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Water Quality and Gross Anatomical Assessment of Clarias Gariepenus: An Ecotoxicological Evaluation of Mgbuoba Fish Pond

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Original Research Article

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Abstract: This study examined the ecotoxicological evaluation of commercial fish pond in Mgbuoba, Port Harcourt, Rivers State Nigeria using water quality and gross anatomical assessment of Clarias gariepenus, using African Aquaculture Centre (ARAC) as a reference site. In this research the following were done: Gross anatomical assessment of the farmed fish which comprised of condition factor (CF), Organo-Somatic Index (OSI) and Health Assessment Index (HAI) and physico-chemical properties of pond water. The chemical assessment involved environmental water quality index (EWQI) and heavy metal assessment. The gross anatomical assessment involved the sampling of twenty table-sized fish harvested from the Mgbuoba commercial fish pond which were compared with ten table-sized fishes of the same specie harvested from an aqua-cultured centre, ARAC. The EWQI of the commercial fish pond was considered to be of a fair quality, condition factor recorded showed that fishes from Mgbuoba were in better condition than that of ARAC, health assessment index was poor in fishes harvested from ARAC. The organosomatic index showed that Mgbuoba fishes had a high mean values for Gills and Liver respectively. Using one-way Anova statistical analysis, no significant difference ($p>0.05$) were noted between Mgbuoba commercial fish pond and ARAC. It is suggested that at intervals the health status and water control should be examined/analyzed to keep a suitable habitat for the fishes and make consumption safe humans.

Keywords: Ecotoxicology, Bio-monitoring, Pollutant, Gross anatomy, ARAC, Mgbuoba

INTRODUCTION

Ecotoxicological investigation of fish farms have become paramount as fish farming is growing by the day. Fish farming is a very prominent practice in Nigeria and lucrative as owners of such farms make fortunes from it. There are speculations that some of these farms operate below standard and breed unhealthy fishes for commercial consumption.

Some of the likely reasons for production of such fishes from the farms could be 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 [1-3].

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].

Researchers have to a large extent done works on ecotoxicology and commercial fish ponds which have been reported by different authors [3-22].

AIM

The aim of this study was to carry out a qualitative histological analysis of the vital organs of *clarias gariepinus* (catfish), the semi-quantitative histological analysis and the pollution status of the fish pond.

MATERIALS AND METHODS

STUDY DESIGN

Analytical and descriptive study.

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].

PHASES OF STUDY

PHASE 1 (Preliminary study): The experimental site was visited and enquiries were made on the quantity of fish 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 Fishes) for the study
ARAC FISHES (GROUP 1)

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 was so that the original aquatic habitat of the fishes will remain the same after harvesting as it was before. 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.

MGBUOBA FISHES (GROUP 2)

Group 2 fishes were harvested following the standard procedure. 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 involves tissue processing procedures through which tissues are prepared to be viewed under a microscope. The processes are 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. This method was preferred because the organs were required alive.

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 them to harden and preserved the tissues. 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:

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

- 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-QUANTITATIVE HISTOLOGICAL 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 neutraised); 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. Organ Index, The organ index was calculated for each sample group (Mgbuoba Group and ARAC or Control Group) and was used to compare the liver, kidney and gill between the groups. This index indicates the combined histological response of the liver for the individual fish. A mean fish index was calculated for the total sample group per species.

RESULTS

ORGAN HISTOPATHOLOGY

Histological alterations noted in the organs were circulatory disturbances (CD) and regressive changes (RC). These alterations included vacuolation, structural alterations and MMCs.

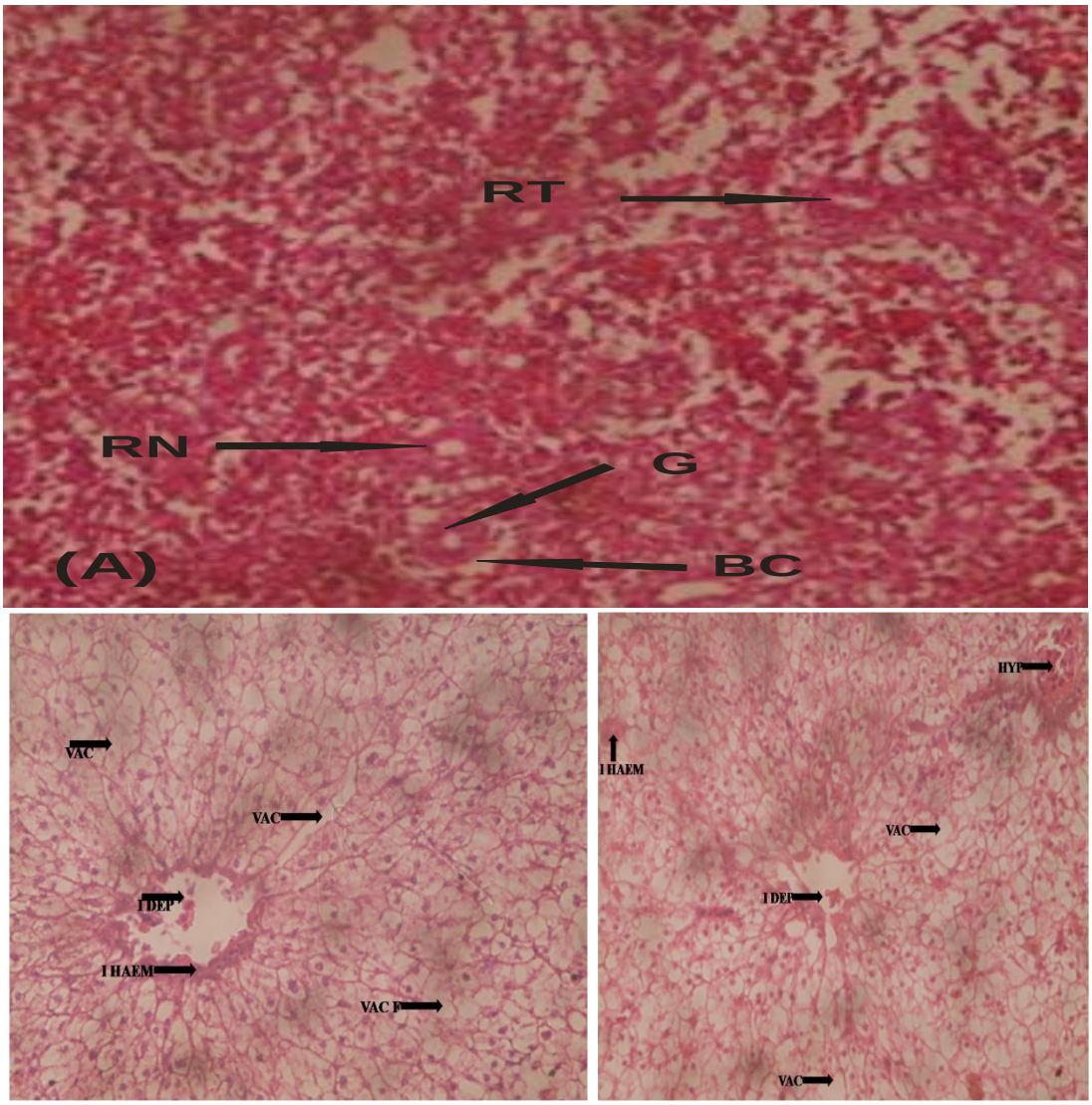
DISCUSSION

ORGAN HISTOPATHOLOGY (KIDNEY, LIVER AND GILL)

In this study the prevalence of haemorrhage was more in Mgbuoba and absent in ARAC. This result indicates that there was damage in the morphology of fish kidney which ultimately led to a distortion of the blood supply to the organ. This distortion evidently was a result of contamination of the aquatic habitat of the fish (pond) which could be attributed to poor hygiene and management of the fish farm. This result agrees with the findings of other authors as have been reported in their works [9, 14-16].

Considering the prevalence vacuolation, Mgbuoba fish farm had higher prevalence than ARAC which means that the rate of contamination of the farm was high to have elicited that such magnitude of vacuolation. The vacuolation prominent in Mgbuoba farm was directly proportional to the rate of the damage to the water body which suggest that the fish could be unhealthy for consumption taking into account the level of distortion on the kidney. Again, the results obtained here in this study, reaffirms the stated findings of other authors in their reports [8, 19, 20, 22].

Furthermore, the prevalence of necrotic cells in the kidney was higher in the Mgbuoba farm than ARAC which again points to the fact that the kidney cells of the fishes in Mgbuoba farm had very little or minimal nutrient supply which could be interpreted that they do not have enough protein to give when consumed. The level of necrosis seen in Mgbuoba fishes in comparison with what is seen in ARAC could be said to be insignificant at the moment but in the nearest further it could become significant and outrageous to cause enormous harm on consumption and lethal to human health if adequate precautionary measures are not taken to curb the contamination in the water body. This result agrees with the findings of other authors as have been reported in their works [1-3].



(B) (C)

Fig-2: Normal and histopathologic tissue micrograph of the kidney A) Normal kidney tissue at 400 X magnification showing the Renal Corpuscles (RC) (with Bowman's space (BS) and Glomerulus (G)) and renal tubules (RT). B) Histopathologic tissue at 400 X magnification showing Intracellular Deposit (IDEP), Vacoulation (VAC) and Intracellular haemorrhage (IHAEM). C) histopathologic tissue at 400 X magnification showing Hyperplasia (HYP), Intracellular Deposit (IDEP), Vacoulation (VAC) and Intracellular haemorrhage (IHAEM).

Table-1: The percentage prevalence of Kidney Histopathology for ARAC and Mgbuoba Fishes.

Alteration	**Prevalence (%)**	
	ARAC (n=10)	MGBUOBA (n=20)
Circulatory Disturbance (CD)		
Haemorrhage	0	7.6
Vacuolation	14.3	22.7
Regressive Change (RC)		
Necrosis	19.0	20.2
Structural Alteration	28.6	26.1
Melano-Macrophage Centers	38.1	23.5
AVERAGE % PREVALENCE	**20.0**	**20.02**

Structural alterations and melano-macrophage centres were more prevalent in ARAC than in Mgbuoba fishes which negate the trend of results seen in this study where Mgbuoba fishes always had more. It therefore suggests that the alterations were not pathologic but physiologic to maintain normal and optimal cellular function in the kidney. Comparing the alterations seen in the ARAC fishes and that of Mgbuoba suggest that the Mgbuoba fishes do not have what it takes to withstand adverse conditions since they do not experience much structural alteration or adjust to cope with changes which means that they may not survive when conditions are unfavourable compared to what is seen in ARAC. This result agrees with the findings of other authors as have been reported in their works [3-7].

CONCLUSION

There were distortions seen in the kidney and these distortions evidently were a result of contamination of the aquatic habitat of the fish (pond) which could be attributed to poor hygiene and management of the fish farm. However, it is important to note that these alterations were not toxicant specific as such could have resulted from lots of sources as pathogens, metal pollution in the water and gas flaring which is common in the south-south region of Nigeria where this study was done.

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CONFLICT OF INTEREST

We write to declare that there is no conflict of interest

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