Histology Based Ecotoxicity Assessment: using the Gills of *Tympanotonus fuscatus* to Evaluate the Environmental Status of Bodo and Creek Road Water Bodies in Rivers State, Nigeria

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Abstract

This study involves the use of the histology of the gills of a periwinkle species, Tympanotonus fuscatus, as a biomarker tool, to investigate and evaluate the ecological pollution status of two different water bodies, Bodo and Creek Road water-sides, in River State. The study involved the sampling of ten (10) feral table sized fish from two stations along the creeks of the experimental sites (Bodo and Creek Road water bodies), with similar specie, size and number from a reference site (ARAC – African Aqua-culture Centre). The test fish gills were harvested from the fish caught from both experimental sites for histological evaluation, while the control gills were harvested from fish harvested from ARAC. The percentage prevalence of histological alterations showed that fish from Bodo water-body had the worse outcome (25%), followed by Creek Road water-body (21.7%) and ARAC having the best outcome (6.6%). Gills histology has proven to be an explorable biomarker for evaluation of environmental status.

Keywords: Histology, Gills, Biomarker, Ecotoxicology, Periwinkle and Aquatic Environment.

Introduction

Preserving untouched natural environments is crucial because they have a significant impact on supplying necessary resources like fresh air, clean water, fertile land, and materials for production. Emphasizing the importance of human health is essential for the perpetuation of life, the facilitation of successful reproduction, and the enhancement of overall well-being. An optimal environment is crucial for attaining economic prosperity and eliminating poverty.

Ecotoxicology is the study of the impact of toxicant in the natural environment. The testing environment is distorted, and the test organism is harvested from its natural habitat. It is an indicate of the ecological impacts of an exposed toxicant effect. Ecotoxicology is different from laboratory toxicological studies; in the former, the testing environment is distorted to suit the testing goals or aim. Ecotoxicology is therefore a field of study that focuses on evaluating the negative impacts of chemicals on ecosystems and the living organisms that inhabit them (Allison & Paul, 2018). It focuses on understanding how pollutants, such as pesticides, heavy metals, and industrial chemicals can impact the environment, including plants, animals, and micro-organisms.

Histology is a tool meant to determine the ecological integrity and pollution status of an aquatic. The terms "Pollution" and "contamination" have often been used erroneously as synonymous entities by environmental analysts (Allison & Paul, 2018). In environmental health, contamination is the presence of foreign substance beyond the pristine or allowable level for that environment,

while pollution is the presence of foreign substance beyond the pristine or allowable level for that environment with evidence of biological effect (Allison & Paul, 2018). The mere presence of a foreign substance above the natural level for that environment alone can trigger contamination; however, it requires a much high concentration to pollute the same environment. Pollution can only be scientifically proven to have occurred when there is evidence that the presence of hazardous chemicals in any environmental medium (water, soil, sediment or atmosphere) has resulted in biochemical, philological and ultimately in structural alteration of any organism within a given environment or toxicological system. Biochemical and physiological alteration are usually early signs of toxicity. Their sustained and sublethal effects ultimately lead to morphological alteration, which leaves a biological characteristic lesion that is pathognomonic for the diagnosing any diseases. In so far that histology is the microscopy study of biological structure, it is therefore a sensitive diagnostic tool for structural alteration, and by extension, a gold standard for the certification and stratification of pollution, in an ecotoxicological setting or toxicity in a laboratory toxicological setting. Organisms also adapt or tolerate some level of contaminants after chronic exposure. This is why the concentration of contaminant to cause pollution changes from organism to organism and from one environment to the other. Contamination can sometimes affect the process, whereas pollution is usually the products of the process being interactive with the environment and hence causing problems. Pollution is after the end process, and contamination is during the process. Contamination happens to the substance in use, whereas pollution happens to nature, soil, water, air, light, etc (Allison & Paul, 2018).

Representative species of the environment must be studied to assess the effects of pollutants in a matrix (Connell, 1984; Cengiz, 2006). Recognizing the significance of examining different species in the environment is crucial for accurately evaluating the impacts of pollutants in a particular setting. A biomonitor is a term used to describe an organism or group of organisms that are essential for assessing the magnitude and consequences of environmental pollution (Crouch et al., 1997; Eggleton & Thomas, 2004).

Environmental degradation has a significant impact on human health and overall social welfare. Current ecotoxicological evaluations primarily concentrate on conducting chemical analyses of hydrocarbons found in soil and water. Hence, the significance of histological techniques in comprehending the effects of harmful substances on aquatic organisms and human health when consuming periwinkle is frequently disregarded by these assessments. By conducting this study, we can assess the pollution levels in the ecosystem of our chosen area using the naturally occurring periwinkle. Given the prevalence of periwinkle in the everyday meals of the Niger Delta region in Nigeria, it is essential to investigate the potential health risks associated with the presence of trace metal contaminants in periwinkle. The aim of this study was to carry out a qualitative assessment on the histo-morphometry of the gills of *Tympanotonus fuscatus* (periwinkle), the semi-quantitative histological analysis and the pollution status of the Bodo and Creek Road waterfront.

Materials and Method

Study Area

Experimental Sites

Bodo Water Front: The water body is located at Bodo Water Front is located in Bodo-city, Gokana Local Government Area, Rivers State, Nigeria. Bodo Water Front is at the coastline of Bodo River, containing densely populated shack houses with an open water front market. The people living at the Bodo water front and in hamlets around its creek are basically fishers, farmers and traders. The Bodo river has been a means of survival for the people of Gokana Kingdom, It is a source of food for a vast number of artisanal and subsistent fisher. Fishes from the rivers form a major commerce in the market along the Bodo water front. In 2008 and 2009, two incidents of massive oil spills from the trans Niger pipeline devastated the Bodo River and coastline, destroying every living thing in the river, opportunities, made fishing impossible, and thereby ruining income that could have come from aquatic resources. Bodo and other communities mangroves and farmlands were reportedly contaminated with raw petroleum spill, which annihilated its environs, making it difficult to fish (UNEP, 2011). The area is also a historic zone for catching periwinkle which is now reported to be having dwindling yield.

Creek Road Water Front: The water body of Creek Road Water Front is located at about Lat 040 45025 N and 070 01027.7 E. The areas has riverside jetty, which is a bay for small boat craft used in ferrying people and large locally fabricated cargo boats for hauling building materials, commercials goods and people to the hinterland which are not connected by road. The area is host to one of the largest open market in Port Harcourt, where all manner of commercial goods are sold with a major seafood section. On its east-side, the water body receives wastewater from its metropolis, market, discharges from clinical, domestic and agricultural sectors and effluents from motor boats engines in the area. On its west-side, it receives wastewater from dockyard of the Ibeto cement factory and from transportation of timber and other goods using speed boat. It is a site for artisanal and subsistent fishing, especial an area for catching periwinkle which is reported to be dwindling in yield.

Reference Area

African Regional Aquaculture Center (ARAC) was chosen as the control site. It is situated at the training center, Buguma in Asari-Toru Local Government Area of Rivers State. It is a centre that focuses on multidisciplinary approach to user-driven aqua cultural research, development and training in sub-Saharan Africa geared towards sustainable fish production in the region. ARAC is affiliated to Rivers State University (RSU) for the award of masters of Science (M.Sc.) and post graduate diploma (PGD) in aquaculture.

Study Species

Tympanotonos fuscatus, the West African mud creeper, is a species of snail living in brackish water, a gastropod mollusk in the family Potamididae. *Tympanotonos fuscatus* is the only extant species in the genus *Tympanotonus* (Enuneku et al., 2013). The periwinkle species *Tympanotonus fuscatus* is known for its role as a deposit feeder, playing a crucial part in the breakdown of detritus and other organic matter in estuarine ecosystems that are both highly productive and often heavily polluted (Fränzle, 2006). The sensitivity of this organism to pollutants has made it highly sought after for scientific research purposes (Marigómez et al., 2003; Markert, 2007). This organism has been extensively utilized as a bio-indicator of aquatic pollution because of its remarkable capacity to accumulate and bio-magnify various contaminants, including heavy metals and polycyclic aromatic hydrocarbons, in its surrounding environment.

Preliminary Study

The reference site was visited and enquiries were made on the identification of the specific species of periwinkle by the taxonomist after been harvested at ARAC marine aqua culture centre at Buguma.

Sampling

Test periwinkle was caught from the experimental water bodies of Bodo and Creek Water Fronts. Samples were taken at stations approximately at a transect of 100; 200; 500; 800; 1,500; 2,500; and 5,000 metres from the water front in the direction of the most persistent bottom current of the nearby river (EGASPIN, 2002). The control periwinkle was harvested from ARAC marine aqua culture at Buguma. A group of ten (10) periwinkle specimens of the target species, each from each study area, was randomly collected at the water's edge near the sampling stations. Periwinkle samples were meticulously gathered by hand picking at the intertidal flats of mangrove swamps. The collection occurred during the mid-tide level (MTL) and mean low tide level (MLTL). The specimen were subjected to a meticulous cleaning process and subsequently sealed in clean plastic bags containing samples of soil and water from their natural environment and transported to the laboratory for further evaluation. The shells of the periwinkle samples from each station were carefully cracked and removed to access the edible tissue inside. The tissue that was separated and underwent multiple rinses using distilled water before detailed anatomical and histopathological examinations.

Histological Analysis

Harvested fish organs were preserved in 10% buffered formalin solution and taken to the laboratory for histological tissue preparation (Drury and Wallington, 1980; Allison and Paul, 2014). Light microscopy (Olympus BH2) was used to identify and interpret tissue slides and micrograph specimens at 40X, X100 and X400 magnification. The percentage prevalence of tissue histopathology from various target organs of the different sites were observed and compared with the control (Allison & Paul, 2014).

Results





Plate 1. Photomicrograph (H&E x400) of the gills: **A**) **ARAC:** Normal architecture showing Primary lamella (PL), Cartilage of primary lamella (CPL) Secondary lamella (SC), Chloride cells (CC), Pavement cells (PVCs) and Pillar cells. **B**) **BODO:** Diffuse vacuolization (DVL), focal area of coagulative necrosis foci (CNF), diffuse interstitial haemorrhage (IH), Pillar Cell Atrophy (PCA) and pavement cells hyperplasia. **C**) **CREEK:** Diffuse necrotic with necrotic foci leading to the degenerative granulation (DG), diffuse interstitial haemorrhage (IH), Vascular congestion, and vascular aneurysm.

	% Prevalence		
Alterations	ARAC (n=10)	Bodo (n=10)	Creek (n=10)
Circulatory Disturbance (CD)			
Intercellular haemorrhage	0	10	20
Interstitial Oedema	10	20	30
Progressive Change (PC)			
Hyperplasia	0	30	30
Regressive Change (RC)			
Architectural & Structural alterations	10	30	20
Nescrosis	10	40	20
Melano-macrophage centres (MMC)	10	20	10
Average % Prevalence	6.6	25	21.7

Table 1: Showing the Percentage prevalence of harvested periwinkle Gills histopathology from

 BODO, CREEK and **ARAC**

DISCUSSION

The gills of the control periwinkle exhibit a clear illustration of the standard structure, including primary lamella, cartilage of primary lamella, secondary lamella, chloride cells, pavement cells, and pillar cells. Various abnormal alterations were detected in the gills of periwinkle in Bodo. Several changes were noted, including the presence of vacuolization, areas of necrosis, interstitial hemorrhage, atrophy of pillar cells, and hyperplasia of pavement cells. Similarly, in Creek, there were observations of necrotic changes accompanied by granulation, interstitial hemorrhage,

vascular congestion, and vascular aneurysm. According to a study conducted by Salami et al., 2023, they investigated the distribution of tissues and the harmful impacts of hexavalent chromium on periwinkles under controlled laboratory conditions. Irregular blood and tissue fluid flow in certain medical conditions are linked to disruptions in circulation. The study has identified circulatory disturbances, specifically intercellular hemorrhage, that were observed in both Bodo and Creek. These areas were discovered to have interstitial oedemas, with the most notable change observed in Bodo, followed by Creek. However, there was no significant effect observed in ARAC. A recent study has revealed notable alterations in the periwinkles found in Bodo and Creek. These changes are characterized by a noticeable surge in the growth of mucous and epithelial cells. According to a study conducted by Cengiz (2006), there is evidence suggesting that gills hyperplasia could potentially function as a protective mechanism. This may result in a reduction in the respiratory surface area and an increase in the distance that harmful substances need to travel to reach the bloodstream. There is a downside to this defense mechanism as it decreases the respiratory efficiency of the gills. As time goes on, the effect on breathing becomes more noticeable compared to the ability to protect against the intake of pollutants. Noted changes include architectural and structural modifications, necrosis, and melano-macrophage centers. ARAC had the lowest levels, while Bodo had the highest levels, followed by Creek. Various responses occur as primary protective mechanisms, occasionally leading to severe disease in specific regions. Marigomez et al. (2003), conducted a study that suggested a potential connection between the occurrence of Melano-macrophage centres (MMC) and a rise in parasitic infestation. According to a study conducted by Proskuryakov et at. (2003), necrosis is a condition where cellular damage does not result in immediate fatality. Reversing the changes is often possible once the cause of damage is removed. Multiple scientific studies have established a correlation between necrotic changes and the presence of algae and heavy metals (Reid et al., 2008; Rajeshkumar et al., 2017; Salami et al., 2023). This discovery aligns with the findings of the EQWI study, suggesting that the development of lesions may be caused by impurities in surface water. Nevertheless, the discovery did not align with the outcome of the sediment quality assessment. There are several factors in surface water hydrology that could potentially account for this difference, such as rain recharges, water currents, and flow rates of the water body. These factors have the potential to decrease the amount of contaminants in water, while simultaneously increasing their buildup in sediment (Zhou et al., 2008; Vinodhini & Narayanan, 2009; Udiba et al., 2020).

Conclusion

The study showed that the comparative higher percentage of prevalence of histological alterations of the kidney of fishes caught from Creek road and Bodo water front water bodies as compared to the control indicates potential pollution at the experimental sites. It would be recommended to further carry out a semi-quantitative histological analysis to properly quantify and stratify the level pollution. A complementary water and sediment quality analysis would give a broader forensic spectrum of the source of pollution and remediation options

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