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THE HISTOLOGICAL ASSESSMENT OF *CLARIAS GARIEPINUS'* GILLS: AN ECOTOXICOLOGICAL EVALUATION OF MGBUOBA FISH POND

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ABSTRACT
Background: Aquatic toxicology is the study of the effects of manufactured chemicals, other anthropogenic, natural materials and activities on aquatic organisms at various levels of organization from subcellular through individual organisms to communities and ecosystems. **Materials and Methods:** The sampling involved harvesting table-sized fish: twenty fishes from MGBUOBA and ten fishes from ARAC. Histological assessment involved determination of the qualitative and semi-quantitative analysis of the gills of the harvested fishes. **Results:** the results got showed that tissue samples of gills had a higher level of histological alterations in MGBUOBA as compared to ARAC. The alterations observed were based on circulatory disturbances (CD) which includes hyperemia, haemorrhage, vacuolation. Regressive change (RC) which includes necrosis and progressive change (PC). The Histological Alteration Index, that is, Fish Index MGBUOBA (12.3) was greater than ARAC (12.0). **Conclusion:** Alterations in the gill of the fish indicates that the fishes were exposed to a mixture of various pollutants though at low concentrations and these alterations cannot be regarded as toxicant specific.

KEYWORDS: Gill, ARAC, Pond, Mgbuoba.

INTRODUCTION
Fish eating has been part of our staple food and a very special delicacy to us Nigerians, which has resulted in people going into fish farming as a major occupation. It is very unfortunate it appears like there is inadequate or non-regulation of the activities of commercial fish farming for the protection of public health. Hence the wholesomeness of fish consumed from commercial fish farms in Nigeria is highly questionable.

Poor management practices which include impure water constituting the fish habitat, dirty surrounding, incomplete draining of pond due to lack of proper drainage, inadequate water supply, infrequent inspection and repairs are major ways of unconsciously exposing commercial fishes to contaminants. With time, pollution sets in leading to changes in the gross anatomy of the fish, histological changes specifically. These changes include: hypertrophy, hyperplasia and even degeneration of the whole cell. This ultimately leads to growth and sales of unhealthy fishes which pose a subtle but great threat to consumers.[1-3]

STUDY AREA
Experimental Site (Commercial Pond in Mgbuoba Community, Port Harcourt, Rivers State, Nigeria). Mgbuoba is a community located in Obio/Akpor local government area of Rivers state. It's geographical coordinates are 4o 50' 51" North, 60 58' 47" East. The surrounding communities are Ozuoba and Rumuokwuta.[3]

The commercial fish pond located in Mgbuoba community is made of concrete. There are two sections of the pond, one for fingerlings and the other for mature fishes of 600grams to 1kilogram. Each pond section has demarcations made of concrete which divide each column into two compartments each. Approximately 800 fishes inhabit both sections of the pond per time.[3]

Fishes cultivated in the pond are fed with a product of Livestock Feeds Plc-Aquamax. The nutrient composition of this feed includes 40% crude protein, 12% fat, 2.6% fibre, 1.0% ash, 2.0% calcium, 2.4% lysine, 1.1% methionine, 12.0% moisture. The antibiotic-Fish Cure is administered to the fishes suffering from ill health. Signs like pale white patches on the head or body of the fishes and shortened barbells indicate ill health.[3]

REFERENCE AREA (ARAC)
The chosen reference centre, African Regional Aquaculture Centre is situated in Omuihuechi Aluu,

Ikwerre Local Government Area, Rivers State. It covers an area of 81hectares. The activities done in this centre comprise research, training and development of sustainable aquaculture options in sub-Saharan Africa.[3]

The Aquaculture was established in 1980 as a result of recommendations of the Aquaculture Planning Regional Workshop that was held in Accra, Ghana in 1975. ARAC develops scientific databank, builds partnerships and linkages across local regional and international boundaries, monitor research outcomes regularly with the view of studying impacts and providing quality of technologies developed.[3]

STUDY SPECIES

Kingdom: Animalia, Phylum: Chordata, Class: Actinopterygii, Order: Siluriformes, Family: Clariidae, Genus: clarias, Species: Clarias gariepinus

Fig. 1: African Sharptooth Catfish, Clarias gariepinus (Source: Food and Agricultural Organization of the United Nations).

STUDY SPECIES DESCRIPTION

Clarias gariepinus is a large, eel-like catfish of African origin. It is a sharptooth catfish with dark gray or black colouration on the back which seemingly becomes faded towards the belly, giving a white belly.[4-6]

This specie of catfish reaches a maximum length of 1.7m and can weigh up to 60kg (130lb).[4-6] They possess slender bodies, flat bony heads and broad terminal mouths with four pairs of barbels. They also possess large accessory breathing organs which comprise modified gill arches and only the pectoral fins have spines.[4-6]

HABITAT

Clarias gariepinus is indigenous to the inland waters of Africa. This specie of catfish is also endemic in Asia Minor in countries such as Israel, Syria, and the south of Turkey.[4-6]

Clarias gariepinus can be reared in areas with a tropical climate, areas with access to geothermal waters or with the use of heated recirculating water systems. This specie of fish can be densely stacked in low oxygen waters because of its hardy nature, making it ideal for culture in areas with a limited water supply.[4-6]

Due to its ability to respire, high fecundity, fast growth rate, resistance to disease and high feed conversion efficiency of this specie of catfish, it is notably the freshwater specie with the widest latitudinal range in the world.[4-6]

There have been works on ecotoxicology and commercial fish ponds which have been reported in different ways and with peculiar results.[3-26]

AIM

To determine the impact of the commercial fish pond located in Mgbouba Community on the health of cultivated fish, using a resident fish Clarias gariepinus as biomarker.

OBJECTIVES

The objectives are to determine qualitative histological analysis of the Gill, the semi-quantitative histological analysis of the Gill and the pollution status of the fish pond.

MATERIALS AND METHODS

PHASES OF STUDY

PHASE 1 (Preliminary study): The experimental site was inspected and vital information gotten as questions were asked. Questions like: the number of fishes

contained in the pond, type and frequency of fish feed used, treatment administered to fish in poor health condition, mode and frequency of changing the water content of the pond. A sample fish was harvested and taken to the African Regional Aquaculture Center for identification by a taxonomist.

PHASE 2 (Sampling of fish)

CONTROL

Control fishes were harvested. This was done by first collecting some water content of the pond into a plastic container which would contain the fishes from the control site to the laboratory. The essence is to maintain the original aquatic habitat of the fishes. Failure to do this would have led to alteration of the fish habitat and questionability of the results obtained. Afterwards, the remaining water content of the pond was drained and with the aid of a seine; ten table-sized cat fishes were harvested, put into the plastic container in which had exactly the same water content of the pond.

EXPERIMENTAL

Experimental fishes were harvested. This was done by first getting a good quantity of water from the pond into a well-aerated plastic container designed for the purpose of transporting the fishes to the laboratory. The water content of the pond was drained in order to make the fishes more accessible for a good harvest. Twenty table-sized cat fishes were harvested from the pond.

HISTOLOGICAL ANALYSIS

This analysis involved the microscopic study of the choice tissues gotten from the harvested fishes. The analysis is thus divided into two: a qualitative and semi-quantitative assessment.

QUALITATIVE HISTOLOGICAL ASSESSMENT

This involved tissue processing and microscopy. The processes were as follows:

STEP 1 (RESECTION)

This is the surgical excision of an organ or tissue, either partially or wholly. Using a dissecting kit, the fishes were sacrificed using pithing method. Pithing is the process of sacrificing a laboratory animal by severing the spinal cord in order to immobilize it and then harvesting the target organs.

STEP 2 (FIXATION)

This was done by immersing the organs in 10% formal saline (10mls formaldehyde in 90mls of water) after excision. The formalin solution slowly penetrated the tissues, caused it to be hardened and preserved. They were left in the fixative for about 24hours to allow the fixative penetrate into every part of the tissue.

STEP 3 (DEHYDRATION)

The tissue samples were dehydrated to remove their water content. Alcohol was used. Dehydration is commonly carried out by immersing specimen in different grades of alcohol (50%, 70%, 90%, 95% and absolute alcohol) of increasing concentrations until 100% (absolute) alcohol. In this step, the alcohol penetrates the tissue quickly and the water is replaced with alcohol.

STEP 4 (CLEARING)

The alcohol used for dehydration of the tissue had to be cleared off the tissues; therefore xylene was used for the clearing process. The solvent (xylene) displaced the alcohol content in the tissue.

STEP 5 (IMPREGNATION)

After clearing, the tissues were transferred into molten paraffin wax for about 30 minutes. Paraffin wax is the most common infiltration and embedding medium. A typical wax is liquid at 60°C and can be infiltrated into tissues at this temperature then allowed to cool to 20°C where it solidifies to a consistency that allows sections to have a uniform cut.

STEP 6 (EMBEDDING)

The tissue samples which had been thoroughly infiltrated with wax were formed into “tissue blocks” which could be clamped into a microtome for sectioning. This step was carried out using an embedding mould which was filled with molten wax and the specimen placed into it. The specimen was carefully orientated in the mould because its placement would determine the “plane of section”, an important consideration in both diagnostic and research histology.

STEP 7 (SECTIONING)

The first step to sectioning is trimming; this is to reduce the excess solid paraffin wax in which the tissue was embedded. It is done to expose tissue before sectioning to ensure fine thin sections, trimming is done by setting the microtome machine to10- 20 microns. This was done to the already embedded tissues thereafter the tissues were sectioned at 3-5 microns and were picked up on a glass microscopic slide. The glass slides were placed in a warm oven for about 15 minutes to help the section adhere to the slide.

STEP 8 (STAINING)

The process was reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Therefore, Routine H&E (haematoxylin and eosin) was done using the following procedures:

i. The tissues were dewaxed in xylene 1 and 2.
ii. Then hydrated in descending grades of alcohol and brought to water.
iii. They were stained in haematoxylin for 25-30minutes, brought to water.
iv. Differentiation in 1% alcohol was done thereafter, rinsed in water immediately.
v. The slides were rinsed in 1% ammonia water, rinsed in water & stained in eosin for 2mins.

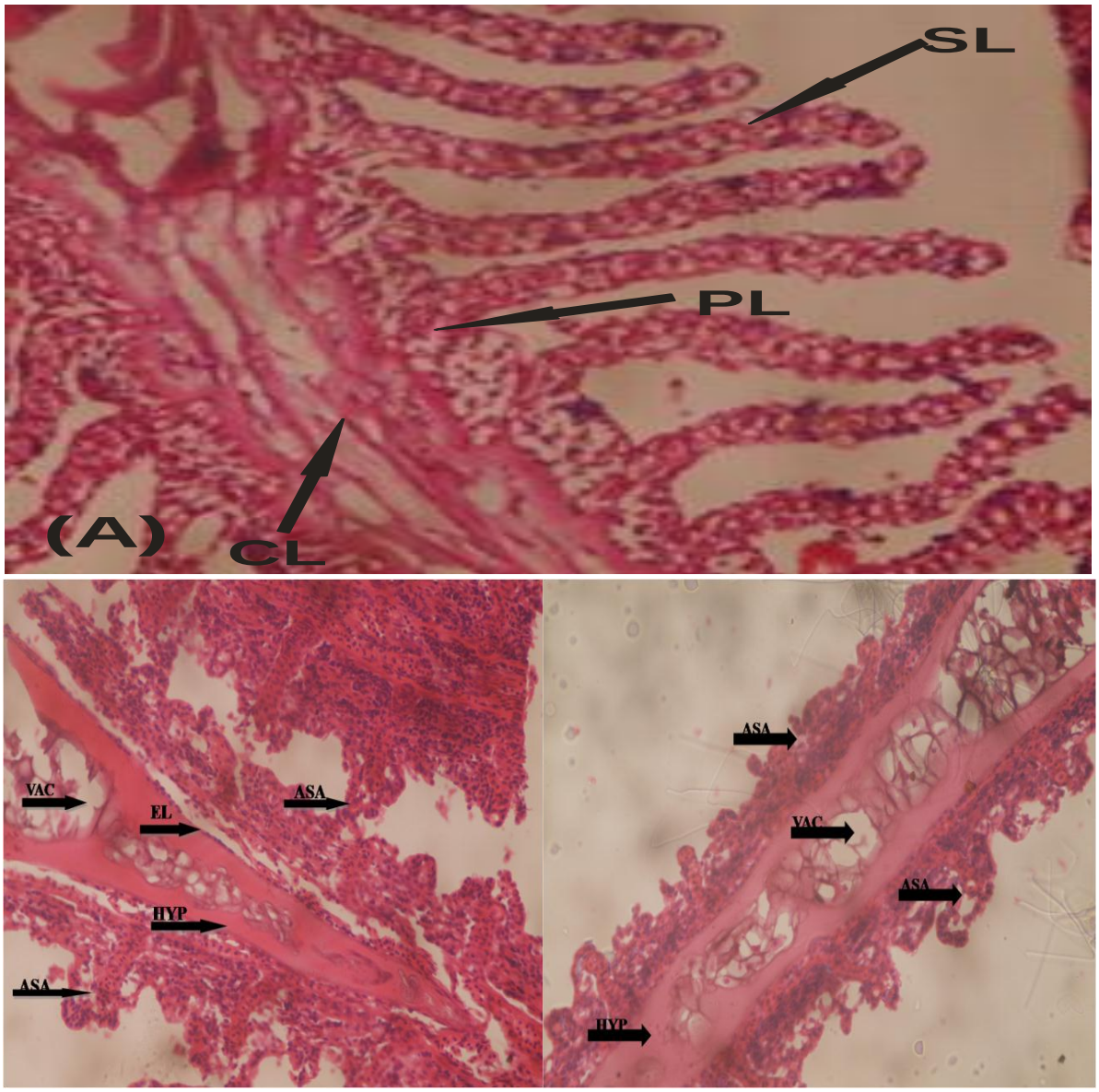
vi. The slides were placed in the oven to dry.

STEP 9 (MOUNTING/COVER SLIPPING)
The stained section on the slide were covered with a thin piece transparent plastic or glass to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the processed tissues. Thereafter, the stained tissues on the slides were covered using a plastic coverslip.

SEMI-QUANTITATIVEHISTOLOGICAL ASSESSMENT
A qualitative assessment protocol was used to qualify histopathological alterations observed in the sections of each of the organs. A qualitative histopathological assessment was done using CX31 Olympus light microscope. Tissue sections were scanned on 400x magnification. Tissue sections were semi-quantitatively assessed using part of a scoring system[18] modified from the protocol.[19] In brief, the tissue samples were assessed by identifying histopathological alteration in terms of reaction patterns including: circulatory disturbance, regressive changes, inflammatory responses, neoplasia.

Neoplasia, if identified, the alteration was given an importance factor which represents the potential of the alteration to affect fish health: 1 (alteration is reversible); 2 (alteration is reversible if the stressor is neutralized); 3 (alteration is irreversible). A score value representing the occurrence of the alteration throughout the tissue was also assigned: 0 (absent), 2 (mild), 4 (moderate), and 6 (severe).[18-20] The score value and the importance factor for the each alteration were multiplied and these results for all the alterations identified in a single organ were summed to give an organ index per fish. Gill Index, The organ index was calculated for each sample group (experimental and control group) and was used to compare the same organ between the groups. This index indicates the combined histological response of the sampled organs for the individual fish. A mean fish index was calculated for the total sample group per species.

RESULTS
GILL HISTOPATHOLOGY
Qualitative histological assessment results showed that circulatory disturbances (CD) and regressive changes (RC) were identified in gill tissue. The circulatory changes were in the form of hyperaemia, haemorrhage, vacuolation and epithelial lifting while regressive changes were seen as architectural and structural alterations of epithelial cells which were seen to be high with samples from experimental site and necrosis. Structural changes in the form of fusion of secondary lamellae and fusion of adjacent lamella were also noted. Secondary lamella with hyperaemia was identified more in fish specimens from MGBUOBA while there was no necrosis identified in ARAC specimens.



(B) **(C)**

Fig 1: Showing normal and histopathologic tissue micrograph of the Gills. A) Normal Gills at 400 X magnification showing Capillary lumen (CL), Primary lamella (PL) and secondary lamella (SL). B) histopathologic tissue at 400 X magnification showing Hyperplasia (HYP), Vacuolation (VAC), Architectural and Structural Alteration (ASA) and Epithelial lifting (EL). C) histopathologic tissue at 400 X magnification showing Vacuolation (VAC), Architectural and Structural Alteration (ASA) and Epithelial lifting (EL).

Table 1: The percentage prevalence of Gills histopathology.

Alteration	Prevalence (%)	
	ARAC (n=10)	MGBUOBA (n=20)
Circulatory Disturbance (CD)		
Hyperaemia	7.4	11.9
Haemorrhage	16.3	2.4
Vacuolation	28.1	20.6
Regressive Change (RC)		
Structural alterations	38.5	36.5
Necrosis	0	11.9
Progressive Change (PC)		
Epithelial Lifting	8.9	17.5
AVERAGE % PREVALENCE	16.5	16.8

DISCUSSION

GILL HISTOPATHOLOGY

The gills are sensitive indicators of environmental stress, including exposure to harmful compounds present in aquatic ecosystems as a result of anthropogenic activities. Gills in fishes are vulnerable to toxicants and irritants because they are in direct contact with the surrounding water and have a rich blood supply to pick up oxygen for respiration from the water. In this very study, histological alterations in varying degrees were identified in gills. These were mostly circulatory disturbances and regressive changes. Circulatory disturbances are related to pathological conditions of blood and tissue fluid flow (hyperaemia, haemorrhage, vacuolation and epithelia lifting) and primarily regressive changes such as structural alterations and necrosis were also seen. Epithelial lifting in focal areas was noted in both fish species. These alterations have been observed in various other studies and the result agrees with the previous findings.[10-26]

Structural alterations as lamella fusion were also noted. Fusion of lamellae is the result of hyperplasia of undifferentiated gill epithelial cells. According to Mallat[23] lamella fusion could be protective in that it diminishes the amount of vulnerable gill surface area. This alteration has previously been identified in fish exposed to pesticides Fish.[19-22]

CONCLUSION

Histopathological alterations in the gill of the fish indicate that the fishes were exposed to a mixture of various pollutants though at low concentrations and these alterations cannot be regarded as toxicant specific. These alterations were not toxicant specific but could be associated with pathogens, and metal pollution in the water. This implies that the pond in which the fishes reside have some levels pollution and attention should be paid to address it holistically.

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CONFLICT OF INTEREST

We write to declare that there is no conflict of interest

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