International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2022.8.12

Research Article
Histopathological Assessment of Gill Tissues in Two Selected *Clariid* Species Sampled from Asa River, Kwara State, Nigeria

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Abstract

Background and Objective: There is a need for sensitive bio-monitoring tools intoxicant impact assessment to indicate the effect of toxicants on fish health in polluted aquatic ecosystems. Histological aberrations were evaluated in *Clarias buthupogon* and *Heterobranchus longifilis* sampled from Asa River Nigeria. **Materials and Methods:** The fish required for this investigation were sampled from the downstream portion of the river which was chosen for their suspected levels of toxicants. A quantitative and qualitative histology-based health assessment protocol was employed to determine the adverse health effects in fish gills. **Results:** The most notable alterations were congestion of blood vessels, desquamation, swelling of pillar cells, hyperplasia and hypertrophy of the gill epithelium. Epithelial lifting of the basement membrane was common in virtually all sampled fish species, while circulatory disturbances were slightly pronounced. **Conclusion:** However, the observed histological aberrations in the gill tissue could be due to increased concentrations of chemical pollutants in the water body. Therefore, proper assessment of the Asa River is necessary with strict vigilance because of ensuring the protection and conservation of the organisms therein.

Key words: Histology, contaminant, *Clarias buthupogon*, *Heterobranchus longifilis*, protection, conservation, Asa River, histopathology

Citation: Akinloye, O.M., F.O. Olatunde and A.O. Olasunmibo, 2022. Histopathological assessment of gill tissues in two selected *Clariid* species sampled from Asa River, Kwara State, Nigeria. Int. J. Zool. Res., 18: 8-12.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Histological analysis is the gold standard for tissue examination, either for research or diagnostic purposes, for both qualitative and quantitative measures. It is used to assess the inflammation or healing stage and to monitor the presence and distribution of degradation products that dissolved into the surrounding tissue. The decline in the populations of important fish species have become a severe problem in many Nigerian Rivers over the past few decades and the reasons for this remained unclear. Water contaminants have been suspected to be possible contributors to visible abnormalities in aquatic organisms. Asa River in Nigeria is one of the rivers that has continued to experience a decline in the population of aquatic organisms[1,2]. Histological studies are used in forensic investigations, autopsy and diagnosis and education. In addition, histology is used extensively in medicine especially in the study of diseased tissues to aid treatment[3].

Histological staining is a series of technique processes undertaken in the preparation of sample tissues by staining using histological stains to aid in the microscope study[4]. The process of histological staining takes 5 key stages which involve, fixation, processing, embedding, sectioning and staining[5]. Great changes have been done on techniques used for histological staining through chemical, molecular biology assays and immunological techniques collectively and have facilitated greatly in the study of organs and tissues[6]. Histopathological biomarkers are valuable indicators of the harmful effects of pollutants which are very suitable for the assessment of the harmful effects of various pollutants[7,8]. The aim of this study was to evaluate the histological degradation in the gill tissues of *Clarias buthupogon* and *Heterobranchus longifilis* sampled in the Asa River, Nigeria.

MATERIALS AND METHODS

Study area: The study was conducted on the Asa River segment at the exit of the industrial estate around Unity Road, Ilorin, North Central Nigeria (8°28'N, 4°38'E to 8°31'N, 4°40'E). It was carried out between April and October, 2017. Asa River is the major water body in Ilorin, its course enters the southern end of the industrial estate from Asa Dam and it runs Northwards through residential and commercial areas of Ilorin city. Apart from the containment of industrial effluents from several manufacturing plants within the estate, the river also serves as a recipient of domestic (sewage) wastes and agricultural waste runoffs along the bank of the river. Along the area of the river, the segment is shopping complexes, a hospital, banks, a car park and a mini-market for the sale of fresh vegetables and fish.

Materials used: Table sizes of both *Clarias buthupogon* and *Heterobranchus longifilis* were collected from the downstream section of the river with the aid of fishermen using conventional fish net traps in the early hours of the day. After dissection of fish samples in the laboratory, parts of the gills were carefully removed and prepared for histological studies. Such other materials used include dissecting sets, Leica Rotary Microtome to section thickness of 4 µm from the paraffin wax blocks, stains formalin, ethanol, xylene, paraffin wax and so on.

Protocol: The collected fish samples were sacrificed and dissected immediately, the target organs (gills) were obtained and fixed in 10% formaldehyde. Haematoxylin and eosin staining were done. The cut-out sections were dewaxed in xylene and hydrated through descending grade of alcohol (absolute: 80%-70% water). The slides were stained in Harris haematoxylin for 10 min and then rinsed in water. The slides were dipped in 1% HCl in 70% alcohol for 1 min for differentiation and then rinsed in water. Blueing was done for 10 min in tap water after which counter staining was done with 1% eosin for 1 min. The slides were then rinsed in water, dehydrated, cleared and mounted for examination on the microscope followed by photomicrography under a light microscope and photographed (Labomed).

RESULTS

***Clarias buthupogon* observation:** The gill structure of *C. buthupogon* showed the varying proportion of histological degradations ranging from hypertrophy, desquamation, oedema, swelling of pillar and chloride cells, disappearance or obliteration of secondary lamellae and cellular necrosis in Fig. 1. Figure 1a showed a normal gill structure with no degeneration of gill composition. Figure 1b showed severe epithelial lifting of secondary lamellae and Swelling of Pillar Cell (SPC) and there were varying degrees of more pronounced desquamation of secondary lamellae and hypertrophy in Fig. 1c. In Fig. 1d the epithelial lifting of secondary lamellae are seen to be distorted, distortion of the pillar cells and highly hypertrophic degeneration.

***Heterobranchus longifilis* observation:** A normal gill structure is presented in Fig. 2a showing unpronounced epithelial distortion. In Fig. 2b, there were more pronounced Curling of Secondary Lamellae (CSL), epithelial lifting with the total Disappearance of Primary and Secondary Epithelial (DEPL) and somewhat severe hypertrophy which is an indication of cellular necrosis. While Fig. 2c showed a severe epithelial lifting of secondary lamellae and hypertrophy which are fore-play of cellular necrosis.

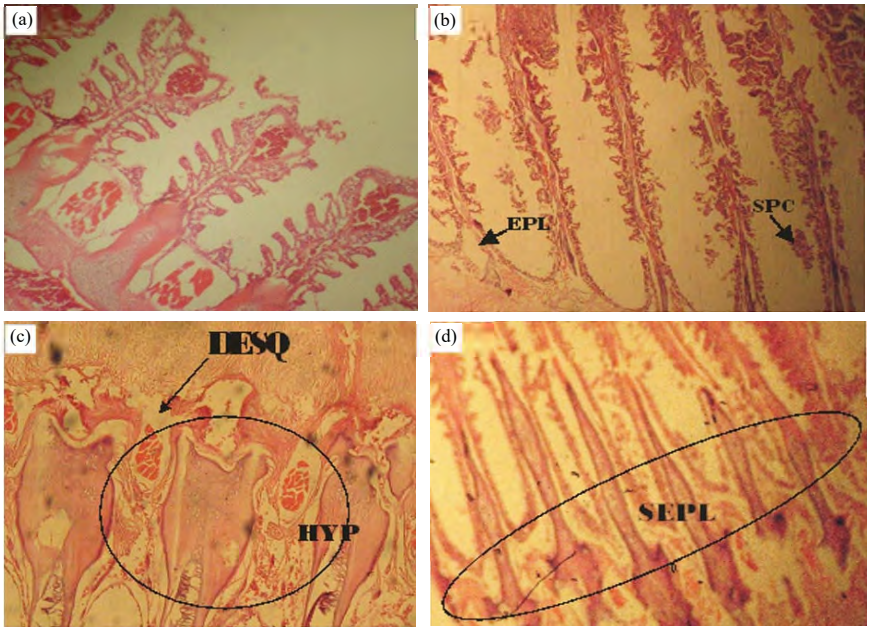

Fig. 1(a-d): (a) Photomicrograph of a normal gill tissue of *C. buthupogon* with mild congestion of blood vessels, (b) Gill tissue of *C. buthupogon* showing severe epithelial lifting of secondary lamellae and Swelling of Pillar Cell (SPC), (c) Gill tissue of *C. buthupogon* revealing severe desquamation of secondary lamellae and hypertrophy and (d) Gill tissue of *C. buthupogon* showing severe epithelial lifting of secondary lamellae (X400 mg)
EPL: Epithelial lifting, SEPL: Severe epithelial lifting, HYP: Hypertrophy and DESQ: Desquamation of secondary lamellae

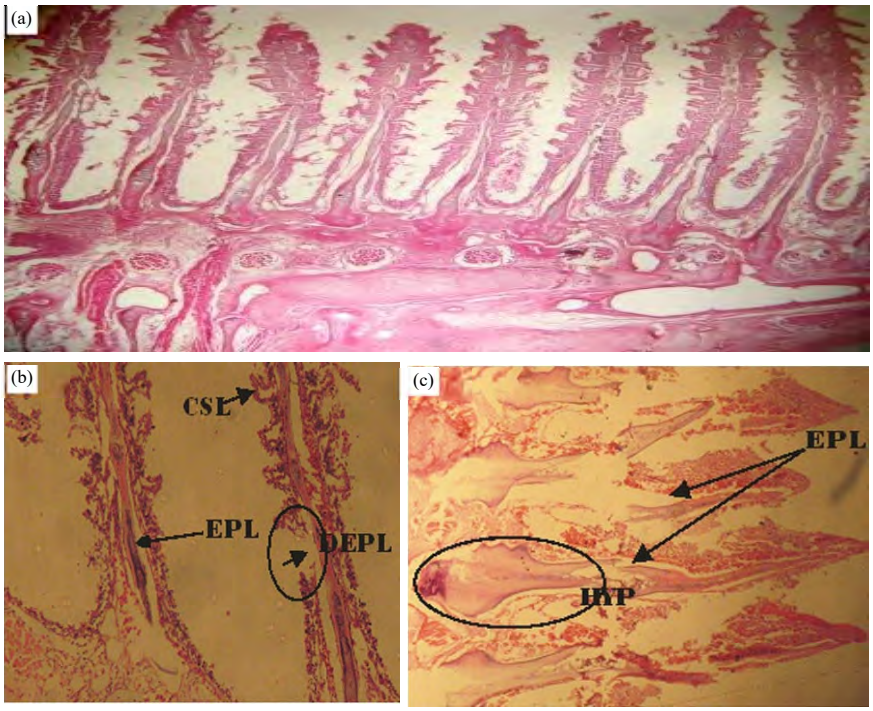

Fig. 2(a-c): (a) Photomicrograph of gill tissue of *Heterobranchus longifilis* showing normal gill architecture with very unpronounced epithelial distortion, (b) Gill tissue of *H. longifilis* showing severe Hypertrophy (HYP), an indication of necrosis and (c) Gill tissue of *H. longifilis* showing more pronounced Curling of Secondary Lamellae (CSL), epithelial lifting with the total Disappearance of Primary and Secondary Epithelial (DEPL), (X400 mg)
EPL: Epithelial lifting, DEPL: Disappearance of primary and secondary epithelial, HYP: Hypertrophy and CSL: Curling of secondary lamellae

DISCUSSION

Histological changes recorded in the gills of the two fish species examined in this work may be an indication of the reaction of toxicants such as metal intake or adaptation to prevent the pollutants entry through the gill surface. Similar findings have been reported for other species that are exposed to different pollutants, Olurin *et al.*[9] documented similar histological aberrations in the gill tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. Likewise, Camargo and Martinez[10] observed hypertrophy and hyperplasia of the epithelium cell, the fusion of secondary epithelium, lifting of lamellae epithelium and congestion of blood vessels in the gills of *P. lineatus* being caged in Brazilian Cambe stream polluted by the industrial, domestic and agricultural wastes. Triebskorn *et al.*[11] reported epithelial lifting, proliferation, necrosis and hyperplasia of mucous cells in gills of *C. nasus* and *L. cephalus* from river Merus in Western Romania which was polluted by heavy metals, faecal coliforms and streptococci bacteria. These histological alterations were recorded in the gills tissues examined and this may be attributed either directly or indirectly to the impact of the river water pollution as fish gills are very sensitive to changes in the composition of their environment especially the waterborne toxicants. Consequently, injury to the gill epithelium is a common response observed in the fish exposed to varieties of contaminants. Arellano *et al.*[12] submitted a gross epithelial lifting of the secondary lamellae after exposure to heavy metals, such as cadmium and copper which agreed well with the present finding. Lifting of lamellae epithelium and lamellae fusion could be protective as it diminishes the extent of vulnerability of the gills surface area[13].

As a consequence of epithelial lifting, there is an increase in the distance between the water and blood, impairing oxygen uptake and increase the rate of respiration because of compensating for the low entrance of oxygen[14]. According to Winkaler *et al.*[15], recorded similar histopathologic lesions recorded in this work, it was opined that these lesions are an indication that the fish responds to the effects of toxic agents present in the water and the sediment, it was observed in his work that alterations such as hyperplasia, lamellae aneurysm and epithelial lifting in specimens of *Astyanax fasciatus* and *Astyanax altiparanae* of the mucosal cells occur as a consequence of infection or due to the presence of pollutants.

CONCLUSION

Conclusively, the two analyzed fish samples are indeed responding to some stressors whose exact nature could be anthropogenic. Concentrations of iron, copper, cobalt, arsenic, lead, chromium and chlorine were at elevated levels and these substances could be responsible for the observed histopathological alterations. The present study represented an additional reason to proceed with detailed monitoring of this river and the wildlife within it. Therefore, consumption of river foods from the Asa River should be discouraged and an urgent water monitoring system is required in the river.

SIGNIFICANCE STATEMENT

This study established that the downstream portion of the Asa River is grossly polluted with varied levels of toxicants that can be injurious or harmful to human health. This is an eye-opener to the residents of the study area to the relative effects that this water body may impact on public health. The implications of these findings may be that people dependent on this river water for domestic use including cooking, bathing, washing and even drinking or for agricultural uses like fishing and farming may be exposed to public health risks. Proper treatment is imperative for the river to be appropriate for potable, domestic and industrial purposes. It is therefore suggested that, efforts should be made at the level of the community dwellers and the government to rescue the downstream Asa River segment and its aquatic life from the current hazard-posing environmental problems.

ACKNOWLEDGMENT

We hereby acknowledge the technical and laboratory staff of the Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria for their kind support in terms of materials supplies.

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