SKIN AND GUT MICROBIOTA OF THE AFRICAN SNAKEHEAD, *PARACHANNA OBSCURA*, GUNTHER, 1861 FROM RIVER OGUN, SOUTHWEST NIGERIA.

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ABSTRACT

Baseline information on the microbiota associated with the skin and gut of potential aquaculture fish candidates is needed to ensure predictive health management under culture. Therefore, the microbial investigation of eighty-eight live specimens of Parachanna obscura caught with Malian traps was done. The skin bacteria species differed from that of the gut. Organisms predominantly present on the skin were E. coli, Bacillus subtilis, Klebsiella sp., Pseudomonas aeruginosa, Micrococcus sp., Staphylococcus aureus, Staphylococcus sp., Enterobacter aerogenes, Bacillus sp., Aeromonas hydrophylia, Enterobacter sp., and Proteus sp. Major gut bacteria were Bacillus licheriforms, Flavobacterium sp., Pseudomonas aeruginosa, Klebsiella sp., Bacillus megaterium, Aeromonas hydrophilia, Pseudomonas sp., Enterobacter aerogenes, Aeromonas sp., and Bacillus sp. Total Bacterial Count (TBC) and Total Coliform Count (TCC) were significantly higher ($P<0.05$) in the gut of the young fish than the adult but the Total Escherichia Coli Count (TEC) was significantly higher ($P<0.05$) in the adult fish. Bacterial load on the skin of the young P. obscura was significantly higher ($P<0.05$) than that of the adult fish. The TBC, TCC and TEC obtained for wet season water were: TBC $5.92\pm1.03 \times 10^5$ cfu/ml, TCC $5.8\pm0.36 \times 10^3$ cfu/ml, TEC $4.05\pm0.35 \times 10^2$ cfu/ml while for dry season; TBC $1.44\pm0.02 \times 10^5$ cfu/ml, TCC $6.12\pm0.32 \times 10^3$ cfu/ml, $2.05\pm0.36 \times 10^2$ cfu/ml. Aspergillus sp., Aspergillus niger, Aspergillus flavus, Penicillium spp. were the fungi isolated from the water, skin and gut of P. obscura. Antibiotics sensitivity test revealed that most of the bacteria were resistant to Tetracycline but susceptible to Ciprofloxacin and Augmentin.

***Keywords**: Microbial analysis, P. obscura, Bacteria, Fungi, Antibiotic sensitivity*

INTRODUCTION

In order to cut down on fish importation and meet the demands of fish consumers in Africa, the quantity of fish produced on the continent must increase by 267% relative to the 2006 production level by 2020 (FAO, 2006). This has precipitated the need to continue investigation on indigenous fish species for their potential use in aquaculture and species such as *Parachanna obscura* with great aquaculture potentialities have been

discovered (Osho and Usman, 2019). The species is of high commercial value because of its good taste and high quality flesh (Abassi and Ogar, 2013). Natural stocks of *P. obscura* are overexploited leading to gross depletion in natural waters. Successful domestication and culture of this species will help to preserve natural stock, continuously produce fingerlings for stocking, and most importantly provide food fish for the populace (Kpogue *et al.*, 2013).

However, inadequate knowledge about prevention, treatment and control of fish diseases is a problem that has limited the growth of aquaculture in many countries. In attempts to curb this, farmers resort to indiscriminate use of antibiotics. The fact that most fish diseases are caused by opportunistic microbes has led to the postulation that a good knowledge of fish microbiota will reduce the incidence of fish diseases in aquaculture (Romero *et al.*, 2012). The microbiota of the skin and gut of fish modulate the host's innate immune system which prevents diseases, metabolize cellulose and other complex polysaccharides that fish are unable to digest on their own due to the lack of endogenous cellulase production (Ganguly and Prasad, 2012). The microbiota of the skin of fish gives information on the microorganism concentration and the state of pollution by biological wastes giving rise to pathogenic microorganism. The gastrointestinal (GI) microbiota of vertebrates plays critical roles in nutrition, development, immunity and resistance against invasive pathogens (Wang *et al*., 2007). In-depth knowledge of the structure and relationships between GI microbiota and their host fish can provide insight into both the function and dysfunction of the host organism.

In spite of the substantial roles that skin and intestinal microbiota play in host metabolism, immunity and health, the composition and structure of microbial communities on the skin and gut of *P. obscura* have not been sufficiently documented. A number of researches have been conducted on the ecology (Ama-Abasi and Affia, 2010), feeding habit (Bolaji *et al.*, 2011), proximate composition (Ama-Abasi and Ogar, 2013), haematology (Adebayo *et al.*, 2007), parasitic helminths infections (Osho, 2017) and digestive enzymes (Odedeyi, 2007). A baseline information on the microbiota associated with the skin and gut of this species is therefore needed to ensure predictive and preventive health management of the species in culture.

MATERIALS AND METHODS

Study Area

The Ogun River is located in Southwest Nigeria, latitudes 6º26' N and 9º10′N and longitudes 2º28′E and 4º8′E. Fish were procured from fishermen catch and transported to the Microbiology

Laboratory of the University of Ibadan for analysis.

Microbiological sampling and analysis

Fish (gut and skin)

A total of sixty live samples of *P. obscura* were collected from fishermen's Malian trap catches bimonthly for 12 months. The samples were split into two sizes; 24.5-30 cm (Adult) and 15-24 cm (Young) (Kpogue *et al.,* 2013). All the fishes were apparently healthy and had no lesions on them. The fishes were killed and aseptically dissected after percussive stunning. Bacteria was isolated from the gut and skin, aliquots of 1ml of serial dilutions were inoculated using pour plate technique on Nutrient Agar, MacConkey Agar, Salmonella Shigella agar, and thiosulphate citrate bile salt sucrose agar (TCBS). The plates were incubated at 37^{o}C for 24 – 48 hrs. Biochemical tests (Gram staining, Motility test, Indole test, Methyl red test, Starch hydrolysis, Oxidase test, Catalase test, Sugar fermentation, Antibiotics Sensitivity test) were done (Olutiola *et al.,* 1991).

Isolation of Fungi

The fungi cultures were isolated using Potato Dextrose Agar (PDA) and identified using microscopic and morphological features.

Antibiotics Sensitivity Test

Four antibiotics were used which includes ciprofloxacin, Tetracycline, Augmentin and chloramphenicol (Tusevljak *et al.,* 2013).

DATA ANALYSIS

Data were analysed using the one-way analysis of variance. T-test was also used to compare and evaluate the difference between means of the two seasons. SPSS Version 22.0 was used for analysis. Significance level was taken at ($P<0.05$)

TABLE 1: BACTERIA ISOLATED FROM *P. OBSCURA* AND WATER OF RIVER OGUN

Sample	Bacteria isolates
Skin	*Escherichia coli, Bacillus subtilis, Klebsiella sp., Pseudomonas aeruginosa, Micrococcus sp., Staphylococcus aureus, Staphylococcus sp., Enterobacter aerogenes, Bacillus sp, Aeromonas hydrophylia, Enterobacter sp.* and *Proteus sp*
Gut	*Bacillus licheniiforms, Flavobacterium spp., Pseudomonas aeruginosa, Klebsiella sp., Bacillus megaterium, Aeromonas hydrophylia, Pseudomonas sp., Enterobacter aerogenes, and Aeromonas spp.* and *Bacillus sp.*
Water	*Pseudomonas aureginosa, Klebsiella spp, Miccococus sp, Staphyloccocus aureus, Enterobacter sp, Bacillus sp, Aeromonas sp, Esherchia coli* and *Proteus sp*

RESULTS

The checklist of bacteria isolated from the skin and gut of *P. obscura* as well as the water of River Ogun are given on Table 1. *Escherichia coli, Bacillus subtilis, Klebsiella sp., Pseudomonas aeruginosa, Micrococcus sp., Staphylococcus aureus, Staphylococcus sp., Enterobacter aerogenes, Bacillus sp, Aeromonas hydrophylia, Enterobacter sp. and Proteus sp* were isolated from the skin of *P. obscura.* The bacteria isolated from the gut of the fish were *Bacillus licheniiforms, Flavobacterium spp., Pseudomonas aeruginosa, Klebsiella sp., Bacillus megaterium, Aeromonas hydrophylia, Pseudomonas sp., Enterobacter aerogenes, and Aeromonas spp. and Bacillus spp.* while *Pseudomonas aureginosa, Klebsiella spp, Miccococus sp, Staphyloccocus aureus, Enterobacter sp, Bacillus sp, Aeromonas spp, Escherichia coli, Proteus sp* were isolated from the bacteria cultured from the water samples from River Ogun.

TABLE 2: GUT MICROBIAL LOAD OF ADULT *P. OBSCURA* IN RAINY AND DRY SEASONS (CFU/ML**)**

Parameter	Fish Class	Season		*p-value*
		Dry	Rainy	
TBC (x10^5)	**Juvenile**	2.06±0.96	3.94±2.47	0.02
	Adult	1.36±0.39	1.94±1.41	0.26
TCC (x10^3)	**Juvenile**	2.27±0.74	3.57±0.36	0.00
	Adult	1.91±0.46	2.75±2.92	0.47
TEC (x10^2)	**Juvenile**	7.29±1.98	4.27±2.64	0.12
	Adult	7.25±2.12	5.38±1.33	0.15

TBC: Total Bacteria Count; TCC: Total Coliform Count; TEC: Total E. Coli Count

TABLE 3: SKIN MICROBIAL LOAD OF ADULT *P. OBSCURA* IN RAINY AND DRY SEASONS (CFU/ML**)**

Parameter	Fish Class	Season		*p-value*
		Dry	Rainy	
TBC (x10^5)	**Juvenile**	4.01±2.33	4.94±3.65	0.03
	Adult	3.38±0.76	3.27±2.55	0.92
TCC (x10^3)	**Juvenile**	3.21±2.39	4.69±2.78	0.38
	Adult	6.63±1.39	2.43±1.65	0.00
TEC (x10^2)	**Juvenile**	5.80±3.17	3.59±3.13^a	0.24
	Adult	7.13±2.03	6.63±1.48	0.67

TBC: Total Bacteria Count; TCC: Total Coliform Count; TEC: Total E. Coli Count

The Total Bacteria Count (TBC), Total Coliform Count (TCC) and Total *Escherichia Coli* from the gut and skin of young and adult *P. obscura* from River Ogun are shown in Tables 2 and 3. For the young fish, TBC and TCC of gut bacteria were significantly different between the seasons while TEC was not. Although the TEC values of dry season (7.29±1.98 CFU/ml) was higher than that recorded for the rainy season (4.27±2.64 CFU/ml), the values were not significantly different ($p>0.05$). For the bacteria isolated from the skin, TBC was significantly different for juvenile fishes across the season while TCC and TEC were not. For the adult fishes, only the TCC was significantly different among the three parameters. Table 4 indicates the seasonal variation in the bacteria load of the water body determined using the student's T-test and expressed as mean ± standard deviation. From the table, the comparison of total bacteria counts in the dry and rainy season showed that the TBC and TEC were significantly higher in the rainy season than in the dry season p value of 0.000 ($p<0.05$). The reverse however is the case for total coliform count which is significantly higher in the dry season than the rainy season, with p-value of 0.000 ($p < 0.05$).

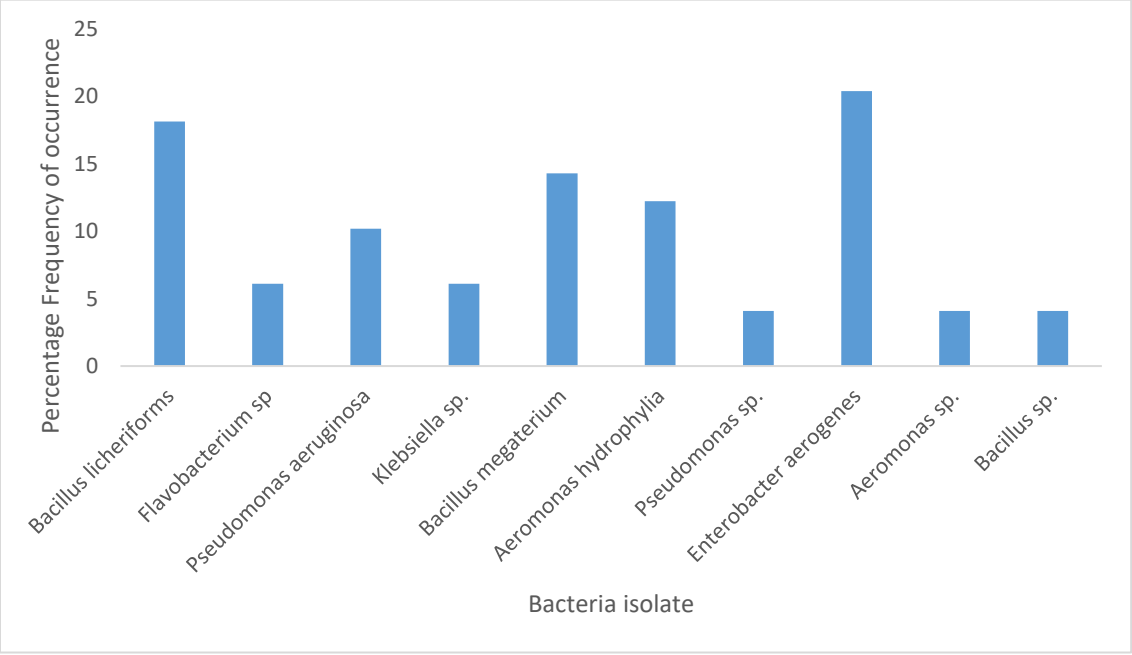

FIGURE 1: PERCENTAGE FREQUENCY OF BACTERIA OCCURRENCE IN THE GUT OF *P. OBSCURA* FROM OGUN

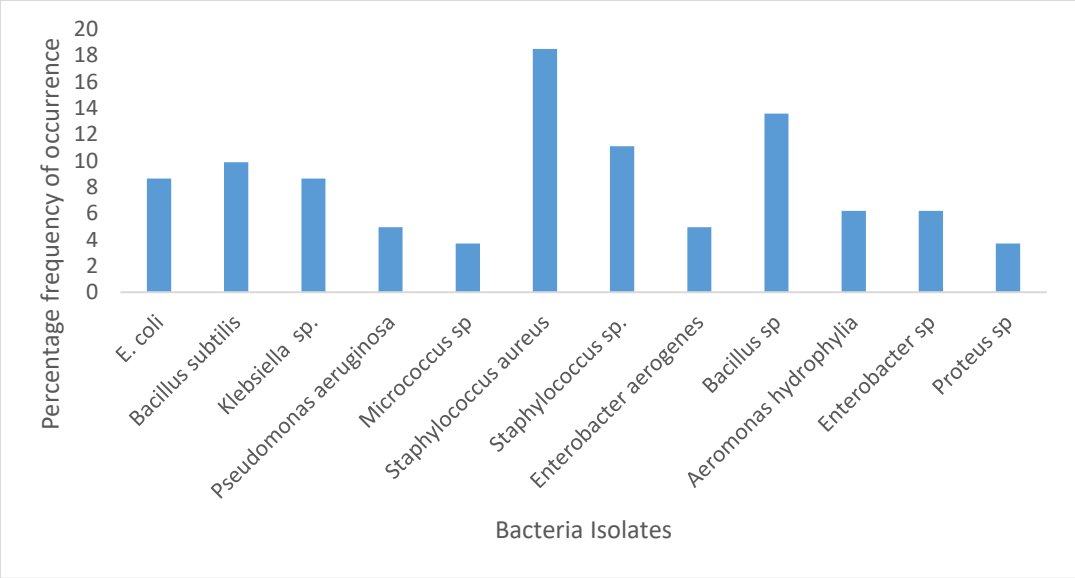

FIGURE 2: PERCENTAGE FREQUENCY OF BACTERIA OCCURRENCE ON THE SKIN *P. OBSCURA* FROM OGUN

The frequency of occurrence of encountered bacteria on the gut and skin of *P. obscura* from River Ogun can be seen on Figures 1 and 2, respectively. In the gut of the fishes, *Enterobacter aerogens* was the most frequently encountered while *Aeromonas sp.*, *baccilus sp.* and *Pseudomonas sp.* were the least frequently encountered. On the skin, *Staphylococcus aureus* was found to be the highest occurring bacteria while the least frequently encountered was *Proteus sp.* As shown in Table 5, Fungi species isolated from the fishes were *Aspergillus sp., Aspergillus niger, Aspergillus flavus, Aspergillus flavus, Pennicilium sp., and Fusirium sp.* while from the gut of the fish, *Aspergillus niger, Aspergillus flavus, Pennicillium sp* were isolated. Tables 6 and 7 gives the antibiotic susceptibility patterns of organisms. They give indications of how bacteria within the skin and gut react to four commonly used antibiotics.

TABLE 4: BACTERIOLOGICAL PROFILE OF WATER (CFU/ML**)**

Seasons	TBC(x10^5)	TCC (x10^3)	TEC (x10^2)
Dry	1.44±0.02^a	6.12±0.32^a	2.05±0.32^a
Rainy	1.44±0.02^a	5.83±0.36^b	4.05±0.35^b

Values with same different superscript down the column are not significantly different p>0.05

TABLE 5: FUNGI SPECIES ISOLATED FROM THE SKIN AND GUT OF P. OBSCURA FROM R. OGUN

Sample	Fungi isolates
Skin	*Aspergillus spp., Aspergillus niger, Aspergillus flavus, Aspergillus flavus, Pennicilium spp, and Fusirium spp.*
Gut	*Aspergillus niger, Aspergillus flavus, Pennicillium sp*

TABLE 6: ANTIBIOTIC SENSITIVITY TEST OF BACTERIA FROM GUT OF *P. OBSCURA* FROM R. OGUN

Isolate/antibiotic	Tetra	Chloram	Cipro	Aug
Bacillus licheriforms	1(9.1)	6(54.5)	11(100)	5(45.5)
Flavobacterium sp	2(50)	3(75)	4(100)	2(50)
Pseudomonas aeruginosa	3(60)	5(100)	5(100)	5(100)
Klebsiella sp.	0	6(100)	6(100)	1(16.7)
Bacillus megaterium	8(80)	10(100)	10(100)	3(30)
Aeromonas hydrophylia	4(57.1)	7(100)	5(71.4)	7(100)
Pseudomonas sp.	2(33.3)	7(100)	7(100)	7(100)
Enterobacter aerogenes	4(66.7)	5(83.3)	3(50)	5(83.3)
Aeromonas sp.	12(100)	12(100)	12(100)	12(100)
Bacillus sp.	1(100)	1(100)	1(100)	1(100)

Keys: Tetra= Tetracycline, Chloram= Chloramphenicol,*Cipro= Ciprofloxacin, Aug= Augmentin

TABLE 7: ANTIBIOTIC SENSITIVITY TESTS OF BACTERIA FROM SKIN OF *P. OBSCURA* FROM R. OGUN

Isolate/antibiotic	Tetra	Chloram	Cipro	Aug
Escherichia coli	1(12.5)	3(37.5)	8(100)	8(100)
Bacillus subtilis	9(60)	8(53.3)	12(80)	15(100)
Klebsiella sp.	2(20)	5(50)	10(100)	10(100)
Pseudomonas aeruginosa	2(40)	3(60)	5(100)	5(100)
Micrococcus sp.	0	2(100)	2(100)	2(100)
Staphylococcus aureus	0	6(85.7)	1(12.5)	0
Staphylococcus sp.	4(80)	4(80)	0	0
Enterobacter aerogenes	6(18)	13(72.2)	18(100)	18(100)
Bacillus sp.	5(45.5)	4(36.4)	11(100)	11(100)
Aeromonas hydrophylia	3(50)	4(66.7)	6(100)	6(100)
Enterobacter sp.	2(100)	2(100)	2(100)	1(50)
Proteus sp.	2(100)	2(100)	2(100)	2(100)

Keys: Tetra= Tetracycline, Chloram= Chloramphenicol,*Cipro= Ciprofloxacin, Aug= Augmentin

DISCUSSION

From this study, there were variations in the bacteria load on skin, gut and water tested. The gut harboured both pathogenic and non-pathogenic organisms (Tiamiyu *et al.,* 2011). Fishes pick microbes in the digestive tract from their environment through sediment, water and food that are populated with bacteria, fungi and other microorganisms (Saha *et al.,* 2006). The microbial counts (TBC, TCC) in the gut of juveniles *P. obscura* were significantly higher ($P<0.05$) during the rainy season than at the dry season. This could be as a result of increased coliform in water in rainy season (Wilson *et al.,* 2008). However, the microbial load in adult fish guts was not significantly different across season, indicating non-transience. This also shows better adaptation to their microbial environment and capability of forming symbiotic associations within the digestive tract of fish (Jimoh *et al.,* 2014). It was also observed that the bacteria load was significantly higher ($P<0.05$) in the adult fish gut than in the young fish gut, thereby supporting the report of Fessehaye (2006) that bacterial diversity increases with age. This difference can also be attributed to the difference in food fed on by the different sizes of fish (Dimitroglou *et. al.,* 2009). Kpogue *et al.* (2013), reported that juveniles of *P. obscura* are omnivores while the adults are purely piscivores.

The skin had higher bacteria load than the gut or water. Highest total bacteria count of 4.94±3.65 cfu/ml was found on the skin of juvenile fishes during the raining season, highest total coliform count (6.63±1.39 cfu/ml) was recorded from the skin of adult fishes during the dry season. The only exception was the total *E. coli* where the highest value was recorded in the gut of juvenile fishes. It has been posited that the skin of fishes is colonised by large numbers of microorganisms that form commensal or mutual relationships with their hosts (Spor *et al.,* 2011). The bacteria identified in water were similar to those identified in the gut and skin. Fishes are in continuous contact with microorganisms in the water and sediment, influencing the microbial species diversity on their skin, gills and alimentary tract.

Apart from the environment, seasonality is also another factor that is thought to affect the microbial load. However, there was no statistical difference ($P > 0.05$) in the bacterial composition (TBC, TCC and TEC) on the skin across seasons. There was also no significant difference in the bacterial TBC and TEC within the length group across the seasons. The above findings are in tandem with the report of Al-Harbi and Uddin (2005), who reported no marked statistical difference in the bacterial load of Tilapia within the same size range across seasons. The TBC of fish skin across lengths was not

significantly different (P>0.05) in both seasons. Rivers in Nigeria are subjected to both domestic and industrial pollution and fishes inhabiting these waters may become infected by pathogenic organisms. The effect of seasonal variation was observed as the values obtained for microbial load during the rainy season for both size groups were higher than those observed during the dry season. This may be as a result of surface run-offs, erosion and leaching of nutrient and untreated sewage into the river in rainy season.

The dominant fungi species Identified are *Penicillium sp. and Aspergillus sp. Penicillium sp. and Aspergillus sp.* was identified in both seasons in the gut. With *Aspergillus* having a dominance of up to 60%. The result gotten from this study is similar to that of Tilapia reported by Thillaimaharani *et al.* (2012). *Fusirium Aspergillus sp.* and *Penicillium sp.* were identified on the skin during the dry season. The fungi species obtained from the skin and gut were similar obtained from *Clarias gariepinus* but the occurrence pattern differ (Jimoh *et al*., 2014). *Aspergillus niger, Aspergillus flavus* are associated with disease outbreak in fish (Olayemi *et al*., 1990).

The antibacterial sensitivity gives the level at which the organisms are susceptible to the antibiotics used in the study. A value of 0 means that the level of susceptibility to that antibiotics is 0. Hence the organism is strongly resistant to that particular antibiotics. While 100 means that the organisms are totally susceptible to the antibiotics used. Results show that most of the organisms are resistant to Tetracycline while most are susceptible to Ciprofloxacin and Augmentin. The prevalence of antimicrobial resistance among food pathogens has increased during recent decades (Chu *et al.,* 2016). The organisms isolated and identified were majorly opportunistic and pathogens. These organisms could cause serious disease outbreak in culture during stress conditions and poor water quality. Indiscriminate use of antibiotics especially tetracycline in culture should be avoided.

CONCLUSION

This research revealed the impact of seasonality on the microbial load of the skin of *Parachanna obscura* and the water of River Ogun. There was a marked increase in the microbial load of the water and of the skin during the raining season. The antibiotic sensitivity test revealed that tetracycline does not have the capability of inhibiting the growth of most of the bacteria isolated while many of the bacteria were susceptible to Ciprofloxacin and Augmentin. This research will serve as a predictive and preventive tool for health management of *P. obscura* during its culture.

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