fish from these ponds are important source of animal protein supply to the entire employee at PSTU. Moreover, most of the employees are very selective to choose or buy fish from some fish pond due to poor water quality and off flavor found in fish. Success of aquaculture almost completely depends on the different water quality parameters (Davis et al., 2009). The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. Good water quality is characterized by adequate oxygen and limited levels of metabolites. This is because large quantities of feed is loaded in ponds and excess feed, fecal matter and then metabolites can cause drastic changes in water quality parameters and sediment chemistry which may affect the growth of fish (Kadir et al., 2007). As fish is a cold-blooded animal, its growth, reproduction, maturity and survival mostly depends on water temperature. Inadequate maintenance of water quality might cause severe problems in fish production (Roy, 2001). The main water quality parameters (physico-chemical) are transparency, temperature, dissolved oxygen, pH, ammonia etc. All of these parameters are essential for cultured organisms (DoF, 2009).

Water quality parameters and microbial load are closely interrelated with each other. Aquatic microorganisms influence the water quality and are closely associated with the fish physiology and diseases. The bacterial content of the water used for growing fish affects the quality of the fish and fishery products production. The bacterial content of the sediments also affects the quality of fish and fish products. Evidence indicates that the gastrointestinal micro flora of fish is highly variable and is a reflection of their aqueous environment especially the food and sediments. Knowledge about bacterial community structure and diversity is essential to understand the relationship between environmental factors and ecosystem functions. Such Knowledge can be used to assess the effect on ecosystem of environmental stress and perturbations like pollution, agricultural exploitation and global changes. Fish take a large number of bacteria into their gut from water, sediment and food (Fierer and Jackson, 2006).

Sometimes bacterial contents in fish may cross the safe limit for human consumption. So, it is essential to investigate the quantitative and qualitative aspects of bacterial flora associated with cultured fish and particularly those of public health concern in order to develop a "Good Aquaculture Practice" (GAP) for the production of fish safe for human consumption and for prevention of fish diseases (Hossain, 2002). Prevention or control of fish disease is essential to the success of the large-scale production of fish. Therefore, to enrich the knowledge of the fish farmers and to develop a good management practice for the production of safe fish for human consumption and for the prevention of diseases, it is important to study the bacterial contents of fish, water and sediment of the pond during culture. Considering the above factors, the bacteriological study of the fish, water and sediments of pond in southern districts of Bangladesh, especially Patuakhali district focusing PSTU campus are time demanded to supply quality and safe fish to the people in PSTU and its surrounding area. Therefore, the main aim of the research was to determine total bacterial load in fish, water and sediments in accordance with water quality parameters such as temperature, pH, transparency and dissolved oxygen of different ponds situated in PSTU campus.

MATERIALS AND METHODS

Ethical approval: The study was conducted as per international guidelines.

Sources and collection of sample: The present study was carried out in six fish pond named Lalkomol, Nilkomol, Tarangotanu, Salsabil, Jolpoddo and Nildhighi in the PSTU campus. The ponds were well exposed to sunlight, interconnected by inlet and outlet and the main sources of water were rainfall and water supplied from a pump using a flexible plastic pipe whenever needed. The triplicate samples for fish, water and sediments were collected every four month interval in summer, winter and rainy season. Bottom soil samples were collected by submerging sterile wide mouth glass bottles at three representative locations in each pond and mixed together. Fish sample were also collected from different corner of pond with randomly. Sterile glass bottles with stopper having a volume of 250 ml each and marked with pond number were used for collection of water samples. Water samples were collected from all layers of the water column (vertical haul of the tube sampler covering about 3-4 feet depth). Each representative sample of fish, water and sediments were marked properly and wrapped with aluminum foil and transported to the laboratory of Dept. of Fisheries Technology immediately and subjected to further bacteriological analysis. Water sample which was collected for bacteriological analysis also used for further physico-chemical analysis. The experiment was conducted for a period of 12 months from April 2016 to March 2017.

Determination of water quality parameters: The water sample were collected and recorded in three separate seasons from existed six (6) fish pond in the PSTU campus. Water sample collection and water quality measurement were made between 08:00 to 11:00 AM on each sampling day using standard techniques (APHA, 2005). During the study period, water temperature was recorded with the help of an ordinary thermometer graduated in centigrade scale (LCD Multi-Thermometer, Model WT-2, Jiangsu, China). Water transparency was recorded in ponds by using secchi disk (20 cm diameter) and measuring tape. Dissolved oxygen value of the pond water was recorded with the help of a dissolved oxygen (DO) meter (Lutron, DO-5509, Taipei, Taiwan). pH of the water samples were measured by using pH meter (HI 98107, Resita, Romania).

Bacteriological study: Bacteriological study was conducted following standard plate count (SPC) method (AOAC, 2007). The following procedure was adopted in preparing media for bacteriological study:

Preparation of nutrient media: For bacteriological analysis, nutrient media were prepared by the following composition and procedure. For preparation of media, the estimated quantities (28g) of nutrient plate count agar media were weighed and suspended into one liter of distilled water in a conical flask. Then the mixture was boiled on hot plate (Model: HP-100A, Yongkang Mars Cooker Factory, Zhejiang, China) to dissolve completely and sterilized.

Sterilization: Media were sterilized before using them in order to kill any bacterial and fungal cells or spores present in the media or in the glass wares containing them. Sterilization was accomplished by placing the media in an autoclave (BIOBASE, BKQ-B50II, Biobase Biodustry Co., Ltd, Shandong, China) for 20 minutes at a temperature of 121°C under 15 lbs/sq. inch pressure. Then it was cooled down to around 50°C and was poured into previously sterilized petridish.

Sample preparation and dilution: Standard plate count expressed as colony forming units per gram (cfu/g) was determined by using consecutive decimal dilution technique using spread plate method. Five gram of fish samples were then homogenized in 200 ml sterile physiological saline. Then shaking was done properly. In order to get 10^{-1} dilution of original sample solution, one ml sample (fish, water and sediments stock solution) was transferred with a micropipette to test tube containing 9 ml of physiological saline. The test tube was shaken thoroughly on a vortex mixture (Fisher scientific, FB 15012, Waltham, Massachusetts, USA). The similar process was followed for dilutions of 10^{-2} to 10^{-6}, 10^{-2} to 10^{-6} and 10^{-2}-10^{-8} for fish, sediment and water, respectively.

Culture for bacteria: After dilution of fish, water and sediment sample, aliquots of 0.1 ml of the serial dilutions was inoculated onto previously prepared nutrient media in triplicate using the spread plate method as this medium recovered most of the bacteria. The samples were spread homogenously and carefully by sterile flamed L-shaped glass rod into laminar air flow (BBS-1300HGS, Biobase, China). The plates were then kept inverted position at 38^0c in an incubator (INC108med, Innovation Diagnostic, Germany) for bacterial culture in the laboratory of Fisheries Technology, PSTU for 24 hours. Similar process was done for each sample.

Aerobic plate count (APC): Bacterial colonies were counted after 24 hours of incubation. Plates containing 30-300 colonies were used to calculate bacterial load results, recorded as Cfu per unit of sample by using following equation:

For water sample:

$$\frac{Cfu}{ml} = No.of\ colonies\ in\ petridis \times 10 \times dilution\ factor$$

For fish sample:

$$\frac{Cfu}{g} = \frac{No.of\ colonies\ in\ Petridis \times 10 \times dilution\ factor \times volume\ of\ total\ stock\ solution}{Wt.of\ fish\ sample\ (g)}$$

For sediments sample:

$$\frac{Cfu}{g} = \frac{No.of\ colonies\ in\ Petridis \times 10 \times dilution\ factor \times volume\ of\ total\ stock\ solution}{Wt.\ of\ sediment\ (g)}$$

RESULTS AND DISCUSSION

Water quality parameters in different ponds in PSTU Campus

Various water quality parameters of different fish ponds (Lalkomol, Nilkomol, Salsabil, Jalpoddo, Tarangatonu and Nildighi) in PSTU campus were observed during the study period. Water and air temperature (ºC), transparency (cm), dissolved oxygen (mg/l) and pH were measured in three consecutive seasons (rainy, winter and summer). The findings of the present study on water quality parameters of three consecutive seasons are presented in Table 1 and 2, and correlation coefficient among water quality parameter in different seasons are presented in Table 3, 4 and 5.

Air temperature mean (±SD) values of in the six fish ponds (Lalkomol, Nilkomol, Salsabil, Jalpoddo, Tarangatonu and Nildighi) varied from 29.10±0.22 to 30.60±0.82, 18.53±5.88 to 21.37±2.73, 31.15±0.31 to 32.00±0.22°C during the rainy, winter and summer seasons, respectively (Table 1). The air temperature in the six fish ponds throughout the study periods was found to vary from 29 to 31.5°C, 13.50 to 24.90°C and 30.80 to 32.30°C in the three consecutive seasons (rainy, winter and summer). Among the six ponds, the highest air temperature were 31.5°C in Lalkomol, 25°C in Salsabil, 32.30°C in Nildighi and the lowest air temperature were 29°C in Jalpoddo, Tarangatonu and Nildighi, 13.50°C in Salsabil, 30.80°C in Jalpoddo during the rainy, winter and summer seasons respectively. Significant difference in air temperature was observed for the same ponds in different seasons ($p<0.05$) but non-significant difference ($p>0.05$) was found in the same season among the six different ponds when ANOVA was performed (Table 1). The reasons behind the variation in results might be due to variation in temperature of the season, sampling time of the day, water depth, meteorological and environmental condition of the ponds. Correlation matrix showed that there was strongly positive ($r= 0.874$, $p= 0.01$) correlation between air temperature and water temperature during rainy season and moderate positive correlation was found during winter ($r= 0.638$, $p= 0.01$) and summer season ($r= 0.624$, $p= 0.01$) which indicated that water temperature was increased with increasing air temperature (Table 3, 4 and 5). Nupur et al. (2013) and Begum (2013) observed that the optimum air temperature for aquatic production were 27.00 to 27.32°C and 29.00 to 31.50°C respectively. In the present study the results are similar to those studies. Air temperature is the principal factor affecting the water temperature as well as the rate of metabolic process (Battes et al., 2004). Temperature has powerful effect on growth both through its influence rates of biochemical processes and hence metabolism and through associated factors such as feed intake. The higher the air temperature, the greater the water temperature and finally the more metabolic rate.

Water temperature closely related to air temperature. In the present study, the mean (±SD) of water temperature in the six fish ponds were 31.60±1.56 to 33.00±0.94, 19.27±1.44 to 20.73±3.00, and 28.43±0.46 to 30.10±0.57°C with ranged from 29 to 34°C, 17.50 to 24.20°C and 27.90 to 30.80°C during the rainy, winter and summer seasons, respectively (Table 1). The highest value of water temperature were 34°C, 24.20°C, 30.80°C in Lalkomol and the lowest water temperature were 29°C, 17.90°C, 27.90°C in Nildidhi during the rainy, winter and summer seasons, respectively. ANOVA showed that there was non-significant difference of water temperature in the same season among the six different ponds ($p>0.05$) but significant difference was observed in the same pond in different seasons ($p<0.05$) (Table 1).

Result might fluctuated due to seasonal changes, time and length of the day, difference in sampling time of the day and water depth, meteorological and environmental condition and location of the ponds. Correlation matrix showed that there was positive significant correlation between water temperature and dissolved oxygen during rainy ($r=0.171$), winter ($r=0.061$) and summer ($r=0.161$) seasons, respectively which indicated that dissolved oxygen was increased with increasing water temperature during all seasons (Table 3, 4 and 5). Aminul (2003) stated that the water temperature ranged from 28 to 35°C is suitable for fish culture and bacterial population. Microbes can grow at wide range of temperature. The suitable temperature for the growth of bacteria is 30 to 40°C, which is similar to the results of the present study in rainy and summer seasons but different in winter season. Other reason of higher level of bacterial load in a temperature lower than optimum range may be the poor water quality due to semi-intensive culture system in fish ponds. The mean values of water temperature were more or less close to suitable range for fish culture. The water temperature of experimental ponds ranged from 29 to 34°C, 17.90 to 24.20°C, 27.90 to 30.80°C during the experiment in rainy, winter and summer seasons respectively, which was more or less similar to the findings of Kohinoor et al. (2001), Maghna et al. (2016) and Akter (2010) and observed that the optimum temperature for aquatic production were 18.5 to 32.9°C, 30 to 34.30°C and 25.20 to 26.73oC, respectively.

Water transparency during the study period, in the six fish ponds (Lalkomol, Nilkomol, Salsabil, Jalpoddo, Tarangatonu and Nildighi) throughout the study periods ranged from 32 to 36, 24 to 48 and 19 to 28 cm with the mean (±SD) of 32.60±0.55 to 35.20±0.84, 23.67±2.52 to 44.67±3.05 and 22.25±2.22 to 24.00± 1.83 cm during the rainy, winter and summer seasons, respectively (Table 2). The highest water transparency was 36 cm in Salsabil and Jolpoddo, 48 cm in Lalkomol, 28 cm in Jalpoddo and the lowest water transparency was 32 cm in Tarangatonu, 21 cm in Jalpoddo, 19 cm in Jalpoddo during the rainy, winter and summer seasons, respectively. In case of same pond in different seasons significant difference ($p<0.05$) in water transparency was found. On the other hand, significant difference in water transparency was found during rainy and winter season ($p<0.05$) but non-significant difference was observed during summer season ($p>0.05$) when ANOVA was performed in the same season among the six different ponds (Table 2). Silt, microscopic organisms, suspended organic matter, sampling time, intensity of light, application of manure, grazing pressure of fishes and rainfall might be the major causes behind these variation. According to correlation matrix, transparency showed positive relationship with pH and negative relationship with DO during rainy and winter season. On the other hand, transparency showed negative relationship with pH and DO (Table 3, 4 and 5). Transparency between 15-40 cm is considered appropriate for fish culture. The result from the present study suggested that transparency of the experimental ponds is suitable for fish culture. Uddin et al., (2012) recorded transparency values ranging from 12.0 to 45.5 cm. Azim et al. (2002) and Begum (2013) suggested that the transparency of productive water should be 36.00 to 46.00 and 34.38 to 36.63 cm respectively which support the findings of the present study.

The highest pH value was 9.00 in Jalpoddo, 8.70 in Salsabil, 9.10 in Tarangatonu and the lowest pH value was 6.80 in Tarangatonu, 7.20 in Traangatonu, 7.20 in Nildidghi during the rainy, winter and summer seasons, respectively. pH ranged from 6.80 to 9.00, 7.20 to 8.70, 7.20 to 9.10 with the mean (±SD) values of 7.58±0.47 to 8.62±0.27, 7.40±0.20 to 8.50±0.20 and 7.60±0.42 to 8.80±0.32 in the six fish ponds during the rainy, winter and summer seasons, respectively (Table 2). There was significant difference in water pH observed both in same season among the six ponds and same pond in the different seasons

Table 1: Air and water temperature recorded from different ponds in PSTU campus in three consecutive seasons.

Pond name	Air temperature (°C)			Water temperature (°C)		
	Rainy season	**Winter season**	**Summer season**	**Rainy season**	**Rainy season**	**Rainy season**
Lalkomol	30.60 ± 0.82^{aA}	18.87 ± 3.00^{aB}	31.65 ± 0.47^{abA}	33.00 ± 0.94^{aA}	33.00 ± 0.94^{aA}	33.00 ± 0.94^{aA}
Nilkomol	30.60 ± 0.42^{aA}	19.90 ± 4.35^{aB}	31.93 ± 0.25^{aA}	32.70 ± 0.27^{aA}	32.70 ± 0.27^{aA}	32.70 ± 0.27^{aA}
Salsabil	30.00 ± 0.35^{abA}	18.53 ± 5.88^{aB}	31.60 ± 0.39^{abA}	32.30 ± 0.84^{aA}	32.30 ± 0.84^{aA}	32.30 ± 0.84^{aA}
Jalpoddo	29.40 ± 0.42^{bcA}	21.37 ± 2.73^{aB}	31.15 ± 0.31^{bA}	31.90 ± 0.65^{aA}	31.90 ± 0.65^{aA}	31.90 ± 0.65^{aA}
Tarangatonu	29.40 ± 0.42^{bcA}	18.90 ± 4.22^{aB}	31.75 ± 0.34^{aA}	31.90 ± 1.24^{aA}	31.90 ± 1.24^{aA}	31.90 ± 1.24^{aA}
Nildighi	29.10 ± 0.22^{cB}	20.23 ± 2.00^{aC}	32.00 ± 0.22^{aA}	31.60 ± 1.56^{aA}	31.60 ± 1.56^{aA}	31.60 ± 1.56^{aA}

Values given are mean ± standard deviation (n=4); Different small alphabet in the same column represent significant difference ($p<0.05$) in same season of different ponds; and different capital alphabet in the same row represent significant difference ($p<0.05$) in same pond of different seasons.

Table 2: Water transparency, pH and dissolve oxygen recorded from different ponds in PSTU campus in three consecutive seasons.

Pond name	Water Transparency (cm)			pH			D.O (mg/L)		
	Rainy	**Winter**	**Rainy**	**Rainy**	**Winter**	**Summer**	**Rainy**	**Winter**	**Summer**
Lalkomol	34.60 ± 0.55^{abB}	44.67 ± 3.05^{aA}	7.32 ± 0.64^{aA}	8.38 ± 0.36^{aA}	8.33 ± 0.20^{abA}	8.30 ± 0.37^{abA}	7.32 ± 0.64^{aA}	6.67 ± 1.30^{abA}	7.93 ± 0.56^{aA}
Nilkomol	34.20 ± 0.45^{abB}	37.33 ± 1.53^{bA}	7.70 ± 0.44^{aA}	8.54 ± 0.15^{aA}	7.50 ± 0.10^{cB}	8.13 ± 0.54^{abcA}	7.70 ± 0.44^{aA}	5.93 ± 0.91^{bB}	8.20 ± 1.13^{aA}
Salsabil	35.20 ± 0.84^{aA}	31.00 ± 1.00^{cB}	7.30 ± 0.57^{aB}	8.08 ± 0.41^{abA}	8.50 ± 0.20^{aA}	8.45 ± 0.31^{abA}	7.30 ± 0.57^{aB}	8.40 ± 0.61^{aA}	7.60 ± 0.51^{aAB}
Jalpoddo	34.00 ± 1.22^{bA}	23.67 ± 2.52^{dB}	6.76 ± 0.68^{aB}	8.62 ± 0.27^{aA}	8.10 ± 0.10^{bA}	7.98 ± 0.54^{bcA}	6.76 ± 0.68^{aB}	8.40 ± 0.53^{aA}	7.30 ± 0.37^{aB}
Tarangatonu	32.60 ± 0.55^{cA}	24.00 ± 1.00^{dB}	6.36 ± 1.01^{aAB}	7.58 ± 0.47^{bB}	7.40 ± 0.20^{cB}	8.80 ± 0.32^{aA}	6.36 ± 1.01^{aAB}	5.43 ± 1.42^{bB}	7.85 ± 0.76^{aA}
Nildighi	33.20 ± 0.45^{cA}	27.67 ± 2.08^{cB}	7.20 ± 0.44^{aA}	8.42 ± 0.37^{aA}	8.30 ± 0.20^{abA}	7.60 ± 0.42^{cB}	7.20 ± 0.44^{aA}	6.80 ± 0.60^{abA}	7.30 ± 0.59^{aA}

Values given are mean ± standard deviation (n=4); Different small alphabet in the same column represent significant difference ($p<0.05$) in same season of different ponds; and different capital alphabet in the same row represent significant difference ($p<0.05$) in same pond of different seasons.

($p<0.05$) when ANOVA was performed Table 2. According to correlation matrix, pH showed positive relationship with DO during rainy (r= 0.260), winter (r= 0.529, p=0.05) and summer (r= 0.356) season, respectively which indicated that dissolve oxygen was increased with increasing water pH Table 3, 4 and 5. The pH level of ponds water during different seasons was approximately neutral or alkaline. The pH value 7 to 9 is suitable for pond fish culture (Boyd, 2001). The results of Boyd (2001) clearly support the present findings. Most bacteria prefer neutral pH (6.5-7.5) for growth. This also agreed with the present study pH 7.0 or nearly neutral which was suitable for bacterial growth. The pH value recorded from the experimental ponds agreed with the findings of Azim et al., (2002), Akter (2010), Uddin et al. (2012) and Maghna et al. (2016) who found the ranges of pH from 6.5 to 9.3, 7.24 to 8.80, 6.26 to 8.42 and 7.60 to 8.30, respectively. Hossain and Paul (2007) found pH values ranges from 6.40 to 7.7 which were slightly different with the findings of the present study as well.

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. The dissolved oxygen (DO) value in the six fish ponds (Lalkomol, Nilkomol, Salsabil, Jalpoddo, Tarangatonu and Nildighi) was found to vary from 5.50 to 8.40, 3.80 to 8.80, 6.50 to 9.10 mg/l with mean (±SD) values of 6.36±1.01 to 7.70±0.44, 5.43±1.42 to 8.40±0.53 and 7.30±0.59 to 8.20±1.13 mg/l during the rainy, winter and summer seasons, respectively (Table 2). The highest DO value was 8.40 mg/l in Lalkomol, 8.80 mg/l in Salsabil, 9.10 mg/l in Nilkomol and the lowest DO value was 5.5 mg/l in Tarangatonu, 3.80 mg/l in Traangatonu, 6.50 mg/l in Nildidghi during the rainy, winter and summer seasons, respectively. From the result, non-significant difference in dissolve oxygen was found among the six ponds during rainy and summer seasons (p>0.05) but significant difference was observed during winter season (p<0.05) in the same season among the six different ponds. Significant difference was also observed in case of same pond in different seasons when ANOVA was performed (Table 2). Temperature fluctuation, water depth, meteorological condition and poor pond management may be the major causes behind this variation. DoF (2011) reported that the range of dissolved oxygen suitable for fish culture would be 5.0 to 8.0 mg/l. The result of present findings of Tarangatonu in winter (DO value 3.80 mg/l) did not agree with the findings of DoF (2011). The concentration of dissolved oxygen in the present study was similar to the findings of Hossain and Paul (2007), Nupur et al., (2013) and Begum (2013) who recorded dissolved oxygen ranged from 5.1 and 8.1mg/l, 5.86 to 7.86 mg/l and 3.21 to 7.29 mg/l, respectively.

Bacterial load of fish, water and sediment in different ponds in PSTU Campus

The growth of aquatic microorganisms is effected by a great variety of physical and chemical factors which, in a multitude of ways, may also act with or against one another. Bacteriological analysis including aerobic plate count e.g.; determination of bacterial load in pond water, sediment and fish were done.

Table 3: Correlation coefficient between water quality parameters of different ponds during rainy season.

	Air Temperature (^{0}C)	**Water temperature (^{0}C)**	**Transparency (cm)**	**pH**	**DO (mg/l)**
Air temperature	1				
Water temperature	0.874**	1			
Transparency	-0.283*	0.046	1		
pH	0.141	0.216	.076	1	
DO	0.273**	0.171	-0.277*	.260*	1

Correlation is significant at the (**) 0.01 level (2-tailed) and (*) 0.05 level (2-tailed).

Table 4: Correlation coefficient between water quality parameters of different ponds during winter season.

	Air Temperature (^{0}C)	**Water temperature (^{0}C)**	**Transparency (cm)**	**pH**	**DO (mg/l)**
Air temperature	1				
Water temperature	.638**	1			
Transparency	-.175	.012	1		
pH	-.239	-.218	.200	1	
DO	.074	.061	-.150	.529*	1

Correlation is significant at the (**) 0.01 level (2-tailed) and (*) 0.05 level (2-tailed).

Table 5: Correlation coefficient between water quality parameters of different ponds during summer season.

	Air temperature (^{0}C)	**Water temperature (^{0}C)**	**Transparency (cm)**	**pH**	**DO (mg/l)**
Air temperature	1				
Water temperature	.624**	1			
Transparency	.339	-.139	1		
pH	.043	.511*	-.033	1	
DO	.177	.161	-.138	.356	1

Correlation is significant at the (**) 0.01 level (2-tailed) and (*) 0.05 level (2-tailed).

The present study results of bacterial load in fish ranged from 5.60×107 to 1.19×108, 3.80×107 to 4.76×107 and 7.12×107 to 1.16×108 cfu/g during rainy, winter and summer seasons, respectively (Table 6). In water, total viable count of bacteria ranged from 5.80×104 to 1.12×105, 4.70×104 to 6.10×104 and 9.10×104 to 1.48×105 cfu/ml and in sediment it was ranged from 1.10×108 to 1.17×109, 5.92×107 to 9.28×107 and 3.64×108 to 9.96×108 cfu/g during rainy, winter and summer seasons, respectively (Table 6). Total bacterial count of the ponds showed that the bacterial load varied slightly from one season to another season, one sample to another sample (fish, water and sediments) and also one pond to another pond. Among the three samples (fish, water and sediments), bacterial load was highest in sediment (1.17×109 cfu/g in Salsabil during summer season) followed by fish (1.19×108 cfu/g in Lalkomol during rainy season) and water (1.48×105 cfu/ml in Tarangatonu during summer season) samples. Among the three seasons, the bacterial content in fish was highest during rainy season (1.19×108 cfu/g in Lalkomol) than winter (4.76×107 cfu/g in Tarangatonu) and summer seasons (1.16×108 cfu/g in Tarangatonu). In ponds water analysis, bacterial load was highest during summer (1.48×105 cfu/ml in Tarangatonu) season followed by rainy (1.12×105 cfu/ml in Lalkomol) and winter (6.10×104 cfu/ml in Tarangatonu) seasons. On the other hand, rainy (1.17×109 cfu/g in Salsabil) season contain highest number of bacterial load than summer (9.96×108 cfu/g in Tarangatonu) and winter (9.28×107 cfu/g in Tarangatonu) seasons from sediment analysis. In case of fish, the bacterial load was lowest in Salsabil (5.60×107 cfu/g in rainy season) and Jolpoddo (3.80×107 cfu/g in winter and 7.12×107 cfu/g in summer) among the six ponds.

Table 6: Bacterial content in fish, water and sediment in three consecutive seasons of different ponds in PSTU campus.

Pond name	Fish (cfu/g)			Water (cfu/ml)			Sediment (cfu/g)		
	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer
Lalkomol	1.19×10^{8}	4.12×10^{7}	1.02×10^{8}	1.12×10^{5}	5.30×10^{4}	1.31×10^{5}	1.15×10^{8}	8.84×10^{7}	7.48×10^{8}
Nilkomol	6.32×10^{7}	4.52×10^{7}	8.96×10^{7}	6.90×10^{4}	5.00×10^{4}	1.23×10^{5}	1.15×10^{9}	7.56×10^{7}	5.08×10^{8}
Salsabil	5.60×10^{7}	4.28×10^{7}	8.12×10^{7}	5.80×10^{4}	5.10×10^{4}	1.27×10^{5}	1.17×10^{9}	7.64×10^{7}	6.68×10^{8}
Jalpoddo	1.17×10^{8}	3.80×10^{7}	7.12×10^{7}	8.50×10^{4}	4.70×10^{4}	9.10×10^{4}	1.17×10^{8}	5.92×10^{7}	3.64×10^{8}
Tarangatonu	1.16×10^{8}	4.76×10^{7}	1.16×10^{8}	9.20×10^{4}	6.10×10^{4}	1.48×10^{5}	1.10×10^{8}	9.28×10^{7}	9.96×10^{8}
Nildighi	9.36×10^{7}	4.40×10^{7}	9.32×10^{7}	8.70×10^{4}	4.90×10^{4}	1.09×10^{5}	1.16×10^{8}	7.84×10^{7}	7.12×10^{8}

The quantitative changes of bacterial load in different samples with various conditions showed a characteristic pattern of increase in number with time. Depending on the result of bacterial load in fish, it could be suggested that Salsabil hold better condition among the six ponds and fish of this ponds safe for human consumption concerning public health. The variation also may influenced by nutritional composition between the ponds. This difference between the samples was also occurred due to the composition of samples, time interval, water depth and meteorological condition, environmental condition, location of the ponds and poor pond management. In this case, temperature may be the major factor in decreasing or increasing bacterial loads in the ponds water, sediment and fish. The suggestion that increase in the bacterial load might be associated with the increase of water temperature has been documented in the literature of Al-Salim et al. (2009) and Rekhari et al. (2014). Al-Harbi and Uddin (2005) reported that total viable counts were 2.8±2.4×107 to 1.0±1.6×108 cfu/g in intestine of tilapia. Total viable counts of bacteria (measured as colony-forming units, cfu) were in the range of 5.6 ± 0.8×103 to 2.4 ± 1.2×104 cfu/ml in pond water; 9.3 ± 1.1×106 to1.9 ± 1.5 × 108 cfu/g in sediments (Al-Harbi and Uddin 2003). These results agreed with our findings.

The results of present study was more or less similar to the results of Middelboe and Glud (2003) and Haque (2012) who reported total viable count of bacteria were 0.1×108 to 4×108 cfu/g in sediments and 4.90±0.10×107 cfu/g in the intestine of fish. Our results also agreed with the findings of Osman (2014) who reported total viable count of bacteria were 4.84±0.8×108 cfu/g, 4.54±0.5×105 cfu/ml and 3.65±0.5×109 cfu/g in fish, water and sediments respectively. The organic matter influences the load and composition of microbial population. Sediment bacterial composition and load greatly influence by effluent characteristics. On the other hand bacterial flora in fish is the reflection of aquatic environments (Bisht et al., 2013). It affects the storage life and quality of fishery products. The result from the present study and findings from the previous related study suggested that the composition of the bacterial flora differs widely dependent not only on the water's content of organic and inorganic material, its pH, temperature and turbidity, water quality but also on the sources from where organisms could enter into the water. In every habitat the nutritional competition between organisms plays an important role and influences decisively the composition of the micro flora.

CONCLUSION

The present study on the six fish ponds in PSTU campus was very useful for gathering valuable information regarding the water quality parameters of pond water and the bacterial load of pond water, sediment and fish samples. Bacterial load increased significantly in pond water, sediment and fish samples during summer than rainy season followed by winter season. Depending on the result of water quality parameter and bacterial load in fish, it could be conclude that Salsabil hold better condition among the six ponds in PSTU campus during the study period. Bacterial load was highly influential by the water quality parameter irrespective of the seasons. The apparent difference in the bacteriological condition of the six ponds makes it justifiable to assume that the bacterial load in the fish sample was higher than the recommended limit for human consumption. Therefore, in view of quality and safe fish and fishery products supply to the people in PSTU and its surrounding area, it is important to carry out the further identification and characterization of bacteria in the fish, water and sediments of pond in southern districts of Bangladesh, especially in Patuakhali district focusing fish farm in PSTU campus.

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CONFLICT OF INTERESTS

No conflict of interest.

AUTHORS' CONTRIBUTION

Md. Shahin: Conduct entire experiment and data analysis and prepare first draft of manuscript. Biplob: Helping during sampling, conduct experiment and preparing manuscript. Md. Sazedul: Plan and design of the experiment, scientific and technical support and supervise the research and, comments, corrections and finalize the manuscript.

REFERENCES

1. Akhter H. Comparative study of supermale tilapia and monosex tilapia in earthen mini pond, MS thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 2013;
2. Akter R. Culture potential of Climbing perch, *Anabas testudineus* (Bloch) under different stocking densities in BFRI research ponds, MS thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 2010;
3. Al-Harbi AH, Uddin MN. Quantitative and Qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) cultured in earthen ponds in Saudi Arabia. Journal of Aquaculture Research. 2003; 34 43-48.
4. Al-Harbi AH, Uddin N. Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brakish water in Saudi Arabia. Journal of Aquaculture Research. 2005; 35 88-97.
5. Ali MM, Hossain MB, Minar MH, Rahman S. Socio-Economic Aspects of the Fishermen of Lohalia River, Bangladesh. Middle-East Journal of Scientific Research. 2014; 19 (2) 191-195.
6. Al-Salim NK, Khamees NR, Al-Niaeem KS. Intestinal bacteria in the University of Basrah fish ponds, Iraq. Journal of al-anbar University for pure science. 2009; 3(1) 42-48.
7. Aminul IM. Qualities of water and Soil in Aquaculture, Fish Week Compilation. 2003;

8. AOAC. Official methods of analysis, 16th edn. Association of Official Analytical Chemists, MD, USA. 2007;
9. APHA. Standard methods for examination of water and waste water. 21st ed. The American public Health Association, Washington D.C., USA. 2005;
10. Azim ME, Wahab MA, Verdegem MCJ, Vandam AA, Van Rooij JM, Beveridge MCJ. The effects of artificial substrates on freshwater pond productivity and water quality and the implications for periphyton-based aquaculture. Aquatic Living Resource. 2002; 15 231–241.
11. Battes KL, Constaintia N, Valencive L, Tarus T. Carp culture in floating cages. Bull. Cerect. Piscis. 2004; 1(1-2) 68-95.
12. Begum A. Assessment of water quality parameter of monosex tilapia (*Oreochromis niloticus*) ponds, MS thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 2013;
13. Bisht A, Singh UP, Pandey NN. Comparative study of seasonal variation in bacterial flora concomitant with farm raised fingerlings of *Cyprinus carpio* at tarai region of Uttarkhand. Journal of Environmental Biology. 2013; 5 363-367.
14. Boyd CE. Water quality management for pond fish culture. Elsevier Scientific Publication CO. 2001; Amsterdam-Oxford-New York. pp. 318.
15. Davis DT, Nguyen ML, Gatlin DM, Okeebe. Advantages in aquaculture nutrition: catfish, tilapia and carp nutrition. New technologies Q.E. management Important Production of Effective Aquaculture. 2009; 24(19) 440-458.
16. Delgado CL, Wada N, Rosengrant MW, Meijer S, Ahmed M. Supply and demand in changing global markets. World Fish Centre Technical Report, 62. Washington DC: International Food Policy Research Institute. 2003;
17. DoF 2011: Fishery Statistical Yearbook of Bangladesh, 2010-2011, Fisheries Recourses Survey System, Department of Fisheries, Bangladesh, Dhaka. pp.41.
18. DoF. National fish week compendium. Department of Fisheries, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh, Matshya Bhaban, Park Avenue, Ramna, Dhaka. 2016; pp. 1-144.
19. FAO. The state of world fisheries and aquaculture. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations, Rome. 2016;
20. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. National Academy of Sciences of the USA. 2006; 103(3) 626-631.
21. Gary B. Microbial ecology of gastrointestinal tract of fish. Journal of world Aquaculture Society 2005; 36(4) 126-132.
22. Haque SA. Effects of antibiotic treated fish diet on bacterial flora under laboratory condition, MS thesis, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. 2012;
23. Hossain KM. Characterization of bacteria isolated from diarrhoeic calves, MS Thesis, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. 2002;
24. Hossain MA, Paul L. Low-cost diet for monoculture of giant freshwater prawn (*Macrobrachium rosenbergii* de Man) in Bangladesh. Journal of Aquaculture Research. 2007; 38 232 - 238.
25. Kadir A, Wahab MA, Milstein A, Hossain MA, Seraji MTI. Effects of silver carp and the small indigenous fish mola, *Amblypharyngodon mola* and Punti, *Puntius sophore* on fish polyculture production. Aquaculture. 2007; 273 (4) 520-531.
26. Kohinoor AHM, Wahab MA, Islam ML, Thilsted SH. Culture potentials of mola (*Amblypharyngodon mola*); chela (*Chela cachius*) and punti (*Puntius sophore*) under monoculture system. Bangladesh Journal of Fisheries Research. 2001; 5(2) 123-134.
27. Maghna SA, Alam MJ, Islam SR, Khatun A, Jaman N 2016: Studies on the stocking density of hormone treated tilapia (*Oreochromis niloticus*) fry. Academic Research International 7(1) 18-22.
28. Middelboe M, Glud RN. Distribution of viruses and bacteria in relation to diagenetic activity in estuarine sediment. Journal of American Society of Limnology and Oceanography. 2003; 48(4) 1447-1456.
29. Nupur N, Shahjahan M, Rahman MS, Fatema MK. Abundance of Macrozoobenthos in relation to bottom soil textural types and water depth in aquaculture ponds. Int. J. Agril. Res. Innov. & Tech. 2013; 3(2) 1-6.

30. Rekhari YC, Agrawal R, Trakroo MD, Tiwari H. Qualitative and quantitative study on bacterial flora of farm raised common carp (*Cyprinus carpio*) in India. African Journal of Microbiology Research. 2014; 8(11) 1125-1129.
31. Roy K. Effects on Stocking Density on the Growth of Carps in Ponds, MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 2001;
32. Siddiq MA, Miah MI, Ahmed ZF, Asadujjaman M. Present Status of Fish, Fishers and Fisheries of Dogger Beel in Hajigonj Upazila, Chandpur, Bangladesh. Journal of Aquatic Science. 2013; 1(2) 39-45.
33. Uddin MN, Shahjahan M, Haque MM. Manipulation of species composition in small scale carp polyculture to enhance fish production. Bangladesh Journal of Progressive Science & Technology. 2012; 10(1) 9-12.