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Abstract

Despite the important roles of coastal and inland waters, there are no established biomonitoring protocols for evaluating environmental, wildlife and human health for these environments. In the present study, contaminants tissue burden and effects at molecular and physiological levels were investigated in *Tilapia guineensis* from a man-made lake (Eleyele Lake) that is generally used for municipal domestic water supply. Gene expression of phase I and II biotransformation systems, oxidative stress and obesogen responses were analyzed using real-time PCR, and these results were related to general health index (condition factor: CF) and muscle burden of trace metals, aliphatic and polycyclic aromatic hydrocarbons (PAHs). We observed a significant increase in phase I and II biotransformation systems, oxidative stress and obesogen responses in male and female fish from Eleyele lake compared with the reference site (Igboho Lake). Overall, our data showed significant relationships between biological responses and tissue concentrations of metals and PAHs for the Eleyele lake. Given that a positive influence on genes and pathways associated with metabolic status has been previously associated with peroxisome proliferator activated receptors (PPARs), xenobiotic compounds that activate PPARs may produce changes in energy and metabolic processes, leading to obesity. The high CF (>1 = good health condition) observed, coupled with the high muscle burden of inorganic and organic contaminants in fish from Eleyele lake suggest a potential obesogenic effect in these fishes. These findings represent co-relational evidence that the Eleyele lake is presumably contaminated and consequently affecting biological and physiological integrity of inhabiting organisms. These findings also suggest potential health risks for humans, since the lake is extensively used for domestic water supply and fisheries.

Keywords: Contaminants, Biomarkers, Aquatic environment, Fish, Human health, Developing country.

Introduction

Persistent organic pollutants (POPs) and trace metals are ubiquitous environmental contaminants with documented wildlife and human health effects (Barrie et al., 1992; Griswold, 1997; Loos et al., 2013). These chemicals are known to modulate the expression and activity levels of phase I and II biotransformation systems through the aryl hydrocarbon receptor (AhR) (Adeogun et al., 2016b; Ibor et al., 2017). Such compounds can also produce an imbalance in the anti-oxidant capacity of cells, either by acting as reactive molecules that enhance intracellular production of oxyradicals or reducing the efficiency of antioxidant defences, inhibition of mitochondrial electron transport chain with subsequent accumulation of reduced intermediates, and by inducing peroxisome proliferation (Livingstone, 2001; Zhou et al., 2006; Regoli and Giuliani, 2014). Thus, several of these cellular responses have also been shown to affect lipid β-oxidation and the expression of peroxisome proliferator-activated receptors (PPARs) (Cocci et al., 2015; Adeogun et al., 2016b, d).

The phase I and II biotransformation pathways include a battery of genes that are controlled by the AhR in several species (Gu et al., 2000) and activated by various endogenous and exogenous compounds (Nelson et al., 1996). Cytochrome P450 (CYP) enzymes play a central role in the oxidative metabolism of a wide range of substrates (Benedetti et al., 2015). Particularly, the CYP1, 2 and 3 enzyme super-families metabolize a wide range of chemical compounds, whose metabolites are substrates for phase II enzymes such as uridine diphosphate glucuronosyltransferase (UDPGT) and glutathione *S*-transferase (GST), resulting in the final inactivation and elimination of lipophilic compounds (Leaver et al., 1992). The expression of *cyp1* isoforms, *udpgt* and *gst* are regulated by ligand-dependent AhR, through which agonists produce altered gene expression and toxicity (Bradshaw et al., 2002; Nelson et al., 1996). Due to their roles in the detoxification and activation of xenobiotics, changes of the expression of biotransformation enzymes markedly affect the potential risks and benefits of xenobiotics and are important from environmental monitoring standpoint (Williams et al., 1998).

The complicated nature of effects after exposure to contaminants requires a broader examination of several and potential interacting pathways. Thus, exposure of organisms to POPs and reduced level of dissolve oxygen (hypoxia) is known to produce reciprocal effects due to competition for the same nuclear translocator (Gnaiger et al., 1995; Mansfield et al., 2005; Nathan and Cunningham-Bussel, 2013; Regoli and Giuliani, 2014). Hypoxia produces the stabilization of

hypoxia-inducible factor-1α (*hif*-1α), which heterodimerizes with *hif*-1β (or AhR nuclear translocator: *arnt*) to form *hif*-1, the transcription factor that affects the expression of a variety of genes (Wenger, 2002). The *arnt* is also the heterodimerization partner to the AhR, and the AhR-*arnt* complex translocates to the nucleus where it transactivates mRNA transcription of genes containing XRE (xenobiotic responsive elements) in their upstream regions, including increases in the expression of CYPs, *gst* and *udpgt* (Mortensen and Arukwe, 2007). Hypoxia and biotransformation pathways are intricately connected to oxidative stress processes, formation of ROS and reactive nitrogen species (RNS), and efficiency of antioxidant defences (Farber, 1994; Sies, 1997). ROS can be further generated by metabolic processes, such as β-oxidation and oxidative phosphorylation and by the auto-oxidation of small molecules in the cell (Regoli et al., 2011). Based on sequence homology and molecular weight, heat shock proteins (hsp) have been divided into gene families that include hsp20, hsp40, hsp60, hsp70, hsp90, hsp100, and hsp110 (Craig and Gross, 1991), where the inducible hsp70 is widely used as a biological marker for environmental changes due to its rapid response to various stressors including thermal shock, trace metals, POPs, free radicals and microbial infection (Whitelaw et al., 1995; Yoo and Janz, 2003; Young and Craig, 1993).

Changes in lipid composition due to exposure to contaminants, will affect many biological processes such as lipid synthesis, transport, deposition and storage, peroxisome proliferation and membrane fluidity (Sheridan, 1988). During FA β-oxidation in the liver, the entry of long-chain FAs into the mitochondria is mediated by the carnitine palmitoyltransferase system - carnitine palmitoyltransferase I (CPT I) whose regulation by malonyl-CoA play a pivotal role in the regulation of FA oxidation (Schmidt and Herpin, 1998). PPARs are critical regulators of lipid homeostasis and control the balance between burning and storage of long chain FAs (Shi et al., 2002). PPARs are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily (Dreyer et al., 1992; Shi et al., 2002). PPARs produce pleiotropic responses by regulating energy homeostasis, adipose tissue differentiation and maintenance, cell proliferation and tissue repair (Qi et al., 2000; Blanquart et al., 2003). These complex interactions have led to the proposition of the environmental obesogen hypothesis that describes the roles of environmental contaminants in the development of obesity by activating the PPARγ, a key regulator of adipogenesis (Jordao et al., 2015; Riu et al., 2014).

Coastal and inland waters, such as lakes, rivers and streams represent highly vulnerable ecosystems in developing countries, due to fluctuations in the flow rates and contamination pressures from extensive urban, waste disposal, industrial and agricultural activities. Despite these pressures, there are no established biomarkers or monitoring protocols for feral fish species in Nigerian rivers and streams (Eruola et al., 2011; Onyeike et al., 2002). For the purpose of sustainable management and protection of aquatic resources and fisheries for developing countries, the potential consequences to wildlife and human health of contaminants exposure have been the subjects of societal concern. Therefore, the present study was designed to investigate exposure and response biomarkers for environmental monitoring, and to validate these biomarkers in *Tilapia guineensis* in relation to levels of selected groups of environmental contaminants, using the Eleyele lake as a model environment.

Materials and methods

Study sites. Eleyele lake is located at Eleyele catchment area (Ido local Government Area, Ibadan, Oyo State, Nigeria). The Ona River, the streams of Awba, Otaru, Yemoja and Alapo empty into the lake through a damming process (Fig. 1). Eleyele lake is at an altitude of 125m above sea level and lies within Latitude 7°25'–7°26'N and Longitude 3°51'–3°52'E. Complete description of the study lake is presented in the supplementary information (SI) file.

Sample collection. A total of 83 (55 males and 28 females) and 75 (49 males and 26 females) *Tilapia guineensis* were samples from the Eleyele lake and Igboho lake (reference site). Fish were collected from 4 randomly picked locations within the lakes between May-October 2013 with the aid of artisanal fishermen using cast net (mesh sizes 50-55mm). Fish were anaesthetized on ice and morphometric data were recorded, then liver, kidney and spleen were harvested and used for calculating organ somatic index. A small portion of the liver was preserved in RNA*later* (Ambion, USA) for transcript analysis.

Morphometric measurements and condition factor (CF). Wet weight (W) total length (TL) and standard length (SL) were measured with an Ohaun digital weighing balance (Mettler Instruments) and an Absolute digital caliper (Tresna Instruments), respectively. The CF was calculated as:

condition factor (k) = 100 x W/L^3, where W = wet weight of fish and L = total length. Organ somatic indices were estimated as percentage (%) of organ weight relative to body weight: (organ weight/total body weight) x 100. The mean total length (± standard error: ste) was 14.0±3.8 cm for males,16.0±2.4 cm for females and 22.6±0.4 cm for males, 20.5±0.4 cm for females *T. guineensis* from Eleyle lake and the reference site (Igboho lake), respectively. The mean body weight (± stdev) was 234.3±9.2g for males; 184.2±7.9 g for females and 181.3±11.8 g for males and 232.5±10.7 for female *T. guineensis* from Eleyle Lake and the reference site (Igboho Lake), respectively.

Quantitative (real-time) PCR: Liver samples were homogenized in TRI-Reagent and RNA isolated using Direct-zol RNA MiniPrep Kit according to the manufacturers protocol (Zymo Research Corporation, Irvine, CA USA). Total cDNA was generated from 1 µg total RNA using a combination of oligo (dT) and random hexamer primers from iScript cDNA synthesis kit, as described by the manufacturer (Bio-Rad, Oslo Norway). Detailed description of real-time PCR analysis is presented in SI.

Chemical analysis. Trace metals (As, Ba, Cu, Hg, Cd, Cr, Fe, Mn, Ni, Pb, V, Zn), aliphatic hydrocarbons (C10-C40) and polycyclic aromatic hydrocarbons (PAHs), were analyzed in fish muscle tissue by conventional procedures based on atomic absorption spectrophotometry, gas-chromatography with flame ionization detector, electron capture detector and mass detector, high performance liquid chromatography (HPLC) with diode array and fluorometric detection. Details on analytical methods and procedures for quality assurance/quality control are given in Supplementary Material (S1).

Statistical analysis. All data were presented as mean ± standard error of mean and analyzed with unpaired t-test to determine sex and sites differences in fish parameters and sediment contaminant load using the Prism GraphPad 5 (GraphPad software, La Jolla, USA). Statistical differences between respective sexes (male and female) from Eleyele Lake and reference site was analyzed using t-test. Values were considered significant at $p<0.05$. Sites and sex dependent relationship between biological variables (biotransformation, oxidative and obesogenic response pathways) and group of contaminants (trace metals, aliphatic hydrocarbons and PAHs) were analyzed for both

Eleyele lake and reference site using principal component analysis (PCA). Extraction of principal components and biplots was achieved using Statistica (TM) for windows version 8.0 (Statsoft. Inc. USA).

Results

CF and organ somatic indices. The biometric measurements showed respective non-significant k-factor of 2.3 ± 0.12 and 2.14 ± 0.1 (mean ± stdev), for male and female *T. guineensis* from Eleyele lake. At the reference site, k-factor of 1.8 ± 0.1 and 2.0 ± 0.0 was recorded for male and female fish, respectively. Generally, k-factor was significantly different in male fish from Eleyele, compared with the reference site (Table 2). The Eleyele lake female fish k-factor was higher than the control site female fish, but this difference was not significant. The hepatosomatic index (HSI) decreased significantly in female fish at Eleyele lake compared with the reference site, with a sex related significant decrease in females compared with males at Eleyele. For spleen somatic index (SSI) a significant increase was recorded in male and female fish from Eleyele lake, compared with the reference site, while kidney somatic index (KSI) significantly decreased in females from Eleyele lake compared with the reference site with a sex related significant difference between male and female fish (Table 2).

Contaminants. Muscle tissues of *T. guineensis* were analyzed for trace metals and several classes of aliphatic and aromatic hydrocarbons in male and female fish from Eleyele lake and the reference site (Tables 3-5). All measured trace metals except Ni, were significantly and markedly higher in male and female fish from Eleyele lake, compared with the reference site (Table 3). Generally, Ba, Cu, Hg, Cd, and Mn showed sex related significant increases (higher in females compared to males: Table 3).

The sum of aliphatic hydrocarbons (∑TAHs) was 272.0±56.0; 200.54±18.2, μg/g (dry weight) in Eleyele lake and 336.0±7.5; 709.6±11.2 μg/g (dry weight) at the reference site for male and female fish respectively (Table 4). Significant increases in C10-C12, C12-C14 concentrations was observed in female *T. guineensis* from Eleyele lake compared with the reference site. Also, significant increases in C20-C22, C26-C28, C28-C30, C30-C32 was recorded in male *T. guineensis* from Eleyele Lake compared with the reference site (Table 4). Generally, most PAHs (naphthalene,

2-methylnaphthalene, benzo(a)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene) significantly increased in male and female fish from Eleyele lake, compared with the reference site (Table 5). Among the PAHs, low-molecular weight (LMW) hydrocarbons were generally higher than high-molecular weight (HMW) compounds. In addition, concentrations were significantly higher in females compared to males from Eleyele lake and the reference site (Table 5). The HMW PAHs significantly increased in female *T. guineensis* from Eleyele, compared with the reference site (Table 5).

Biotransformation and oxidative stress responses. The phase I- and II biotransformation responses were analyzed by *cyp*1 gene family (*cyp*1a, *cyp*1b and *cyp*1c), *gst* and *ugt*1. The *cyp*1a, *cyp*1b and *cyp*1c genes were significantly higher in both male and female *T. guineensis*, from Eleyele lake compared with the reference site (Fig. 2A-C). For *cyp*1a, sex-related significant increase (higher in males) was recorded in fish from Eleyele and the reference site (Fig. 2A), while no significant sex related differences were recorded for *cyp*1c and *cyp*1b in Eleyele lake (Fig. 2C). For the phase II genes (gst and *ugt1*), higher expression levels of *gst* and *ugt1* were measured in male and female *T. guineensis* from Eleyele lake compared with reference site (Fig. 3A-B), although a significant sex related difference (higher in males) was only measured for *ugt1* in fish from Eleyele lake (Fig. 3B). Also, *CuZn-sod* and *Mn-sod* mRNA expressions were significantly higher in male and female *T. guineensis* from Eleyele lake, compared with the reference site, showing significant sex related differences (higher in males) from both Eleyele lake and the Igboho reference site (Fig. 3C and D, respectively).

Changes in ppars, cpt, hif-1α and hsp70. The *ppar* isoforms (*ppar*-α, *ppar*-β and *ppar*-γ) and *cpt* were strongly expressed and increased significantly in fish from Eleyele lake compared with the reference site. (Fig. 4A-D), with only *ppar*-β showing a sex related significant increase in males at the reference site (Fig. 4B). For *cpt* mRNA expression, a significant sex-related increase in mRNA levels were recorded at Eleyele lake male fish, compared to females (Fig. 4D). Similarly, *hif*-1α and *hsp*70 mRNA, were strongly expressed (Fig. 5A and B, respectively) with a significant increase in male and female fish from Eleyele lake, compared with the reference site for *hsp70* (Fig. 5B).

However, a trend towards higher mRNA levels in males (albeit not significant), compared to females was observed for both sites.

Principal component analysis (PCA). The extracted principal component (PC), percentage variation, PCA biplot of the relationship between sites and sex-related biological responses and contaminant burden in fish muscle are shown in Fig. 6. Four (4) principal components were extracted accounting for 85% of the total variation in the entire dataset and showing different groupings for sites and sex on the different sides of the plot (SM 2). PC1 accounted for 56% showing an arrangement of variables on ordination space with strong indications that sites and sex were major factors determining the uptake and biological effects of contaminants in fish. For example, male fish from Eleyele lake showed positive correlation with biological variables (BW, CF, *cyp*-1a, b, c, *gst*, *ugt1*, *sod*, *ZnCu-sod*, *ppar*-α, β γ**,** *hif*-1α*, hsp70*), all PAHs (except anthracene and chrysene) and metals (As, Hg, Cr, Ni, Pb, V and Zn), while female fish from reference site showed positive relationships with biological variables (TL), PAHs (anthracene and chrysene), aliphatic hydrocarbons (C12-C40) and metals (Ba, Cd, Cu, Fe and Mn). PC2 accounted for 15% of the total variance, with male fish from the reference site showing positive relationships with biological variables (TL, *ZnCu-sod*, *sod*, *ugt1* and *cyp1a*), aliphatic hydrocarbons (C12-C34, C36-C40) and metals (Hg and As), while females from Eleyele lake showed positive relationships with biological variables (BW, CF, *hif*-1) and groups of contaminants; metals (Ba, Fe, Mn, Ni, Pb and V), aliphatic hydrocarbons (C34-C36) and PAHs (naphthalene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, 7,12-dimethylbenzo(a)anthracene, benzo(b)fluoranthene, dibenzo(ah)anthracene, benzo(k)fluoranthene, BaP, benzo(ghi)perylene and indeno(123-cd)pyrene).

Discussion

Marine and freshwater environments in Nigeria are presumably contaminated with organic and inorganic pollutants that consequently affect ecosystems integrity, as well as the biological and physiological wellbeing of organisms. As in many other developing countries, the use of biological response for environmental monitoring is either not established or validated (except for our recent publications). The Eleyele lake provides a huge amount of water volumes that are used for municipal domestic water supply and fisheries. Thus, the present study was designed to investigate

biotransformation, oxidative stress and obesogen responses in *fish* as biomarkers of environmental pollution and biota health. We showed that fish inhabiting the Eleyele lake are eliciting elevated growth (high BW and CF) that parallel increases in tissue contaminant burden (metals and PAHs) with a resultant downstream transcriptional activation of phase I and II biotransformation, oxidative stress and obesogenic responses, compared with fish from the reference site. Also, our results show significant sex related differences in *cyp1* isoforms, *ZnCu-sod*, *sod*, *ugt*1 and *cpt* (higher in males) suggesting sexual dimorphic responses on genes regulating phase I and II biotransformation responses.

Biotransformation and oxidative stress responses

In establishing phase I and II biotransformation responses as biomarkers of exposure to dioxins, planar PCBs, PAHs and related chemicals, it has been shown that several abiotic (temperature, oxygen concentration, pH) and biotic factors (species, age, sex, genetic population, feeding status, reproductive stage) may confound these responses (Forbes et al., 2006). Particularly, biotransformation responses are known to show clear gender-related differences, decreasing in maturing fish, probably due to cellular changes in sex steroids (Andersson, 1990; Roselli and Resko, 1997). Also, the CYP isozymes belonging to the 1, 2 and 3 subfamilies and phase II enzymes (GST and UGT) have been shown to metabolize several xenobiotic compounds in fish (Buhler and Williams, 1988; Goksøyr and Förlin, 1992), following the induction of mRNA transcription, increased levels of proteins, and catalytically active enzymes (Goksøyr and Förlin, 1992). The molecular and biochemical responses of these steps, ranging from mRNA, protein, and enzyme activity are established and validated biomarkers of exposure to organic contaminants both in field and laboratory conditions (van der Oost et al., 1996).

Herein, mRNA analysis was performed for *cyp*1a, *cyp*1b and *cyp*1c showing that all three CYP isoforms were detected in both male and female fish, with significant differences observed only for *cyp1a* mRNA according to sites (higher in Eleyele lake, compared with the reference site) and sex (higher in males). The significant increase in cyp1 isoforms in fish from Eleyele lake compared with the reference site paralleled the significant increase in PAHs, suggesting a positive relationship between increases in PAH concentrations and cyp1 expression. Consistent with our results, some reports have demonstrated the induction of CYP1 responses in fish species sampled

from PAHs contaminated environments (van der Oost, 1996; Regoli et al., 2001). Therefore, the increases in cyp1 isoform mRNA observed in this study suggest that the Eleyele lake fish are exposed to cyp1 inducing contaminant, which is in accordance with recent field studies showing the induction of phase I biotransformation responses in several fish species (Sanchez et al., 2011; Jung et al., 2011; Adeogun et al., 2016b, d). Interestingly, the lower expression of *cyp*1a in females is in contrast to the higher concentrations measured for several PAHs, but consistent with previous laboratory and field studies showing gender-related differences and lower expression of *cyp* genes in vertebrate females (Andersson, 1990; Arukwe and Goksøyr, 1997). These sex related effects are generally attributed to changes in sex steroid levels and exposure to estrogenic compounds that significantly decrease hepatic *cyp*1a1 transcriptional levels, with subsequent decrease in functional responses such as protein levels and 7-ethoxyresorufin O-deethylase, EROD activity (Arukwe et al., 2000; Navas and Segner, 2000). There are several proposed hypotheses that explain the sex steroid reduction of CYP1 responses, and these include - the binding of steroid hormones and/or metabolites on CYP1A1 protein (Chan and Hollebone, 1995) and consequent inhibition of the catalytic activity through competition mechanisms (Arukwe and Goksøyr, 1997). Furthermore, the inhibitory effects of hormones can also be mediated through the estrogen receptor (ER), where the ER-E2 complex interferes with the *cyp*1a gene directly or through interaction with the AhR complex, that regulates *cyp*1a gene expression. In addition, estrogens or estrogen mimics may influence ER recruitment and other co-activators, besides activating the biotransformation pathways (Chan and Hollebone, 1995; Arukwe and Goksøyr, 1997; Navas et al. 2000). In a separate study on estrogenic responses, using the same fish samples from the present report, we also observed that the female *T. guineensis* enhanced expression of Vtg and Zrp significantly more than males (Adeogun et al. 2016a). All of these mechanisms may explain the sex-differences observed in cyp1a expression in *T. guineensis* from Eleyele lake.

The phase II biotransformation responses (*gst* and *ugt1*) also showed significant site related differences (higher in Eleyele), with *ugt*1 mRNA expression showing a sex related significant increase (higher in males). The mRNA expression also paralleled the significant increase in contaminant burden (PAHs and metals), suggesting the involvement of UGT and GST in xenobiotic metabolism and excretion. Similarly to the CYPs, phase II biotransformation enzymes are widely used as xenobiotic biomarker responses, whose isoforms display expression

differences and overlapping substrate specificities to endogenous and xenobiotic compounds (Clarke et al., 1991). Although in the present study, *ugt1* and *gst* mRNA expression followed the same expression pattern with *cyp*1a, other investigations have described conflicting results in wild fish (Gadagbui and Goksøyr, 1996; Vogelbein et al., 1990). The observed sex-related significant differences in *ugt*-1 mRNA expression may have several physiological explanations due to their integral roles in regulating steroid hormone homeostasis in organisms (Clarke et al., 1991).

The mRNA expression of *CuZn-sod* and Mn-*sod* mRNA expression showed site (higher at the contaminated site) and sex (higher in males) related significant increases and these responses also paralleled the general contaminant burden (PAHs and trace metals) in fish from these sites. Such increases in oxidative stress markers reflect the need for a higher protection against contaminant induced oxidative stress in fish from Eleyele lake (Regoli and Giuliani, 2014). These enzymes are responsible for the conversion of superoxide anion (O_2^-) to H_2O_2 (Benedetti et al., 2014; Giuliani et al., 2013). It has been previously reported that trace metals such as Cd produced a time and dose-dependent increase of *Cu/Zn-sod* mRNA levels in clam (*M. veneriformis*), while an initial increase in SOD activity was followed by a progressive decrease to basal levels (Fang et al., 2012; Fang et al., 2010). ~~The significant increases in male fish of *gst, CuZn-sod* and Mn-*sod* expression observed in this study should be viewed as beneficial since these enzymes play significant roles in conjugating reactive cellular molecules against oxidative stress (Buhler and Williams, 1988).~~ Further, the observed higher increase in males of Eleyele lake of *ugt*-1, *CuZn-sod*, Mn *sod* may probably suggest that both phase II and oxidative stress enzymes are also involved in the regulation of sex steroids levels.

The expression of *hif*-1α and *hsp*70 also revealed a site related significant difference (higher in contaminated Eleyele lake) with no gender-related differences, although a tendency to higher mRNA levels was observed in male fish. These significant increases in *hif*-1 and *hsp70* transcript levels may confirm an environmental stress experienced by fish of Eleyele lake.

Induction of *hsp70* is a general stress response and hypoxia inducible factors, beside their response to dissolved oxygen, are closely related to the biotransformation pathways (Regoli and Giuliani, 2014). It was shown that PAHs reduced hypoxia pathways and supported the hypothesis that *hif*-1α can compete with AhR for a shared *arnt* pool in fish cells, limiting the

activation of the AhR pathways (Bel Aiba and Gorlach, 2003; Rytkonen et al., 2008). In general, the significant expression of *hif*-1α and hsp70 in fish from Eleyele lake compared with reference site, together with the biotransformation responses and estrogen responses (Adeogun et al., 2016a) indicate that the Eleyele lake biota are experiencing a clear biological stress.

CF and obesogenic responses

In this study, we observed that the growth and health condition of *T. guineensis* were high with a k-factor ≥2. In addition, the HSI and KSI significantly decreased in female fish from Eleyele lake compared with the reference site, indicating a potentially deleterious effect of the higher burden of some metals and PAHs in fish liver and kidney. A similar reduction in HSI and KSI has been reported in Tilapia species exposed to an array of environmental contaminants from Ogun River, Nigeria (Ibor et al., 2017). In addition, *ppar* isoforms and *cpt* mRNA expression significantly increased in fish from Eleyele lake compared with the control site. The regulation of target gene expression and pathways associated with metabolic status have been associated with xenobiotic compounds that activate PPARs thus producing changes in metabolic homeostasis (Auwerx, 1999), leading to obesity (Janesick and Blumberg, 2011). Although *T. guineensis* from Eleyele lake were in good health condition (i.e. very high k-factor), severe endocrine disruptive effects with high frequency of intersex and elevated muscle burden of inorganic and organic contaminants were observed in these fish (Adeogun et al. 2016a). While *ppar* isoforms were equally expressed in both males and females, we showed higher levels in males of *cpt* mRNA, a gene involved in fatty acid (FA) β-oxidation. The good CF recorded at Eleyele lake despite the high contaminant burden may suggest obesogenic responses in fish from the Lake. Recently we reported a high CF in Tilapia species from Ogun river and Awba dam and proposed that fish CF may not be a conclusive measure to determine health status of contaminated aquatic biota as previously anticipated (Adeogun et al., 2016b,d).

It has been proposed that environmental contaminants with endocrine disruptive effects may act as obesogens, altering the regulation of lipid metabolism and adipogenesis (Grun and Blumberg, 2006), particularly by interacting with PPARs and subsequently acting as metabolic sensors, with pivotal roles in lipid homeostasis (Grun and Blumberg, 2009). The obesogenic hypothesis, relating the interaction of pollutants, drugs and PPARs have generally focused on the activation of *ppar*-γ

isoform for mediating obesogenic effects (Janesick and Blumberg, 2011). The CPT system mediates the uptake of long-chain FA to the mitochondria, where the PPARs are key mediators during FA β-oxidation process (Relat et al., 2004). In mammals, it was shown that *cpt*1 mRNA expression was increased by PPARs (Baldan et al., 2004). A relationship between *cpt*1b and *ppar*-β expression patterns were observed in both white muscle and liver of sea bream suggesting the key role of *ppar*-β isoform in controlling FA metabolism through *cpt*1b activation (Boukouvalas et al., 2010b; Yoon et al., 2006). Elsewhere, clofibric acid and bezafibrate produced *ppar*-γ mediated increase in FA β-oxidation in salmon hepatocytes (Ruyter et al., 1997). Combined exposure of tributyltin (TBT) with second (cAMP) messenger activator (forskolin) produced *ppar* isoform-specific effects when forskolin was given singly or in combination with TBT (Pavlikova et al., 2010). Recently, we reported that di-isodecyl phthalate (DiDP) bound with high efficiency to piscine PPARs demonstrating a greater preference for RXRα, in addition to coordinated increase in the expression of PPARs and *rxr*-α, as well as their downstream target genes in fish *in vitro* system (Cocci et al., 2015). Relevant to the present data, it should be noted that we recently reported high phthalate esters (PEs) concentrations in Eleyele lake water, sediment and biota samples (Adeogun et al. 2015). Further, a parallel increase in *cpt*1 and *ppar*-α with triacylglycerol content in the liver of rats exposed to high fat diet was observed (Boukouvalas et al., 2010a). When viewed together, our present findings suggest the potential compensatory mechanism for counteracting the contaminant-induced disturbance of lipid metabolism (*e.g.* increased expression of adipogenic genes and high CF). While these findings provide an insight into the probable physiological, oxidative and obesogenic effects of contaminant mixtures in *T. guineensis*, we recommend further systematic and differently designed studies in laboratory-controlled experiments to discern mechanistic scenarios.

In conclusion, fishing is a significant part of recreational activity for several classes of people, and fish constitute a major source of proteins, low cholesterol and other nutrients important for good health,. The Eleyele lake provides a significant quantity of water for municipal domestic use. In addition, the Fisheries division of the Ministry of Agriculture and Natural Resources in Oyo State, Nigeria took over the management of fishing activities in the Lake with the aim of encouraging intensive artisanal fisheries. Herein, trace metals and PAHs were shown to accumulate in the edible tissue (muscle) of the studied fish, consistent with recently reported levels of phthalate

esters (PEs) in sediments and biota at Eleyele lake (Adeogun et al., 2015). Overall, given that the Eleyele lake plays some significant roles in the livelihood of the neighboring communities through domestic water supply and fisheries, the high concentration and occurrence of PAHs, trace metals and other environmental contaminants measured in edible fish should be viewed as significant health concerns. Therefore, the establishment of a biomonitoring protocol for evaluating the environment and human health of the lake, for food and water safety is urgently needed.

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References

Adeniyi, A., Dayomi, M., Siebe, P., Okedeyi, O., 2008. An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa. Chem. Central J. 2, 9.

Adeogun, A.O., Ibor, R.O., Omiwole, R.A., Hassan, T., Adegbola, R.A., Adewuyi, G.A., Arukwe, A., 2015. Occurrence, species and organ differences in bioaccumulation patterns of phthalate esters in municipal domestic water supply Lakes in Ibadan, Nigeria. J. Toxicol. Environ. Health Part A Current Issues 78(12):761-777.

Adeogun, A.O., Ibor, O.R., Adeduntan, S. D., Arukwe, A., 2016a. Intersex and alterations in reproductive development of a cichlid, *Tilapia guineensis,* from a municipal domestic water supply lake (Eleyele) in Southwestern Nigeria. Sci. Total Environ. 541, 372-382

Adeogun, A,O., Ibor, O.R., Regoli, F., Arukwe, A., 2016b. Peroxisome proliferator activated receptors and biotransformation responses in relation to condition factor and contaminant burden in tilapia species from Ogun River, Nigeria. Comp. Biochem. Physiol. Part C. 183-184, 7-19.

Adeogun, A.O, Ibor, O.R., Onoja, A. B., Arukwe, A., 2016d. Fish condition factor, peroxisome proliferator activated receptors and biotransformation responses in Sarotherodon melanotheron from a contaminated freshwater dam (Awba Dam) in Ibadan, Nigeria. Mar. Environ. Res. 121:74-86.

Andersson, T., 1990. Sex differences in cytochrome P-450-dependent xenobiotic and steroid metabolisme in the mature rainbow trout kidney. J. Endocrinol. 126, 9-16.

Arukwe, A., Celius, T., Walther, B.T., Goksoyr, A., 2000. Effects of xenoestrogen treatment on zona radiata protein and vitellogenin expression in Atlantic salmon (Salmo salar). Aquat. Toxicol 49, 159-170.

Arukwe, A., Eggen, T., Moder, M., 2012. Solid waste deposits as a significant source of contaminants of emerging concern to the aquatic and terrestrial environments - a developing country case study from Owerri, Nigeria. Sci. Total Environ. 438, 94-102.

Arukwe, A., Goksøyr, A., 1997. Changes in three hepatic cytochrome P450 subfamilies during a reproductive cycle in Turbot (Scophthalmus maximus L.). J. Exp. Zool. 277, 313-325.

Asakura, H., Matsuto, T., Tanaka, N., 2004. Behavior of endocrine-disrupting chemicals in leachate from MSW landfill sites in Japan. Waste Manag. 24, 613-622.

Auwerx, J., 1999. PPARγ, the ultimate thrifty gene. Diabetologia 42, 1033-1049.

Baldan, A., Relat, J., Marrero, P.F., Haro, D., 2004. Functional interaction between peroxisome proliferator-activated receptors-alpha and Mef-2C on human carnitine palmitoyltransferase 1beta (CPT1beta) gene activation. Nucleic Acids Res. 32, 4742-4749.

Barrie, L.A., Gregor, D., Hargrave, B., Lake, R., Muir, D., Shearer, R., Tracey, B., Bidleman, T., 1992. Arctic contaminants: sources, occurrence and pathways. Sci. Tot. Environ. 122, 1-74.

Bel Aiba, R.S., Gorlach, A., 2003. Regulation of the hypoxia-inducible transcription factor HIF-1 by reactive oxygen species in smooth muscle cells. Adv. Exp. Med. Biol. 536, 171-178.

Benedetti, M., Gorbi, S., Fattorini, D., D'Errico, G., Piva, F., Pacitti, D., Regoli, F., 2014. Environmental hazards from natural hydrocarbons seepage: integrated classification of risk from sediment chemistry, bioavailability and biomarkers responses in sentinel species. Environ. Pollut. 185, 116-126.

Benedetti M., Giuliani M.E., Regoli F., 2015. Oxidative metabolism of chemical pollutants in marine organisms: molecular and biochemical biomarkers in environmental toxicology. Ann. N.Y. Acad. Sci. 1340 (1), 8-19.

Blanquart, C., Barbier, O., Fruchart, J.C., Staels, B., Glineur, C., 2003. Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation. J. Steroid Biochem. Mol. Biol. 85, 267-273.

Boukouvalas, G., Gerozissis, K., Kitraki, E., 2010a. Fat feeding of rats during pubertal growth leads to neuroendocrine alterations in adulthood. Cell. Mol. Neurobiol. 30, 91-99.

Boukouvalas, G., Gerozissis, K., Markaki, E., Kitraki, E., 2010b. High-fat feeding influences the endocrine responses of pubertal rats to an acute stress. Neuroendocrinol. 92, 235-245.

Bradshaw, T.D., Trapani, V., Vasselin, D.A., Westwell, A.D., 2002. The aryl hydrocarbon receptor in anticancer drug discovery: friend or foe? Curr. Pharm. Des. 8, 2475-2490.

Breitburg, D.L., Hondorp, D.W., Davias, L.A., Diaz, R.J., 2009. Hypoxia, nitrogen, and fisheries: integrating effects across local and global landscapes. Ann. Rev. Mar. Sci. 1, 329-349.

Buhler, D.R., Williams, D.E., 1988. The role of biotransformation in the toxicity of chemicals. Aquat. Toxicol. 11, 19-28.

Chan, Z., Hollebone, B., 1995. A QSAR for steroidal compound interaction with cytochrome P4501A1. Environ. Toxicol. Chem. 14, 597-603.

Clarke, D.J., George, S.G., Burchell, B., 1991. Glucuronidation in fish. Aqua. Toxicol. 20, 35-56.

Cocci, P., Mosconi, G., Arukwe, A., Mozzicafreddo, M., Angeletti, M., Aretusi, G., Palermo, F.A., 2015. Effects of diisodecyl phthalate on PPAR:RXR-dependent gene expression pathways in seabream hepatocytes. Chem. Res. Toxicol. 25, 935-947.

Craig, E.A., Gross, C.A., 1991. Is hsp 70 the cellular thermometer? TIBS 16, 135-140.

Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G., Wahli, W., 1992. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68, 879-887.

Eruola, A.O., Ufoegbune, G.C., Ojekunle, Z.O., Makinde, A.A., Ogunyemi, I.O., 2011. Analytical Investigation of Pollutants in Lagos Coastal Waters, Nigeria. Adv. Analyt. Chem. 1, 8-11.

EU Regulation, 2002. Polycyclic Aromatic Hydrocarbons – Occurrence in foods, dietary exposure and health effects. SCF/CS/CNTM/PAH/29 ADD1 Final.

Fang, Y., Yang, H., Liu, B., 2012. Tissue-specific response of metallothionein and superoxide dismutase in the clam Mactra veneriformis under sublethal mercury exposure. Ecotoxicol. 21, 1593-1602.

Fang, Y., Yang, H., Wang, T., Liu, B., Zhao, H., Chen, M., 2010. Metallothionein and superoxide dismutase responses to sublethal cadmium exposure in the clam Mactra veneriformis. Comp. Biochem. Physiol. A, 151, 325-333.

Farber, J.L., 1994. Mechanisms of cell injury by activated oxygen species. Environ. Health Perspect. 102 Suppl 10, 17-24.

Forbes, V.E., Palmqvist, A., Bach, L., 2006. The use and misuse of biomarkers in ecotoxicology. Environ. Toxicol. Chem. 25, 272-280.

Gadagbui, B.K.-M., Goksøyr, A., 1996. CYP1A and other biomarker responses to effluents from a textile mill in the Volta River (Ghana) using caged tilapia (*Oreochromis niloticus*) and sediment-exposed mudfish (*Clarias anguillaris*). Biomarkers 1, 252-261.

Giuliani, M.E., Benedetti, M., Arukwe, A., Regoli, F., 2013. Transcriptional and catalytic responses of antioxidant and biotransformation pathways in mussels, Mytilus galloprovincialis, exposed to chemical mixtures. Aquat. Toxicol. 134-135, 120-127.

Gnaiger, E., Steinlechner-Maran, R., Méndez, G., Eberl, T., Margreiter, R., 1995. Control of mitochondrial and cellular respiration by oxygen. J. Bioenerg. Biomembr. 27, 583-596.

Goksøyr, A., Förlin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology, and environmental monitoring. Aquat. Toxicol. 22, 287-312.

Griswold, B., 1997. Fisheries and pollution. Transact. Amer. Fisher. Soc. 126, 504-505.

Grun, F., Blumberg, B., 2006. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinol. 147, S50-55.

Grun, F., Blumberg, B., 2009. Endocrine disrupters as obesogens. Mol. Cell. Endocrinol. 304, 19-29.

Gu, Y.Z., Hogenesch, J.B., Bradfield, C.A., 2000. The PAS superfamily: sensors of environmental and developmental signals. Annu. Rev. Pharmacol. Toxicol. 40, 519-561.

Hassell, K.L., Coutin, P.C., Nugegoda, D., 2008. Hypoxia impairs embryo development and survival in black bream (Acanthopagrus butcheri). Mar. Pollut. Bullet. 57, 302-306.

Ibor, O.R. Adeogun, A.O., Chukwuka, A.V., Arukwe, A., 2017. Gross pathology, physiological and toxicological responses in relation to metals and persistent organic pollutants (POPs) burden in tilapia species from Ogun River, Nigeria. Mar. Environ. Res. 129,245-257

Imevbore, A.M.A., 1967. Hydrology and Plankton of Eleiyele reservoir, Ibadan, Nigeria. Hydrobiol. 30, 154-176.

Jaji, M.O., Bamgbose, O., Odukoya, O.O., Arowolo, T.A., 2007. Water quality assessment of Ogun river, South West Nigeria. Environ. Monit. Assess. 133, 473-482.

Janesick, A., Blumberg, B., 2011. Minireview: PPARγ as the target of obesogens. J. Steroid Biochem. Mol. Biol. 127, 4-8.

Jordao, R., Casas, J., Fabrias, G., Campos, B., Pina, B., Lemos, M.F., Soares, A.M., Tauler, R., Barata, C., 2015. Obesogens beyond Vertebrates: Lipid Perturbation by Tributyltin in the Crustacean. Environ. Health Perspect. 123, 813

Jung, J., Kim, M., Yim, U.,.H., Ha, S.Y., An, J.G., Won, J.H., Han, G.M., Kim, N.S., Addison, R.F., Shim,W.J., 2011. Biomarker responses in pelagic and benthic fish over 1 year following the Hebei spirit oil spill (Taean, Korea). Mar. Pollut. Bull. 62, 1859–1866.

Langenbuch, M., Bock, C., Leibfritz, D., Portner, H.O., 2006. Effects of environmental hypercapnia on animal physiology: a 13C NMR study of protein synthesis rates in the marine invertebrate Sipunculus nudus. Comp. Biochem. Physiol. Part A, 144, 479-484.

Leaver, M.J., Clarke, D.J., George, S.G., 1992. Molecular studies of the phase II xenobiotic-conjugating enzymes of Pleuronectid flatfish. Aquat. Toxicol. 22, 265-278.

Liu, R.Y., Corry, P.M., Lee, Y.J., 1994. Regulation of chemical stress-induced hsp70 gene expression in murine L929 cells. J. Cell Sci. 107, 2209-2214.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656-666.

Loos, R., Carvalho, R., Antonio, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475-6487.

Mansfield, K.D., Guzy, R.D., Pan, Y., Young, R.M., Cash, T.P., Schumacker, P.T., Simon, M.C., 2005. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-α activation. Cell Metab. 1, 393-399.

Mortensen, A.S., Arukwe, A., 2007. Modulation of xenobiotic biotransformation system and hormonal responses in Atlantic salmon (Salmo salar) after exposure to tributyltin (TBT). Comp. Biochem. Physiol. Part C 145, 431- 441.

Nathan, C., Cunningham-Bussel, A., 2013. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nature reviews. Immunol. 13, 349-361.

Navas, J.M., Segner, H., 2000. Antiestrogenicity of beta-naphthoflavone and PAHs in cultured rainbow trout hepatocytes: evidence for a role of the arylhydrocarbon receptor. Aquat. Toxicol. 51, 79-92.

Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, R., Waxman, D.J., Waterman, M.R., Gotoh, O., Coon, M.J., Estabrook, R.W., Gunsalus, I.C., Nebert, D., 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenet. 6, 1-42.

Onyeike, E.N., Ogbuja, S.I., Nwinuka, N.M., 2002. Inorganic ion levels of soils and streams in some areas of Ogoniland, Nigeria as affected by crude oil spillage. Environ. Monit. Assess. 73, 191-205.

Pavlikova, N., Kortner, T.M., Arukwe, A., 2010. Peroxisome proliferator-activated receptors, estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and second messenger activator. Aquat. Toxicol. 99, 176-185.

Qi, C., Zhu, Y., Reddy, J.K., 2000. Peroxisome proliferator-activated receptors, coactivators, and downstream targets. Cell Biochem. Biophysics 32 Spring, 187-204.

Relat, J., Nicot, C., Gacias, M., Woldegiorgis, G., Marrero, P.F., Haro, D., 2004. Pig muscle carnitine palmitoyltransferase I (CPTI β), with low Km for carnitine and low sensitivity to malonyl-CoA inhibition, has kinetic characteristics similar to those of the rat liver (CPTI alpha) enzyme. Biochem. 43, 12686-12691.

Regoli F., Giuliani M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106-117.

Regoli, F., Giuliani, M.E., Benedetti, M., Arukwe, A., 2011. Molecular and biochemical biomarkers in environmental monitoring: a comparison of biotransformation and antioxidant defense systems in multiple tissues. Aquat. Toxicol. 105, 56–66.

Riu, A., McCollum, C.W., Pinto, C.L., Grimaldi, M., Hillenweck, A., Perdu, E., Zalko, D., Bernard, L., Laudet, V., Balaguer, P., Bondesson, M., Gustafsson, J.A., 2014. Halogenated bisphenol-A analogs act as obesogens in zebrafish larvae (Danio rerio). Toxicol. Sci. 139, 48-58.

Roselli, C.E., Resko, J.A., 1997. Sex differences in androgen-regulated expression of cytochrome P450 aromatase in the rat brain. J. Steroid Biochem. Mol. Biol. 61, 365-374.

Ruyter, B., Andersen, O., Dehli, A., Ostlund Farrants, A.K., Gjoen, T., Thomassen, M.S., 1997. Peroxisome proliferator activated receptors in Atlantic salmon (Salmo salar): effects on PPAR transcription and acyl-CoA oxidase activity in hepatocytes by peroxisome proliferators and fatty acids. Biochim. Biophys. Acta 1348, 331-338.

Rytkonen, K.T., Ryynanen, H.J., Nikinmaa, M., Primmer, C.R., 2008. Variable patterns in the molecular evolution of the hypoxia-inducible factor-1 alpha (HIF-1alpha) gene in teleost fishes and mammals. Gene 420, 1-10.

Sanchez,W., Sremski,W., Piccini, B., Palluel, O., Maillot-Marechal, E., Betuolle, S., Jaffal, A., Ait-Aissa, S., Brion, F., Thybaud, E., Hinfray, N., Porcher, J., 2011. Adverse effects inwild fish living downstream from pharmaceutical manufacture discharges. Environ. Int. 8, 1342–1348.

Schmidt, I., Herpin, P., 1998. Carnitine palmitoyltransferase I (CPT I) activity and its regulation by malonyl-CoA are modulated by age and cold exposure in skeletal muscle mitochondria from newborn pigs. J. Nutr. 128, 886-893.

Sheridan, M.A., 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol. B, 90, 679-690.

Shi, Y., Hon, M., Evans, R.M., 2002. The peroxisome proliferator-activated receptor delta, an integrator of transcriptional repression and nuclear receptor signaling. Proc. Nat. Acad. Sci. USA 99, 2613-2618.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291-295.
Stoner, M., Saville, B., Wormke, M., Dean, D., Burghardt, R., Safe, S., 2002. Hypoxia induces proteasome-dependent degradation of estrogen receptor alpha in ZR-75 breast cancer cells. Mol. Endocrinol. 16, 2231-2242.
van der Oost, R., Goksøyr, A., Celander, M., heida, H., vermeulen, N.P.E., 1996. Biomonitoring of aquatic pollution with feral eel (Anguilla anguilla) II. Biomarkers: pollution-induced biochemical responses. Aquat. Toxicol. 36, 189-222.
Vogelbein, W.K., Fournie, J.W., van Veld, P.A., Huggett, R.J., 1990. Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res. 50, 5978-5986.
Wenger, R.H., 2002. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. Faseb J 16, 1151-1162.
Whitelaw, M.L., McGuire, J., Picard, D., Gustafsson, J.A., Poellinger, L., 1995. Heat shock protein hsp90 regulates dioxin receptor function in vivo. Proc. Nat. Acad. Sci. USA 92, 4437-4441.
Williams, D.E., Lech, J.J., Buhler, D.R., 1998. Xenobiotics and xenoestrogens in fish: modulation of cytochrome P450 and carcinogenesis. Mutat. Res. 399, 179-192.
Wu, R.S.S., 2009. Effects of hypoxia on fish reproduction and development. Fish Physiol. 27, 79-141.
Yoo, J.L., Janz, D.M., 2003. Tissue-specific HSP70 levels and reproductive physiological responses in fishes inhabiting a metal-contaminated creek. Arch. Environ. Contam. Toxicol. 45, 110-120.
Yoon, M.J., Lee, G.Y., Chung, J.J., Ahn, Y.H., Hong, S.H., Kim, J.B., 2006. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 55, 2562-2570.
Young, M.R., Craig, E., 1993. Saccharomyces cerevisiae HSP70 heat shock elements are functionally distinct. Mol. Cell. Biol. 13, 5637-5646.
Zelibe, S.A.A., Fagade, S.O., A.A., A., 1990. Food and feeding interrelationships of jóvenes of cichlids in Eleiyele reservoir, Ibadan, Nigeria. J. Exp. Appl. Biol. 2, 70-81.
Zhou, B., Liu, W., Siu, W.H., O'Toole, D., Lam, P.K., Wu, R.S., 2006. Exposure of spermatozoa to duroquinone may impair reproduction of the common carp (Cyprinus carpio) through oxidative stress. Aquat. Toxicol. 77, 136-142.

Figure legends

Figure 1. Map of Eleyele Lake indicating the sampling sites and surrounding environments.

Figure 2. Changes in transcript levels of *cyp*1a (A), *cyp*1b (B) and *cyp*1c (C) in male and female *Tilapia guineensis* from Eleyele Lake and a reference site (Igboho Lake) in Southwestern Nigeria. Messenger RNA (mRNA) was analyzed by real-time PCR with gene-specific primer pairs and presented as mRNA copies (ng/μL). All values represent the mean ± standard error of the mean (SEM). Different symbols (#) denotes significant differences across individual sexes between

different sampling sites and asterisk (*) denotes significant difference between male and female from each sampling site. The level of significance was set at $p<0.05$.

Figure 3. Changes in transcript levels of *gst* (A), *ugt1* (B), *CuZn-sod* (C) and *sod* (D) in male and female *Tilapia guineensis* from Eleyele Lake and a reference site (Igboho Lake) in Southwestern Nigeria. Messenger RNA (mRNA) was analyzed by real-time PCR with gene-specific primer pairs and presented as mRNA copies (ng/μL). All values represent the mean ± standard error of the mean (SEM). Different symbols (#) denotes significant differences across individual sexes between different sampling sites and asterisk (*) denotes significant difference between male and female from each sampling site. The level of significance was set at $p<0.05$.

Figure 4. Transcriptional changes of *ppar*-α (A), *ppar*-β (B), *ppar*-γ (C) and *cpt* (D) in male and female *Tilapia guineensis* from Eleyele Lake and a reference site (Igboho Lake) in Southwestern Nigeria. Messenger RNA (mRNA) was analyzed by real-time PCR with gene-specific primer pairs and presented as mRNA copies (ng/μL). All values represent the mean ± standard error of the mean (SEM). Different symbols (#) denotes significant differences across individual sexes between different sampling sites and asterisk (*) denotes significant difference between male and female from each sampling site. The level of significance was set at $p<0.05$.

Figure 5. Changes transcript levels of *hif*-1α (A) and *hsp70* (B) in male and female *Tilapia guineensis* from Eleyele Lake of Southwestern Nigeria. Messenger RNA (mRNA) was analyzed by real-time PCR with gene-specific primer pairs and presented as mRNA copies (ng/μL). All values represent the mean ± standard error of the mean (SEM). Different symbols (#) denotes significant differences across individual sexes between different sampling sites and asterisk (*) denotes significant difference between male and female from each sampling site. The level of significance was set at $p<0.05$.

Figure 6. Biplot of biotransformation, oxidative and obesogenic pathways and concentration of environmental contaminants (trace metals, aliphatic and polycyclic aromatic hydrocarbons) measured in edible fish tissue (muscle) from Eleyele Lake and reference site.

Figures

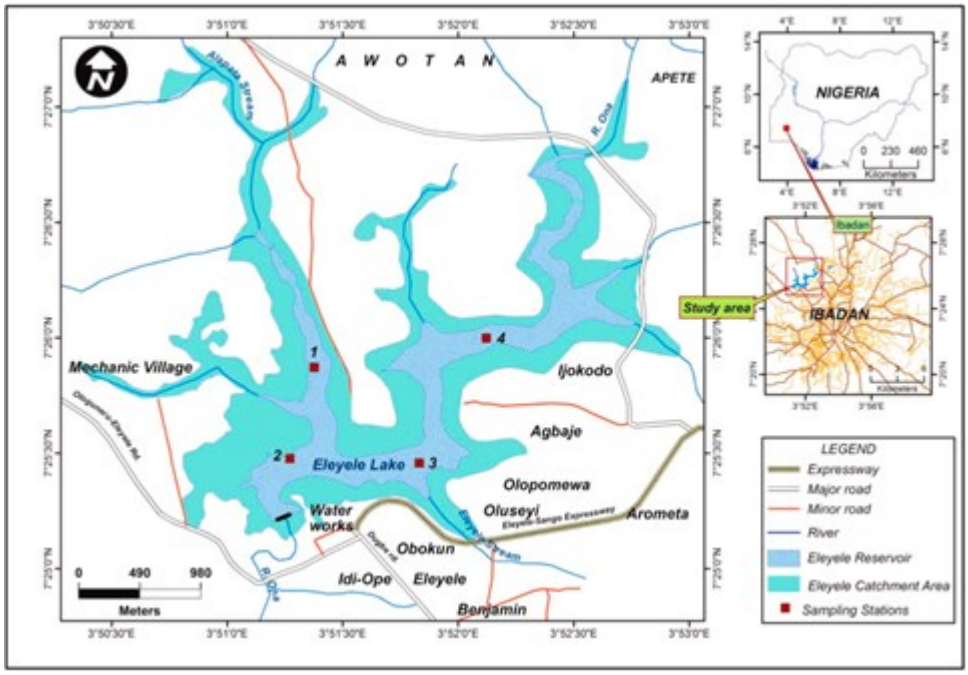


Figure 1.

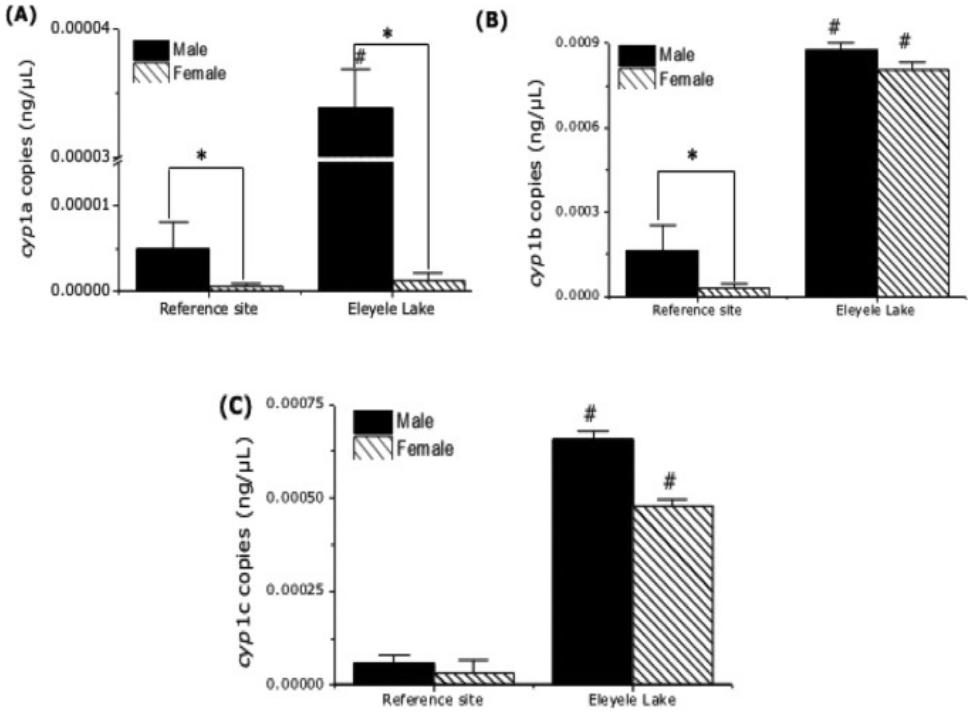


Figure 2.

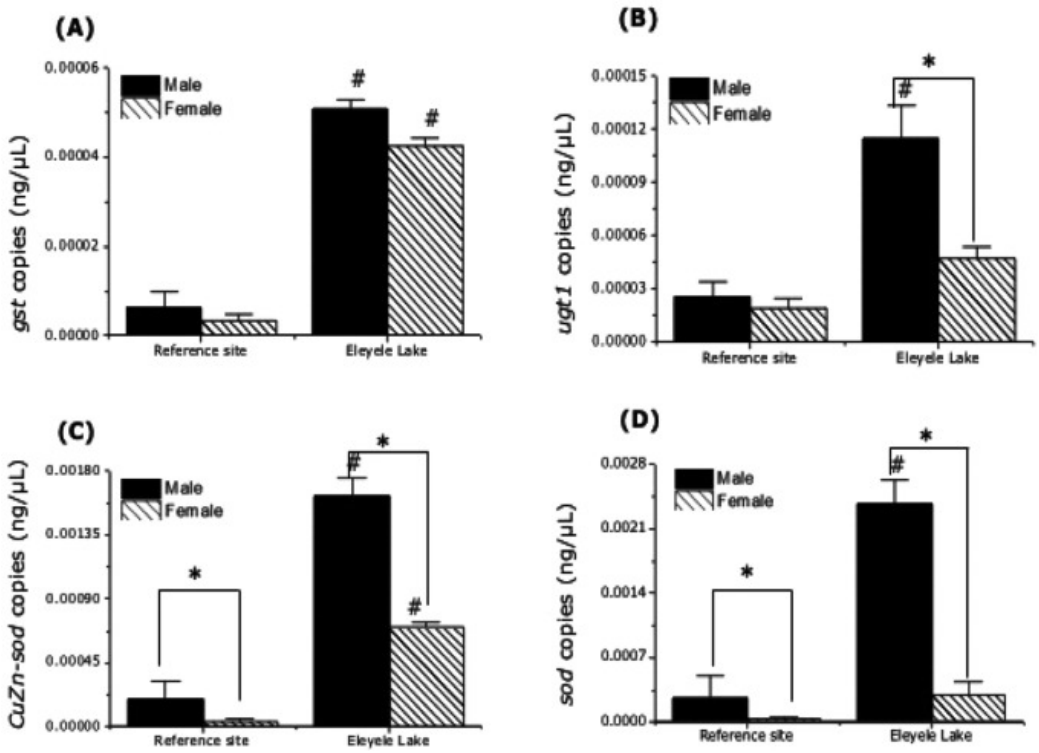


Figure 3.

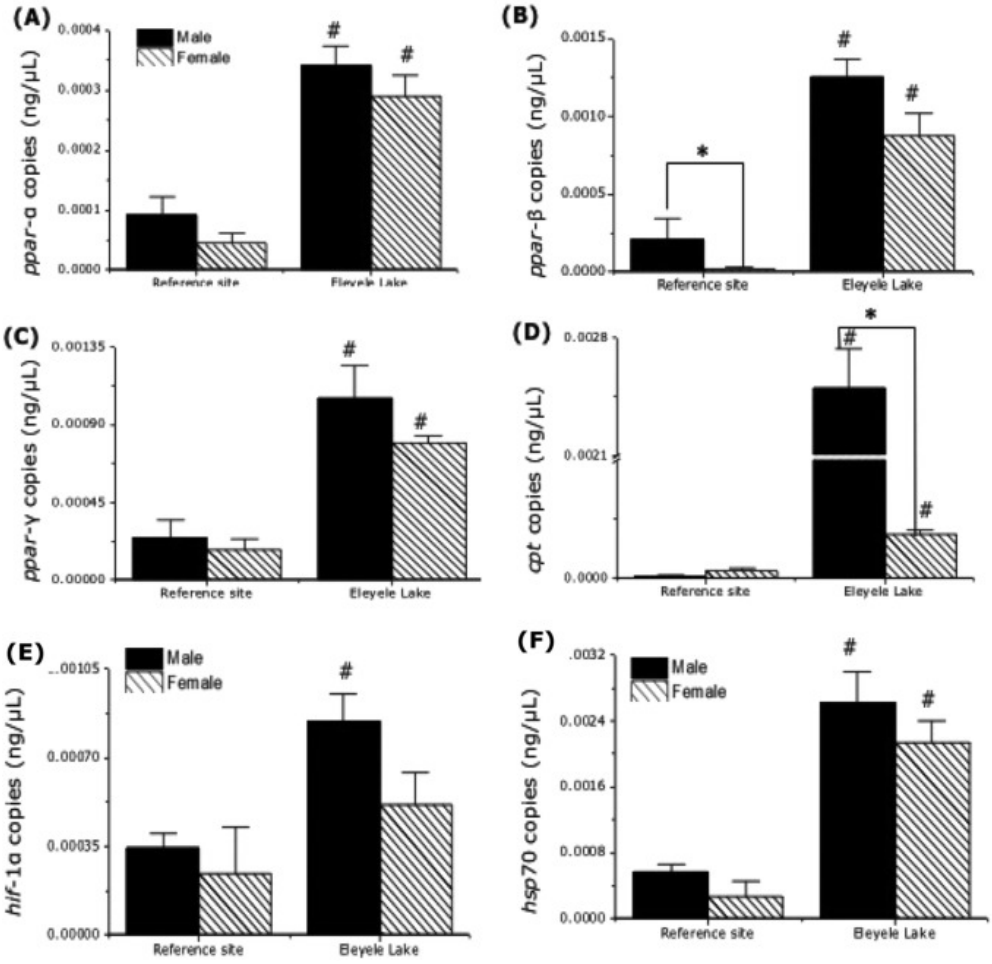
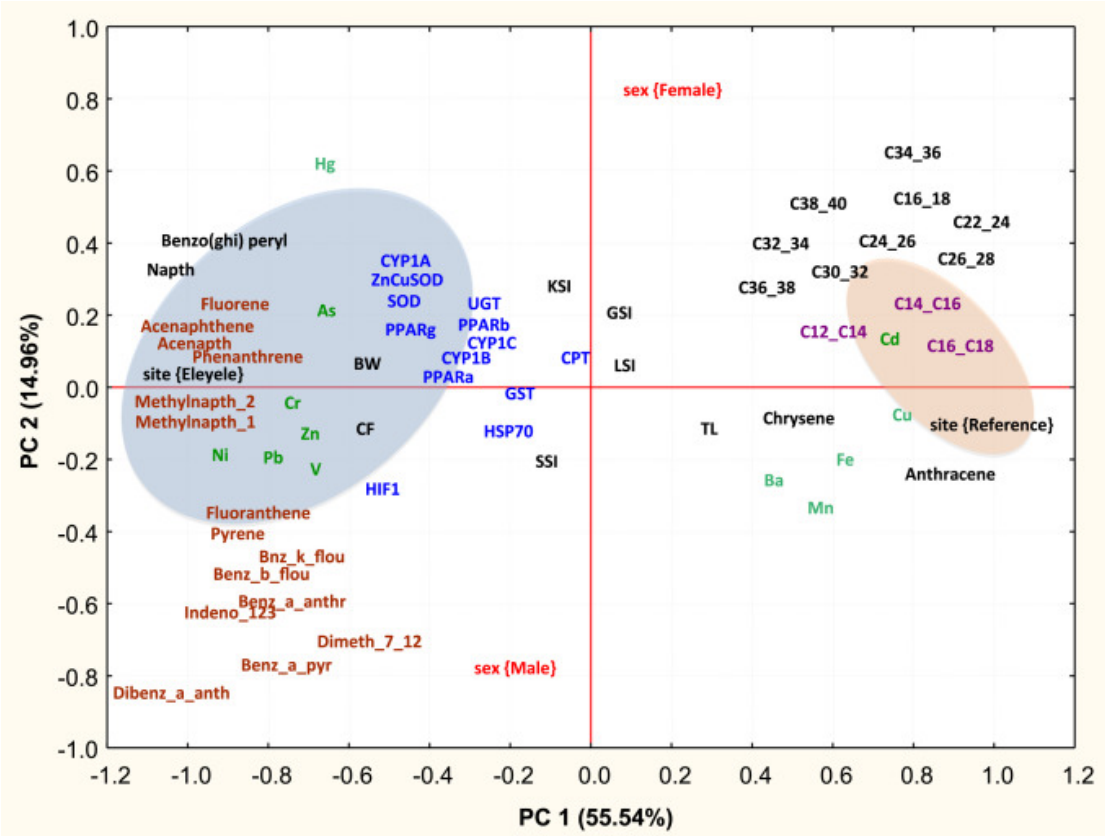

(A)
Male
Female
ppar-α copies (ng/μL)
0.0004
0.0003
0.0002
0.0001
0.0000
#
#
(B)
ppar-β copies (ng/μL)
0.0015
0.0010
0.0005
0.0000
*
#
#
Reference site
Eleyele Lake
(C)
ppar-γ copies (ng/μL)
0.00135
0.00090
0.00045
0.00000
#
#
Reference site
Eleyele Lake
(D)
cpt copies (ng/μL)
0.0028
0.0021
0.0000
*
#
#
Reference site
Eleyele Lake
(E)
Male
Female
hif-1α copies (ng/μL)
0.00105
0.00070
0.00035
0.00000
#
Reference site
Eleyele Lake
(F)
Male
Female
hsp70 copies (ng/μL)
0.0032
0.0024
0.0016
0.0008
0.0000
#
#
Reference site
Eleyele Lake

Figure 4.


PC 2 (14.96%)
PC 1 (55.54%)
1.0
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-1.0
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-0.8
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-0.4
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0.2
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0.8
1.0
1.2
sex {Female}
sex {Male}
Hg
Benzo(ghi) peryl
Napth
Fluorene
Acenaphthene
Acenapth
Phenanthrene
site {Eleyele}
Methylnapth_2
Methylnapth_1
Ni
Pb
Cr
Zn
V
As
BW
CF
CYP1A
ZnCuSOD
SOD
PPARg
UGT
PPARb
CYP1C
CYP1B
PPARa
HIF1
GST
HSP70
SSI
KSI
CPT
GSI
LSI
TL
Fluoranthene
Pyrene
Bnz_k_flou
Benz_b_flou
Benz_a_anthr
Indeno_123
Dimeth_7_12
Benz_a_pyr
Dibenz_a_anth
C34_36
C38_40
C16_18
C22_24
C32_34
C24_26
C26_28
C30_32
C36_38
C14_C16
C12_C14
Cd
C16_C18
Chrysene
Cu
site {Reference}
Fe
Ba
Anthracene
Mn

Figure 5.

Table 1. Biometric data of *Tilapia guineensis*, sampled at Eleyele Lake and at the reference site (Igboho Lake).

Empty Cell	Reference site		Eleyele Lake	
	Male	**Female**	**Male**	**Female**
Total length (TL)	22.6 ± 0.4[a]*	20.5 ± 0.4[a]	14.0 ± 3.8[a]	16.0 ± 2.4[a]
Body weight (BW)	232.5 ± 10.7[a]*	181.3 ± 11.8[a]	234.3 ± 9.2[a]*	184.2 ± 7.9[a]
Condition factor (CF)	1.8 ± 0.1[a]	2.0 ± 0.0[a]	2.3 ± 0.12[b]	2.14 ± 0.1[a]
Hepatosomatic index (HSI)	0.4 ± 0.0[a]	0.6 ± 0.1[a]	0.51 ± 0.1[a]*	0.32 ± 0.1[b]
Spleen somatic index (SSI)	0.06 ± 0.0[a]	0.08 ± 0.0[a]	0.2 ± 0.0[b]*	0.10 ± 0.01[b]
Kidney somatic index (KSI)	0.21 ± 0.0[a]	0.4 ± 0.1[a]*	0.31 ± 0.05[a]*	0.19 ± 0.02[b]

Values are given as mean ± SE (standard error of the mean). Different letters denote significant differences ($p < 0.05$) across individual sexes between different sampling station and asterisks (*) denote significant difference between sexes (male vs female) from each sampling site. NA = Not available.

Table 2. Biometric data of Tilapia guineensis, sampled at Eleyele Lake and at the reference site (Igboho Lake).

	Reference site		Eleyele Lake	
	Male	**Female**	**Male**	**Female**
Total length (TL)	22.6±0.4[a]*	20.5±0.4[a]	14.0±3.8[a]	16.0±2.4[a]
Body weight (BW)	232.5±10.7[a]*	181.3±11.8[a]	234.3±9.2[a]*	184.2±7.9[a]
Condition factor (CF)	1.8±0.1[a]	2.0±0.0[a]	2.3±0.12[b]	2.14±0.1[a]
Hepatosomatic index (HSI)	0.4±0.0[a]	0.6±0.1[a]	0.51±0.1[a]*	0.32±0.1[b]
Spleen somatic index (SSI)	0.06±0.0[a]	0.08±0.0[a]	0.2±0.0[b]*	0.10±0.01[b]
Kidney somatic index (KSI)	0.21±0.0[a]	0.4±0.1[a]*	0.31±0.05[a]*	0.19±0.02[b]

Values are given as mean ± SE (standard error of the mean). Different letters denote significant differences ($p<0.05$) across individual sexes between different sampling station and asterisks (*) denote significant difference between sexes (male vs female) from each sampling site. NA = Not available

Table 3: Concentration of heavy metals (µg/g) in the muscle of Tilapia guineensis from Eleyele Lake and at the reference site (Igboho lake).

Trace metal (µg/g)	**Reference site**		**Eleyele Lake**		**NESREA[1]**	**WHO[2]**
	Male	**Female**	**Male**	**Female**		
As	0.02±0.0[a]	0.05±0.0[a]*	0.26±0.02[b]	0.24±0.03[b]	NA	0.003
Ba	0.06±0.0[a]	0.11±0.0[a]*	0.23±0.07[b]	4.73±1.05[b]*	NA	NA
Cu	0.12±0.0[a]	0.22±0.0[a]*	1.12±0.29[b]	1.53±0.66[b]	0.10	0.20
Hg	0.01±0.0[a]	0.02±0.0[a]	0.03±0.00[b]	0.07±0.00[b]*	0.005	0.003
Cd	0.001±0.0[a]	0.001±0.0[a]	0.003±0.000[b]	0.01 ±0.001[b]*	0.005	0.003

Cr	0.1±0.0[a]	0.1±0.0[a]	0.61±0.04[b]	0.62±0.02[b]	0.10	0.05
Fe	1.1±0.3[a]	1.3±0. 3[a]	5.03±2.97[b]	14.63±8.14[b]	0.10	0.40
Mn	0.41±0.1[a]*	0.21±0.0[a]	3.74±0.92[b]	19.1± 5.4[b]*	0.10	0.40
Ni	0.01±0.0[a]	0.01±0.0[a]	0.03±0.00[a]	0.03±0.00[a]	0.01	0.07
Pb	0.01±0.0[a]	0.01±0.0[a]	0.07±0.01[b]	0.12±0.04[b]	0.05	0.01
V	0.01±0.0[a]	0.01±0.0[a]	0.20±0.02[b]	0.32± 0.1[b]	NA	0.003
Zn	1.0±0.4[a]	0.8±0.5[a]	32.63±4.32[b]	36.2 ±6.63[b]	0.10	0.40

Values are given as mean ± SE (standard error of the mean). Different letters denote significant differences ($p<0.05$) across individual sexes between different sampling station and asterisks (*) denote significant difference between sexes (male vs female) from each sampling site. NA = Not available. Permissible limits in food: 1National Environmental Standards and Regulations Enforcement Agency; 2World Health Organization

Table 4: Levels of aliphatic hydrocarbons (µg/g) in the muscle of Tilapia guineensis from Eleyele lake and at the reference site (Igboho lake).

Aliphatic hydrocarbon (µg/g)	**Reference site**		**Eleyele Lake**	
	Male	**Female**	**Male**	**Female**
>C10-12	2.5±0.8[a]*	0.9±0[a]	3.3±0.2[a]	2.9±0.5[b]
>C12-14	7.7±2.3[a]*	3.9±0.0[a]	8.8±2.1[a]	6.5±1.7[b]
>C14-16	7.4±0.2[a]	9.1±2.1[a]	8.1±2.1[a]	7.31±1.6[a]
>C16-18	9.8±0.2[a]	7.5±1.0[a]	8.0±2.1[a]	8.34±0.7[a]
>C18-20	32.3±3.6[a]	43.4±1.4[b]	39.8 ±8.52[a]	29.7±1.5[a]
>C20-22	36.3±1.1[a]	61.4±4.3[b]*	69.8± 14.9[b]*	36.4 ±1.7[a]
>C22-24	7.9±1.8[a]	17.0±1.0[b]*	11.7±4.5[a]	12.8±0.6[a]
>C24-26	4.2±0.3[a]	8.2±2.0[a]	7.5±2.2[a]	5.2±0.7[a]
>C26-28	2.7±0.4[a]	2.6±0.4[a]	4.2±0.1[b]*	2.0±0.7[a]
>C28-30	1.2±0.4[a]	3.6±1.0[a]	3.80±0.43[b]	2.56±0.71[a]
>C30-32	39.4±3.9[a]	151.3±52.3[b]*	85.7±16.4[b]	63.0±6.8[a]
>C32-34	4.6±2.1[a]	8.6±3.0[b]	2.0±0.4[a]	1.5±0.5[a]
>C34-36	4.2±2.4[a]	4.5±0.1[a]	7.0±1.5[a]	2.6±1.7[a]
>C36-38	6.8±0.1[a]	7.1±3[a]	5.3±0.6[a]	10.5±5.0[a]
>C38-40	4.4±1.7[a]	27.1±1.5[b]*	7.4±0.5[a]	9.3±2.5[a]
∑TAHs	171.4±7.5[b]	356.2±11.2[b]*	272.0±56.0[a]	200.5±18.2[a]*

Values are given as mean ± SE (standard error of the mean). Different letters denote significant differences ($p<0.05$) across individual sexes between different sampling station and asterisks (*) denote significant difference between sexes (male vs female) from each sampling site.

Table 5: Concentrations of polycyclic aromatic hydrocarbons (PAHs: ng/g) in the muscle of Tilapia guineensis from Eleyele lake and and at the reference site (Igboho lake).

PAH (ng/g)	**Reference site**		**Eleyele Lake**	
	Male	**Female**	**Male**	**Female**
Naphthalene	41.2±3.1[a]	71.2±3.0[a]*	80.2 ±8.43[b]	132.9 ±53.3[b]*
1-Methylnaphthalene	89.2±14.4[a]	81.1±21.0[a]	97.9±2.0[a]	99.4±18.5[a]
2-Methylnaphthalene	73.8±2.3[a]	81.3±38.0[a]	86.8 ± 1.7[b]	94.9±11.9[a]
Fluorene	6.2±0.2[a]	8.3±3.5[a]	8.8±0.8[b]	7.82±2.0[a]
Phenanthrene	3.5±0.1[a]	37.5±17.0[b]*	3.6±0.0[a]	3.6 ±0.7[a]
Anthracene	0.6±0.1[a]*	1.1±0.2[b]	0.7±0.1[a]	0.9±0.2[a]*
Fluoranthene	4.1±0.1[a]*	3.13±3.0[a]	8.80±5.3[a]	6.2 ±0.2[a]
Pyrene	2.5±0.3[a]*	1.0±0.9[a]	0.9±0.4[b]	1.1±0.2[a]
Benzo(a)anthracene	0.2±0.1[a]	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]
Chrysene	0.6±0.1[a]	0.2±0.1[a]	0.3±0.0[b]	0.1±0.0[a]
7,12-Dimethylbenzo(a)anthracene	4.6±0.4[a]*	0.6±0.5[a]	2.1±0.1[b]	14.5±12.2[b]*
Benzo(b)fluoranthene	0.7±0.0[a]	0.2±0.1[a]	0.49±0.3[b]	0.2±0.0[a]
Benzo(k)fluoranthene	0.2±0.0[a]	0.2±0.0[a]	0.8±0.0[b]*	0.03±0.0[a]
Benzo(a)pyrene	0.2±0.0[a]	0.1±0.0[a]	0.4±0.1[b]	0.6±0.5[b]
Dibenzo(ah)anthracene	0.3±0.1[a]	0.5±0.1[a]	1.11±0.7	6.90±6.3[b]
Benzo(ghi)perylene	0.1±0.0[a]	0.6±0.1[b]	0.81±0.0[b]*	0.3±0.2[a]
LMW PAHs	214.5±9.1[b]	280.5±8.1[b]	278 ± 7.5[a]	339.5 ± 86.6[b]*
HMW PAHs	13.5±0.1[a]	6.63±0.3[a]	13.4 ± 3.3[a]	27.0 ± 16.3[b]*
Total PAHs	228.2±5.0[b]	287.1±4.2[a]*	291.4 ± 4.2[a]	366.5 ± 103.1[a]*

Values are given as mean ± SE (standard error of the mean). Different letters denote significant differences ($p<0.05$) across individual sexes between different sampling station and asterisks (*) denote significant difference between sexes (male vs female) from each sampling site.