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Occurrence of bacteria in the buccal cavity, gill and skin of *Chrysichthys nigrodigitatus, Sardinella maderensis, and Mugil cephalus* from Lagos lagoon Nigeria

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ABSTRACT

Morphometric characteristics of *Chrysicthys nigrodigitatus, Sardinella maderensis and Mugil cephalus* species collected from Lagos lagoon were studied. Swab samples were taken from the gills, buccal cavity and skin of the three fish species. The cultural, morphological and biochemical characteristics of the bacterial isolates was done. The morphometric characteristics revealed that there is significant difference between the weight of *Chrysicthys nigrodigitatus* (404.00 ± 11.28 g), *Sardinella maderensis* (173.68 ± 2.69 g) and *Mugil cephalus* (87.50 ± 4.11 g). *Chrysicthys nigrodigitatus* had the highest count occurring in the buccal cavity (2.97 x 10^4 cfu/ml) the bacteria count ranged from 2.0 × 10^3 -2.90 × 10^4, 1.64 ×10^4-2.99 × 10^4 and 1.1 × 10^3 -2.56 × 10^4cfu/ml in the skin, buccal cavity and gill of *Chrysicthys nigrodigitatus* respectively; 1.6 × 10^3 -2.78 × 10^4, 1.5 × 10^3 -2.95 × 10^4 and 1.1 × 10^3 -2.71 × 10^4 in the skin, buccal cavity and gill of *Sardinella maderensis* respectively; 5.7 × 10^3 -2.58 × 10^4, 6.3 × 10^3 -2.83 × 10^4, 2.1 × 10^3 - 2.78 × 10^4 in the skin, buccal cavity and gill of *Mugil cephalus* respectively. Bacteria counts were significantly higher ($p<0.05$) in the skin of *Chrysicthys nigrodigitatus* (2.0 × 10^5 -2.90 × 10^4 $P<0.05$). *Pseudomonas aeruginosa, Salmonella aereus, Escherichia coli, Enterobacter spp Bacillus spp, Alcaligen spp, Citrobacter spp, Shigella spp, Klebsiella spp, Micrococcus spp, Serratia spp, Proteus spp, Salmonella spp, Streptococcus spp, Enterobacter aeruginosa,* and *Staphylococcus epidermis* were isolated and identified from the three fish sample. The bacteria organisms isolated from the fish were pathogenic and of public health importance.

Keywords: Morphometrics, bacteria, culture, lagoon and fish species.

INTRODUCTION

Fish is a generally accepted cheap source of protein for human consumption both for the rich and poor. In addition to proteins, some types of fish contain omega-3 fatty acids, a type of fat that helps decrease the risk of cardiovascular disease (CVD). Cold-water fish such as salmon, tuna, and mackerel have omega-3 fatty acids most. While meat is a good source of protein, it can also be rich in fat and cholesterol which is practically low in fishes (Eyo, 2001). Proteins are found in muscles, bones, skin, hair, and virtually every other body part or tissue. They are used to make hemoglobin, to build heart muscle, and to keep the immune system functioning. Infectious diseases of fishes occur when susceptible fishes are exposed to virulent

pathogens under certain environmental stress conditions. Very little research has been carried out to show the effect of pollution on outbreaks of infectious diseases of fishes. Therefore, examples taken from the literature were selected and reviewed to show the coincidence of infectious diseases with stress caused by temperature, eutrophication, sewage, metabolic products of fishes, industrial pollution, and pesticides.

The Lagos Lagoon is an area of salt and brackish water separated from the adjacent sea by a low-lying sand or shingle barrier. The high level of urbanization and industrialization of the city of Lagos and its environs with inevitable generation of domestic and industrial wastes have led to biological consequences in the coastal aquatic environment (Ajao, 1990). The Lagos lagoon catchment is located in Lagos State, south-western Nigeria with geographical coordinates of latitude 6°27′N and 6.45°N and longitude 3°23′E and 3.383°E. Its Maximum length is 50 km, Maximum width is 13 km while its surface area is 6,354.7 km² and surface elevation is 0 m. The lagoon is more than 50 km long and 3 to 13 km wide, separated from the Atlantic Ocean by long sand spit 2 to 5 km wide, which has swampy margins on the lagoon side. Its surface area is approximately 6,354.7 sq km. The lagoon is fairly shallow and is not plied by ocean-going ships, but by smaller barges and boats. The lagoon is a dump site for sewage disposal around Lagos metropolis (Obafemi, 2008). *Chrysichthys nigrodigitatus* is a common silver coloured African catfish occurring in Nigeria and several West African countries. It is a highly valuable fish species amongst the indigenous African populations. Considerations for the culture of the fish have resulted in several biological studies on the growth and fecundity of the fish species. *Mugil cephalus*, the mullets or grey mullets are a family (Mugilidae) of ray-finned fish found worldwide in coastal temperate and tropical waters, and in some species in fresh water also (Johnson and Gill, 1998). Mullets have served as an important source of food in Mediterranean Europe since Roman times. The family includes about 80 species in 17 genera, although half of the species are in just two genera (*Liza* and *Mugil*). *Sardinella maderensis* is one of the most important families of commercial fishes, processed for food, oil, or fish meal. The family Clupeidae belongs to the class Actinopterygii (ray-finned fishes) and the order Clupeiformes. It contains 66 genera and 216 species. It may be found in marine, brackish, and freshwater environments and is primarily marine. Members of this family are not used in the aquarium trade.

Bacteria are microscopic organisms whose single cells have neither a membrane-bounded nucleus nor other membrane-bounded organelles like mitochondria and chloroplasts. Another group of microbes, the archaea, meet these criteria but are so different from the bacteria in other ways that they must have had a long, independent evolutionary history since close to the dawn of life (Zillig, 1991). Bacteria occur almost everywhere in nature. The bacteria in fish intestines are somewhat depending on the food being consumed but normally contain *Vibrio, Achromobacter, Pseudomonas* and *Peromonas* in addition to smaller numbers of Gram-positive bacteria including *Clostridium.* Since bacteria are living things they require a source of food, moisture and suitable temperature to grow, when these conditions are adequate, bacteria will grow by a process known as binary fission in which one cell divides into two new cells (Eyo, 2001). The bacteria of fish are mostly *Psychrophilic,* growing between 0°C and about 30 °C with some strain growing as low as -75 °C. A very large effect in growth rate of temperature ranges was noted.

In understanding the relationships amongst the host, agents and natural environment, we will be able to investigate disease in natural population. These relationships determine the eventual observed pattern of disease both in time and space. The

study of the relationship among animals, pathogens and their environment in a natural situation without intervention is ecology of disease. When humans intervene in natural ecological relationship such as applying fertilizers to farmlands close to rivers which eventually enter into the estuaries, may result in alterations to the local ecosystem with disease occurring such as with epizootic ulcerative syndrome in wild fish. This relationship is often interpreted as a particular set of conditions relating to the agent, host and environment coming together for disease to occur. Freshly caught fish and shellfish generally contain in the same *genera* of bacteria but in different proportions. They are the Gram negative *genera Pseudomonas, Moraxella, Acinotobacter* (formally called *Achromobacter*), *Vibrio, Aeromonas, Flavobacterium* and *Cytophaga,* and the Gram positive genus *Micrococcus* and *Coryneform* group. The dominant floras of tropical fish are the Gram positive forms (Robert, 2010). When the fish are caught close to land, organisms of terrestrial origin mainly of the aerobic spore forming bacilli may be isolated.

Marine fish caught in open sea away from pollution never have any normal food poisoning organism unless they are subsequently contaminated as a result of poor handling (Gottesfeld et al., 2007). If the water is polluted with sewage then the fish may carry water borne pathogens such as *Salmonella, Aeromonas hydrophilia, Shigella, Vibrio cholera* and the Hepatitis A virus (Eyo, 2001).

However fishes are not the main source of the disease associated with intake of these organisms. Two types of food poisoning bacteria, *Clostridium botulinum* and *Vibrio parahaemolticus* can be present in freshly caught fish from unpolluted marine waters. The organisms though small in number have been responsible for many deaths resulting from the consumption of infected fish in different parts of the world and are regarded as organisms of public health importance. Growth and toxin production is most often associated with poor handling and processing. According to Horsley (1973), the variability of the kinds of bacteria present in fresh water fish is attributed to the micro-flora of the water. The composition of the micro flora of the different species of fish tested were mostly reported to be dominated by Gram-negative bacteria usually identified as *Achromobacter, Pseudomonas* and *Flavobacterium* or less frequently *Vibrio* or *Enterobacterial genera*, however there were few reports on large numbers of Gram-positive bacteria. In earlier studies, fish were obtained from commercial sources and the heterogeneous nature of the micro flora and the presence of organisms associated with animals including *micrococcus* is not unexpected. The main aim of this study is to provide information on the significance of morphometrics of three fish species to occurrence and prevalence of bacteria flora from the gills, buccal cavity and skin of *Chrysichthys nigrodigitatus, Mugil cephalus, Sardinella maderensi* collected from Lagos lagoon.

MATERIALS AND METHODS

Live or dying *Chrysichthys nigrodigitatus, Mugil cephalus* and *Sardinella maderensi* fish samples were collected from the fishermen from Lagos lagoon at Falomo fishing jetty in Victoria Island, Lagos. On the field, identification of fish and measurement of morphometric characteristics such as standard length, head length, gill length and buccal cavity in centimetres (cm) were done after weighing the fish specimen in grams (g). Samples were swabbed from three spots on each fish i.e. skin, gills and buccal cavity. The swabbing was done with the aid of sterile swab stick and after swabbing it was cocked back in the case that already contains peptone water that serves as transport media. The swab sticks were preserved with ice parks before getting to the laboratory for necessary bacteriological analysis.

Preparation of media

The media used were Nutrient agar, Salmonella-Shigella agar, Pseudomonas agar, and eosine methylene blue agar. They were all weighed following the manufacturer's description and dispense into conical flask (250 ml), distilled water was added to the media so as to dissolve easily after which the conical flask was covered with foil before it was sterilized in autoclave at a temperature of 126 °C.

Serial dillution

Each sample was separately analysed by ensuring homogeneity of the samples using a sterile pipette. 1 ml of each sample was suspended into 9 ml sterile water aseptically in a MacCartney bottle which was then shaken together. Further dilution of 10^{-1}, 10^{-2}, 10^{-3} were carried out after which 10^{-2} dilution was later used.

Pour plate method

Aliquots of 1 ml of different dilution of the sample type were pipetted into disposable Petri dishes and the plates were labelled. Thereafter, sterilized media were added respectively onto the samples and swirled gently. This method allows for the growth of anaerobic and facultative anaerobic organisms.

Microbiological analysis

Analysis of samples

Each swab stick was initially cultured on Nutrient agar (NA) for growth and subcultured on Pseudomonas agar (PSA), Salmonella-Shigella agar (SSA), and Eosine methylene blue agar (EMBA) and incubated at 37 °C.

Total bacteria count (cfu/ml)

The total bacteria count for each sample was determined with the pour plate technique using the necessary agar. The plates were incubated between 24 hours at 37 °C. All colonies appearing at the end of the incubation period were counted using digital illuminated colony counter and the counts were expressed in colony forming unit per ml (CFU/ml) of the sample.

Identification of microorganism

The organisms were identified using the following biochemical tests to confirm the presence of the suspected microorganism by their reaction to the tests (Gram staining, catalase, oxidase, coagulase, indole, citrate utilization, motility and sugar fermentation tests).

Statistical analysis

Statistical analysis was carried out using statistical packages like SPSS, Microsoft Excel and Epi Info 2002. The results are presented in tables.

RESULTS

Morphometric characteristics

The summary of the morphometric characteristics of *Chrysichthys nigrodigitatus, Sardinella maderensis* and *Mugil cephalus* is illustrated with significance differences. The weights are in grams while the lengths are in centimetres. In Table 1, the means of morphometric characteristics with the significant differences were shown. From the table, the weight of *Chrysichthys nigrodigitatus, Sardinella maderensis* and *Mugil cephalus* are significantly different from each other at $P<0.05$. Also, the head lengths of the three species were significantly different at $P<0.05$. The standard length, gill length of the three fish samples were significantly different at $P<0.05$. The buccal depth of *Chrysicthys nigrodigitatus* and *Sardinella maderensis* were not significantly different at $P<0.05$, but significantly different from *Mugil cephalus.*

Viable bacteria count

Table 2 shows the viable bacteria count in the three fish samples (cfu/ml). *Chrysichthys nigrodigitatus* had a range of 2.0×10^{3} -2.90×10^{4} in the skin, 1.64×10^{4}-2.99×10^{4} in the buccal cavity and 1.1×10^{3}-

2.56×10^4 in the gill. For *Sardinella maderensis* the range in the skin was 1.6×10^3-2.78×10^4, 1.5×10^3-2.95×10^4 in the buccal cavity and 5.7×10^3-2.58×10^4 in the gills. While for *Mugil cephalus*, the skin had a range of 5.7×10^3 -2.58×10^4; 6.3×10^3 -2.83×10^4 in the buccal cavity and 2.1×10^3- 2.78×10^4 in the gills.

Percentage occurrence of bacteria isolates

Table 3 shows the occurrence of bacteria flora from gills, buccal cavity and skin of the three fish samples. It indicated that *Escherichia coli* had 11%, 8% and 8% occurrence in the skin, buccal cavity and gills of *Chrysichthys nigrodigitatus* respectively. *Pseudomanas aeruginosa* had the highest percentage occurrence in the buccal cavity and gills of *Sardinella maderensi* and *Mugil cephalus* also respectively. *Enterobacter aeruginosa*, *Staphylococcus epidermis* recorded the least percentage occurrence in the buccal cavity of *Mugil cephalus* recording 6%. *Enterobacter aeruginosa* recorded in the gills, none in the skin and buccal cavity of *Chrysicthys nigrodigitatus* and in other fish species used, while *Staphylococcus epidermis* had 6% in the gills of *Mugil cephalus* and none in the buccal cavity and skin, and in other species used.

Table 1: Summary of morphometric characteristics.

Morphometrics	*Chrysichthys nigrodigitatus*	*Sardinella maderensis*	*Mugil cephalus*	*F statistic*
Weight (g)	404.00 ± 11.28^a	173.68 ± 2.69^b	87.50 ± 4.11^c	530.90
Standard length (cm)	35.50 ± 1.32^a	9.05 ± 0.14^c	12.18 ± 0.63^b	289.06
Head length (cm)	4.70 ± 0.32^a	3.56 ± 0.12^b	2.50 ± 0.07^c	30.178
Gill length (cm)	3.45 ± 0.10^a	2.67 ± 0.05^b	2.08 ± 0.05^c	91.72
Buccal depth (cm)	1.50 ± 0.07^b	1.58 ± 0.05^b	1.78 ± 0.05^a	14.15

Table 2: Summary of viable bacteria count (cfu/ml).

Fish species	Body parts	Range
Chrysichthys nigrodigitatus	Skin	2.0×10^3 -2.90×10^4
	Buccal cavity	1.64×10^4-2.99×10^4
	Gill	1.1×10^3-2.56×10^4
Sardinella maderensis	Skin	1.6×10^3-2.78×10^4
	Buccal cavity	1.5×10^3-2.95×10^4
	Gill	1.1×10^3 -2.71×10^4
Mugil cephalus	Skin	5.7×10^3-2.58×10^4
	Buccal cavity	6.3×10^3 -2.83×10^4
	Gill	2.1×10^3- 2.78×10^4

Table 3: Percentage occurrence of bacteria flora.

Bacterial species	***C. nigrodigitatus***			***S. maderensis***			***M. cephalus***		
	S	B	G	S	B	G	S	B	G
Pseudomonas aeruginosa	6	11	6	6	11	11	6	8	11
Salmonella aereus	11	3	8	Nil	Nil	Nil	6	3	11
Escherichia coli	11	8	8	11	11	6	11	11	6
Enterobacter spp	8	3	6	7	Nil	11	6	Nil	11
Bacillus spp	6	8	3	Nil	Nil	Nil	11	Nil	7
Alcaligen spp	7	3	Nil	7	Nil	Nil	7	Nil	7
Citrobacter spp	7	7	7	Nil	7	Nil	6	11	Nil
Shigella spp	Nil	6	Nil	Nil	11	6	Nil	11	Nil
Klebsiella spp	Nil	6	Nil	6	11	Nil	11	Nil	Nil
Micrococcus spp	6	6	6	11	6	11	Nil	Nil	11
Serratia spp	Nil	6	6	11	6	Nil	Nil	Nil	6
Proteus spp	3	6	6	6	6	6	6	6	8
Salmonella spp	Nil	6	Nil	Nil	8	6	Nil	11	Nil
Streptococcus spp	Nil	Nil	3	6	Nil	Nil	Nil	Nil	Nil
Enterobacter aeruginosa	3	Nil	3	Nil	Nil	Nil	Nil	Nil	Nil
Staphylococcus epidermis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	6	Nil
Mean bacteria counts (cfu/ml)	8.43 ± 0.31^{b}	13.64 ± 0.71^{a}	8.81 ± 0.38^{a}	9.49 ± 0.41^{a}	12.24 ± 0.05^{a}	8.98 ± 0.22^{a}	8.68 ± 0.21^{ab}	$13.25\pm0.28a^{a}$	7.49 ± 0.47^{a}
$F_{statistic}$		2.69			3.33			2.34	

Key: G=gill, B=buccal cavity, S=skin, Nil= not present

[a, b, c] Means along the same row with different superscript are significantly different at ($P<0.05$).

DISCUSSION

The morphometric features evaluated were significantly different (P<0.05) for all the fish species used. *Chrysichthys nigrodigitatus* recorded the highest mean weight (grams), standard length, head length (cm) and gill length (cm) at (P<0.05). While *Mugil cephalus* had the highest buccal depth. In this study, a total of seventeen (17) species of bacteria were recovered from the gills, skin and buccal cavity of *Chrysichthys nigrodigitatus,Mugil cephalus* and *Sardinella maderensis.* Thirteen (13) out of the isolated bacteria species were Gram negative *Enterobacter spp., Pseudomonas spp, Proteus spp, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Alcaligens spp, Citrobacter spp, Shigella spp, Salmonella spp, Serratia spp, Enterobacter aerogenes,* and *Staphylococcus epidermis.* This also confirmed the findings of Ahmed and Naim (2002) who reported that the bacteria identified from the brackish pond water, sediments and healthy tilapia cultured in Saudi Arabia were predominantly Gram negative; being in support of this study that recorded the highest for *Escherichia coli.*

Chrysichthys nigrodigitatus had the highest bacteria count range of 2.0×10^3 - 2.90×10^4 in its skin, this disagree with the argument of Jara and Chodyniecki (1999) that gills come into direct contact with the environment and any of the pathogen. Ashokkamaar (2000) observed $350x10^3$ CFU/g for *Upeneus spp*. in South East Coast of India.

In terms of mean percentage bacteria occurrence, there was a significant difference (P>0.05) in the gills of Chrysichthys *nigrodigitatus, Mugil cephalus* and *Sardinella maderensis.* From the result, the morphological characteristics showed that four bacterial isolates were Gram positive *Staphylococcus aureus, Micrococcus spp, Bacillus spp* and *Streptococcus* spp while other bacteria isolates were Gram negative.

Conclusion

This work provided information on the bacterial flora from the gills, buccal cavity and skin of three commercially important brackish water fish species such as *Chrysichthys nigrodigitatus, Mugil cephalus,* and *Sardinella maderensi* which support huge artisanal and culture fisheries in Nigeria. Hence, this study confirms the existence of pathogenic bacterial organisms which are of public health importance. The findings have confirmed that fish can be infected with a variety of microbial species especially those of bacteria in the brackish environment. The isolates from the gills, buccal cavity and skin can be accounted for mainly by the filter effect of the gills, the feeding habit or the slime layer of the skin, also partly as a result of the active bacteria multiplication and adaptation. The isolates have the potentials to cause serious infections to fish, to the animals that feed on them and finally to man. Based on the findings of this study, the following recommendations are suggested.

-The monitoring of standards should be ensured for the fish meant for consumption as well as good fish handling and sanitation practices should be encourage among the fisher folks and fish processors with the intention to reduce the occurrence of food poisoning as a result of pathogenic bacterial contamination.

-The Lagos lagoon should be prevented from indiscriminate disposal of human sewages, industrial waste and agricultural effluents from the land (soil) to reduce environmental stress on fish as a result of the disproportionate load of pathogenic bacteria in water that could be transferred to fish external organs such as the gills, buccal cavity and epithelium (skin).

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