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Seasonal variations in the intestinal bacterial flora of *Oreochromis niloticus* cultured in farms

Shakeela Parveen[1], Fayyaz Rasool[1*], Ehsan Mahmood Bhatti[2], Andlib Anwar[3], Muhammad Ismail Chaughtai[4]

[1]*Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[2]*Fisheries Research and Training Institute, Manawan-Lahore, Department of Fisheries, Punjab, Pakistan*

[3]*Department of Zoology and Fisheries, Faculty of Sciences, University of Agriculture Faisalabad, Pakistan*

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Abstract

Microbial contamination in different food items is becoming a hazardous problem now a day. The present study was conducted to examine intestinal bacterial flora of tilapia (*Oreochromis niloticus*) in different seasons of the year. Fish and water samples collected from fresh water fish farms were tested for the determination of microbial load by culturing on different bacterial growth media. The water samples were analyzed for physico-chemical properties. The results obtained were compared for microbial population in the intestine of fish samples collected in winter, spring and summer seasons from the same locality. Data was tested to appropriate statistical model to determine significance and non-significance among various parameters. The result showed that bacterial flora was more in autumn and spring season than in winter season. Bacterial load was also dependent on fish size; larger fish had more bacterial content.

***Corresponding Author:** Fayyaz Rasool ✉ fayyazrasool@uvas.edu.pk

Introduction

Fish and fish yields are highly perishable and disposed to vast variations in quality due to differences in species, ecological habitats and nursing ways. In addition, they can also play a role as transporters of many microbial and other health hazards (Yagoub, 2009). Although only a few infective representatives in fish are able to contaminate humans, some exceptions occur that may result in mortalities. However, the highest risk to human health is owing to utilization and consumption of raw or insufficiently processed fish and fish stuffs (Yagoub, 2009). According to the Center for Food Safety and Applied Nutrition in Washington, most of the diseases related to fish food are produced by *Salmonella*, *Staphylococcus* spp., *Vibrio parahemolyticus*, *Clostridium perfringens*, *Clostridium botulinum E*, and *Entreoviruses* (Yagoub, 2009). In the tropics, the fish is most promising source of animal protein and has been broadly established as a good source of protein and other essentials for the maintenance of health. The less exploited countries capture 50% of the world yield and a large number of the catch are consumed. Over 50% of the protein is consumed by the fish in many countries of Asia while this consumption is 17.50% in Africa (Adebayo *et al.*, 2012). Fish and fish goods constitute an imperative part in the worldwide trade, recently value more than 50 billons US$ representing customer attention in product. Fish is generally considered as a good source of vitamins B_{12} and B_6. The fluorine and iodine are also found in the fish which are required for the development of strong teeth and the prevention of goiter in humans (Adebayo *et al.*, 2012). Though, the accessibility of these vital nutrients depends to a great degree on the procedures of storage such as salting, roasting, drying and freezing.

Animal and human wastes have traditionally been used in Asia as sources of fertilizer for fish culture ponds. The use of waste stabilization ponds is common throughout the world where land is available. This is most imperative and cost effective method of treating wastes (Feachem *et al.*, 1977). Experiments have demonstrated that the addition of some species of fishes to specially designed waste stabilization pond increases the treatment efficiency. The possibility that fish raised for market in this manner can transmit disease is a current research concerned. The purpose of such research is to examine the primary immune response of a fish to a pathogenic bacterium and to determine the antibody titer in the absence of viable and active agents. *O. niloticus* or tilapia (family: *Cychlidae*), an omnivorous, filter feeding, freshwater fish can be selected for such research because of its potential for culture in wastewater-fertilized ponds (Baker and Smitherman, 1983).

The epithelial surface of fish, such as the skin and gill provide environment for potential pathogen. Fish lives in a microbe-rich environment and is vulnerable to invasion by pathogenic micro-organisms. The coliform bacteria accepted as the indicators of fecal pollution in water over the past years. All coliforms may be considered as of fecal origin but they are also present in various sources like plants, sediment, etc. (Ebran *et al.*, 2000). The researchers in the present era have been paid much consideration to the fecal coliform bacteria as pollution indictors. They can flourish well at 44°C (Anbuchezhian *et al.*, 2011). Micro-organisms are present typically on the skin, gills, operculum and intestines of alive and freshly trapped fish. The microbial flora differs contrarily in different parts of fishes and described the normal range of 10^{-2} -10^{7} on skin surfaces. Contamination of fish can also be associated to raw material, personnel, processing tools such as forks. The problem associated with identification of pathogens from fish products demand development of accurate and rapid identification methods (Young-Jun *et al.*, 2000). Microbial contamination in different food items is becoming a hazardous problem now a day. The present study was conducted to examine intestinal

bacterial flora of tilapia (*Oreochromis niloticus*) in different seasons of the year to address such issues.

Materials and methods

Collection of samples

Fish and water samples were collected from Fisheries research Farms, University of Agriculture, Faisalabad. Fish samples were collected aseptically in clear and sterilized polythene bags and were immediately transported to laboratory and promptly inoculated in order to save microbial population and avoid contamination.

Water Analysis

pH and Water temperature were noted by pH meter at site and samples were analyzed for calcium, magnesium and total hardness by titration method in Lab following A.P.H.A. (1998).

Microbial examination of fish samples

All the microbiological work was done in laminar air flow to avoid contamination.

Following steps were followed for microbiological studies:

(A) Sample preparation

Intestine of fish was separated by using a sharp scissor and forceps. The separated organs were immediately transferred to sterilized water in conical flask and were shaked well to allow the bacteria to be shifted to water.

(B) Total viable counts

Total viable count of bacteria from different organs of fish samples was done through visual counting under microscope (OLYMPUS, BH-2).

(C) Total plate count

Different growth media *viz.* blood agar and MacConkey agar were used to study the growth of different types of bacterial colonies. Growth of bacteria was monitored by counting the bacterial colonies at different time intervals i.e. 24 hours and 48 hours. Colony forming unit was calculated by the following formula:

CFU = Average number of colonies x Dilution factor

(D) Staining and identification

Different dyes like Gram-iodine, safranine, nigrosine, crystal-violet and malachite-green were used for staining and identification of different types of bacterial colonies. Slides of selected bacterial colonies were prepared, stained and examined under the compound microscope (OLYMPUS, BH-2).

Data Analysis

All the samples were analyzed individually in triplicate and were statistically analyzed by using appropriate statistical method (Steel *et al.,* 1996). Data obtained was statistically analyzed by using Tukey's test to know the significant and non-significant differences among seasons.

Results

Water Analysis

Temperature

Temperature is considered as one of the most important ecological factor. The minimum ambient temperature during autumn season was observed as 18°C while maximum value was 28°C. The minimum temperature during winter season was observed as 11°C while maximum value was 18°C and during spring season the observed minimum value of temperature was 20°C while maximum value was 29°C.

Water, Calcium and Magnesium Hardness

There are many different divalent salts; however, calcium and magnesium are the most common sources of water hardness. The mean of the total hardness observed during the autumn season was 164 mg/L, during winter season was 116.8 mg/L and during spring season was 134 mg/L. The mean value of calcium observed during the autumn season was 93 mg/L, during winter season was 93.6 mg/L and during spring season was 98 mg/L. The Mg^{++} is often

associated with calcium primarily to its similar chemistry. The mean value of magnesium observed during the autumn season was 89 mg/L, during winter season was 85.8 mg/L and during spring season was 79 mg/L. The carbon dioxide was absent during all my research period.

pH

pH is an important factor in analysis of water. The most favorable pH value for fish growth lies slightly on alkaline side. During this experiment the observed mean values of pH during autumn, winter and spring season were 7.45, 7.34 and 8.15 respectively.

Parameters for Fish

In this experiment, the different parameters of fish like wet weight, head weight, eye diameter, snout length, standard length, forked length, and total length, weight of intestine and length of intestine were observed.

Microbial Counts

Twenty samples of *Oreochromis niloticus* were collected from fresh water Fisheries Research Farms, UAF and were subjected to microbiological examination. The samples were analyzed for total plate count.

Microbial counts in autumn

The total microbial count in autumn on Blood agar and MacConkey agar in the intestine of tilapia (*Oreochromis niloticus*) ranged from 1.85 to 2.09 from 4 Oct, 2012 to 22 Nov, 2012.

Microbial counts in winter

The total microbial count in winter on Blood agar and MacConkey agar in the intestine of tilapia (*Oreochromis niloticus*) ranged from 1.72 to 2.08 from 6 Dec, 2012 to 28 Feb, 2013.

Microbial counts in spring

The total microbial count in spring on Blood agar and MacConkey agar in the intestine of tilapia (*Oreochromis niloticus*) ranged from 1.81 to 2.14 from 7 Mar, 2013 to 25 Apr, 2013.

Table 1. Mean comparison of bacterial load in the intestine of *Oreochromis niloticus* during autumn, winter and summer season.

Dates	Seasons	Mean Viable bacterial counts in the intestine (Log cfu ml^{-1})
4 Oct to 22 Nov, 2012	Autumn	1.98
6 Dec, 2012 to 28 Feb, 2013	Winter	1.69
7 Mar to 25 Apr, 2013	Spring	2.31

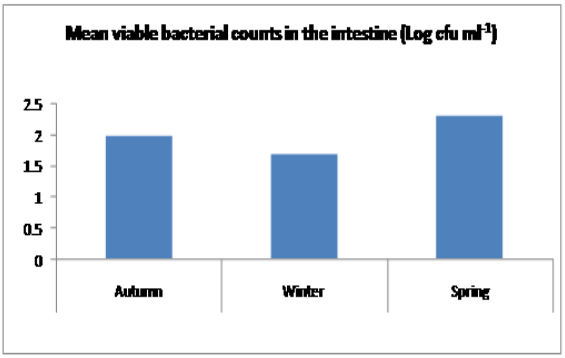

Fig. 1.

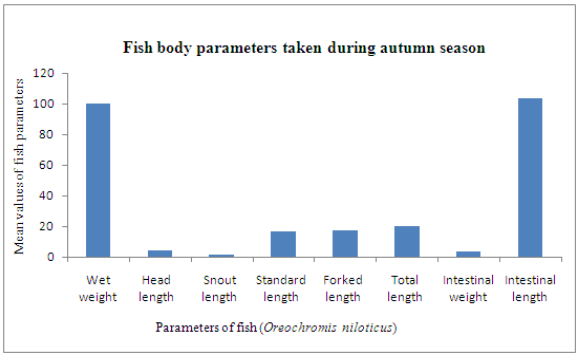

Fig. 2. Mean values of fish body parameters in autumn season.

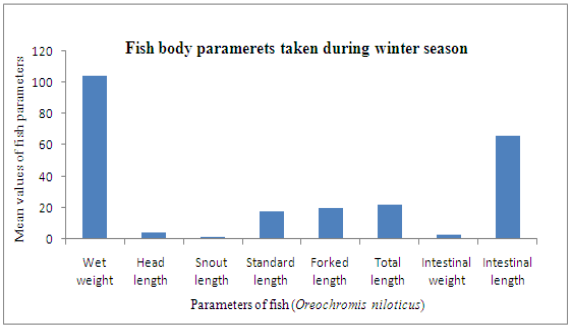

Fig. 3. Mean values of fish body parameters in winter season.

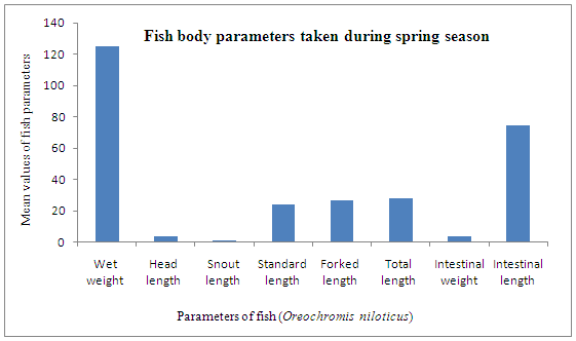

Fig. 4. Mean values of fish body parameters in spring season.

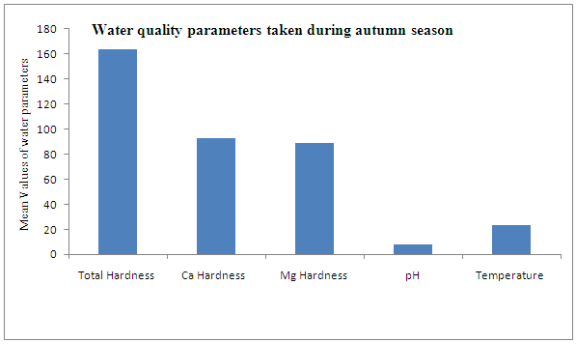

Fig. 5. Mean values of water quality parameters in autumn season.

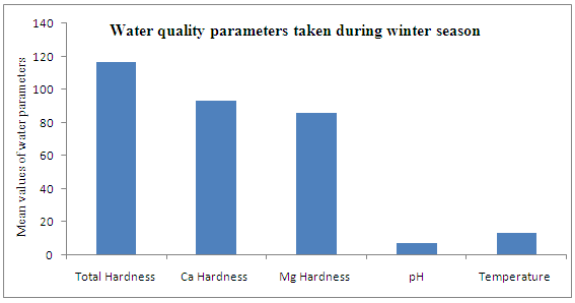

Fig. 6. Mean values of water quality parameters in winter season.

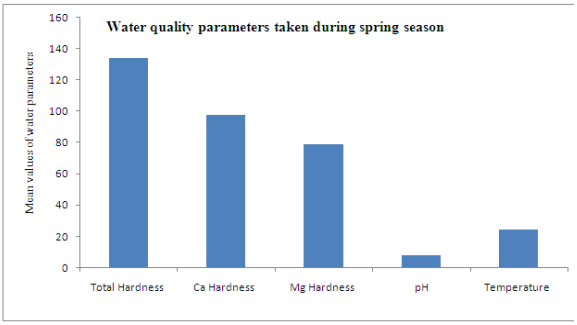

Fig. 7. Mean values of water quality parameters in spring season.

Discussion

The present study was conducted to determine the effects of autumn, winter and spring seasons on the intestinal bacterial flora of *Oreochromis niloticus*. The results of present research showed higher bacterial load in autumn and spring seasons, and lower in winter season. These results are similar to the findings of Nahiduzzaman *et al.* (2000) who reported a ten months investigation to observe the bacterial flora in the farmed hybrid catfish (*Clarias hybrid*) and respective pond water. They concluded that the numbers of bacteria were more in the months of September and October. Holben *et al.* (2002) also analyzed the intestinal micro flora which showed that the bacterial fermentation levels were much higher in the fish digestive tract due to the food contamination. Comparison of fish body parameters showed no significant change due to seasonal change. In the present experiment, culturing of bacterial population was done in two media i.e. McConkey and blood agar. After culturing, the bacterial colonies were Gram stained. The results obtained by observing the stained slides showed significantly higher microbial counts during autumn and spring seasons as compared to the winter season. The mean values of viable bacterial counts in the intestine of *O. niloticus* during the autumn, winter and spring seasons were 1.98 log cfu ml^{-1}, 1.69 log cfu ml^{-1} and 2.31 log cfu ml^{-1} respectively. Some workers suggested that the bacterial load in fish might be increased with the increase of water temperature due to seasonal change. Hossain *et al.*, 1999 reported intestinal bacterial load of tilapia as 5.5 x 106 to 9.8 x 109 cfu g^{-1}. After staining the cultured colonies of bacteria, it was observed that these colonies of bacteria had different morphologies. Some were Gram-Positive while others were Gram-Negative. Gram-Positive bacteria were those that were stained dark blue or violet by Gram staining. That was in contrast to Gram-Negative bacteria, which could not retain the crystal violet stain, instead of taking up the counterstain (safranin) and appeared red or pink. Regarding shape, some were spherical

(cocci) while others were rod shaped (bacilli), but all were capsular.

The parameters of water quality were measured during present study. During present investigation, mean values of water, calcium and magnesium hardness, pH, electrical conductivity, dissolved oxygen, alkalinity, carbonates, bicarbonates and temperature were measured in autumn, winter and spring seasons. The results showed significant fluctuation of pH and temperature through spring season while other parameters showed no significant variation in all the seasons. Kumari *et al.* (2011) examined the seasonal changes in the physico-chemical parameters of fish pond water, which reported that the abundance of divalent salts in surface and bottom water was highest in the rainy season followed by winter and modest in summer. Total dissolved solids showed mean values of 164 mg/L, 116.8 mg/L and 134 mg/L during autumn, winter and spring respectively. The mean values of calcium hardness during my research period were from 93 to 98 mg/L and the mean values of magnesium hardness were from 79 to 89 mg/L. The mean values of electrical conductivity, dissolved oxygen, alkalinity, carbonates and bicarbonates throughout my study were ranged from 3.80 to 3.81 mS/cm, 5.30 to 5.43, 322.81 to 323.9 mg/L, 34 to 36.75 mg/L and 286.44 to 287.68 mg/L respectively. The results showed that in all the three seasons, the water quality parameters remained within a suitable range as described by Kozisek (2003) who reported the levels of total hardness, calcium and magnesium hardness in freshwater which were usually in the range of 15 to 375 mg/L, 10 to 250 mg/L and 5 to 125 mg/L respectively. During present experiment, temperature range of all the three seasons was 11-29^{o}C. The winter season showed the low temperature as compared to the other two seasons. Several scientists said that the increase in temperature increased the growth of bacterial colonies as in the case of present study that the spring season which possessed the high temperature as compared to others, therefore, spring season showed high microbial counts. Dixon *et al.* (2012) studied the effect of temperature on the inactivation of fish viral and bacterial pathogens and their results showed that low temperature slow down the growth of fish and also microbes. The observed pH range during present study was from 6.5-8.5 which was according to the WHO standards. According to the experiment of Fakayode (2005), the pH of a water body is very important in determination of water quality since it affects other chemical reactions such as solubility and metal toxicity. El-Sherif and El-Feky (2009) reported that the suitable range of pH for tilapia culture is 7 to 8 to get maximum growth and survival rate. According to my results, the value of pH was from 7.5 to 8.5 during the spring season which showed the favorable alkaline environment for fish and bacterial growth according to the Stevens (2009). Therefore, my results showed significant microbial load in the spring season. In the light of my results, present study provides conclusive evidence that the composition of intestinal micro flora changed under the effect of seasonal factors and environmental factors.

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