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Histopathology of Selected Economically Important Fish Species from the Escravos Estuary, Nigeria: A Baseline Study for Environmental Monitoring

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Abstract

The estuarine environment is a major sink for potentially hazardous chemical pollutants emitted from industrial and domestic sources. Particularly, the Escravos estuary is vulnerable to crude oil spillage due to the numerous crude oil exploration and transportation activities. This could lead to the contamination of water and affect the health of aquatic organisms. In the present study, the histopathological structures of the liver and gills of three selected economically important fish species (Ethmalosa fimbriata, Pseudotolithus elongatus, and Caranx senegallus) from the Escravos estuary were studied. Fish samples were collected through fishers operating on the estuary using purse seine and gill nets. After the catch, the liver and gills were harvested, labeled, and prepared for photomicrography. Liver histopathology showed a diffused preponderance of macrovesicular steatosis, ballooning degeneration, and mononuclear inflammatory infiltrates in all target fish species. Whereas no observable lesion was observed in the gills of P. elongatus and C. senegallus, the gill histopathological evaluation of E. fimbriata indicated that the lamella appeared acellular with no evidence of epithelial cells. The observed discrepancy in alterations in the gills among the selected fish species could be attributed to the evolutionary history of fish species, age, and differences in species behavior, diet, or habitat preference. The study revealed that the liver was mostly affected. This is could be largely due to the recent government intervention in combating vandalism of crude oil facilities and oil theft which has played a crucial role in the significant improvement of water quality in the estuary

Keywords: *Estuarine ecosystem, crude oil, liver, gills, fish species*

1. Introduction

Nigeria is endowed with abundant aquatic resources, including rivers, lakes, estuaries, and a vast coastline. These ecosystems harbour a wide variety of fish species, playing a vital role in food security, nutrition, and economic well-being[1]. However, human activities such as industrial discharges, agricultural runoff, and oil spills pose significant threats to the health of these fish populations. These contaminants can cause water pollution, habitat destruction, and ultimately, a decline in fish populations[2, 3]. To protect and preserve these valuable aquatic resources, it is crucial to assess the impact of such pollutants on the health of aquatic resources using biomarkers.

Biomarkers are responses to any exposure demonstrated in physiological, histological, genetic biochemical, and behavioral alteration[4]. They function as early warning signals of the presence of potentially harmful xenobiotics and are veritable instruments for assessing exposure to or effects of these compounds, and provide information on the bioavailability of toxic substances[5]. Numerous biomarkers have been developed for biomonitoring the environment and assessment of risk programmes. However, histopathology is a veritable biomarker because alterations in organs are sensitive biomarkers, and provide a better assessment of the impact of aquatic pollution than other biomarkers[6]. Histopathology involves the inspection of cells and tissues of an organism with the aim of detecting histological abnormalities[7]. The assessment of histopathological alterations in different tissues has been a reliable instrument in aquatic toxicology[8, 9] and are a reflection of the general health of the ecosystem[10].

Fish are crucial in assessing aquatic ecological status and are sensitive to water contamination due to their trophic levels, sizes, and ages[11, 12]. They adapt their metabolite functions to environmental toxic changes. Fish are preferred in toxicological research due to their well-developed osmoregulatory, nervous, endocrine, and immune systems[13, 14]. They can absorb toxicants from water and sediments or ingest them through contaminated food[5].

In fish species, the liver, gills, muscles and kidney are frequently studied for toxicological, ecological, and pathological aspects due to their metabolic activity and higher accumulation of toxicants[15]. The pollutant enters the fish mainly through the gills and reaches the parenchymal organs along with the blood, where it remains for a long time[16]. The gills can also reflect the level of the pollutant in the water where the fish live; while the level in the liver and kidney represent storage of pollutant. Fish exposure to chemical contaminants can cause organ lesions and injuries, with liver and gills being crucial targets for histopathological examination to detect tissue and cell damage[17]. The liver detoxifies fish, performs metabolism, and excretes poisonous substances, whereas the gills are multifunctional organs that transport ions, exchange gases, regulate acid-base balance, and manage waste[18]. Gills are also crucial for detecting water toxicants' effects on fish organisms, as they are the first to come into contact with environmental pollutants[19].

The Escravos estuary is characterized by high landings of commercially important fish species in Nigeria. This estuary is an important transportation route for waterborne activities and shipping, particularly for Atlantic Ocean vessels involved in the oil and gas industry [20]. The Escravos estuary is susceptible to crude oil spillage due to pipeline leakage, rupture, engineering failure, accidental discharges, vandalism, artisanal refining (*bunkering*) or oil theft[21, 22]. Despite the abundance and species composition of this water body, the impact of these anthropogenic activities has not been carried out on fish species using histopathological techniques. This study aims to understand the histopathological status of economically significant fish species from the Escravos estuary, providing a baseline resource for managing aquatic resources and assessing environmental changes' impact on fish health.

2. Materials And Methods

2.1. Description of the Study Area

Escravos Estuary (Figure 1), is a distributary of the Niger River, Nigeria. Its 56-kilometer westerly course flows through mangrove swamps and coastal sand ridges before entering the Gulf of Guinea.

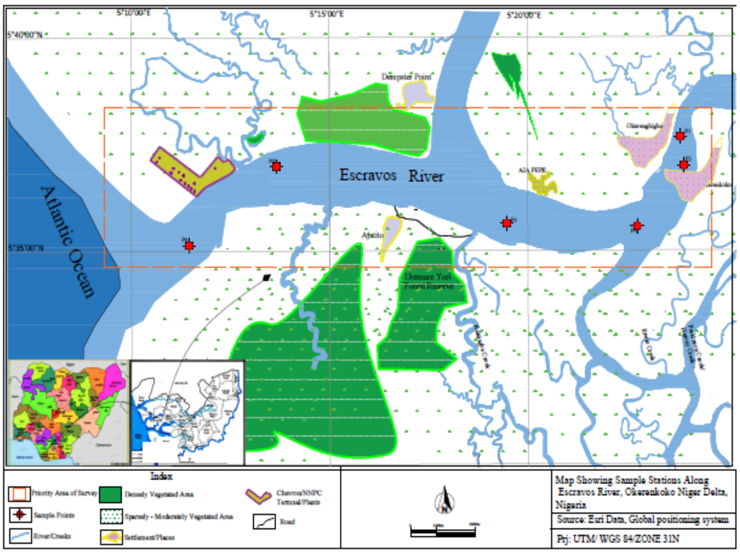

Figure 1. *Map of the Escravos Estuary showing sample stations*

The Escravos River, connected to the Forcados, Warri, Benin, and Ethiope rivers, has replaced the Forcados as the main approach to Delta ports (Warri, Sapele, Burutu, Forcados, and Koko) since the completion of the Escravos Bar Project in 1964. The estuary provides the only route for oceangoing vessels to these ports and serves a petroleum shipping station 18 kilometer offshore[23]. Located in latitude 5° 34' 56'' N and longitude 5° 10' 49'' E, the Escravos estuary is noted for waterborne transportation and crude oil activities[21, 22].

The Creek, a tropical equatorial type is dominated by *Rhizophora mangle*, *Rhizophora racemose*, *Achrostichum aureum*, and *Paspalum viginatum*. It has a wet season (May-October) and a dry season (November-April). The average temperature ranges from 26°C in the wet months to 33°C in the dry season, with an average humidity of 80%. The region receives approximately or sometimes over 3000mm of annual rainfall[24].

2.2. Sample Collection

Three dominant fish species from the Escravos estuary, namely, *Ethmalosa fimbriata*, *Pseudotolithus elongatus*, and *Caranx senegallus* were used for the study. Fish samples were collected through indigenous fishers operating on the estuary using purse seine and gill nets (mesh size ranging from 20 – 50 mm). After the catch, fish samples were transported to the laboratory in an iced pack for a thorough species identification using Idodo-Umeh[25]. Fresh samples of liver and gill of each of *E. fimbriata*, *P. elongatus*, and *C. senegallus* were harvested and preserved in 10% ethanol for histopathology.

2.3. Histopathological Analysis

Histopathology was carried out following the method of[26] with little modification. The liver and gills were dehydrated and cleared using chloroform, then embedded in paraffin wax with a melting point of 60-70°C. Blocks were prepared for sectioning, cut to a width of 5 µm, and fixed on slides. Samples were air-dried overnight, stained with Eosin and Hematoxylin, and mounted in DPX (a mixture of distyrene, a plasticizer, and xylene) to extend their durability. The slides were examined using a digital light microscope at 400 × magnifications, and photographs were taken using a digital microscopic camera. Histopathological analysis was carried out at the Department of Histopathology, University of Uyo Teaching Hospital.

3. Results And Discussion

3.1. Histopathology of Liver

The photomicrographs of liver sections of *E. fimbriata*, *P. elongatus*, and *C. senegallus* are presented in Figures 2, 3, and 4, respectively. Liver histopathological evaluation showed a diffused preponderance of macrovesicular steatosis and ballooning degeneration as well as the mononuclear inflammatory infiltrates in all target fish species. The liver is a crucial organ, responsible for detoxification and biotransformation, due to its location, function, and blood supply[27, 28]. It is a major detoxification organ, and the effects of continued exposure to pollutants are visible at the cellular and tissue level[29]. The liver, a key organ for heavy metal storage[30] has also been considered one of the organs that is mostly affected by pollutants in the aquatic ecosystem[31]. The histopathological parameters of fish liver are critical for assessing the impacts of persistent pollutants in effluent-affected waters[32].

Macrophage aggregates (Melanomacrophages) and liver necrosis are the most frequent histopathological alterations in the liver of fish species from polluted sites [33]. Melanomacrophages, a type of immune cell, can indicate environmental stress due to exposure to degraded environments. Increased melanomacrophage numbers indicate inflammatory responses and detoxification of substances. Hyperemia causes liver infiltration, enhancing blood flow and transporting macrophages to damaged areas, potentially indicating an auxiliary detoxification mechanism[34]. The observed hepatocellular vacuolation of the hepatocytes, diffused preponderance of macrovesicular steatosis, ballooning degeneration, mononuclear inflammatory infiltrates are all toxicological responses in fish. Santos *et al*.[35] have reported necrosis in the liver of fish species during the assessment of the impacts of bioaccumulation of butyltins in Paranagu´a Bay, Brazil. Similarly, Khoshnood[36] has observed that various provenance of contaminants can lead to mild or serious damage to liver cells. The histopathological damage in the liver observed in this study was also in agreement with the observations of Olarinmoye *et al*.[37]. that reported hydropic degeneration, sinusoidal congestion, and hepatic necrosis in fish samples obtained from the Lagos lagoon.

The alterations observed in the liver samples of all target fish species could be attributed to the frequent crude oil spillage that is endemic in the region. This observation is supported by the work of Eriegha and Sam[9] and Eriegha *et al*.[22] that reported that Crude oil can cause deleterious damage to histopathological components in fish. Various forms of alterations such as congestion of capillaries, atrophied and necrotic lamellae have been observed in fish exposed to crude oil[10]. Water pollution can impact the histopathological status of fish by causing tissue damage, behavioral issues, immunotoxicity, altered growth, and reproductive damage.

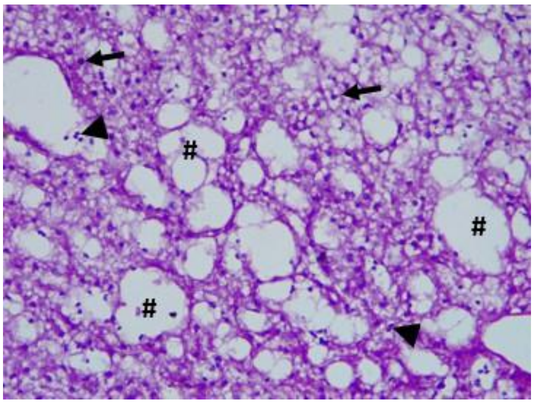

Figure 2. *Photomicrograph of Liver section of E. fimbriata showing hepatocytes (**thick arrow**), diffused preponderance of macrovesicular steatosis and ballooning degeneration (#), and the mononuclear inflammatory infiltrates (**arrowhead**). H&E stain, x400 magnification*

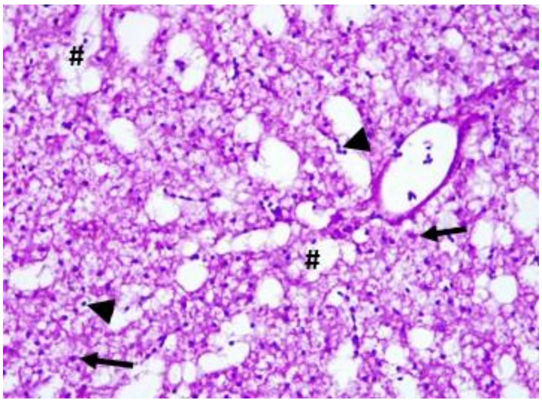

Figure 3. *Photomicrograph of Liver section of P. elongatus showing hepatocytes (**thick arrows**), diffused preponderance of macrovesicular steatosis and ballooning degeneration (#) and the mononuclear inflammatory infiltrates (**arrowhead**). H&E stain, x400 magnification*

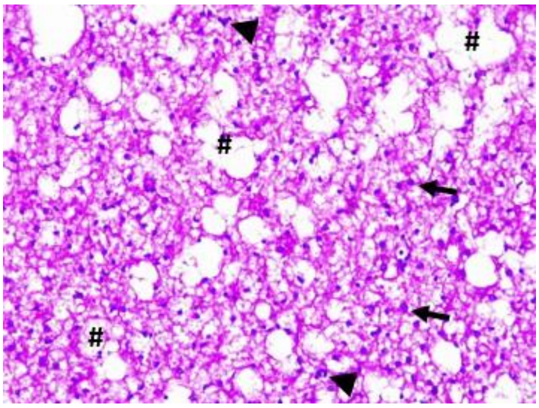

Figure 4. *Photomicrograph of Liver section of C. senegallus showing hepatocytes (**thick arrow**), diffused macrovesicular steatosis, and ballooning degeneration (#) mononuclear inflammatory infiltrates (**arrowhead**). H&E stain, x400 magnification*

3.2. Histopathology of the Gill

The photomicrographs of liver sections of *E. fimbriata*, *P. elongatus*, and *C. senegallus* are presented in Figures 5, 6, and 7, respectively. Gill histopathogical evaluation of samples of *E. fimbriata* showed acellular lamella with no evidence of epithelial cells. No observable histopathological alterations were observed in *P. elongatus* and *C. senegallus*. Fish gills, due to their stable external environment, are the first organs to come into contact with waterborne pollutants, making them crucial targets for histopathological evaluation of cellular damage in polluted fish water[17, 38]. This is because Morphological changes in gills can indicate adaptation strategies for physiological functions or evaluate exposure to chemicals in water and sediment[39].

Whereas no observable lesion was observed in *P. elongatus* and *C. senegallus*, histopathological assessment of *E. fimbriata* indicated that the gills were affected by pollutants. The observed discrepancy in alterations among the selected fish species could be due to variation in species behavior, diet, or habitat preference. Species that have evolved in different ecological niches may have different adaptive strategies that affect their histopathological responses to similar stimuli[40]. *Ethmalosa fimbriata* is a pelagic fish species, while *P. elongatus* is a benthic species[41]. The evolutionary history of fish species, the age or life stage of the individuals being studied, and variations in genetic adaptation can influence the ability of a species to cope with environmental stressors[42, 43]. Yancheva *et al.* [5] have reported that some fish species (such as trout and salmon) are more sensitive than others to pollutants while some others (such as carp and perch) are more resilient to contaminated water. Hinton *et al.*[44] have also reported that variations in metabolism, immune system function, or other physiological processes can result in different patterns of histopathological alterations. Various types of damage to gill tissue, including necrosis, edema, and hyperplasia, which can lead to cell death have been reported by Kaoud and El-Dahshan[45]. Fish inhabiting the water of the Nile River, Egypt have been reported to experience edema, lamella fusion, hyperplasia, necrosis, degeneration, congestion, and hypertrophy[26]. Gill tissues, responsible for gas exchange and osmoregulation, are extremely sensitive to water contamination. Pinto *et al.*[46] suggested that the type of histological changes observed depends on the individual exposure time to pollutants, the type and concentration of pollutants.

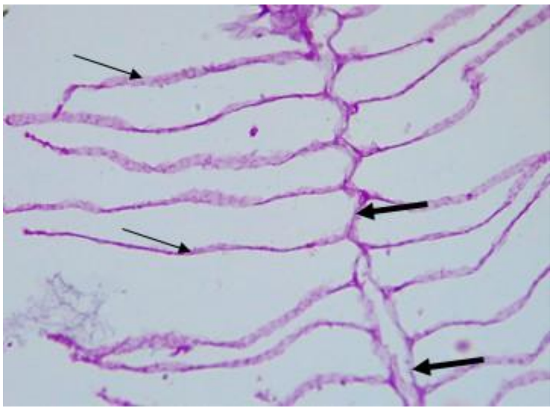

Figure 5. *Photomicrograph of gill arch of E. fimbriata showing gill filaments (primary lamella) (**thick black arrow**) and the several parallel threadlike secondary lamellae (**thin black arrow**) perpendicular to the primary lamellae. The lamella appeared acellular with no evidence of epithelial cells. H&E stain, x400 magnification.*

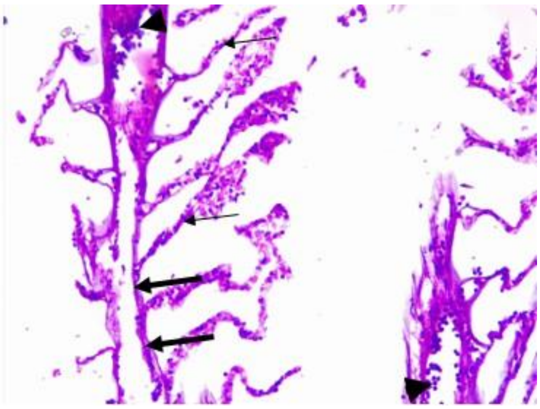

Figure 6. *Photomicrograph of gill arch of P. elongatus showing gill filaments (primary lamella) (**thick black arrow**) and the several parallel threadlike secondary lamellae (**thin black arrow**) perpendicular to the primary lamellae. There are evidence of epithelial cells (**arrowhead**) on the lamella. Also seen were supporting cartilaginous tissue (**blue arrow**) and the brachial muscle (**blue arrowhead**) between the gill filament. H&E stain, x400 magnification*

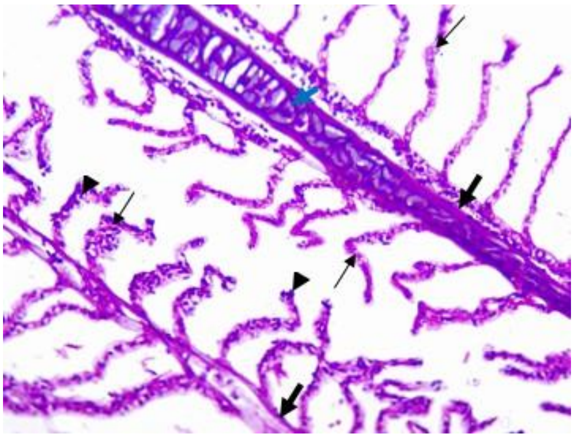

Figure 7. *Photomicrograph of gill arch of C. senegallus showing gill filaments (primary lamella) (**thick black arrow**) and the several parallel threadlike secondary lamellae (**thin black arrow**) perpendicular to the primary lamellae. There are evidence of epithelial cells (**arrowhead**) on the lamella. H&E stain, x400 magnification*

4. CONCLUSION

Histopathological evaluation is an important method for evaluating the health of fish populations in aquatic ecosystems. By identifying pollutant-induced tissue abnormalities, scientists can gain valuable insights into the influence of environmental changes on the health of aquatic organisms. The histopathological evaluation of fish species from the Escravos estuary showed that the impact is both organ- and species-specific. Liver histopathology revealed alterations in all three fish species examined. On the contrary, histopathological alteration was only observed in the gills of *E. fimbriata.* These findings suggest that the impact of water contamination in the Escravos estuary was more severe in the liver compared with the gills. The gills reflect the concentration of xenobiotics in the water, while the liver shows toxicant storage. This could be a positive sign, potentially linked to the recent government efforts to control oil theft and improve the water quality of the Escravos estuary. Efforts should therefore be made to sustain pipeline surveillance to protect the environment, aquatic resources, and the livelihoods of those who depend on it.

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