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Research Article

The Prevalence of occurrence of an opportunistic pathogen in *Gynmnarchus niloticus*, Cuvier (1829) In Anambra River, Nigeria

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ABSTRACT

The available studies have shown that *Gynmnarchus niloticus*, Cuvier (1829) in Nigerian freshwater bodies record low parasitic infections. Therefore, both parasitic and histopathological studies on the liver of *G. niloticus*, Cuvier (1829) as one of the most commonly occurring species were carried out to determine the healthy state of these species in the Anambra River after initial observation in one *G. niloticus* sample from Imo River in 2017. Though no protozoan or helminth infection was detected, there was liver infection by an opportunistic pathogen - *Aeromonas* sp. 115 samples of *G. niloticus* were collected and examined for the presence of a pathogen from January to June 2023, 32 (27.8 %) were infected by *Aeromonas* sp. All the affected *G. niloticus* were asymptomatic. Sections of the liver showed severe, multifocal, degeneration and necrosis of the hepatocytes with varying degrees of leucocytic/inflammatory cell infiltration. The degenerate hepatocytes showed multiple clear vacuoles in their cytoplasm (micro-vesicular lipidosis). Within the necrotic areas, bacterial colonies consisting of slender short to medium-sized filamentous rods were observed. Clear large vacuoles of varying sizes in the tissue (likely gas bubbles as a result of gas-forming bacterial activity) were also observed. Also, soil nematodes from the soil around the water body were analyzed for heavy metals contents using atomic absorption spectrometry (AAS) mg/kg as a possible source of stress to *G. niloticus* involving eight elements but three namely Cd (0.005±0.001) mg/kg, Cr (0.0095±0.0015) mg/kg, Pd(0.008±0.000) mg/kg were detected in low, safe levels.

Keywords: *Aeromonas* sp; Anthropogenic activities; *Gynmnarchus niloticus*; Heavy metals; Histopathology

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INTRODUCTION

Gynmnarchus niloticus, Cuvier (1829) occurs widely in West Africa especially in the Nile, Volta, Niger, Kainji dam and many inland waters of Africa such as Anambra River Basin, Imo River Basin and their tributaries. It grows to a hulking size in length, and it's the only extant species of Gymnarchidae. They are piscivorous as well as carnivorous (Bennett, 1971; Idod-Umeh, 2003). They are widely consumed as they form important food resource for human communities living along the banks of broad tropical rivers. The Nigerian people with a population of over 170 million are perhaps the largest consumers of fish in Africa (Echi, 2005).

Although, *G. niloticus* serves important food resources in Nigeria (Akinsanya *et al.*, 2010), little information exists on the diseases that affect them. For instance, there have been reports of low and no occurrence of parasitic organisms in Imo River Basin (Ugwuozor, 1985; Nnamdi, 2017), where a lot of human activities take place within and around its banks.

Due to this prevailing reality we engender this histopathological study on the liver of *Gynmnarchus niloticus* as one of commonly occurring fish species that inhabit Rivers in order to use entrenchment of disease by any opportunistic bacteria in this species to show possible inducement of stress as a result of anthropogenic activities.

Heavy metal contamination in urban lands can pose serious threats to the soil ecosystem. Soil nematode communities have the ability to respond rapidly to minor alterations in the soil environment over brief time intervals and are extensively used to track changes in the soil environment (Zhongqi Huo *et.al.,* 2024). Nematodes have been used as bioindicators of soil quality for many years, and have been shown to have good potential for assessing the impact of heavy metal pollution on soil. They provide information about the biological condition of soil and can reveal dysfunctions linked to the presence of contaminants. In the case of contamination by multiple pollutants, bioindicators can reveal synergistic toxic effects on organisms living in soil. Anthropogenic pollution, including heavy metal pollution, that can pose a severe threat to humans and the environment ([Duruibe *et al*., 2007; Rhind, 2009).

Free living nematodes indicate direct reactions in expected character to earth ecosystem alterations (Freckman and Ettema, 1993; Moody and Aitken, 1997, Moura and Franzener, 2017). They are secondary consumers in the ecosystem, that readily respond to little chnages in the physicochemical properties of the soil. Their reactivity to environmental changes is associated comparative abundance, unique morphology, diversity, and wide range of trophic survival specialism (Bileva *et al*., 2013; Nzeako *et al.,* 2015). This is important as some studies indicate anthropogenic pressure plays damaging role to aquatic properties in addition to general health implication of high values for heavy metals (Echi *et.al.,* 2018).

MATERIALS AND METHODS

Species of *Gynmnarchus niloticus was* collected from fishers in Imo River and Anambra River in 2017 and 2023 respectively. Description of the study sites have been described by Ugwuozor, 1985 and Echi, 2010 respectively. The freshly caught fish was cut open ventrally to expose the liver. The diseased liver excised, fixed in 10 % formalin – saline solution for days prior to paraffin processing: the fixed tissue was transferred through graded series of alcohol - 70 %, 80 %, 90 %, absolute 1 and 2 solutions for one and half hours in each case to dehydrate the tissues. The alcohol saturated tissue was then transferred to chloroform which cleared the tissue overnight. The chloroform was miscible with both the alcohol and the paraffin wax and also raised the refractive index of the tissue, imparting to it a transparent appearance. Then the tissue was embedded in molten paraffin wax, until the tissue was sufficiently impregnated with the wax, the tissue was embedded in fresh wax which solidified on cooling. The tissue was then sectioned at 5 – 6 microns thick, using microtome and stained using hematoxylin eosin stain. The sections were placed on glass slides and viewed and photographed under microscope at X 100 and X 400 objective lenses (Drury *et.al.,* 1967; Echi *et al.,* 2014 ; Nnamdi, 2017).

10 Samples of soil nematodes were collected from January – June 2023 from the surrounding water body soil in order to determine the level of ecosystem toxicity and randomly divided into two and used for heavy metals profiling. The samples were burnt to ashes in a muffle furnace, after which 1g of it was placed in a beaker 10ml Aqua Regia: (Measure 75ml of Conc. HCl and 25ml conc. HNO_3 into 100ml Volumetric Flask, 3:1 was added. Then, it was stirred to dissolve completely using a glass stirring rod. The solution was then cooled and filtered into a 100ml flask and further diluted to mark with distilled water (Radojevic and Bashkin, 1999), while Atomic Absorption Spectrophotometer (AAS) mg/kg was used to read off the metal level at a particular wavelength (Lenntech, 2012).

RESULT

A total of 115 samples *G. niloticus* were collected from fishers from January to June 2023, in Anambra River after initial observation in one *G. niloticus* sample from Imo River in 2017. Out of this number 32 (27.8 %) were affected by *Aeromonas* sp. All the affected *G. niloticus* were asymptomatic. The excised liver revealed instead dark brown liver lobules covered entirely with white loci surrounded by a bright rim of bloody tissue (Plate 1).

Histopathological Findings

Sections of the liver showed severe, multifocal, degeneration and necrosis of the hepatocytes with varying degrees of leucocytic/inflammatory cell infitration. The degenerate hepatocytes showed multiple clear vacuoles in their cytoplasm (micro-vesicular lipidosis). Within the necrotic areas, bacterial colonies consisting of slender short to medium sized filamentous rods were observed. Clear large vacuoles of varying sizes in the tissue (likely gas bubbles as a result of gas forming bacterial activity) were also observed.

Morphological Diagnosis

Hepatitis (Severe, sub-acute, multifocal, necrotizing, with multifocal bacterial colonies).

Out of the eight different metals namely Cd, Cr, Co, Ni, Pb, V, Hg and As analyzed for the presence of heavy metals in tissues of the soil nematodes only three: Cd, Cr, and Pb were detected in low as well as safe levels. Soil inhabiting organisms appear safe to heavy metals in southeast, Nigeria, it is therefore unlikely that perhaps agricultural chemicals in or on soils around water bodies, constitute source of heavy metals that floats through run-offs during and after rains into water bodies constitute stress to *G. niloticus.* Therefore other anthropogenic activities especially during dry season during contraction of aquatic habitat constitute serious concern to aquatic life forms (Echi, 2016).

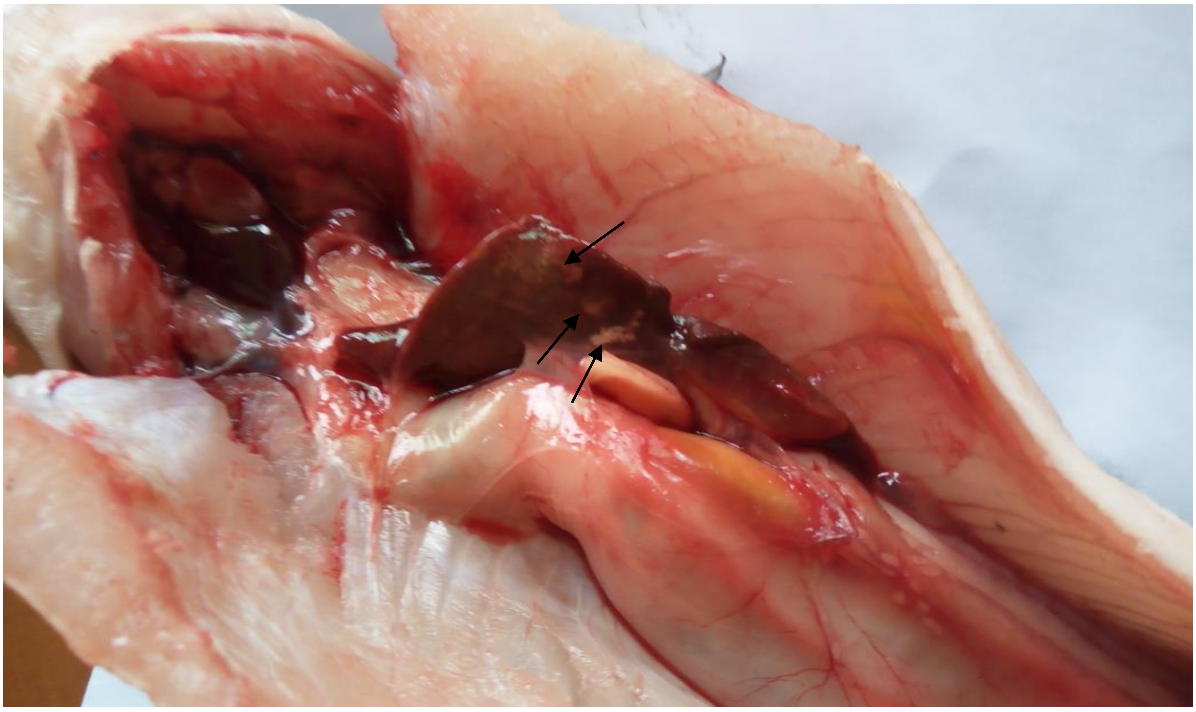

Plate 1: Dissected viscera of *Gynmnarchus niloticus* showing the diseased liver (prominent white loci in the lobules)

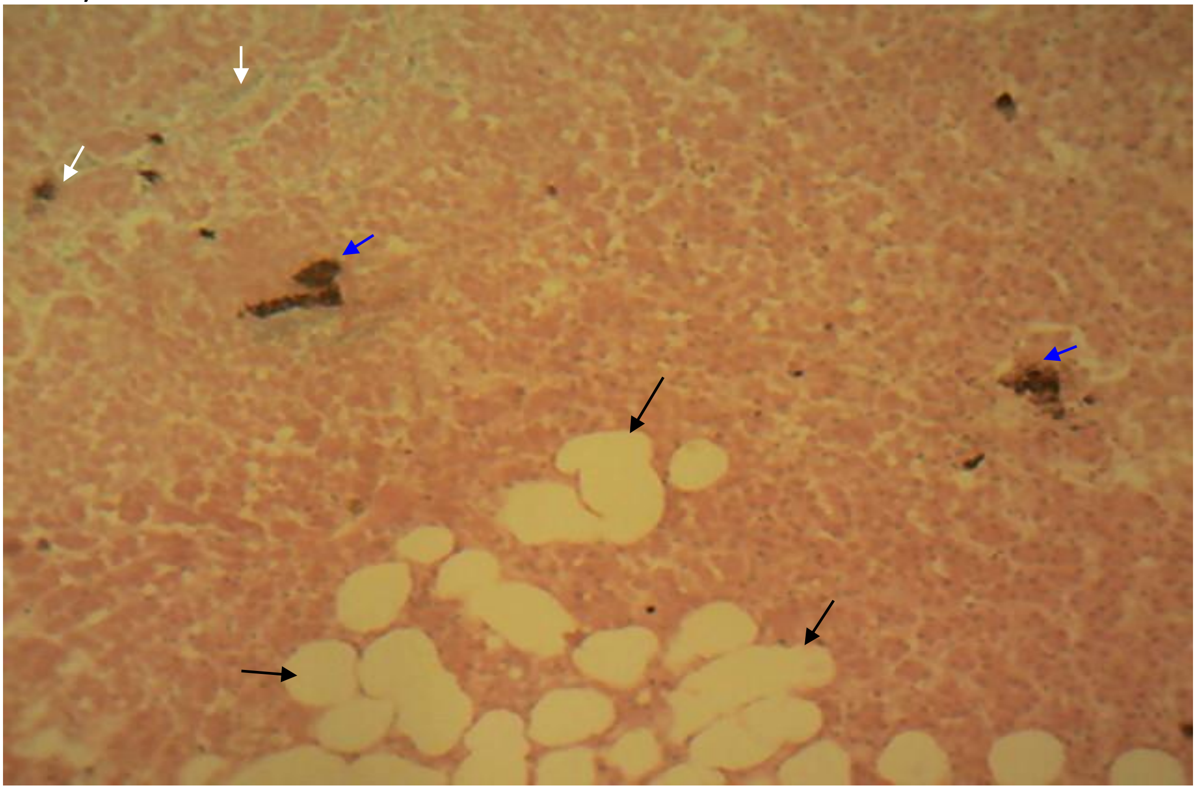

Plate 2: Low power (Mag X100), showing the gas bubbles (black arrow) and the *Aeromonas* sp colonies (white arrow). Hemosiderin pigments (blue arrow)

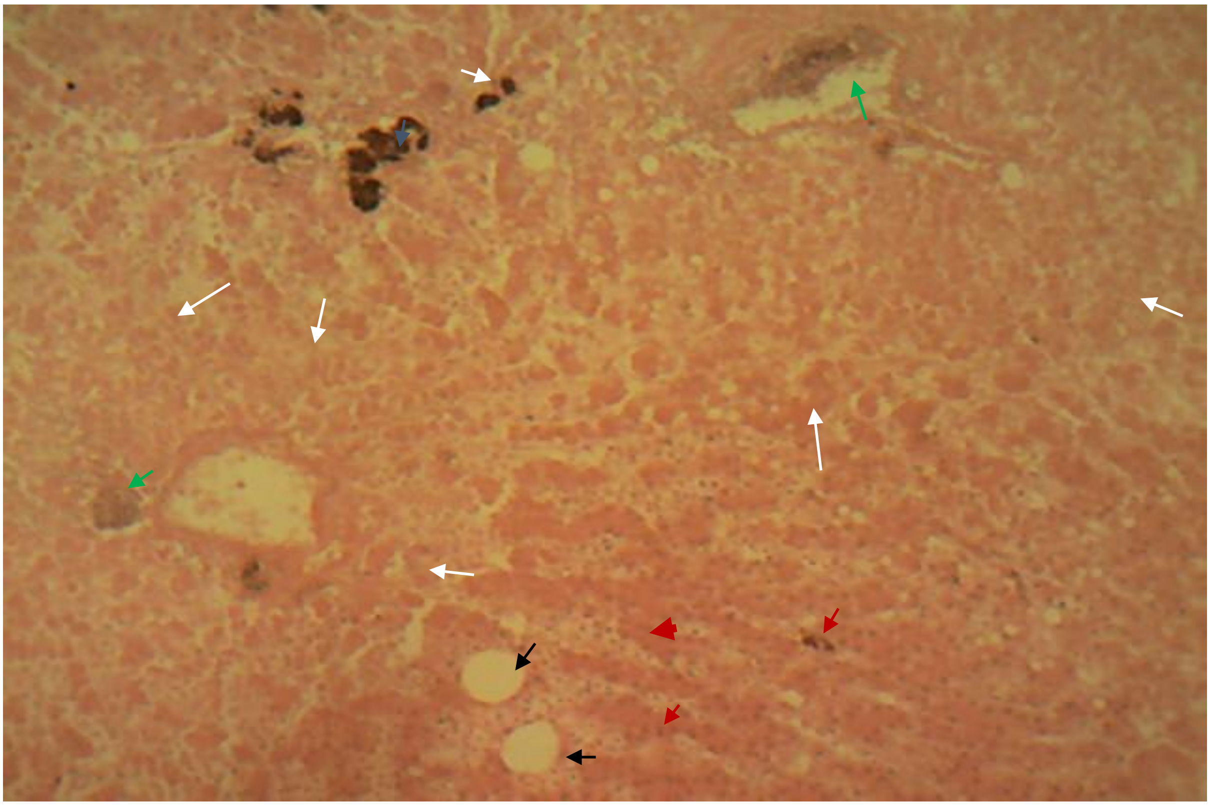

Plate 3: Low power (Mag x100), showing a wide area of degeneration and necrosis of the hepatocytes (white arrow), compare with normal hepatocytes (red arrow). Also present in the photomicrograph are bacteria-induced gas bubbles (black arrow), and a cluster of *Aeromonas* sp (green arrow)

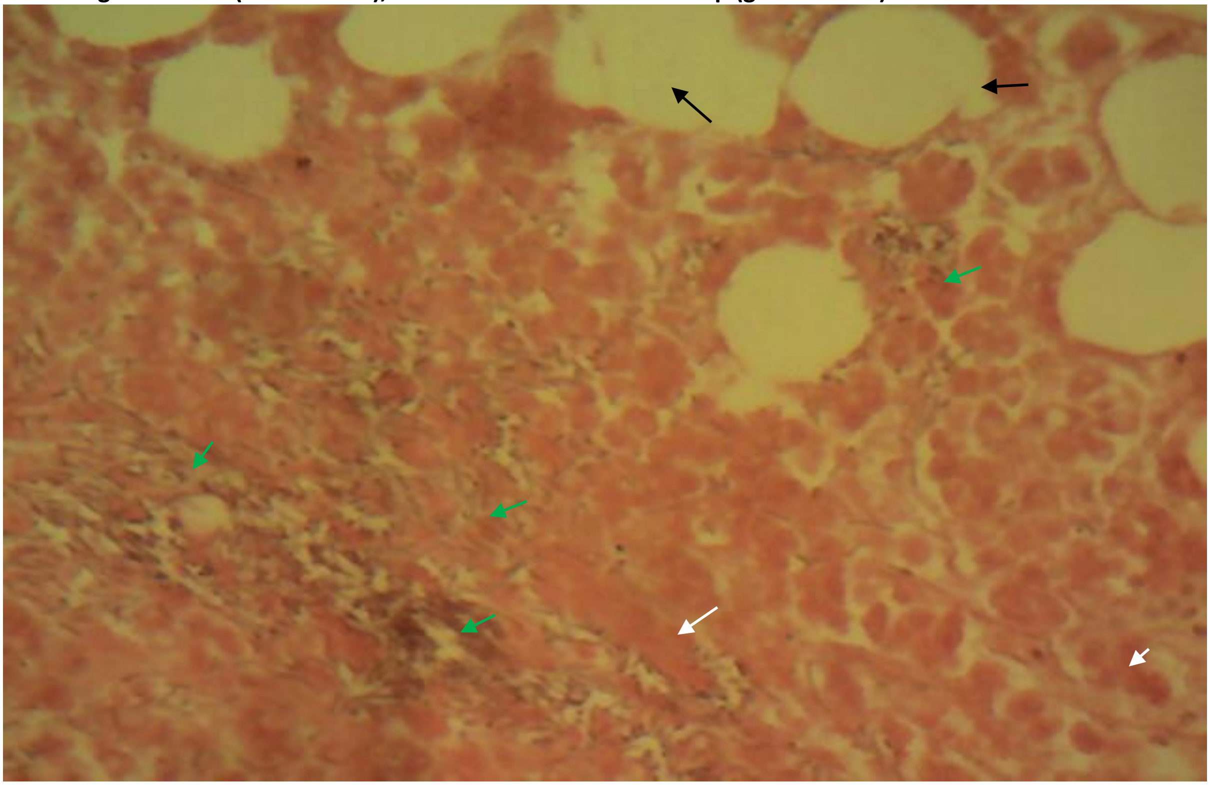

Plate 4: High power (Mag x400) showing bacteria-induced gas bubbles (black arrow); necrotic hepatocytes (white arrow) and aggregates of *Aeromonas* sp (green arrow)

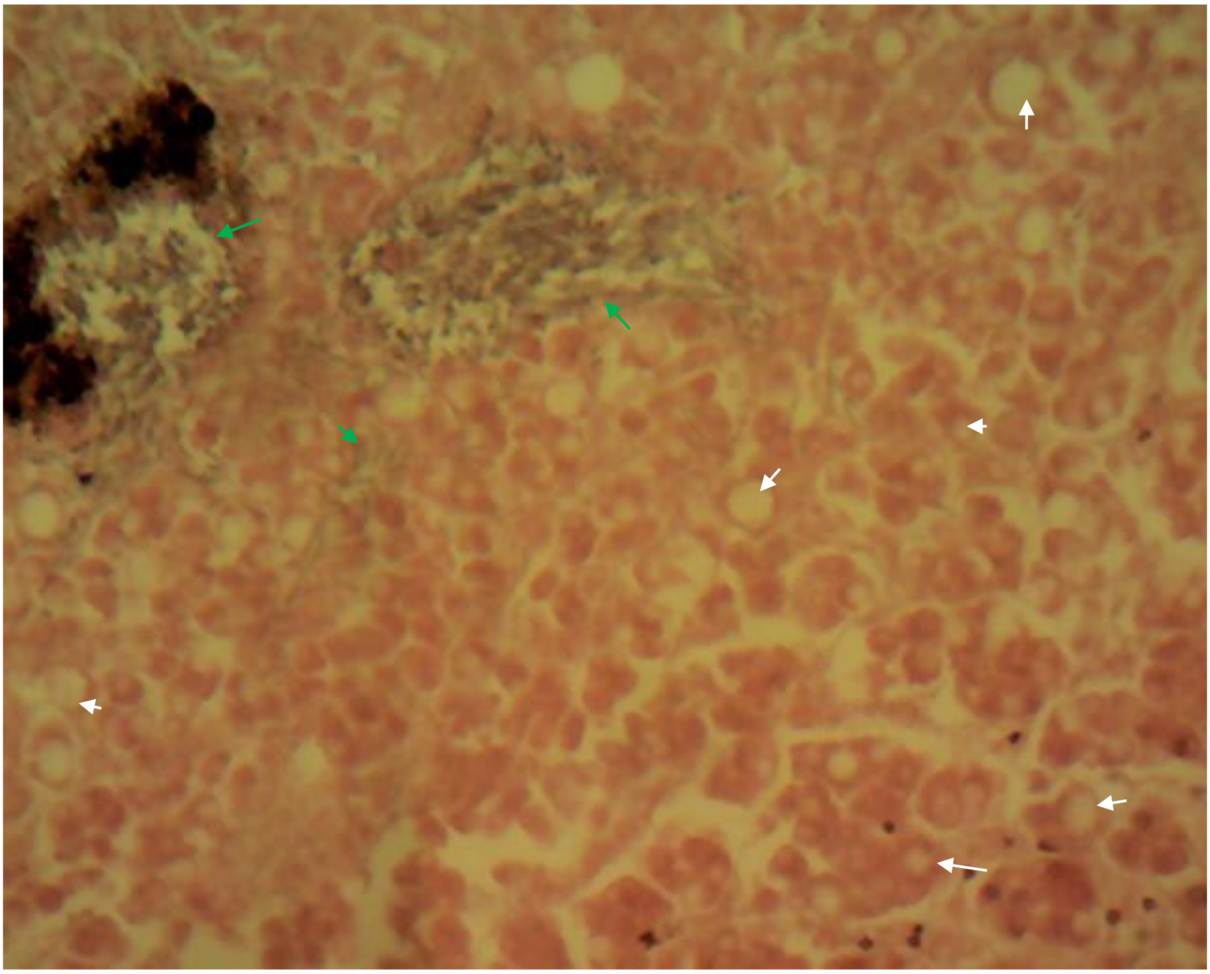

Plate 5: High power (Mag x 400) showing hepatocytes undergoing micro and macrovesicular lipidosis. The white arrow shows the intracytoplasmic vacuoles. *Aeromonas* sp (green arrow)

DISCUSSION

Aeromonas sp is a Gram negative bacterium that causes various forms of diseases in fish organs such as skin, gills, intestine, spleen, liver, gallbladder and kidney (Pech *et al.,* 2017).

Gram negative bacterium is the most important cause of bacterial disease in fish, for instance genus *Aeromonas* sp causes furunculosis and hemorrhagic septicemia in skin (Austin *et al*., 2012). Also, this group of bacteria is listed within the boundaries of public health concerns with high resistance impact to various antibiotics (Calvo *et al*., 2009).

Ergo, epidemic is linked to the existence of a stress factor based on the interaction between fish, pathogens and aquatic environment as a natural habitat of the organism, as well as poor water quality or excess of organic matter factors which allows the incidence of disease to be greater (Huicab-Pech *et al*., 2016).

The active anthropogenic influence on the ecological parameters of tropical Rivers from their banks should be a concern. Human disturbances of aquatic ecosystem reduce water quality and induce stress. When stress is established opportunistic infection causing organism would cause infections due to host factors such as reduced immunity in the host fish. For instance, complex human activities at the bank of Anambra river have increased influx of mainly organic materials example food materials, human excreta, agro- chemicals etc into the River and this keeps the pH range (5.5 – 7.0) at fairly constant (Echi and Ezenwaji, 2010). Stress can permanently affect the organism by increasing the corticoid hormone production of the adrenal gland. The corticoids block the protective mechanisms of the fish phagocytosis and immune responsiveness, thereby making the fish prone to parasitic infections (Woo, 1995).

In such aquatic ecosystems the pH range is an indication of predominating high carbonic acid content. In a human disturbed aquatic ecosystem, low alkalinity value/s is characteristic of water body that is heavily infested with heavy organic materials from its surroundings. The carbonic acid formed after dissolution of carbon dioxide gets dissociated into Bicarbonate ($HCO3$ -) and Carbonate ($CO3$-2) ions (Gupta and Gupta, 2013). *Aeromonas* *hydrophila* is related to sudden changes in temperature, dissolved oxygen and inadequate nutrition (Soto-Rodríguez *et al.,* 2013; Li and Cai, 2011).

Constant variation of physicochemical parameters is a stress factor that benefits the outbreak of disease caused by opportunistic bacteria. In contrast, *Aeromonas hydrophila* is related to sudden changes in temperature, dissolved oxygen and inadequate nutrition, as pointed out by (Li and Cai, 2011; Soto-Rodríguez *et al.,* 2013)

The presence, as an opportunistic pathogen, is due to the conditions of the aquatic environment and stress, as reported by (Burr *et al*., 2012; Palumbo *et al*., 1992; Woo and Bruno, 2010; Johri *et al*., 2006), who consider that organisms under stress conditions are susceptible to the presence of opportunistic pathogens. These pathogens cause hemorrhagic septicemia and clinical signs of erratic or circling swimming, uncoordinated movements, anorexia or decreased appetite, exophthalmia, corneal opacity, visceral cavity extension, bleeding and abdominal inflammation, softening of the brain and liver, hepatomegaly and pallor in the organ, as well as splenomegaly and visceral adhesion commonly found in crops and studies at the experimental level.

Notwithstanding there had been reports that they harboured few parasites; 2 each of different species of nematode; Anisakid and Philometrid nematodes in Lekki, Lagos Lagoon with no perceptible effects on these hosts (Akinsanya *et al.,* 2010).

Low and safe levels of the heavy metals Cd (0.005±0.001) mg/kg, Cr (0.0095±0.0015) mg/kg, Pd (0.008±0.000) mg/kg indicate that other environmental causes other than heavy metals are responsible for stress and possible entrenchment of opportunistic diseases in *G. niloticus.* It is a documented fact that soils in Southeast, Nigeria contain safe levels of heavy metals. This is important because the rarity of non-migratory species such as *Hemisus guttatus* has not been linked to the presence of heavy metals and migratory animals such as *Milvus migrans* parasites that visit the southeast areas seasonally perhaps accumulate such metals from environments other than Southeast environments (Echi *et.al.,* 2025).

Cadmium affects the liver, placenta, kidney, lungs, brain and bones. Experimental data in humans and animals showed that cadmium may cause cancer in humans (Jarup *et al.,* 2000; Nordberg *et al.,* 2002). Elevated levels of chromium can result in reduced reproductive success and impaired immune function in wildlife, which can have cascading effects on ecosystem health (Denny, 1999). Lead, is long been recognized for its harmful effects on both for life forms have been extensively studied in the context of lead poisoning due to the ingestion of lead contaminated food sources (Fisher *et al*., 2006).

CONCLUSION

The results presented here serve as a baseline report on the presnce of rare, opportunistic pathogen in the organs of *Gynmnarchus niloticus* in the wild, which is indicative of increased stress in these aquatic ecosystems where they inhabit perhaps by other anthropegenic activities other than heavy metals.

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