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Research article

Bioaccumulation of Bacterial Indicators of Faecal Contamination in African Catfish (*Clarias gariepinus*) Raised in a Concrete Pond

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ABSTRACT

This study was carried out to determine the rate of bioaccumulation of bacterial indicators of faecal contamination in African catfish (*Clarias gariepinus*) in a concrete pond in Akure, Nigeria. *Clarias gariepinus* and samples of their growing waters were collected weekly over a period of twelve weeks. The concentration of *Escherichia coli*, faecal coliforms, intestinal enterococci, *Salmonella* and *Shigella* were determined by standard microbiological method. The accumulation factor of each parameter was determined by dividing the log concentration of each microorganism in *C. gariepinus* by the corresponding log concentration in their growing waters. Physicochemical properties of the growing waters were determined using standard methods. The accumulation factor of *E. coli* ranged from 0.93 to 1.03; faecal coliforms 0.94 to 1.02 and intestinal enterococci 0.91 to 1.01. Positive correlations were observed between water temperature and the bioaccumulation of faecal coliforms (r = 0.71) in *C. gariepinus.* The findings of this study suggest that the rate of bioaccumulation of faecal indicator bacteria in *C. gariepinus* to a large extent depends on the physicochemical characteristics of the growing waters. Hence, fish raised in faecally impacted waters must be adequately cooked before consumption in order to prevent the occurrence of foodborne illness.

Keywords: *Accumulation factor, foodborne illness, microorganism, physicochemical characteristics, surface water*

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INTRODUCTION

Aquaculture activities are widely relevant at local and regional scales in different environments (coastal, off-shore, natural and artificial enclosed or semi-enclosed basins) and these activities are conducted using different densities of reared species and feeding practices (i.e., semi-intensive or intensive). Intensive fish farming utilizes a minimum land and water sources while providing an economically important source of high quality of protein (Fafioye 2011). In Nigeria, the demand for fish greatly exceeds supply and this problem is aggravated by the low level of domestic fish production against the increase in human population (Eze and Ogbaran 2010). Fish are susceptible to wide variety of bacterial pathogens most of which are capable of causing diseases, hence the consumption of microbially contaminated fish are of great public health significance. The type of microorganisms associated with fish depends largely on its habitat (i.e., the growing waters of the fish) as well as environmental factors (Mhango *et al*. 2010).

The bacterial pathogens associated with fresh fish are classified as indigenous and non-indigenous. The non-indigenous bacterial pathogens contaminate the fish or the habitat from various sources of pollution and examples include *Escherichia coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens*, *Salmonella* sp., e.t.c. The indigenous bacterial pathogens that are found naturally in the fish's habitat include; *Vibrio* species, and *Aeromonas* species (Hudson *et al*. 2005; Fafioye 2011). Bacterial species associated with fresh fish only become pathogenic when fish are physiologically unbalance, nutritionally deficient or there are other stressors i.e., poor water quality, overstocking, etc. Studies have demonstrated the presence of indicator microorganisms of faecal pollution, opportunistic and pathogenic bacteria in groundwater supply wells that may be used for aquaculture and in fish samples (Omojowo and Omojasola 2013; Somaratne and Hallas 2015). There are often bacterial species that are facultative pathogens in both fish and humans and may be isolated from fish without apparent symptoms of the disease (Mhango *et al*. 2010).

Human infections caused by pathogens transmitted from fish or the aquatic environments are quite common and depend on the season, contact with fish and related environment, dietary habits and the immune status of the individual. There have been great economic losses reported due to foodborne illness such as dysentery and diarrhea resulting from consumption of contaminated fish (Ampofo and Clerk 2010).

The physiochemical determinant of good growth of fish in their growing water includes dissolved oxygen, hardness, turbidity, alkalinity, nutrients, temperature, etc. Other parameters such as biological oxygen demand and chemical oxygen demand indicate the level of pollution in their growing waters (Ehiagbonare and Ogunrinde 2010; Bhatnagar and Devi 2013). Aquatic microorganisms not only influence the water quality but are also known to be closely associated with the physiological status of the fish and the postharvest quality of fish (Gram and Huss 2000).

This study was aimed at determining the rate of bioaccumulation of bacterial indicators of faecal contamination in *Clarias gariepinus* in a concrete pond in Akure, Nigeria. This is to gain a better understanding of the risk of foodborne illness associated with the human consumption of *Clarias gariepinus* raised in faecally impacted growing waters.

MATERIALS AND METHODS

Sampling site and collection of samples

The sampling site was situated in Shagari village, Akure, Nigeria. It contains series of concrete ponds used for commercial aquaculture activities in an enclosed environment. The concrete ponds are raised and thus less prone to fluctuating faecal input from farm animals and diffuse pollution from runoffs.

Sampling activities were carried out weekly over a period of twelve weeks i.e., from June to August, 2017. On each sampling occasion, a grab sample of approximately one litre of the growing water was collected at a depth of about 10 – 20 cm in a pre-sterilised plastic bottle in accordance with standard protocol (Anon. 2012). In addition, three to four *C. gariepinus* samples were visually examined to ensure that they were apparently healthy and thereafter picked randomly from the concrete pond into sterile polyethylene bag. The *C. gariepinus* samples and their growing waters were transported in a cool box with ice packs to the laboratory and analysed within one hour.

Microbiological analyses

Freshly collected *C. gariepinus* and samples of growing waters were analysed using standard microbiological methods.

Enumeration of bacterial indicators of faecal contamination in *C. gariepinus* and their growing waters

Preparation of the *C. gariepinus* samples was carried out using 1 g of the intestinal tract of the fish samples. The intestinal tract was dissected using a sterile knife, measured and macerated in a sterile mortar with about 4 ml of distilled water representing the stock solution. One millilitre aliquot was taken from the stock solution into a sterile test tube containing 9 ml of distilled water resulting into 1:10 dilution. Serial dilution was further carried out until the fifth dilution.

The concentrations of *E. coli* in growing waters were determined using the membrane filtration method (ISO 9308-1) (Anon. 2000a). The membrane filters were placed on freshly prepared selective media (membrane lauryl sulphate agar 'MLSA', eosin methylene blue Agar 'EMB'). Similarly, the concentrations of *E. coli* in *C. gariepinus* were determined using the pour-plate technique i.e., 0.1 ml of the prepared serially diluted aliquot were inoculated into sterile plates containing freshly prepared selective media (MLSA, EMB). Agar plates were incubated at 37 °C for 24 hours, and colonies were counted, calculated and expressed as colony-forming units (CFU) per 100 g of *C. gariepinus* or CFU per 100 ml of water. The concentrations of faecal coliforms in growing waters were determined using the membrane filtration method (ISO 9308-1, ISO 7899-2) (Anon. 2000a; 2000b). The membrane filters were placed on freshly prepared selective media (membrane faecal coliforms agar '*m*-FC'). Similarly, the concentrations of faecal coliforms in *C. gariepinus* were determined using the pour-plate technique i.e., 0.1 ml of the prepared serially diluted aliquot were inoculated into sterile plates containing freshly prepared selective media (*m*-FC). Agar plates were incubated at 44 °C for 24 hours, and colonies were counted, calculated and expressed as CFU per 100 g of *C. gariepinus* or CFU per 100 ml of water.

The concentrations of intestinal enterococci in growing waters were determined using the membrane filtration method (ISO 7899-2) (Anon. 2000b). The membrane filters were placed on freshly prepared selective media (membrane enterococci agar '*m*-Ent'). Similarly, the concentrations of intestinal enterococci in *C. gariepinus* were determined using the pour-plate technique i.e., 0.1 ml of the prepared serially diluted aliquot were inoculated into sterile plates containing freshly prepared selective media (*m*-Ent). Agar plates were incubated at 37 °C for 48 hours, and colonies were counted, calculated and expressed as CFU per 100 g of *C. gariepinus* or CFU per 100 ml of water. The concentrations of *Salmonella* and *Shigella* in growing waters were determined using the membrane filtration method. The membrane filters were placed on freshly prepared selective media (*Salmonella Shigella* agar 'SSA'). Similarly, the concentrations of *Salmonella* and *Shigella* in *C. gariepinus* were determined using the pour-plate technique i.e., 0.1 ml of the prepared serially diluted aliquot were inoculated into sterile plates containing freshly prepared selective media (SSA). Agar plates were incubated at 37 °C for 24 hours, and colonies were counted, calculated and expressed as CFU per 100 g of *C. gariepinus* or CFU per 100 ml of water.

Determination of accumulation factor

The rate of bioaccumulation of faecal indicator bacteria in *C. gariepinus* samples was determined by applying accumulation factor (AF) to the dataset i.e., by measuring the ratio of the concentrations of faecal indicator organisms in *C. gariepinus* relative to the concentration of faecal indicator organisms in the growing waters. In this study, the accumulation factor of each parameter was obtained by dividing the log concentration of each organism in *C. gariepinus* (CFU/100 g) by the

corresponding log concentration of organisms in the growing water (CFU/100 ml) at the same point in time.

Determination of the physicochemical properties of the growing water samples

The physicochemical properties of the growing water from the concrete pond were measured using standard methods (Anon. 2012). These include temperature (Celsius), pH, electrical conductivity (micro Siemens per centimetre), alkalinity (milligrams per litre), turbidity (nephelometric turbidity units), total dissolved solids (milligrams per litre), dissolved oxygen (milligrams per litre) and salinity (parts per thousand).

Statistical analysis

Data were transformed to $\log_{10}$, then examined using general descriptive statistics and checked for normality using the skewness and kurtosis statistic. Further analyses were undertaken using Statistical Package for Social Sciences (SPSS) Version 20.0, and all data were subjected to the Pearson's correlation analysis to determine whether there were positive correlations between the rate of bioaccumulation of the bacterial indicators of faecal contamination in *C. gariepinus* and physicochemical properties of the growing waters.

RESULTS

Bioaccumulation of bacterial indicators of faecal contamination in *C. gariepinus*

Levels of *E. coli* were observed to bioaccumulate to densities averaging 0.97 ± 0.03 lower than the levels recorded in the growing waters. Only on two sampling occasions (16%) was the accumulation factor of *E. coli* greater than one. The mean recorded accumulation factor of *E. coli* ranged from 0.93 to 1.03. Levels of faecal coliforms were observed to bioaccumulate to densities averaging 0.99 ± 0.03 lower than the levels recorded in the growing waters. The accumulation factor of faecal coliforms was observed to be greater than one on five sampling occasions (41%). The mean recorded accumulation factor of faecal coliforms ranged from 0.94 to 1.02. Levels of intestinal enterococci were observed to bioaccumulate to densities averaging 0.96 ± 0.03 lower than the levels recorded in the growing waters. Only on one sampling occasion (8%) was the accumulation factor of intestinal enterococci greater than one. The mean recorded accumulation factor of intestinal enterococci ranged from 0.91 to 1.01. Levels of *Salmonella* were observed to bioaccumulate to densities averaging 0.80 ± 0.05 lower than the levels recorded in the growing waters. In this study, the accumulation factor of *Salmonella* was observed to be lesser than one on all sampling occasions (100%). The mean recorded accumulation factor of *Salmonella* ranged from 0.74 to 0.92. Levels of *Shigella* were observed to bioaccumulate to densities averaging 0.83 ± 0.14 lower than the levels recorded in the growing waters. Only on one sampling occasion (8%) was the accumulation factor of *Shigella* greater than one. The mean recorded accumulation factor of *Shigella* ranged from 0.63 to 1.06 (Figure 1).

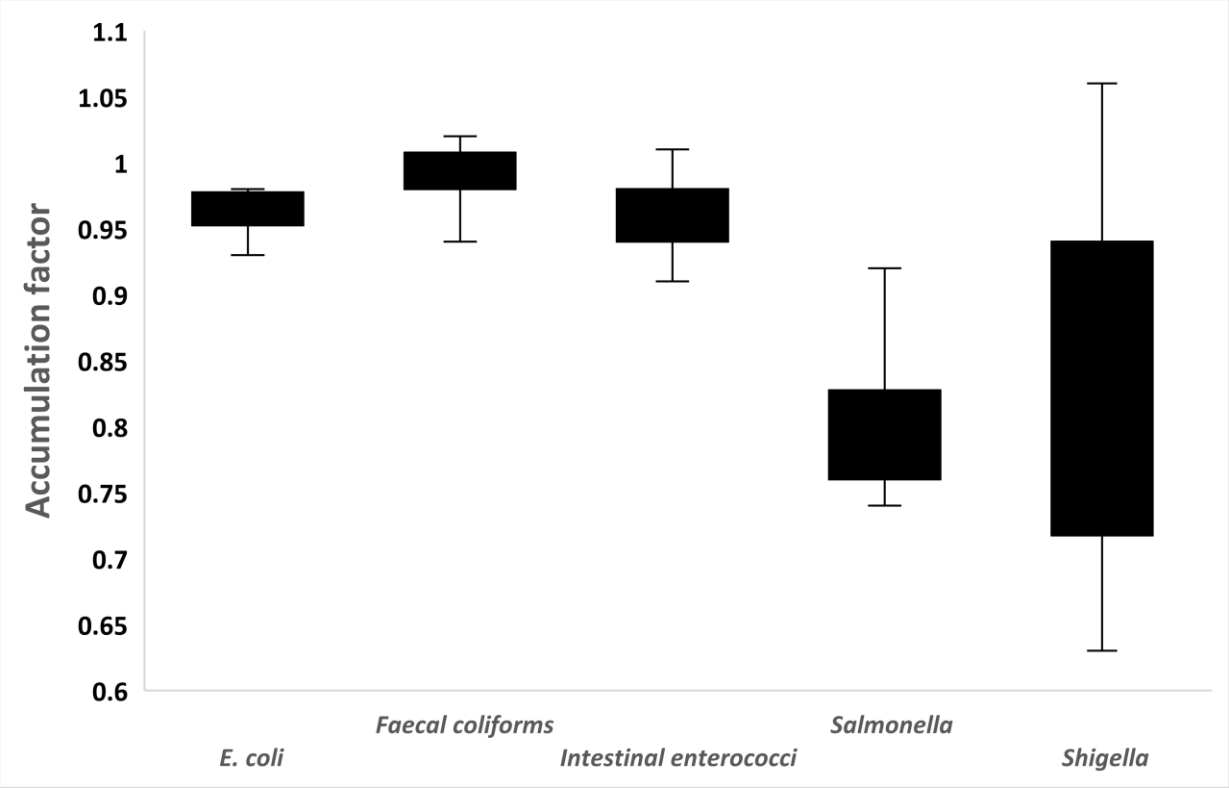

Figure 1
Mean values of accumulation factor of bacterial indicators of faecal contamination in *C. gariepinus*

Physicochemical properties of the growing waters

The temperature of the growing waters from the concrete pond ranged from 21 to 30 ˚C, while the pH values ranged from 6.0 to 8.2. The electrical conductivity ranged from 1.7 to 3.8 µS/m, whereas turbidity values ranged from 3.115 to 8.3 NTU and salinity ranged from 130 to 142 ppt. The total dissolved solids ranged from 146 to 174 mg/l, while the amount of dissolved oxygen ranged from 7.8 to 13.2 mg/l (Table 1).

Similarly, electrical conductivity exhibited a positive correlation with the bioaccumulation of *Shigella* (r = 0.53) in *C. gariepinus.* On the other hand, the bioaccumulation of *E. coli* (r = -0.51) and *Salmonella* (r = -0.50) showed a negative relationship with total dissolved solids, whereas the bioaccumulation of *Shigella* (r = -0.61), intestinal enterococci (r = -0.57) and faecal coliforms (r = -0.50) in *C. gariepinus* showed inverse relationships with turbidity, salinity and the amount dissolved oxygen in the growing waters respectively (Table 2)

Table 1

Physicochemical characteristics of the growing water samples from the concrete pond

Physicochemical parameters	Mean ± Standard Deviation (Minimum – Maximum)
Temperature (˚C)	25.42 ± 2.58 (21-30)
pH	7.08 ± 0.67 (6.0-8.2)
Salinity (ppt)	133.67 ± 4.29 (130-142)
Total dissolved solids (mg/l)	137.58 ± 10.72 (124-158)
Dissolved oxygen (mg/l)	9.19 ± 1.72 (7.8-13.2)
Electrical conductivity(µS/m)	2.59 ± 0.70 (1.7-3.8)
Turbidity (NTU)	5.69 ± 1.76 (3.1-8.3)

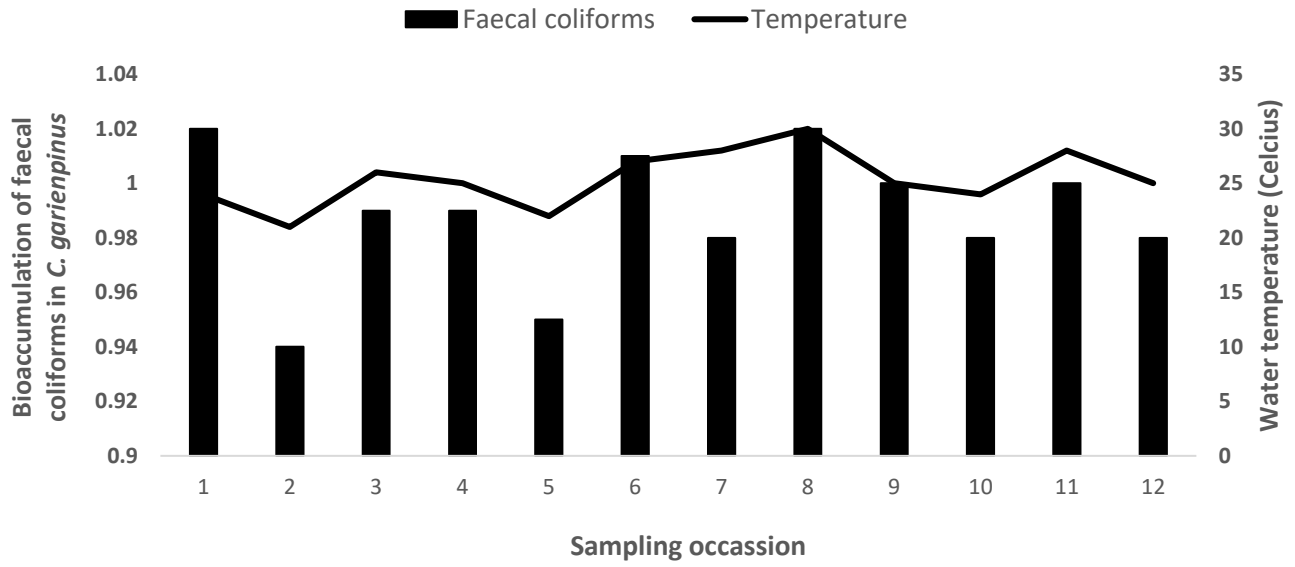

Figure 2

Positive relationship between water temperature and the bioaccumulation of faecal coliforms in *C. gariepinus*

Table 2

Significant Pearson's correlation coefficient (r) between the bioaccumulation of faecal indicator bacteria in *C. gariepinus* and the physicochemical characteristics of the growing water samples

	Temp.	pH	SAL	TUR	DO	EC	TDS	*E. coli*	FC	IE	SA	SHI
Temp.	1.00											
pH	0.18	1.00										
SAL	0.03	0.14	1.00									
TUR	**-0.55**	-0.09	0.02	1.00								
DO	-0.14	0.11	-0.43	0.02	1.00							
EC	-0.28	-0.17	-0.09	-0.38	-0.31	1.00						
TDS	-0.05	-0.40	0.00	0.13	-0.13	0.19	1.00					
E. coli	-0.03	0.28	0.28	0.43	-0.05	-0.42	**-0.51**	1.00				
FC	**0.71**	0.20	0.16	-0.34	**-0.50**	-0.18	-0.18	0.36	1.00			
IE	0.10	0.37	**-0.57**	-0.35	0.20	-0.19	-0.30	-0.11	0.22	1.00		
SA	-0.17	-0.04	-0.04	0.04	-0.11	0.25	**-0.50**	0.01	-0.10	0.25	1.00	
SHI	0.44	-0.42	-0.12	**-0.61**	-0.39	**0.53**	0.17	-0.39	0.31	0.16	0.13	1.00

Relationship between the bioaccumulation of bacterial indicators of faecal contamination in *C. gariepinus* and physicochemical properties of the growing waters

Positive correlations were observed between water temperature and the bioaccumulation of faecal coliforms (r = 0.71) in *C. gariepinus* (Figure 2).

DISCUSSION

This study investigated the rate of bioaccumulation of bacterial indicators of faecal contamination in *Clarias gariepinus* in a concrete pond in Akure, Nigeria, and examined the influence of physicochemical characteristics of the growing waters on the bioaccumulation rate. This is to gain a better understanding of the risk of foodborne illness associated with the human consumption of *Clarias gariepinus* raised in faecally impacted growing waters.

Studies have shown that the bioaccumulation process of microorganisms in aquatic animals such as *C. gariepinus* is affected by a range of environmental factors (Ringo and Strom 1994; Rajiv *et al*. 2012). The bioaccumulation of *E. coli* in *C. gariepinus* showed a negative correlation with total dissolved solids. The levels of *E. coli* that were observed to bioaccumulate to densities averaging 0.97 lower than the levels recorded in the growing waters may not be unconnected with the amount of the total dissolved solids in the growing waters. This was evident on those two sampling occasions (16%) when the accumulation factor of *E. coli* was observed to be greater than one. Similarly, the bioaccumulation of *Salmonella* in *C. gariepinus* exhibited a negative relationship with total dissolved solids. Levels of *Salmonella* were observed to bioaccumulate to densities averaging 0.80, this is much lower than those observed for *E. coli* in *C. gariepinus.*

Furthermore, studies have suggested that water temperature is a major factor responsible for variation of indicator microorganisms in aquatic animals. Olalemi *et al.* (2016) observed a positive correlation between water temperature and the bioaccumulation of faecal indicator bacteria in *Mytilus edulis*. The authors demonstrated that during periods of relatively high temperature faecal coliforms were bioaccumulated to considerably higher levels and during periods of low temperature, levels of bioaccumulated faecal coliforms were lower. This is in agreement with the findings of this study in which the levels of faecal coliforms were observed to bioaccumulate to densities averaging 0.99 lower than the levels recorded in the growing waters and exhibited a positive correlation with water temperature. Again, this may partly be responsible for the accumulation factor of faecal coliforms that was observed to be greater than one on five sampling occasions (41%). On the other hand, dissolved oxygen demonstrated a negative relationship with the bioaccumulation of faecal coliforms in *C. gariepinus*. Dissolved oxygen is important in aquaculture and reduces as a result of increase in water temperature, respiration and organic matter decomposition by aerobic aquatic organisms (Eze and Ogbaran 2010; Njoku *et al*. 2015).

The bioaccumulation of intestinal enterococci in *C. gariepinus* exhibited a negative relationship with salinity and this may be responsible for the observation in this study, whereby only on one sampling occasion (8%) was the accumulation factor of intestinal enterococci greater than one despite the fact that levels of intestinal enterococci were observed to bioaccumulate to densities averaging 0.96 lower than the levels recorded in the growing waters. This agrees with Ringo and Strom (1994) where the authors highlighted the effect of salinity on the microflora of the gastrointestinal tract of a free-living fish. Similarly, the bioaccumulation of *Shigella* in *C. gariepinus* exhibited a negative relationship with turbidity. Levels of *Shigella* were observed to bioaccumulate to densities averaging 0.83 lower than the levels recorded in the growing waters and only on one sampling occasion (8%) was the accumulation factor of *Shigella* greater than one. This observation also agrees with Olalemi *et al*. (2016) where the authors observed a negative correlation between turbidity and the bioaccumulation of faecal indicator bacteria in *Mytilus edulis*. In addition, the bioaccumulation of *Shigella* in *C. gariepinus* exhibited a positive relationship with electrical conductivity.

The results from this study demonstrated that the bioaccumulation of bacterial indicators of faecal contamination in African catfish (*Clarias gariepinus*) raised in a concrete pond show a positive relationship with water temperature and electrical conductivity; and a negative relationship with total dissolved solids, salinity, dissolved oxygen and turbidity. These results demonstrated that the rate of bioaccumulation of faecal indicator bacteria in *C. gariepinus* to a large extent depends on the physicochemical characteristics of the growing waters. Hence, fish raised in faecally impacted waters must be adequately cooked before consumption in order to prevent the occurrence of foodborne illness.

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