Histopathology and Morphometric Characters of *Tilapia Guineensis* as Biomarker to Evaluate Level of Environmental Stress in Badagry and Ojo Creeks, Lagos, Nigeria

Abstract

Forty wild adult *Tilapia guineensis* were sourced from Ojo and Badagry creeks between January and March, 2021, in order to investigate its morphometric character (total length, body weight, standard length, and head depth) and tissues structure (gill, muscle and small intestine) differences. Samples were collected and analysed using standard methods. The results showed no significant difference (P<0.05) between the mean total length, body weight, standard length, and head depth of *Tilapia guineensis* recorded from both creeks. Length- weight relationships of *T. guineensis* obtained from both creeks showed negative allometry(b<3) growth pattern while its condition factor (k) value were greater than 1 which indicated that the fishes are in good condition. Muscle of *T.guineensis* from Ojo creek showed severe lesion of muscle bundle, degeneration of muscular bundle and shortening of bundles. Similarly, the gills structure of *T. guineensis* from Ojo creek showed severe degeneration, hyperplasia of secondary gill lamellae, and curling of secondary lamellae with unclear water channel. It is also characterized by hypertrophy of lamellae and there is an evidence of partial fusion of lamellae and dilation of gill filament. The observation of none lesion or degenerated tissues of *T. guineensis* in Badagry creek could suggest that Ojo creek is more subjected to effluents or other environmental stressors than Badagry creek. In conclusion**,** *T. guineensis* from Badagry creek are less affected by any environmental contaminants in contrast to *T. guineensis* from Ojo creek. Therefore, a collective effort towards ensuring reduction in direct discharge of untreated effluent or avoidance of other unhygienic usage of the creeks is very pertinent.

Keywords: *Tilapia guineensis,* environmental contaminants, growth, histopathology, creeks,

Introduction

Tilapia guineensis (Bleeker, 1862) is a widespread species known to inhabit a variety of habitats such as open and closed estuaries, permanent and temporary rivers, large equatorial lakes, tropical and subtropical rivers, lagoons, swampy lakes, and coastal brackish lakes (Trewavas, 1983, Zengeya *et al*., 2012; Nyirenda, 2017). Tilapia is a highly nutritious food and it is particularly valued for its beneficial qualities which are attributed to its wealth of nutrients, minerals, protein, omega-3 fatty acids, selenium, phosphorous, potassium, vitamin B12, niacin, vitamin B6, and pantothenic acid (Ojutiku *et al*., 2009).

The phenotypic plasticity of fish allows them to respond adaptively to environmental change by modification in their behaviour and physiology which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variation (Meyer, 1987;

Huseyn *et al*., 2015). With the growth of human populations and commercial industries, brackish water has received large amounts of pollution from a variety of sources such as recreation, fish culture and the transport of pollution effluents through river (Muduli and Panda, 2010). These situations have generated great pressure on the ecosystem, resulting in a decrease of water quality and biodiversity loss of critical habitats (Herrera-Silveira and Morales, 2009).

Despite the advent of techniques which directly examines biochemical or molecular genetic variation, the analysis of phenotypic variation in morphometric characters or meristic counts is the method most commonly used to delineate stocks of fish (Yakubu and Okunsebor, 2011; Samaradivankara *et al*., 2012, Bradbeer *et al*., 2018). However, Davis *et al*.(2005) and Moller (1998) reported that environmental disturbances can induce changes in same organism.

Like any other morphometric characters, the length-weight relationship can be used as a character for the differentiation of taxonomic units and the relationship changes with the various developmental events in life such as metamorphosis, growth and onset of maturity (Thomas *et al*., 2003). Condition factor is also a useful index for monitoring of feeding intensity, age, and growth rates in fish (Oni *et al*., 1983). It is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene, 2005).

Some literatures have revealed the use of morphological examinations of fish organs to evaluate effect of pollutants in fish (Poleksic and Mitrovic-Tutundzic, 1994). That is why histopathological assessment of fish allows for early warning signs of disease and detection of long term injury in cells, tissues and organs before obvious manifestations. It is well known that aquatic pollution causes severe physiologic and morphologic alterations in aquatic organisms (Mazon *et al*., 1999, Mekuleyi and Fakoya, 2017). Effluents have been demonstrated to affect fish health, causing a histological lesions and higher susceptibility to infectious diseases (Escher *et al*., 1999; Bernet *et al*., 2001). Thus analysis of biochemical and histopathological changes is often used to assess the effect of effluents on different tissues in field and experimental studies.

Though reports of histopathological evaluation of toxicants effects in *Tilapia spp* exist (Midhila and Chitra, 2015; Patel *et al*., 2016) but there is no information on histopathological assessment of *Tilapia guineensis* in Ojo and Badagry creeks. Therefore the aim of this study is to compare some morphometric characters (Total length, standard length, body weight, and head depth), and histopathology of *Tilapia guineensis* from Ojo and Badagry creeks- as a measure of assessing the impact of environmental contaminants on the well being of this species.

MATERIALS AND METHODS

Study area

The study areas are Ojo and Badagry creeks (Image 1). Badagry creek is approximately 60 km long and 3 km wide and lies between longitude 2^0 42^1 and 3^0 23^1E and latitude $6^0 23^1$ and $6^0 28^1$ N. It is part of a continuous system of lagoons and creeks along the coast of Nigeria from the border with the Republic of Benin to the Niger delta. Its water depth ranges from 1- 3m. The creek experiences two broad seasons: the dry season (December - May) and the wet season (June - November). Most of the year, it is characterized by fresh and slightly brackish water. The creek is approximately equidistant from the entrances of Lagos and Cotonou harbours. As a result, it is influenced by tides and floods from the Lagos Lagoon and Cotonou harbour through Lake Nokue and Lake Porto-Novo (Anyanwu and Ezenwa, 1988). On the other hand, Ojo creek is an extension of Badagry creek which is one of the marginal estuaries' characteristic of the West African coastline (Anyanwu and Ezenwa, 1988).

Collection of Samples

Forty wild adult *Tilapia guineensis* were sourced from Ojo and Badagry creeks between January and March, 2021.They were collected from fishermen at the landing sites and transported to the laboratory of Department of Fisheries, Lagos State University where they were kept in fridge for 2 days.

Morphometric Parameters

The weight and length of each fish were determined using Mettler balance with sensitivity of 0.01g and measuring tape to the nearest 0.1cm, respectively. Prior to measurement, Fishes were defrosted at room temperature (25 °C). Morphometric characters which include head depth, standard length and total length were measured with measuring board of precision of 0.1 cm. Description of dimension of each morphometric distance has already been documented in literature (Bagenal, 1978)

Length weight relationship and Condition factor

The length and weight relationship (LWR) of the fish samples were calculated using the equation $W=aL^b$. $\ln W = \ln a + b\ln L$ (Pauly 1983)Where

W = Weight of fish in (g), L = Total length (TL) of fish in (cm), a = Constant (intercept) i.e a coefficient related to body form, and b = slope (change in weight per unit change in length) and is an exponent coefficient indicating the growth type. The "a" and "b" values were obtained from a linear regression of the length and weight of the fish measured. The data collected were validated by the analysis of the graph corresponding to length-weight relationships (Ecoutin and Albaret, 2003). The degrees of association between the variables were assessed by the determination coefficient (r^2).

Condition factor (k) of the fish specimens in this study was determined by using the formulae:

$k = 100W/L^3$, where: W=body weight of the fish, and L = total length of the fish.

Histopathological Examination

The extracted tissues (gills, small intestine and muscle) of T. *guineensis* were fixed in 10% formalin for 24hour, cleared in xylene, embedded in paraffin wax and sectioned at 5µm. Hematoxylin and Eosin was used for histological evaluation (Cek *et al*., 2001). Photographs were taken using canon digital camera power shot (SX170 IS) with 64-megapixel sensor (focal length: 28-448mm, 7.5cm (3.0") TFT).

Data analysis

Data for Length-weight relationship of the fish samples was computed using Excel 2007. All morphometric data obtained were expressed as mean ± standard deviation. Statistical comparison between morphometric data collected was tested with independent student t-test with values of $p < 0.05$ being considered significant. Also, in order to determine the kind of allometry exhibited by the fish species, slopes of length-weight regressions were compared to 3 using student t- test.

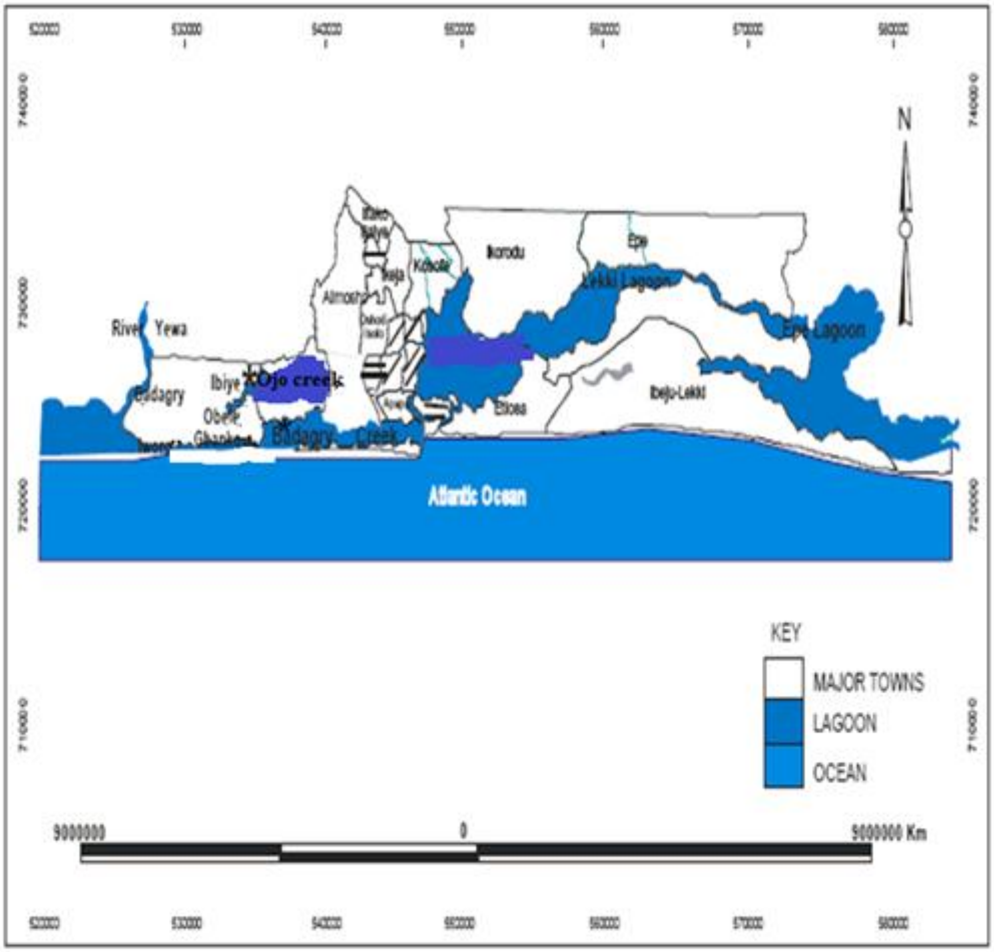

Image 1: Map showing the location of Badagry and Ojo Creeks in Lagos, Nigeria

RESULTS

As shown in Table 1, *Tilapia guineensis* from Ojo creek had total length which ranged from 9-12.9cm (mean=10.57±1.17), the range of its body weight was 20-40g (mean = 29.44±8.56), standard length ranged between 6.8-11.8cm (mean= 8.79±1.52), while the range of its head depth was 1.3-2.2cm (mean =1.88±0.25). On the other hand, *T. guineensis* from Badagry creek, had total length range of 7.6-12cm (mean=10.26±1.21), standard length ranged between 6-10.9cm (mean= 8.53±1.29), the range of its body weight was 20-40g (mean = 29.17±5.75), while the range of its head depth was 1.1-2.3cm (mean =1.79±0.28). However, there was no significant differences ($p>0.05$) between the mean total length, body weight, standard length, and head depth of *T. guineensis* recorded from both creeks.

While *T. guineensis* from Ojo creek had condition factor (K) value of 2.28±0.54 (range: 1.86-2.74), the K value of *T. guineensis* from Badagry creek was 3.59±0.44 (range: 2.31-4.55). The K values for the fish from Badagry creek differ significantly ($p<0.05$) from that from Ojo creeks.

Table 1: Summary of Some Morphometric characters and condition factor of *Tilapia guineensis* from Badagry and Ojo creeks in Lagos, Nigeria

	Ojo Creek				Badagry Creek			
Parameter	Min.	Max.	Mean	SD	Min	Max	Mean	SD
TL(cm)	9	12.9	10.57^a	1.17	7.6	12	10.26^a	1.21
BW(g)	20	40	29.44^a	8.56	20	40	29.17^a	5.75
HD(cm)	1.3	2.2	1.88^a	0.25	1.1	2.3	1.79^a	0.28
SL(cm)	6.8	11.8	8.79^a	1.27	6	10.9	8.53^a	1.29
K	1.86	2.74	2.28^b	0.54	2.31	4.55	3.59^a	0.44

TL = Total Length, BW=Body Weight, HD=Head depth, SL=standard length, K=Condition factor, Min= Minimum, Max= Maximum, SD=Standard Deviation. Mean value with different superscript in the row= significant different ($p < 0.05$)

Length- weight relationships of *T. guineensis* obtained from Ojo creek and Badagry creek is presented in figures 1-2 respectively. The value of b (slope) of the linear regression equation showed that growth pattern of *T. guineensis* were negative allometry (b<3). At Ojo creek, the fish had higher b value (2.002) as shown in figure 1, while the fish from Badagry creek had b value of 0.371 (figure 2). The value of their various intercepts (*a*) ranged from - 1.366 at Ojo creek to 2.490 in Badagry creek. The higher regression correlation values (R^2) was recorded in *T. guineensis* from Ojo creek (R^2 =0.551) as against that from Badagry creek (R^2 =0.052).

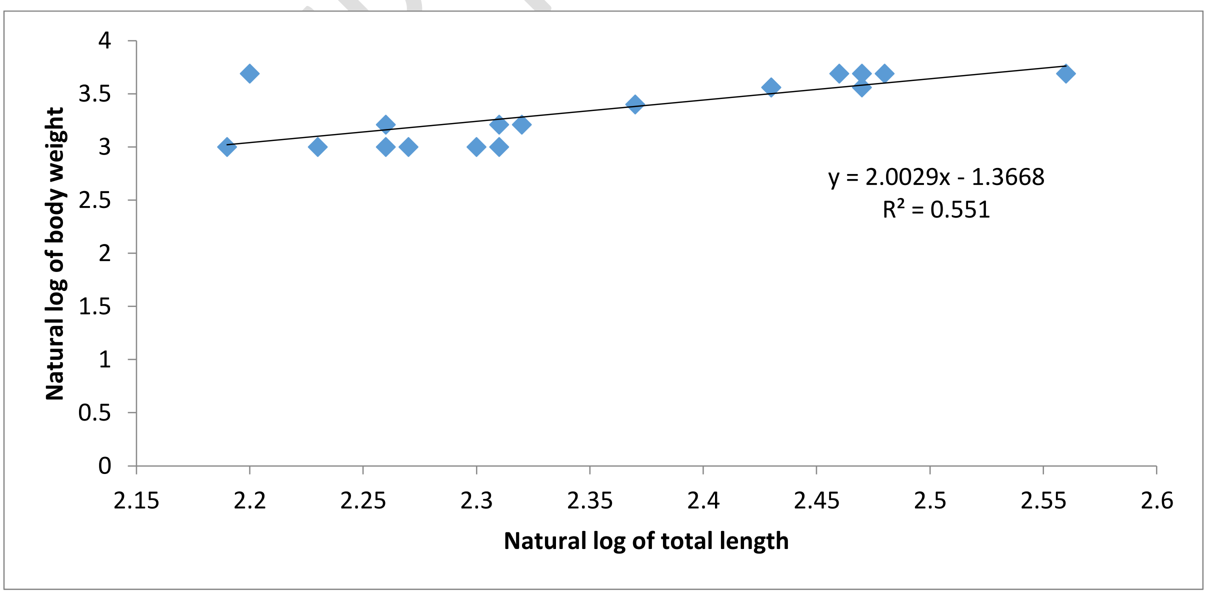

Figure 1: Length-weight relationship of *Tilapia guineensis* from Ojo Creeks

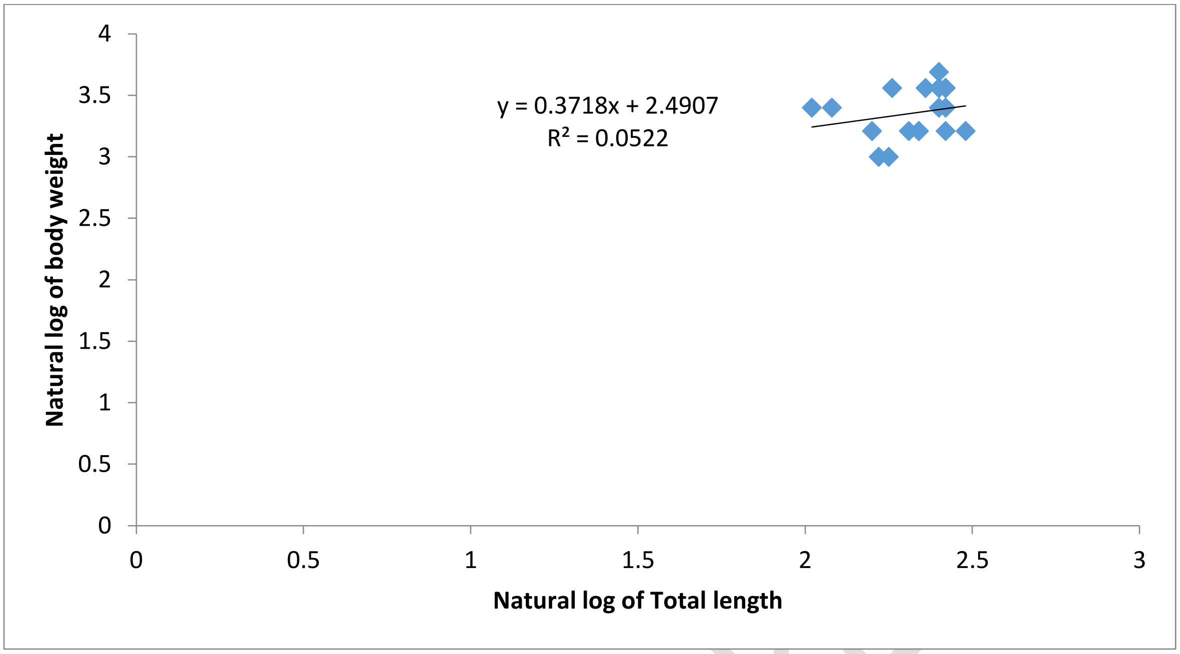

Figure 2: Length-weight relationship of *Tilapia guineensis* from Badagry Creeks

The histopathological results for *Tilapia guineensis* from Badagry and Ojo Creeks are presented in figures 3a-5b. Figure 3a and 3b showed the structures of the gills of *T. guineensis* from Badagry creek and Ojo creek respectively. The gill of the fish from Badagry creek (Fig.3a) showed vasodilation (V) with blood congestion (C) and mild hypertrophy (H) of lamellae. However, most of the secondary lamellae (SL) are free (clear water channel (W)) and intact with no evidence of epithelial lifting and curling of lamellae.

On the other hand, gill structure of *T. guineensis* from Ojo creek (Fig.3b) showed severe degeneration (D) and hyperplasia (HL) of secondary gill lamellae (D), separation of gill fill mate from basement membrane (S), and curling of secondary lamellae (C) with unclear water channel (W). It is also characterized by hypertrophy of lamellae (H). There is also evidence of partial fusion of lamellae (F) and dilation of gill filament (V).

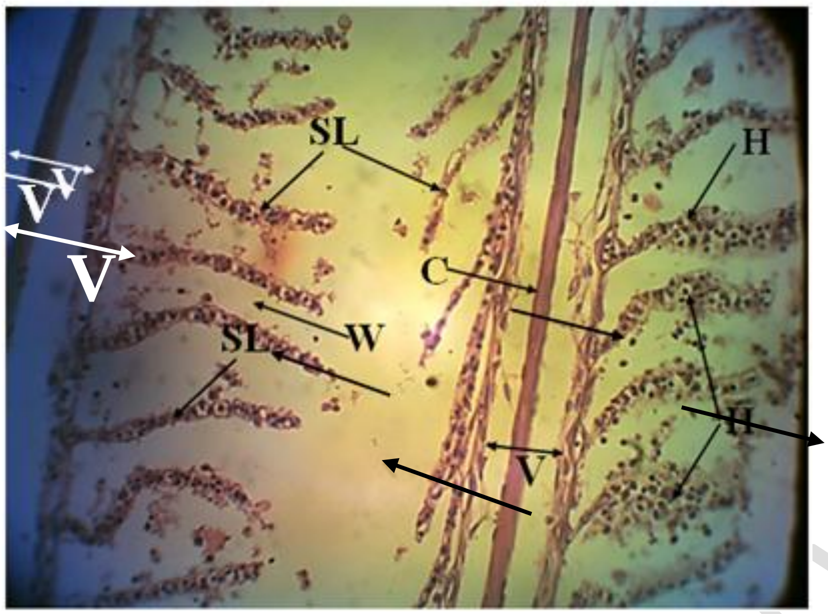

Figure 3a: Showing the gill's structure of *Tilapia guineensis* from Badagry creek

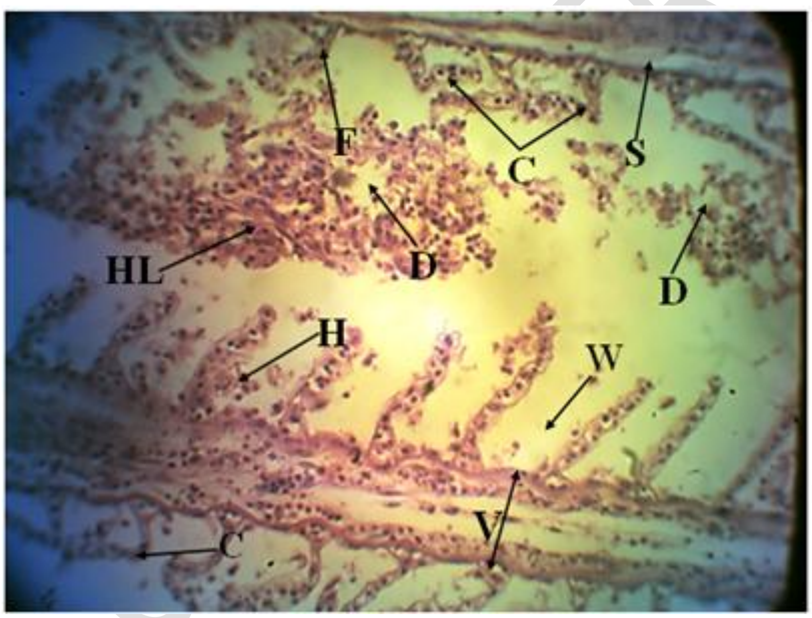

Figure 3b: Showing the gill's structure of *Tilapia guineensis* from Ojo creek

Figures 4a and 4b presented the histopathological results on small intestine of *T. guineensis* from Badagry creek (Fig.4a) and Ojo creek (Fig.4b). For *T. guineensis* from Badagry creek, the cross section of the small intestine showed the intestinal fold (I), Outer muscularis (Om) and inner muscularis layer (Im). There is slight degeneration and vacuolization in Submucosa connective layer (D). However, glandular cell (Gc) are adequately seen. It also showed mild lesion in epithelia lining Serosa(S). As shown in Figure 4b, small intestine of *T. guineensis* from Ojo creek is characterized by few presence of glandular cell (G), vacuolization of connective tissues in the submucosal layer (Vs). However, the intestinal layers were seen. Serosa (S), muscularis (M), sub mucosal (Sm) and intestinal lining epithelium (Ie) were also very clear.

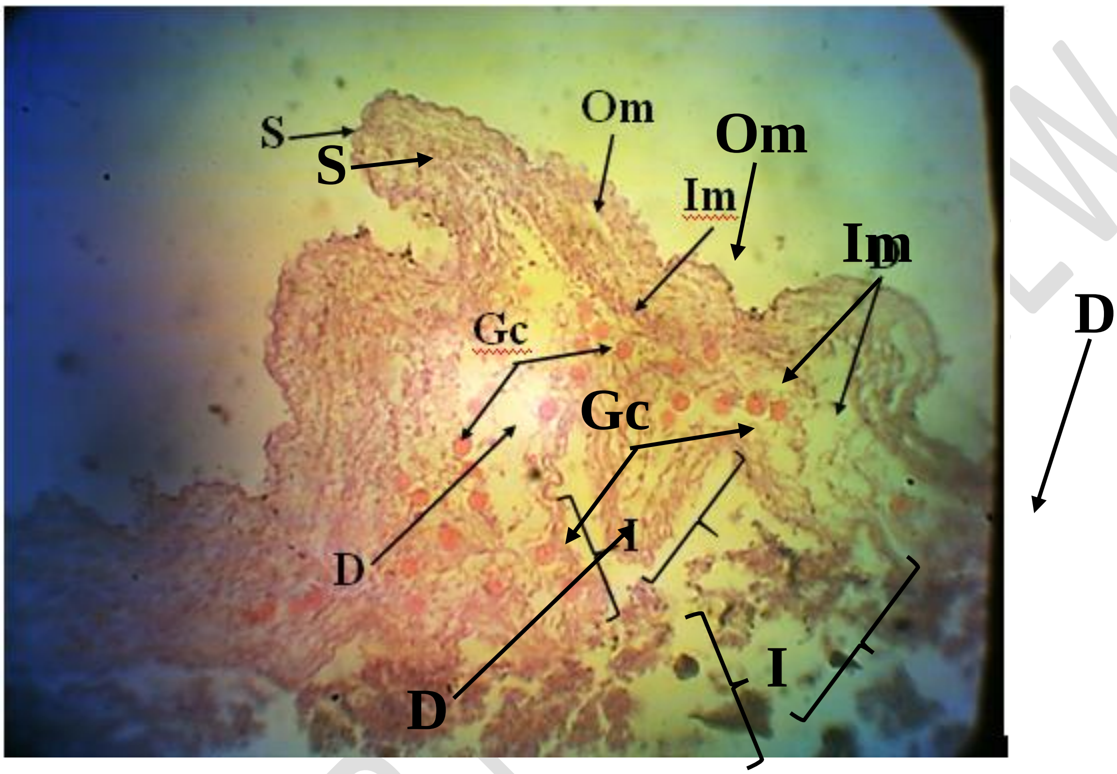

Figure 4a: The cross section of the small intestine of *Tilapia guineensis* from Badagry creek

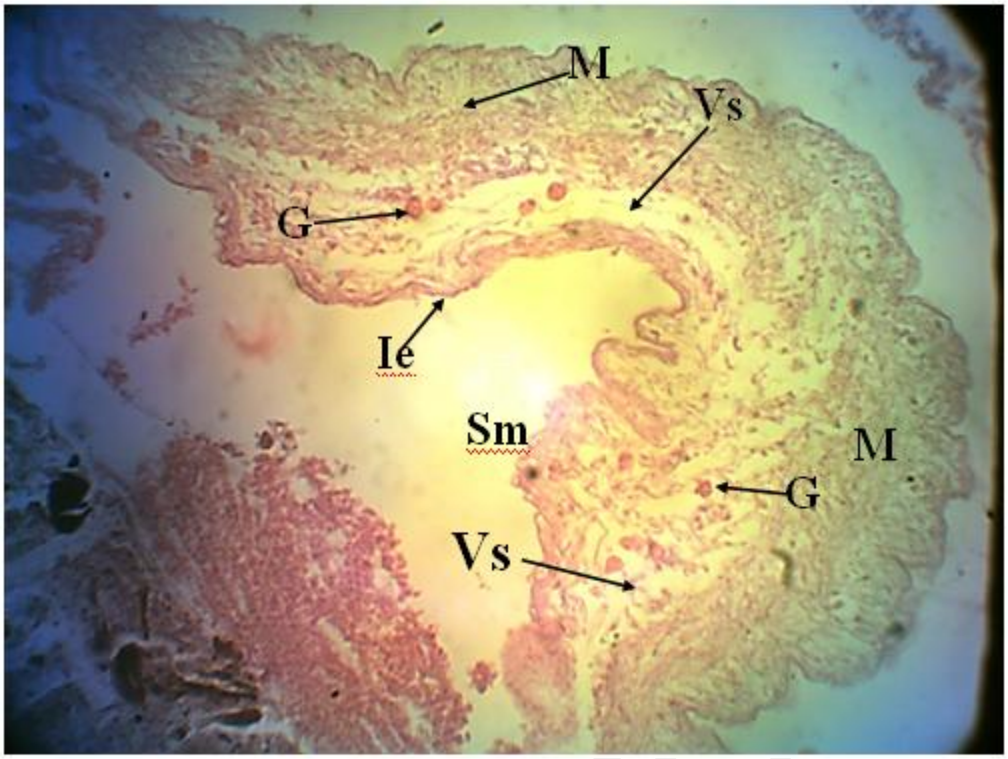

Figure 4b: The cross section of the small intestine of *Tilapia guineensis* from Ojo creek

The cross section of muscle of *T.guineensis* from Badagry creek and Ojo creek are presented in figure 5a and 5b respectively. Muscle of *T.guineensis* from Badagry creek showed intact section of muscle bundles (M) divided with septa spaces (S) and there was no visible degeneration and lesion. On the other hand, cross section of muscle of *T.guineensis* from Ojo creek showed severe lesion (L) of muscle bundle with widen intramuscular spaces (Sw). There is also degeneration of muscular bundle (d) and shortening of bundles (s).

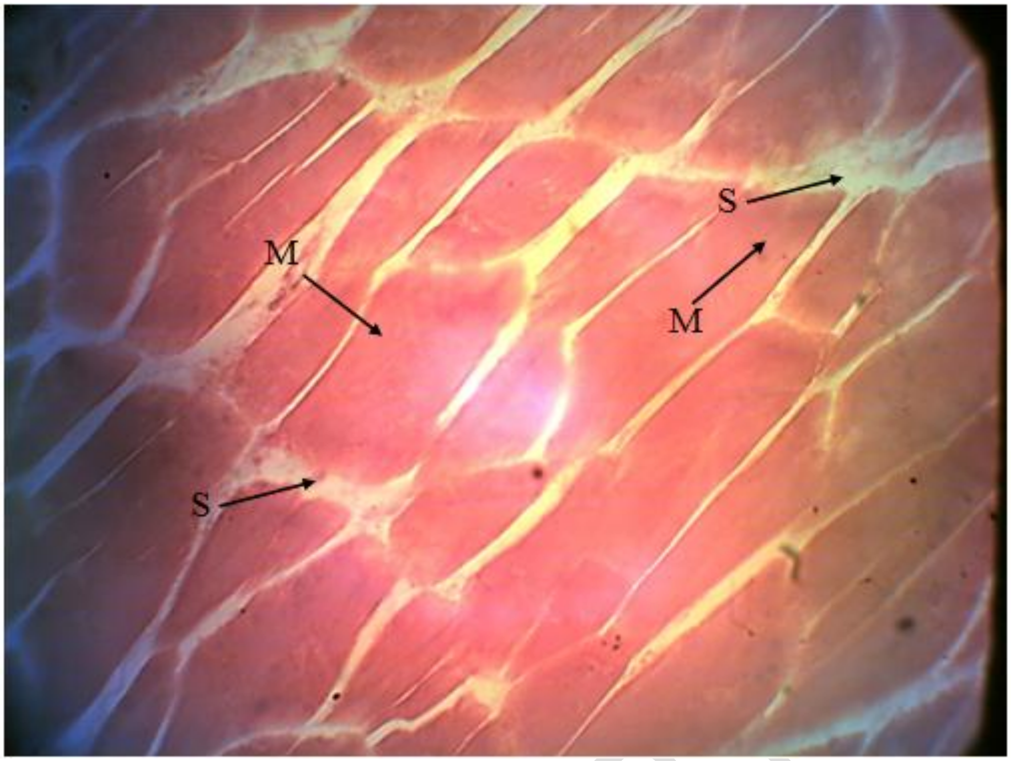

Figure 5a: The cross section of muscle of *Tilapia guineensis* from Badagry creek

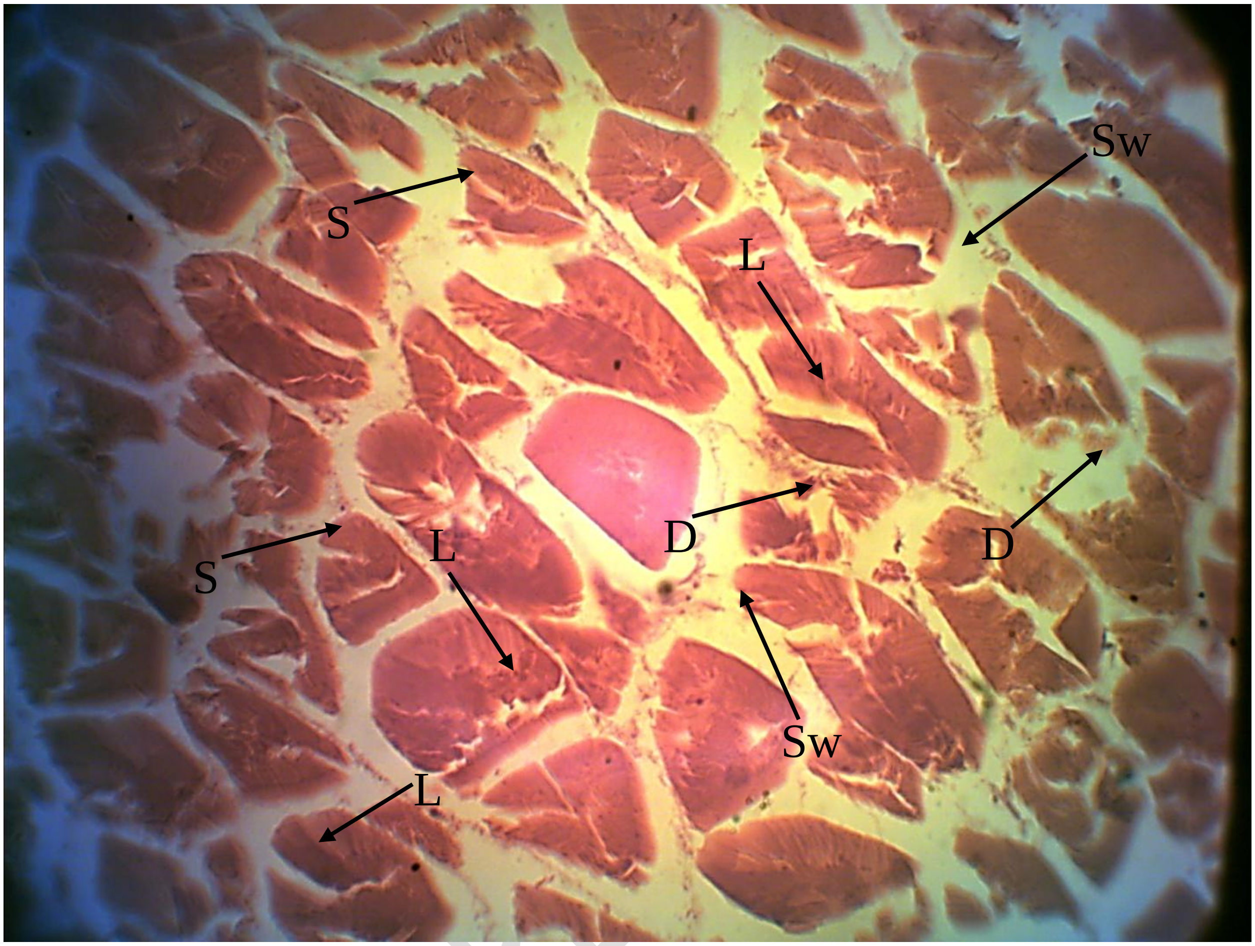

Figure 5b: The cross section of muscle of *Tilapia guineensis* from Ojo creek

DISCUSSION

In this study, there was no significant difference between the mean total length, body weight, standard length, and head depth of *Tilapia guineensis* recorded from both Badagry and Ojo creeks. This indifference in the morphometric characters of *T. guineensis* from both creeks could imply that there was no variation in growth rate, survival and metabolism in the fish as opined by Diaz *et al*. (2000). Davis *et al*. (2005) had reported that environmental disturbances can induce changes in same organism, while Torres *et al*. (2010) divulged that morphometric variation between fish stocks can be applicable for studying short-term, environmentally induced variation geared towards successful fisheries management.

However, the total length and body weight of *T. guineensis* from Ojo and Badagry creeks are comparatively lower than that recorded in *Oreochromis niloticus* and *Lates niloticus* from Doma Dam, north central, Nigeria (Yakubu and Okunsebor, 2011). Similarly, the values of morphometric character recorded for *T. guineensis* in this study was lower than that reported for *Oreochromis niloticus* in Thailand (Piya *et al*., 2014).

Both *T. guineensis* from Ojo and Badagry creeks had condition factor (k) value greater than 1 which indicated that the fishes are in good condition. Similar trend of k factor have been reported by Dan-Kishiya (2013) in *Barbus occidentalis* and *Tilapia marie*. Moreso, k factor greater than 1 was recorded for *Parachana obscura* from River Oshun (Olurin and Savage, 2011). Condition factor is a useful index for monitoring feeding intensity, age, and growth rates in fish (Oni *et al*., 1983) however, k factor is strongly influenced by both biotic and abiotic environmental conditions and thus can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene, 2005, Tah *et al*., 2012).

Length- weight relationships of *T. guineensis* obtained from Ojo creek and Badagry creek showed that *T. guineensis* had negative allometry (b<3) growth pattern. This implies that the fish becomes thinner with increase in length. The value of regression correlation (R^2) recorded in *T. guineensis* from Ojo creek(R^2 =0.551) indicated a strong positive relationship between the length and weight of the fish, while regression correlation (R^2) for *T. guineensis* from Badagry creek (R^2 =0.052) indicated a weak positive relationship. Similar negative allometry growth patterns in different fish species have been documented. For instance, Aderinola *et al*. (2020) had reported negative allometry growth pattern for *Tillapia zilli*, *Sarotherodon galilaeus*, *Sardinella maderensis* and *Pellonula leonesis* from Badagry creek. Similarly, Kumolu-Johnson and Ndimele (2010) reported negative allometric growth for both males and females juvenile of *Sphyraena afra* in the coastal waters of Lagos State, while Caroline and Adedolapo (2019) reported a negative allometric growth for *S. afra* in two water bodies from Lagos State. However, in contrast to results obtained in this study, Coulibaly *et al*. (2018) reported an isometric growth for sub adult *Lutjanus goreensis* caught from Grandlahou Lagoon in Coted' Ivoire. Also, Waly *et al. (*2015) reported an isometric growth pattern for *P. typus* caught from Senegal coast. On the other hand Jaiswar *et al*. (2004) reported positive allometry for juvenile *Sphyraena obtusata* in Bombay waters of the west coast of India, while positive allometry was reported for *Parachanna obscura* population in Anambra River (Odo *et al*., 2012). Length-weight relationships provide useful information for fishery management and for both basic and applied purposes (Dar *et al*., 2012, Ndiaye *et al*., 2015).

As observed in this study, there was no significant variation between the structure of small intestine of *T. guineensis* from Badagry creek and Ojo creek as the glandular cell, serosa, muscularis, sub mucosal and intestinal lining epithelium of *T. guineensis* from both creeks were very clear; although mild lesion was observed in the small intestine of *T.guineensis* from Badagry creek. However, the examined muscle of *T.guineensis* from Badagry creek and Ojo creek are different. While there was no visible degeneration and lesion in the muscle of *T.guineensis* from Badagry creek, muscle of *T.guineensis* from Ojo creek showed severe lesion of muscle bundle, degeneration of muscular bundle and shortening of bundles. This observation could suggest that Ojo creek is more polluted with different contaminants in comparison with Badagry creek. In contrast to the present observation, Mekuleyi and Fakoya(2017) observed no difference in the muscle of *Callinectes pallidus* collected from two coastal waters in Lagos state.

In this study, the gills structure of *T. guineensis* from Badagry creek is better than gills structure of *T. guineensis* from Ojo creek. The reason is that gills structure of *T. guineensis* from Ojo creek showed severe degeneration, hyperplasia of secondary gill lamellae, and curling of secondary lamellae with unclear water channel. It is also characterized by hypertrophy of lamellae and there is an evidence of partial fusion of lamellae and dilation of gill filament. On the contrary, gills structure of *T. guineensis* from Badagry creek showed no evidence of epithelial lifting and curling of lamellae and the secondary lamellae are intact with clear water channel. This different observation could suggest that Ojo creek is more subjected to effluents than Badagry creek.

The observation in this study is similar to the report of Abiona *et al* (2019) which divulged that the most prevalent lesions on gills of *Clarias gariepinus* and *Oreochromis niloticus* were distortion of lamellae, clubbing and fusion of the secondary lamellae due to hypertrophy and hyperplasia of the respiratory epithelium and cells at the base of the secondary lamellae.

As opined by Garcia-Santos *et al*.(2006) and Playle *et al.* (1992), pollutants not only enter the organism through the gills, but also exert their primary toxic effects on the branchial epithelium which in turn, may influence the general gill functions. A very high percentage of gill lesions, especially hyperplasia in *Tautogolabrus adspersus,* exposed to municipal and industrial effluents (Billiard and Khan, 2003) also buttressed the present findings. Moreso, Mekuleyi and Fakoya(2017) had reported that environmental stressors such as brewery effluents- that contains high carbohydrate, nitrogen and washing reagents; high concentration of heavy metals (such as Cu, Zn and Fe) and pharmaceutical effluents causes structural degeneration and lesion in the hepatopancreas and ovaries of *Callinectes pallidus.*

Conclusion

The study have indicated the *T. guineensis* from Badagry creek and Ojo creek had negative allometric growth (b<3), and there was no significant different in their total length, body weight and head depth, Although *T. guineensis* from both creeks had condition factor (k) greater than 1 which signify being in good condition, the histopathological examination revealed that *T. guineensis* from Ojo creek are more threaten by environmental stressors than *T. guineensis* from Badagry. Therefore, collective effort towards ensuring reduction in direct discharge of untreated effluent into the creek is pertinent.

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