MORPHOLOGICAL RELATIONSHIPS, PHYSIOLOGICAL AND OXIDATIVE STRESS RESPONSES OF MALE AND FEMALE AFRICAN SNAKEHEAD (*Parachanna obscura* **Gunther, 1861) FROM EPE LAGOON, LAGOS, NIGERIA**

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ABSTRACT

Sex-specific oxidative stress response in fish is a widely postulated phenomenon but with limited empirical information on most tropical freshwater fishes. This study was carried out to determine morphological relationships among sexes of *Parachanna obscura* from Epe Lagoon, Nigeria. It also assessed the physiological and oxidative stress responses of the sexes. Sixty samples of *Parachanna obscura* were obtained from fishers' catch and examined for sex, morphometric (18) and meristic (6) characters. Blood samples (∼3 ml) were collected from the caudal peduncle vein for haematological analysis. Liver tissues were obtained from male and female samples, homogenized and then assessed for total antioxidant activity $(H_2O_2, CAT, GSH, SOD, GPX,$ GST, MDA and MPO). Data were analysed using descriptive statistics and t-test analysis. Sexual dimorphism was not markedly visible, while all morphometric parameters and meristic counts were consistent between sexes, except for the weight. Females (169.25±52.58 g) were significantly larger than males (159.44±56.78 g). Blood parameters were not significant different between sexes. In addition, the antioxidants and liver enzymes revealed no sex-related differences in activities. These implies that physiological and oxidative responses to environmental stress were similar for both sexes.

Keywords: Snakehead fish**,** Morphology, Blood, Liver tissue, Total antioxidant activity

INTRODUCTION

Pollution of aquatic ecosystems has become a global problem with serious harmful impacts on living organisms including fish (Bashir *et al*., 2020). This contamination of living and non-living components adversely affect the normal optimum environmental processes, resulting in primary or secondary damage (Kemp, 1998; Abaje *et al*., 2020). Primary damage in aquatic systems, can be quantified, and its impact monitored, while secondary damage occurs as a marginal disturbance to the delicately poised

biological food web balance and can be noticed only after prolonged durations (Gheorghe and Ion, 2011). Aquatic organisms in freshwater environment suffer secondary damage with potential human and biota health consequences, being a major cause of concern in sustainable management and protection of aquatic resources and fisheries (Bashir *et al*., 2020).

In Nigeria, inland and coastal waters such as dams, lakes, rivers, and streams are among the most vulnerable aquatic environments, due to high contaminant inputs through effluents resulting from extensive

urbanization, waste disposal, industrial and agricultural activities (Ibor *et al*., 2018).

In these water bodies, there are over 300 indigenous, cultivable freshwater fish species (Olaosebikan and Raji, 2013). These delicate species require appropriate monitoring in order to achieve their sustainable utilisation. African snakehead, *Parachanna obscura* is one of the leading economically important fish resources facing combined danger of environmental pollution and overexploitation (Olanrewaju, 2017). The fishing pressure on this species is a consequence of its nutritional quality, tasty flesh, and consumer preference (Rahman *et al*., 2012). Fortunately, *P. obscura* has been prioritized as a suitable aquaculture candidate due to its zoo-technical performance, high economic value and preference among African consumers (Vodounnou *et al*., 2017).

Although, *Parachanna obscura* occurs in large quantities in Epe lagoon, it is continuously exposed to environmental stressors such as, organic and inorganic compounds from anthropogenic activities within the catchment area (Olukolajo and Hillary, 2012; Akinsanya *et al*., 2019). Heavy metal pollution is a major environmental hazard with increasing severity in aquatic ecosystems as the human society advances (Sevcikova *et al*., 2011; Defo *et al.*, 2014). These contaminants may produce toxic responses in aquatic organisms, even at low concentrations, leading to oxidative stress (Defo *et al*., 2014). These stressors cause an imbalance between the production of toxic Reactive Oxygen Species (ROS) and antioxidant

defence mechanisms, with serious effects on the blood physiology of individual species (Pizzino *et al*., 2017).

Oxidative stress may differ across the sexes because of differences in metabolic rate or mitochondrial efficiency, and may affect rates of ROS production (Magwere *et al*. 2006). Lushchak (2011) emphasized that factors like high levels of contaminants or low levels of dissolved oxygen weaken immune systems and make species to become more vulnerable to sickness and infection. Thus, there is a need to monitor such undesirable changes by comparing oxidative and physiological responses with reference values established for inland water fish species.

Substantial research work has been carried out on *P. obscura* in terms of stock assessment, biology, length-weight relationships, with few reports on the species haematology and serum biochemistry profile (Kpogue *et al*., 2012; Olanrewaju *et al*., 2017; Osho *et al*., 2020). Some studies have documented the seasonal changes in haematological profile of male and female *Parachanna obscura* from Nigeria's inland waters (Odedeyi, 2013; Olanrewaju, 2022). Also, bioaccumulation, histopathological alterations, parasitological, immunological, and oxidative stress responses of *P. obscura* to benzene, toluene, ethylbenzene, and xylenes have been documented in Lekki Lagoon (Akinsanya *et al*., 2019). However, information on the physiological and oxidative stress response of *P. obscura* in Epe Lagoon is scarce. This study, therefore, was carried out to evaluate the morphometric relationships and sex-oriented differences in physiological and oxidative stress responses of *P. obscura* from Epe Lagoon, Lagos, Nigeria.

MATERIALS AND METHODS

Study Area

Parachanna obscura samples were collected from the artisanal fishermen, at their landing site along the banks of Epe Lagoon. This lagoon is sandwiched between the Lekki Lagoon in the east (Freshwater) and Lagos Lagoon (Brackish water) in the west. The lagoon has a surface area of 225 km^2 and maximum depth of 6 m (Olukolajo and Hillary, 2012). It opens into the Gulf of Guinea via the Lagos Harbour and lies at 03050'–04010'N and 005030'–005040'E.

Sample Collection

Sixty live samples of *P. obscura* (23 males and 37 females) were collected twice a week for three months between May and July 2021. All specimens were transported to the laboratory in a cooling van to ensure constant water temperature before analysis.

Morphometric Measurements and Meristic Counts

Eighteen morphometric characters were measured, while six meristic characters were counted in this study. The morphometric variables were measured according to the method described by Olanrewaju (2017) (see Figure 1), while meristic parameters were presented in Table 1.

Morphometry measurements were conducted with a ruler and 60 cm wooden measuring board (manufactured by PENTAIR Aquatic Ecosystem, Florida, United States of America). Each character was measured to 0.1 cm accuracy. After morphological assessment, blood samples were collected before dissection of fishes to determine their sexes.

Blood Component Analysis

The blood (∼3 ml) samples were collected from the caudal peduncle vein of each fish with a 2-ml sterile plastic syringe and dispensed into Ethylene Diamine Tetraacetic Acid (EDTA) tubes for haematology analysis. White Blood Cell (WBC) and Red Blood Cell (RBC) counts were determined using the improved Neubauer haemocytometer after appropriate dilution of blood samples (Schalm *et al*., 1975). Haemoglobin concentration was estimated using cyanmethemoglobin technique, while packed cell volume, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration were calculated according to Blaxhall and Daisley (1973). The differential leukocyte counts were determined by scanning Giemsa's-stained slides in the classic manner (Schalm *et al*. 1975).

Oxidative Stress Analysis

Tissue (liver) was taken from each fish and homogenized in the Physiology Laboratory of Department of Animal Science, University of Ibadan, Nigeria for biochemical analysis. The total protein concentration was calculated using the biurets method as described by Lowry *et al.* (1951) and three replicate measurements were taken for each sample. Hydrogen peroxide (H_2O_2) was measured by staining the samples with 5, 6-carboxy-2,7 dichlorodihydrofluorescein diacetate (DCFDA) and estimated by the fluorescence intensity of DCF of 530 nm, following the method of Ubezio and Civoli (1994). Catalase activity (CAT) was determined by measuring the decrease in the H_2O_2 concentration at absorbance of 240 nm as described by Beers and Sizer (1952). The superoxide dismutase (SOD) activity was calculated based on the procedure of Misra and Fridovich (1972). The reduced glutathione (GSH) was determined according to the method of Jollow *et al*. (1974). The glutathione peroxidase (GPX) was estimated as described by Beutler *et al*. (1963) and optical density was measured at a wavelength of 540 nm. The glutathione transferase (GST) activity was estimated via the conjugation of 1-chloro-2, 4 dinitrobenzene (CDNB) with reduced glutathione (Seyyedi *et al*., 2005). The malondialdehyde (MDA) concentration was determined by the method of Varshney and Kale (1990) and the absorbance of samples was measured at 490 nm. The Myeloperoxidase (MPO) activity was evaluated as described by Xia and Zweier (1997) with the reaction absorbance monitored at 450 nm.

Figure 1: Schematic illustration showing morphometric characters measured in *Parachanna obscura***.** Total Length (TL), Standard Length (SL), Head Length (HL), Pre-Orbital Length (PrOL), Post Orbital Length (POL), Eye Diameter (ED), Inter Orbital Length (IOL), Pre-Dorsal Length (PrDL), Pre-Pectoral Length (PrPL), Pre-Pelvic Length (PrPvL), Pre-Anal Length (PrAL), Dorsal fin Length (DL), Pectoral fin Length (PL), Pelvic fin Length (PvL), Anal fin Length (AL), Caudal fin Length (CL), Caudal Depth (CD), Body Depth (BD), and Gape Length (GL).

Character	Acronym	Description	
Anal fin ray	AFR	Number of soft rays in anal fin	
Dorsal fin ray	DFR	Number of soft rays in dorsal fin	
Pectoral fin ray	PFR	Number of soft rays in pectoral fin	
Caudal fin ray	CFR	Number of soft rays in the caudal fin	
Pelvic fin ray	PvFR	Number of soft rays in the pelvic fin	
Blotches	BL	Number of blotches on either side of the body	

Table 1. Definitions of Meristic counts of *Parachanna obscura*

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) (version 20.0, SPSS Inc., Chicago, IL, USA). All the data obtained were analysed with descriptive statistics (mean and standard deviation). The differences in morphometric, haematological, and oxidative activities between the two sexes were analysed using paired t-test. Statistical difference was accepted at 5% (p<0.05) confidence level.

RESULTS

The morphometric characteristics for male and female *Parachanna obscura* indicated no significant difference (p>0.05) between both sexes of *P. obscura*, except weight (Table 2). However, the result showed that females had higher mean values compared to males in all examined morphometric measurements. The mean body weight of female (169.25 ± 53.58) g) was significantly higher than that of male $(159.44 \pm 56.78 \text{ g})$. Total length was also markedly higher in females $(26.15\pm3.72$ cm) than in males (25.86±3.88 cm). The standard length

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obtained for males ranged from 11.0 to 26.5 cm with a mean of 21.75 ± 3.74 cm, while it varied between 12.0 cm and 26.0 cm $(21.93\pm3.45$ cm) in females. However, there is no significant different between sexes. Similarly, dorsal fin length (14.20±2.72 cm), post-orbital length (1.32±0.30 cm), preorbital length $(2.19\pm0.22$ cm), anal fin length $(10.10\pm1.81$ cm), pelvic fin length (3.39±0.68 cm), caudal length (4.24±0.75 cm), pre-dorsal length $(7.53\pm0.82$ cm), preanal length $(11.49 \pm 1.73 \text{ cm})$, and caudal depth $(2.15\pm0.31$ cm) were slightly higher in the females, while males showed marginally higher inter-orbital length (2.04±0.12 cm), pectoral fin length (2.83±1.39 cm), prepelvic fin length (7.16±1.23 cm), prepectoral length $(8.14 \pm 1.22 \text{ cm})$ and body depth $(5.46 \pm 1.29 \text{ cm})$.

The minimum, maximum, mean, and standard deviation of the six meristic traits indicated no significant differences exists (Table 3) between sexes. Dorsal fin ray count ranged between 41 and 44 in male and female samples. The blotches (6) and pelvic fin ray (6) counts were the same in both sexes. Pectoral fin ray count was lowest in male samples (14.75±0.94) and highest in female samples (15.00±0.97). However, anal

fin ray count was lowest in the female sample (29.33 ± 1.46) and highest in the male sample (30.00 ± 1.01) . Meanwhile, caudal fin ray count ranged from 13 and 15 in males, but varied from 12 – 16 in female samples.

The haematological status of male and female *Parachanna obscura* from Epe Lagoon showed no significant differences (Tabke 4). The females had the highest red blood cells $(2.61\pm0.7910^6 \mu l^{-1})$, white blood cells $(16133.33\pm 2051.98 \ 10^3 \ \mu l^{-1})$, packed cell volume $(28.00 \pm 5.22 \%)$, haemoglobin $(8.91 \pm 1.76 \text{ g } \text{d} \text{)}$, eosinophils $(4.17 \pm 1.47 \text{)}$ %), basophils (0.33±0.49 %), mean corpuscular volume (113.88±26.52 fl), and mean corpuscular haemoglobin (37.34±7.92 pg). Mean corpuscular haemoglobin concentration $(32.36 \pm 1.32 \text{ g } l^{-1})$, platelets $(154937.50\pm37422.75 \qquad 10^3 \qquad \mu l^{-1})$) and monocytes (3.44 ± 0.73) %) were highest in the males. Lymphocytes and heterophils showed similar trends in both sexes.

DISCUSSION

In the study, all the morphometric and meristic characters in male and female *Parachanna obscura* were consistent. Similar observation in morphological relationships had been documented by Vodounnou *et al*. (2017) in African snakehead (*P. obscura*) from a swamp in Sakete, South-eastern District of Benin. These authors however found significant differences in tail length of the fish. Also, the maximum total length of *P. obscura* (30.50 cm) was lower than the maximum length (39.20 cm) reported by Olanrewaju *et al*. (2017) in Eleyele Reservoir, Southwestern Nigeria. This variation could be attributed to differences in seasons,

richness of fishing ground and fishing efforts. The species may not show clear sexual dimorphism in Epe lagoon, but the slight morphological differences between sexes might be an indication of their responses to environmental conditions (Khatun *et al*., 2021). Further, the body weight was significantly higher in female *P. obscura* compared to male individuals. This large and plumper body in female specimens, contradicted the findings of Vodounnou *et al*. (2017) who reported slightly higher body weights in male *P. obscura* over their female counterparts. The contradictions might be attributed to differences in seasons and geographical locations of the two studies.

Most blood haematological indices (red blood cells, white blood cells, haemoglobin, lymphocytes, eosinophils, basophils, mean corpuscular volume and mean corpuscular haemoglobin) were higher in female than male samples. Yousefzadeh and Khara (2014) hinted that these differences in haematology may be related to differential oxygen demand by sexes, which in turn can be linked to reproductive activity. Results of the current study are in agreement with the findings of Olanrewaju (2022) who reported higher red blood cells, haemoglobin, and pack cell volume in male *P. obscura* from Eleyele Reservoir.

The author reported higher white blood cell values in female *P. obscura* as observed in this study. The disparity in results could be linked to water quality and flow. Meanwhile, high white blood cells in female *P. obsura* could mean a strategy to fight short-term stress due to reproduction.

Table 2. Morphometric characteristics of male and female *Parachanna obscura* **from Epe lagoon, Lagos, Nigeria**

Parameter	Male		Female		P-value
	Range	Mean ±SD	Range	Mean ±SD	
Blotches	6.00	6.00 ± 0.00	6.00	6.00 ± 0.00	1.00
DFR	$41.00 - 44.00$	42.14 ± 0.80	$41.00 - 44.00$	42.04 ± 0.90	0.53
PvFR	6.00	6.00 ± 0.00	6.00	6.00 ± 0.00	1.00
PFR	$13.00 - 17.00$	14.75 ± 0.94	$14.00 - 18.00$	15.00 ± 0.97	0.74
AFR	$27.00 - 32.00$	30.00 ± 1.01	$25.00 - 31.00$	29.33 ± 1.46	0.72
CFR	$13.00 - 15.00$	13.97 ± 0.38	$12.00 - 16.00$	13.95 ± 0.69	0.56

Table 3. Meristic analysis of male and female *Parachanna obscura* **from Epe lagoon, Lagos, Nigeria**

DFR *dorsal fin ray*, PvFR *pelvic fin ray*, PFR *pectoral fin ray*, AFR *anal fin ray*, CFR *caudal fin ray*

RBC *Red blood cell*, WBC *White blood cell*, PCV *Packed cell volume*, MCV *Mean corpuscular volume*, MCH *Mean corpuscular haemoglobin*, MCHC *Mean corpuscular haemoglobin concentration, p<0.05*

Parameters	Male	Female
TP (g dL ⁻¹)	$15.87 \pm 2.05^{\text{a}}$	15.66 ± 2.34 ^a
GPx (μ mol l^{-1})	$11.74 \pm 1.69^{\text{a}}$	11.55 ± 1.50^a
GSH $(\mu M \text{ mgProtein}^{-1})$	269.34 ± 34.38 ^a	287.58±73.99 ^a
GST (mmol μ g Protein ⁻¹)	35.85 ± 7.17^a	$40.02 \pm 3.76^{\text{a}}$
$H_2O_2(Mg100g^{-1})$	4.18 ± 0.91 ^a	$3.92 \pm 0.52^{\text{a}}$
MDA (μ M mg Protein ⁻¹)	1.62 ± 0.88 ^a	2.26 ± 1.01^a
MPO (U g Protein $^{-1}$)	2.03 ± 0.20^a	1.20 ± 0.41 ^a
SOD (U mg Protein $^{-1}$)	1.90 ± 0.41 ^a	$1.85 \pm 0.49^{\rm a}$
CAT (U mg Protein ⁻¹)	14.29 ± 1.42^a	15.01 ± 2.41 ^a

Table 5. Antioxidant enzymes measured in male and female *Parachanna obscura* **liver from Epe lagoon, Lagos, Nigeria (Mean±SD)**

TP *Total protein*, GPx *Glutathione peroxidase*, GSH *Glutathione*, GST *Glutathione Stransferase*, CAT *Catalase*, H2O2 *Hydrogen peroxide*, MDA *Malondialdehyde*, MPO *Myeloperoxidase*, SOD *Superoxide dismutase*

In the work of Odedeyi (2013), the influence of seasonality on haematological parameters was pronounced, with higher haemoglobin and packed cell volume in female during dry season, while the same parameters were higher in male, during wet season. This implies that each season comes with different stress loads for aquatic organisms, and thus affecting their physiological responses. The haemoglobin $(8.34 \pm 1.17 8.91 \pm 1.76$ g dL⁻¹) and pack cell volume $(25.81 \pm 3.78 - 28.00 \pm 5.22)$ %) were higher than 5.70 g dL^{-1} and 19.20% reported by Kori-Siakpere *et al*. (2005) for *P. obscura*; but lower to values reported by Olanrewaju (2022). While haemoglobin is the substance in blood that carries oxygen, packed cell volume gives an indication of red cell concentration in blood, and their reduced values indicate stress response to environmental toxins.

Among the white blood cell differential counts measured in the present study, only platelets and monocytes were higher in male *P. obscura*. These results are at variance

with the report of Olanrewaju (2022) who reported higher monocytes and platelets values in females, but higher lymphocytes and basophils in males. Whilst the variations are not significant between both sexes, it could mean a sign of physiological stress threatening the fish in their environment. Olanrewaju *et al*. (2023) disclosed that the increased levels of white blood cell differential counts suggest the prevalence of diseases from different micro-organisms in the environment due to pollution. However, the higher mean platelets, monocytes and mean corpuscular haemoglobin concentrations noticed in male *P. obscura* could be due to higher physiological activeness in addition to metabolic activity [\(Sharma](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10079988/#B47) *et al*., 2017).

Lemos *et al*. (2023) acknowledged that the study of fish stress responses and the assessment of organic pollutants in aquatic environments are vital for the health and sustainability of fish populations and aquatic ecosystems. Fish liver is the main source of antioxidant enzymes that detoxify the environmental pollutants and it has been largely used as an indicator of environmental pollution (Siscar *et al*., 2014). In this study, the biological responses of antioxidant compounds and enzymes in *P. obscura* liver revealed no sex-related differences. However, there were slight variations in the parameters tested. For instance, the activities of glutathione, glutathione S-transferase, malondialdehyde, and catalase were somewhat higher in female sample compared to male. This implies that both male and female *P. obscura* have similar response to water quality and other environmental factors that induce oxidative stress. The finding was however inconsistent with the work of Adeogun *et al.* (2020) who reported sexspecific response to oxidative stress in Black jaw tilapia (*S. melanotheron*) from Awba Dam, Ibadan. Blonska *et al*. (2021) also confirm sex-biased effect of acute heat shock on the antioxidant system of nonnative round goby, *Neogobius melanostomus*. These authors report that the liver of males was more responsive in all tested parameters. The differences cannot be ruled out, since the concentrations at which a compound is lethal could depend upon many contributing factors, including species and water quality (Scott and Sloman, 2004).

Blonska *et al*. (2021) recognized that checking oxidative stress biomarkers has become an important and usual means to evaluate organismal condition and response to endogenous and ecological factors. Total antioxidant activity is a combination of enzymatic (SOD, GPx, CAT) and nonenzymatic (vitamins, bilirubin, glutathione)

et al., 2018). This study indicates that the same total antioxidants activities in both sexes of *P. obscura* portray similar behavioural pattern. Allegra *et al*. (2023) however suggested that heredity, evolution, biological chemistry, and ecological parameters were connected and could cause sex variations. Some clinical studies have shown that the sexes do not respond equally to perturbation in either ROS or antioxidant resources (Magwere *et al*., 2006). However, the role of sexual selection in oxidative stress across the sexes in natural environment is highly varied. Although, it can also be argued that differences in metabolic rate or mitochondrial efficiency in fish of different sex may affect rates of ROS production and show different responses to changes in REDOX status that affect their resistance to oxidative damage (Magwere *et al*., 2006). Meanwhile, the similarities in morphological characteristics between male and female in this study might be an indication that their responses to environmental conditions were similar (Khatun *et al*., 2021). This may also reflect the adaptive responses of *P. obscura* in their natural environment regardless of their sexes.

antioxidants in the biological system (Jimoh

CONCLUSION

The variations in morphometric and meristic traits of *Parachanna obscura,* were not distinct enough to show clear sexual dimorphism. The study also provides a general image of the blood composition parameters and total antioxidant activity in *P. obscura* with no significant variations between sexes. Finally, this suggests no sexrelated physiological and/or oxidative stress on *P. obscura* in Epe Lagoon. Future research will benefit from the blood parameters and total antioxidant activity data generated in this study as reference values in the face of global climate change.

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