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INFLUENCE OF WEIGHT AND SEX ON INTESTINAL BACTERIA OF WILD *Clarias gariepinus* (Burchell, 1822) IN RIVER RIMA SOKOTO STATE, NIGERIA.

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ABSTRACT

This study was conducted to determine the influence of sex and weight on intestinal bacteria of wild *Clariasgariepinus*from River Rima Sokoto North western Nigeria. Twenty (20) samples of *C.gariepinus* were used and were grouped into two groups; ≤100g as juvenile and ≥100 as adults, and this give rise to 9 and 11 juveniles and adults respectively.The bacteria isolated, were investigated using standard microbiological procedures which includes; gram staining, microscopic examination and biochemical tests.Twelve (12) bacteria species were isolated from the sampled fishviz; *Aeromonashydrophila, Bacillus cereus, Bacillus pasterii, Bacillus pantothenticus, Bacillus subtilis, Bacillus megaterium, Enterococcus faecalis, Escherichia coli, Micrococcus luteus, Shigellaflexnerii, Sporosarcinaureae* and *Staphylococcus aureus*from both female and male of *C. gariepinus*. And the most dominant species of the isolated bacteria is *Bacillus* species. The study further revealed that, different fish body weights had no effect on intestinal bacteria of the species of fish, however, difference was observed among bacteria community composition between the sexes.

Keywords: *Clarias gariepinus*, wild, Intestinal bacteria, sex, weight and River Rima

INTRODUCTION

Fish is a vital source of food for people and contributes about 60% of the world's supply of protein (Abisoye *et al*., 2011). The advantage of fish as food is as a result of its easy digestibility and high nutritional value. Fish is viewed not only as food, but also as a ready source of income in the smallholder farming sector (Smith and Yoshida, 2000). *Clarias gariepinus* (BURCHELL, 1822), the African catfish is generally considered to be one of the most important tropical catfish species for aquaculture in West Africa (Clay, 1979). It is widely distributed throughout Africa, inhabiting tropical swamps, lakes, and rivers, some of which are subjected to seasonal drying (Olufemi *et al*., 1999). In Nigeria, the rearing of African catfish (*Clarias gariepinus*) is proving to be a lucrative option for small-scale inland fisheries (Cowx, 1992), and its consumption is on the increase (FDF, 2007). It is also known as the African sharp tooth catfish which is a large eel-like fish, usually of dark grey or black coloration on the back fading to a white belly. It is a highly nutritious fish that contains high quantity of vitamins, proteins, minerals and a little or no saturated fat and is low in carbohydrates (Lee, 1991).Bacterial agents are among the highly encountered causes of diseases in stressed warm water aquaculture. Aquatic micro-organisms not only influence the water quality but are known to be closely associated with the physiological status of the fish and the postharvest quality of fish (Al-Harbi and Uddin, 2003). Not all bacteria are pathogenic, some are beneficial. The gut microbiota can convert feedstuff into microbial biomass and fermentation end products that can be utilized by the animal host (Flint *et al*., 2008; Kong *et al.*, 2010). In the absence of this microbial fermentation, calories present in a diverse array of complex dietary glycans would be unavailable to the host (Costello *et al.*, 2010). Gut bacteria may also play an important role in host health (Ringo *et al*., 2003; Round and Mazmania, 2009).

Understandingthe intestinal microbiota of fishes is largely derived from culture-based approaches (Shiina *et al*., 2006), which usually reveal only a limited range of microbial diversity (Sullam *et al*., 2012). But this study focused mainly on wild fish species. Gender is one of the factors influencing the intestinal microbial composition in mammals, but whether fish also have gender-specific intestinal microbial patterns remains unknown (Liu *et al*., 2016).

The intestinal microbial communities in fish do not only play an important role in the nutrition of fish, but also influences the establishment of pathogenic microorganisms in the fish intestine by preventing their manifestation (Denev, 2000). There is paucity of information on gut microflora in fish (Mondal *et al*., 2008).

MATERIALS AND METHODS

Study Area

The study was conducted in River Rima, Sokoto, North-western Nigeria. Sokoto lies between longitudes 408'E and 605'E, and latitudes 120N and 13058'N (Mamman, 2000). The climate of Sokoto is tropical continental, with much of the rains between June and September, while the long dry season is from October and May (Ita *et al*., 1982). River Rima flows in a South-western direction over 100km and joins the major River Sokoto to form the Sokoto-Rima river system. The Sokoto-Rima River flows South-westerly in a direction up to Zogirma, where it changes direction and run southwards before emptying into River Niger. The River is seasonal, usually over flooding its banks during the rainy season in August and September, and up to October at times (Mock, 1963).

Collection and identification of fish sample

Twenty samples of *Clariasgariepinus* were collected in batches from Kwalkwalawa fish landing site.The samples were divided into two groups according to fish body weight: Less than or equal to 100g (≤ 100g) as juvenile and greater than 100g (>100) as adult in this study and with 9 and 11 juveniles and adults respectively.Fish samples were identified to species level using the guide provided by Reed *et al*. (1967), Holdens and Reed (1972) and Olaosebikan and Raji (1998).

Morphometric Measurement

In the laboratory, samples were examined fresh immediately after collection. On each sample, length parameters such as total length (TL), standard length (SL) were measured using a metric ruler (in cm) and weight parameters the total weight (TW) and gutted weight were weighed using top-loading balance (in g).Sex was determined by physical examination using the presence or absence of genital papilla of *C. gariepinus*.

Laboratory procedures for Bacteria Isolation and Identification

Preparation of Fish Gut for serial dilution

Sterile dissecting tools were used, the whole of the fish intestine and the content were cut into pieces. Sterile spatula was used to collect the cut samples into sterile measuring cylinder. To the measuring cylinder, distilled water was added to top up to 100ml. From the 100ml, 10ml was collected into sterile test tube. Six - fold serial dilutions were made using pipette (10^{-1} to 10^{-6}). To the serially diluted samples, 10^{-4}, 10^{-5} and 10^{-6} sample were inoculated on Nutrient Agar plates using pour plate method in triplicate and incubated at 37^0C for 24hours (Cheesbrough, 2000). Colony counting was conducted using physical counting of the organisms. Distinct colonies with different morphological characteristic were sub cultured using sterile inoculating loop into Nutrient agar plate for further biochemical test.

Gram Staining and microscopic examination

Gram staining was carried out as described by Cheesbrough, (2000). A drop of water was placed on a clean grease free glass slide, and a colony of bacteria was taken over from an overnight culture (24 hours culture) and a thin smear made. The smear was then allowed to air dry and it was flooded with crystal violet for 60 seconds before being washed by tap water. Lugol's iodine was added for 60 seconds and washed with tap water. It was then decolorized with 95% ethanol for 15 seconds. The smear was finally flooded with safranin for 1 minute and then washed with water and allowed to air dry. It was then viewed under oil immersion objective (100X). Gram positive cells stained purple while gram negative cells-stained red.

Identification and classification of the isolates

The following biochemical tests as described by cheesbrough, (2000) were carried out to further identify the bacteria isolated.

Catalase test

A few colonies of bacteria culture were transferred with a straight wire loop to a drop of hydrogen peroxide on a slide and emulsified Formation of bubbles (oxygen) was observed and if present indicates a positive test while lack of bubbles (oxygen) formation indicates the absence of catalase enzyme.

Starch hydrolysis

A loopful of each colony of the gram-positive rods was inoculated into a starch agar medium and incubated at 37^0C 24 hours. The plate was then flooded with iodine solution, blue black indicates positive reaction while yellow colour indicates negative reaction.

Indole test

A colony of each isolate was inoculated using a sterile wire loop onto a 5ml of sterile peptone – water enriched with 1% tryptophan in a test tube and incubated at 37^0C for 48hours. To the mixture, 0.5ml Kovac'sindole reagent was added and then shaken gently. A yellow layer at the surface of the medium

indicates a negative result while a positive result shows red or red-violet layer.

Citrate test
A colony of each isolate was inoculated using a sterile wire loop into Koser's citrate medium and incubated at 37^0C for 48 hours. A positive citrate test was indicated by formation of blue color while the initial green color indicates a negative test.

Urease test
A colony of each isolate was inoculated using a sterile wire loop onto Christensen's urea agar and incubated at 37^0C for 24 hours. This was then followed by observation for the change in colour of the medium from yellow to red which indicates positive result and absence of it indicate a negative result.

Methyl Red Test (MR Test)
A colony of each isolate was inoculated using a sterile wire loop onto glucose phosphate peptone water medium and was incubated at 37^0C for 48 hours. A few drops of methyl red were added to the culture and observe for the formation of red colour

RESULTS
Table 1. Below shows the percentage of occurrence and frequency of the isolated bacteria from the intestine of wild clarias gariepinus from Rima River Sokoto, with *Aeromonas hydrophila*, *Bacillus magaterium* and *Micrococcus luteus* as the highest observed bacteria species with 17.65% while, *Bacillus pasteris*, *Bacillus pantothenticus*, *Enterococcus faecalis*, *Shingella flexnerii* and *Saprosarcina ureae* as the least occurred bacteria species with 2.94%

Table1: Percentage of occurrence and frequency of the bacteria isolated from the intestine of *Clariasg ariepinus*

Organism	Frequency	Percentage of occurrence
Aeromonas hydrophila	6	17.65
Bacillus cereus	2	5.88
Bacillus pasterii	1	2.94
Bacillus pantothenticus	1	2.94
Bacillus subtilis	5	14.71
Bacillus megaterium	6	17.65
Enterococcus faecalis	1	2.94
Escherichia coli	2	5.88
Micrococcus luteus	6	17.65
Shigella flexnerii	1	2.94
Sporosarcina ureae	1	2.94
Staphylococcus aureus	2	5.88
Total	**34**	**100**

Table 2 shows the species of bacteria isolated from the female *Clarias gariepinus* with *Bacillus* species as the highest observed species whereas; *Escherichia*.sp, *Shigella*.sp and *Streptococcus*.sp were the least species observed from the intestine of the female *C. gariepinus*.

Table 2. Frequency and Percentage of Occurrence of Bacteria species common to female fish of Clarias gariepinus from River Rima Sokoto.

Organism	Frequency	Percentage of occurrence
Aeromonas.sp.	5	27.78
Bacillus.sp.	7	38.88
Escherichia.sp.	1	5.56
Micrococcus.Sp.	3	16.66
Shigella.sp.	1	5.56
Streptococcus.sp.	1	5.56
Total	**18**	**100**

Table 3 shows bacteria isolate common to the male of *Clarias gariepinus* as observed from the study area, Bacillus species was observed to be the highest occurred species with 50% occurrence rate while *Aeromonas. Sp*, *Escherichia. sp* and *Enterococcus. Sp*, were the least occurred bacteria species common to male *C. gariepinus*.

Table 3: Frequency and Percentage of Occurrence of Bacteria species common to female fish of *Clarias gariepinus* from River Rima Sokoto.

Organism	Frequency	Percentage of occurrence
Aeromonas. sp.	1	6.25
*Bacillus.*sp.	8	50
Escherichia. sp.	1	6.25
Enterococcus. sp.	1	6.25
Micrococcus. sp.	3	18.75
*Streptococcus*sp.	2	12.5
Total	**16**	**100**

Table 4, below showed the t-test statistics between bacteria load and sex of *C. gariepinus* which showed no significant difference

Table 4: the t-test Between Bacteria load and Sex of *C. gariepinus* in River Rima Sokoto

t	Sig (2 tailed).	DF	Std. Error of Difference
0.321	0.752	18	1830707.240

Table 5, is a t-test statistical table between bacterial load and weight of C. gariepinus which showed no significant difference between bacterial load and weight.

Table 5: the t-test Between Bacteria load and weight of *C. gariepinus* in River Rima Sokoto

t	Sig (2 tailed).	DF	Std. Error of Difference
-1.043	0.311	18	1791819.217

DISCUSSION

Twelve (12) bacteria species were isolated from the sampled fish in the present study which comprises of; *Aeromonashydrophila*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pasterii*, *Bacillus pantothenticus*, *Escherichia coli*, *Enterococcus faecalis*, *Micrococcus luteus*, *Shigella flexnerii*, *Sporosarcina ureae* and *Staphylococcus aureus*. The findings of this study revealed diverse bacteria species as compared to the findings of Tiukaa and Sampson, (2013) who discovered six (6) bacteria species; *Escherichia coli*, *Salmonella* sp, *Klebsiella* sp, *Shigella* sp, *Staphylococcus* sp and *Enterococcus* sp. from the intestine of cultured *Clarias gariepinus* the differences may be attributed to the environment, since this study was carried out in a wild and the other in a control environment (Culture system) and diversity of bacteria is a function of the environment. Though, some of the bacteria isolated from this study were found to be pathogenic as reported by Ponnerassery, (2012), *Aeromonas hydrophila* was found to cause Motile *Aeromonas septicemia* (MAS) in many fresh water fish species.

The independent sample t-test was used to determine the differences between bacteria load and sex of *C.gariepinus*, the result from this study showed no significant difference between bacteria load and sex of *C. gariepinus*(P=0.752), it is important to know that, the

present study revealed, no numerical differences between bacteria load and the sex of the species studied but, there is differences in the bacteria composition between the sexes of the species, moreover, endothermic animal studies have also revealed that the gut microbiota composition between gender are different (Mueller *et al*., 2006; Ding and Schloss, 2014 and Deusch *et al*., 2015) the differences observed between the endothermic animal and aquatic animal may be due to environment. Frequency of occurrence was used to determine the bacteria peculiar to female and male of *C. gariepinus* and the dominant bacteria identified in both sexes is *Bacillus* Sp. but, thereis differences in terms of bacteria composition between the sexes as presented above.

The findings from this study is in line with the findings of Li *et al*. (2016), who showed the abundance of genera *Aeromonas* and *Pseudomonas*, both belonging to phylum *Proteobacteria* with no significant difference between bacteria load and sex of largemouth bronze gudgeon (*Coreiusguichenoti*).The independent sample t-test between bacteria load and weight of *Clarias gariepinus* showed no significant difference, this indicates that, different fish body weights had no effect on intestinal microbiota of the fish species. These findings agreed with the findings of Li *et al.* (2016) who reported no significant different between fish body weights and intestinal microbiota of largemouth bronze gudgeon.

CONCLUSION AND RECOMMENDATION

This study revealed the following bacteria isolated from the intestine of wild *Clariasgariepinus*, *Aeromonashydrophila*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pasterii*, *Bacillus pantothenticus*, *Escherichia coli*, *Enterococcus faecalis*, *Micrococcus luteus*, *Shigella flexnerii*, *Sporosarcina ureae* and *Staphylococcus aureus*, but it is important to note that, *Bacillus* sp. appeared to be the dominant bacteria in the both sexes. The study revealed that there was no significant difference observed between sex or weight and bacterial load but did however reveal a difference in bacterial composition between sexes.

From the result of this study, it is recommend further research to be carried out on Bacillus species as probiotics, since Bacillus species is the highest observed bacteria species in both sexes.

REFERENCE

Abisoye, B. F., Ojo, S. S., Adeyemi, R. S., and Olajuyigbe, O. O. (2011). Bacteriological Assesment of Some Commonly Sold Fishes in Lagos Metropolis Market Nigeria. *Journal of Micobiology Reserve*, **1** (2):23-26.

Al- Harbi, A. H., and Uddin, N. (2003).Quantitative and qualitative studies on the bacterial flora of hybrideOreochromisniloticus and O. aureus cultured in earthen ponds in Saudi Arabia.*Aquaculture Research* **14**: 43 - 48.

Bagenal, T. B., and Tesch, F. W. (1978). Age and Growth In: Bagenal T.B. (Ed.). *Method for the Assessment of Fish Production Fresh Waters.*3rd.edition. Blackwell Scientific Publication, 93 - 123.

Cheesbrough, M. (2000).*Fungal pathogens District laboratory practice in Microbiology*.Cambridge University press.231-301.

Clay, D. (1979). Population biology, growth and feeding of the African catfish, *Clariasgariepinus* with special reference to juveniles and their importance in fish culture.*Archives of Hydrobiology*, **87(**4), 453 - 482.

Costello, E., Gordon, J., Secor, S., and Knight, R. (2010).Postprandial remodeling of the gut microbiota in Burmese python.*Journal of the International society for Microbial Ecology* **4**, 1375 - 1385.

Cowx, G. (1992). Aquaculture development in Africa: Training Reference Manual for Aquaculture Extortionist Hull. Humberside International Fisheries Institute. (1): 1-26.

Denev, S. S., Moutafchieva, R., and Beev, G. (2000).Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics in finfish.*Aquaculture International Aquatic Research*, **1**(1), 1 -29.

Deusch O., O'Flynn C., Colyer A, Swanson K.S., Allaway D., and Morris P. A. (2015) longitudinal study on the feline faecalmicrobiome identifies changes intoearly adulthood irrespective of sexual development. *PLoS One*.**10**:e0144881

Ding T. and Schloss P.D. (2014) Dynamics and associations of microbial communitytypes across the human body. *Nature*.**509**:357–67

FDF.(2007). *Fishries Statistics of Nigeria*, Fourth Edition. Abuja: Federal Department of Fisheries.250-342

Flint, H. J., Bayer, E. A., Rincon, M. T., and White, B. A. (2008). Polysaccharide utilization by gut bacteria:Potential for new insights from genomic analysis. *Nature Reviews Microbiology*, **6,** 121 - 131.

Holdens, M., and Reed, W. (1972).*West African Freshwater Fish*. London: Longman Publisher.234

Ita, E. O., Balogun, J. K., and Ademola, A. (1982). A Preminary Report of Pre-impoundment Fisheries Study of GoronyoReservior, Sokoto State, Nigeria.Sokoto Nigeria: A report submitted to the Sokoto Rima River Basin Development Authority (SRRBDA).

Kong, Y., Teather, R., and Forster, R. (2010). Composition, spatial distribution, and diversity of the bacterial communities in the rumen of cows fed different forages. *Federation of European Microbiological Society Microbiology Ecology*,**74**: 612 - 622.

Lee, J. S. (1991). Commercial catfish farming.Hongkong: Interstate Publishers, Inc.455

Li, X., Yan, Q., Ringo, E., Wu, X., He, Y. and Yang, D. (2016) The influence of weight and gender on intestinal bacterial community of wild largemouth bronze gudgeon(*Coreiusguichenoti*, 1874). *Bio Med Central Microbiology***16**: 191.

Liu, Y., Yao, Y., Li, H., Qiao, F., Wu, J., and Du, Z.-Y.(2016). Influence of Endocrine on Intestinal Microbiota in Zebrafish.*Plos One Journal*, 135 - 139.

Mamman, A. B. (2000).*Nigeria: A People United, A future Assured* (Sokoto State). Lagos: Gabumo Publishing Company Ltd.12

Mock, F. J. (1963). Hydrological study on the flooding of the Rima Fadama.Sokoto: Unpublished report. United Nations Special Funds Intervention F.A.O.12

Mondal, S., Sec, S., and Ray, A. (2008).Distribution of enzyme - producing bacteria in the digestive tracts of some freshwater fish.*ActaIchthyologicapiscatoria*.*Aquaculture*, **38** (1), 1 - 8.

Mueller S., Saunier K., Hanisch C., Norin E., Alm L., Midtvedt T., Cresci A., SilviS.,Orpianesi C., Verdenelli M.C., and Clavel T. (2006). Difference in faecalmicrobiota indifferent European study populations in relation to age, gender, andcountry: a cross-sectional study.*Applied Environmental Microbiology*.**72**:1027–33

Olaosebikan, B. D., and Raji, A. (1998). New Bussa: Federal College of Freshwater Fisheries Technology Nigeria. 23-42

Olufemi, B. E., Akinlabi, B. E., and Agbede, S. A. (1999). Aerobic bacterial pathogen isolated from African catfish *Clariasgariepinus*. *Tropical veterinary Medicine*, 177 - 180.

Ponnerassery, S.S., Al-ghabshi, A., Al-mazrooei, N. and Al-habsi, S. (2012).Comparative pathogenomic of bacteria causing infectious diseases in fish.*International Journal of Evolutionary Biology*.**10**:656 675.

Reed, W. J., Burchard, A. J., Hopson, J., Jenness, J., and Yaro, I. (1967). Fish and Fisheries of Norther Nigeria (1st ed.). Ministry of Agriculture Norther Nigeria.

Ringo, E., Olsen, R., Mayhew, T., and Myklebust, R. (2003).Electron microscopy of the intestinal microflora of fish.*Aquaculture*, **227**, 395 - 415.

Round, J., and Mazmanian, S. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Review Immunology***, 9**, 313 - 323.

Shiina , A., Itoi, S., and Washio, S. (2006). Molecular identification of intestinal microflora in Takifuguniphobles.*Comparative Biochemistry and physiologyD***. 1**, 128 - 132.

Smith, G. A., and Yoshida, I. (2000). Sustainable integrated Kysei nature farming EM technology and food security in africa.

Sullam, K., Essinger, S., and Lozupone, C. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: A meta - analysis. *Molecular Ecology*, **21**, 3363 - 3378.

Tiukaa J.A and Sampson U.N (2013) Bacteria flora of African Catfish (*Clariasgariepinus*) Harvested from Pond in Uyo South-South Nigeria. *Journal of Envrionmental Science, Toxicology and Food Technology***5**:(3) 72-76

Ward, N. L., Steven, B., Penn, K., Methe, B. A., and Detrich III, W. H. (2009).Chacracterization of the intestinal microbiota of two Antarctic *notothenioid* fish Species.*Extremophiles*, **13**, 679 - 685.