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Biomarkers of toxicity in *Clarias gariepinus* exposed to sublethal concentrations of polycyclic aromatic hydrocarbons

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Physiological, biochemical and histological indices in *Clarias gariepinus* broodstock, and teratogenic indices in embryos exposed to sublethal concentrations of naphthalene, phenanthrene and pyrene were investigated in 2014 using a static-renewal bioassay protocol. Phenanthrene (1.41 mg l^{-1}) was the most toxic, followed by pyrene (1.53 mg l^{-1}) and naphthalene (7.21 mg l^{-1}), based on 96 h LC50 values. Hepatosomatic indices were significantly higher in naphthalene- and pyrene-treated males compared with solvent controls, whereas fecundity in females was significantly lower by factors of 2.4 (naphthalene), 2.8 (phenanthrene) and 2.4 (pyrene), compared with controls. Catalase levels were lower in female phenanthrene-treated fish compared with controls. Histological alterations observed in PAH-treated fish include oedema, inflammatory cells, epithelial lifting and hyperplasia in the gills, vacuolation, haemosiderin pigments and sinusoidal congestion in the liver, and degenerated zona radiata in the ovary. Teratogenic effects were not observed, as evidenced by the lack of histological alterations in embryos spawned from pre-exposed broodstock. Sex-specific responses and the utility of biomarkers at cellular and individual levels of organisation are therefore demonstrated for holistic evaluations of polycyclic aromatic hydrocarbons in ecotoxicological studies.

Keywords: African sharptooth catfish, biochemical indices, biological responses, histological alterations, naphthalene, phenanthrene, physiological indices, pyrene

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, some of which are classified as carcinogens, mutagens, and teratogens by the U.S. Environmental Protection Agency (USEPA 2011). Wildlife exposures to PAHs, through contaminated soil, sediment, or food chains, can produce a variety of population-level effects, including impaired growth, increased disease susceptibility, reduced larval survival, and reduced net population fecundity (Heintz 2007). These effects or responses are termed biomarkers that can serve as indicators of stress in the organism, population or ecosystem. The use of biomarkers in ecotoxicology and ecological risk assessment stems partly from a desire for early warning signals that appear before measurable effects on individual performance and population/community dynamics occurs (Munoz and Sabater 2014). Biomarkers of ecotoxicity also help elucidate potential causes of observable population- and community-level effects (Forbes et al. 2006). The prevalence of skin lesions in some fish species has been associated with PAH exposures from the Deepwater Horizon oil spill in the Gulf of Mexico in 2011 (Murawski et al. 2014). According to Tuvikene (1995), a major mode of PAH toxicity, particularly that of the lower molecular weight congeners, is interference with the functions of cell membranes and membranes associated with enzyme systems.

Several studies have reported oxidative stress, measured using selected biomarkers, in PAH-exposed fish (Abdel-Gawad and Khalil 2013). For example, reduced glutathione (GSH) levels, glutathione disulphide ratios, and whole-body vitamin E concentrations were reported in rainbow trout *Oncorhynchus mykiss* larvae exposed to the alkylated PAH, retene (Bauder at al. 2005). Similarly, killifish *Fundulus heteroclitus* inhabiting the PAH-contaminated Elizabeth River in Virginia, USA, exhibited elevated total GSH and mitochondrial lipid peroxidation (Bacanskas et al. 2004). In addition to oxidative stress markers, other indicators of toxicity have been demonstrated in PAH-exposed fish. Phenanthrene exposures at 50–400 µg l^{-1} were shown to have adverse effects on Tilapia *Oreochromis niloticus* (female) × *O. aureus* (male), including a decrease in hepatosomatic index (HSI) after 14 days of exposure (Xu et al. 2009). Developmental abnormalities associated with PAH exposures include jaw reductions, skeletal defects, pericardial and yolk sac oedema, and cardiac dysfunction (Huang et al. 2012).

Biological indices assessed at higher levels of organisation can have more direct ecological relevance, but are

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generally less specific to particular chemical exposures and have less early warning value than cellular or organism-level indices (Hanson et al. 2013). Some researchers advocate exploiting a mixture of cellular, organism, and population-level assays and indices, in order to increase the ecological reliability and to provide a holistic evaluation of contaminants (Manzo et al. 2014). According to the World Health Organization (2010), there is limited data on the toxicity of individual PAHs in experimental animals, as well as wild populations. Also, several studies have shown the influence of confounding factors, such as sex and age of fish on biomarker responses to pollutants in aquatic ecosystems (Fossi et al. 2002; Karami et al. 2015) Hence, there is a need to conduct and document toxicological effects of these substances on various life stages and sex of experimental animals in order to establish appropriate guidelines for environmental and health risk assessments. The African sharptooth catfish *Clarias gariepinus* was selected as the test organism, because of its ecological and commercial importance in several countries especially Nigeria (Esenowo and Ugwumba 2010). Also, it is a model organism that has been utilised extensively for various ecotoxicological studies.

The aim of this study was to investigate the effects of environmentally realistic concentrations of three PAHs naphthalene, phenanthrene and pyrene on an array of physiological, biochemical indices and histological indices in *Clarias gariepinus* broodstock, as well as their effects on teratogenic indices in *C. gariepinus* embryos spawned from exposed broodstock. The physiological indices serve as biomarkers at the individual level of organisation, whereas the biochemical and histological indices are biomarkers at the cellular level.

Materials and methods

Test chemicals

Polycyclic Aromatic Hydrocarbons (PAHs) - naphthalene (grade - 99%, CAS number - 91203), phenanthrene (assay - 98%, CAS number - 85018) and pyrene (assay – 98%, CAS number - 129000) were purchased from Sigma Aldrich, Ghana. The test compounds/PAHs were selected based on the frequency of their occurrence and levels in the surface water, sediment and fish species sampled from the Lagos lagoon in July 2012 (Sogbanmu et al. 2016).

Relative acute toxicity studies with Clarias gariepinus fingerlings

Acute toxicity studies with *Clarias gariepinus* fingerlings (weight range: 6–10 g; length range: 4.8–6.0 cm) were conducted according to the OECD (1992) protocol for fish acute toxicity tests in 2014. Because of the hydrophobic nature of the PAHs, stock solutions of the PAHs were prepared by dissolving them in an organic solvent (acetone). Stock solutions were made by dissolving 100 mg of PAHs in 1 ml of acetone and making up to 1 l with dechlorinated tap water. Test solutions were prepared from the stock solutions. A static-renewal bioassay protocol was employed where test solutions were replaced every 24 h, because of the high volatility of the test compounds (Luis and Guilhermino 2012). After an initial range finding test, definitive tests were conducted with four (4) test animals in triplicates exposed for 96 h to graded concentrations of PAHs as follows: naphthalene - 1.5, 7.5, 16 mg l^{-1}; phenanthrene - 1.2, 1.4, 1.8 mg l^{-1}; pyrene - 1.4, 2, 4, 6 mg l^{-1}; acetone (solvent) - 0.16 ml l^{-1} and untreated (dechlorinated water only).

Experimental design for sublethal toxicity studies with Clarias gariepinus broodstock

Twenty (20) broodstock *C. gariepinus* (male - weight range: 1 ± 0.6 kg; length range: 49.6 ± 1.2 cm and female - weight range: 1.1 ± 0.3 kg; length range: 46.8 ± 3.1 cm) were purchased from the University of Lagos fish farm. Four fish (ratio 1:1, male to female) were exposed to the PAH treatments and controls in 800–900 l water storage tanks containing 600 l of dechlorinated tap water. They were allowed to acclimatise to the new environment for a period of 2 weeks, during which they were fed with Coppens fish feed and the water was changed every 48 h. Sublethal studies with *C. gariepinus* broodstock were conducted according to Afolayan et al. (2014). The test animals were exposed to sublethal concentrations (1/10th of 96 h LC_{50}) derived from results of single action toxicity studies of the PAHs and untreated control for eight weeks and they were fed twice daily, morning and evening. A static-renewal bioassay protocol was employed where test solutions were replaced every 48 h, because of the high volatility of the test compounds. The concentrations used were; naphthalene 0.72 mg l^{-1}; phenanthrene 0.14 mg l^{-1}; pyrene 0.15 mg l^{-1}; acetone (solvent control) 0.0072 ml l^{-1} = 7.2 µl l^{-1} and water only (control medium).

The physicochemical parameters of the test media, including temperature, conductivity, dissolved oxygen, pH, salinity and total dissolved solids, were measured with Hanna instruments and maintained as follows; temperature (27.27 ± 1.51 °C), conductivity (0.19 ± 0.09 mS cm^{-1}), dissolved oxygen (9.29 ± 3.10 mg l^{-1}), pH (7.52 ± 0.42), salinity (0.1 ± 0.02 ppt) and total dissolved solids (0.13 ± 0.03 g l^{-1}).

Assessment of physiological indices in Clarias gariepinus broodstock exposed to sublethal concentrations of naphthalene, phenanthrene and pyrene

After eight (8) weeks of treatment, male and female broodstock fish were harvested and the physiological indices were evaluated as follows;

- Condition factor (K) = $\frac{\text{fish wet weight (g)}}{(\text{fish total length})^3\ (\text{cm})} \times 100$ (Salam and Mahmood 1993)
- Gill somatic index (GiSI) = $\frac{\text{fish gill weight (g)}}{\text{fish body weight (g)}} \times 100$ (Wingfield and Grimm 1977)
- Hepatosomatic index (HSI) = $\frac{\text{fish liver weight (g)}}{\text{fish body weight (g)}} \times 100$ (Wingfield and Grimm 1977
- Cardiosomatic index (CSI) = $\frac{\text{fish heart weight (g)}}{\text{body weight of the fish (g)}} \times 100$ body weight of the fish (g)
- Fecundity (total egg number) (Yelden and Avsar 2000) = $\frac{\text{total weight of ovary (g)} \times \text{no. of eggs in sub-sample}}{\text{weight of sub-sample (g)}}$

Evaluation of biochemical indices in Clarias gariepinus broodstock exposed to sublethal concentrations of naphthalene, phenanthrene and pyrene

Following eight weeks of treatment, male and female broodstock fishes were harvested and euthanised by a single sharp blow to the head with the aid of a hammer

followed by pithing (AVMA 2013). Liver and gill tissues were excised, rinsed in ice cold isolation medium (0.25 M sucrose, 5 mM tris HCL), lightly blotted and weighed. Thereafter, they were cut into fragments, homogenised (9% w/v) in 100 % methanol and centrifuged at 10 000 × *g* for 15 min at 4 °C following Hermes-Lima et al. (1995). The supernatant was collected for substrate and enzyme assays as described in Sogbanmu and Otitoloju (2014). Briefly, protein concentration was determined using the Biuret method (Gonall et al. 1949). 5 ml of blank Biuret reagent was prepared by dissolving $CuSO_4.5H_20$ crystal in 500 ml of distilled water which was added to sample blank. These were mixed well and allowed to stand for 20 min at room temperature (25–27 °C). Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using optical density for standard × concentration of standard. Then, the levels of homogenised tissue malondialdehyde (MDA), an index of lipid peroxidation were estimated by thiobarbituric acid reaction (TBARS Assay) using the method of Yagi (1998), where MDA is measured spectrophotometrically at absorbance levels of 535 nm. The results were expressed in MDA units mg protein^{-1}. Superoxide dismutase (SOD) enzyme activity was determined according to the method of Sun and Zigman (1978).

The assay determined the difference between superoxide anion decomposition and production i.e. its ability to inhibit the autoxidation of epinephrine. Enzyme activity was monitored at absorbance level of 450 nm. Concentrations were expressed as SOD units mg protein^{-1}, where one unit is defined as the amount of enzyme needed to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25 °C and pH 7.8. Serum catalase (CAT) activity was determined according to the method of Beers and Sizer, as described by Usoh et al. (2005) by measuring the decrease in absorbance at 240 nm, because of the decomposition of hydrogen peroxide (H_2O_2) in a UV recording spectrophotometer. The results were expressed in CAT units mg protein^{-1}, where one unit is the amount of enzyme that hydrolyses 1 µmol of H_2O_2 per minute and per milligram of protein at 30 °C and pH 8.0. The reduced gluthathione (GSH) content of liver tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). The absorbance was read at 412 nm. The activity of gluthaione S-transferase (GST) was determined according to the method of Habig and Jakoby (1974). GST activity was measured by monitoring at absorbance level of 340 nm, the formation of a conjugate between 1 Mm GSH and 1 mM 1–chloro-2,4–dinitrobenzene (CDNB). The results were expressed in GST units mg protein^{-1} or U mg l^{-1}, where one unit is defined as the amount of enzyme that conjugates 1 µmol of CDNB per minute and per milligram of proteins at 25 °C and pH 7.4.

Assessment of histological indices in Clarias gariepinus broodstock exposed to sublethal concentrations of naphthalene, phenanthrene and pyrene

After eight weeks of treatment, male and female broodstock fishes were harvested and euthanised by a single sharp blow to the head with the aid of a hammer followed by pithing (AVMA 2013). Livers, gills and ovaries from the exposed fishes were harvested and immediately transferred to glass containers containing the fixative Bouin's fluid. Tissue processing was conducted following Eseigbe et al. (2013). Briefly, after fixing the tissues in Bouin's fluid for 8 h, they were taken through a series of dehydration in graded ethanol followed by clearing in xylene and embedding in paraffin wax (melting point 56 °C). Serial tissue sections were cut in a rotary microtome at 2–5 µm thickness. Subsequently, the tissue sections were serially rehydrated through xylene, followed by absolute ethanol and water. Tissue sections were stained with haematoxylin and eosin, dehydrated in graded ethanol, cleared in xylene and mounted in Canada balsam. Slides were dried on a hot plate for 2 h before imaging. Slides imaging and descriptions of alterations were carried out by a pathologist.

Determination of teratogenic (histological) indices in Clarias gariepinus embryos

Teratogenic indices assessed through histology of the embryos spawned from pre-exposed *C. gariepinus* broodstock were conducted according to Sayed et al. (2012). Briefly, after eight weeks of treatment, one female broodstock *C. gariepinus* from each treatment tank was injected with Ovaprim (Syndel laboratories Ltd, Canada) hormone at 0.5 ml per kg of fish. After 10 hours latency period, slight pressure was applied on the abdomen of the females leading to a running out of the eggs, which were collected in a plastic bowl. Two males from each tank were euthanised and the testes were carefully removed with the aid of a new razor blade. An incision was carefully made on each testis to let out the milt used for fertilizing the eggs. Fertilisation was aided with the addition of saline water to the mixture and the bowl was gently rotated to ensure adequate mixing of the milt with the eggs. Fertilised eggs were identified and confirmed with the aid of a dissecting microscope. Five (5) fertilised eggs (within 1 h post-fertilisation) were subsequently transferred to plain sample bottles containing Bouin's fluid before processing for histological analysis.

Statistical analyses

Toxicity data were evaluated using probit analysis (Finney 1971). One-way analysis of variance (ANOVA) was used to test for significant difference between treatment means and control for the physiological and biochemical indices data. Level of significance was placed at $p < 0.05$ (SPSS version 16.0). Post-hoc tests were conducted using Duncan's Multiple Range Test (Duncan 1955).

Results

Relative acute toxicity studies with Clarias gariepinus fingerlings

The results of the acute toxicity studies with *C. gariepinus* fingerlings showed that, based on the 96 h LC_{50} values, phenanthrene (1.41 mg l^{-1}), pyrene (1.53 mg l^{-1}) were five times more toxic than naphthalene (7.21 mg l^{-1}) (Table 1).

Clarias gariepinus broodstock physiological indices

The assessment of physiological indices in *C. gariepinus* broodstock exposed to sublethal PAH concentrations

Table 1: Single action toxicity of polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and pyrene) against *Clarias gariepinus* fingerlings based on 96 h mortality data

Treatment (mg l^{-1})	LC_{50} (95% CL)	Slope ± SE	Probit line equation	DF	TF
Naphthalene	7.21	2.86 ± 0.80	$y = 2.86x - 2.45$	1	1
Phenanthrene	1.41 (1.13–1.68)	8.10 ± 3.20	$y = 8.10x - 1.20$	1	~5
Pyrene	1.53 (0.01–2.53)	1.75 ± 0.81	$y = 1.75x - 0.32$	2	~5

CL = Confidence limit; DF = Degrees of freedom; SE = Standard error; 96 h LC_{50} - median lethal concentration at 96 h

TF = Toxicity factor = $\frac{\text{96 h } LC_{50} \text{ of least toxic compound}}{\text{96 h } LC_{50} \text{ of most toxic compound}}$

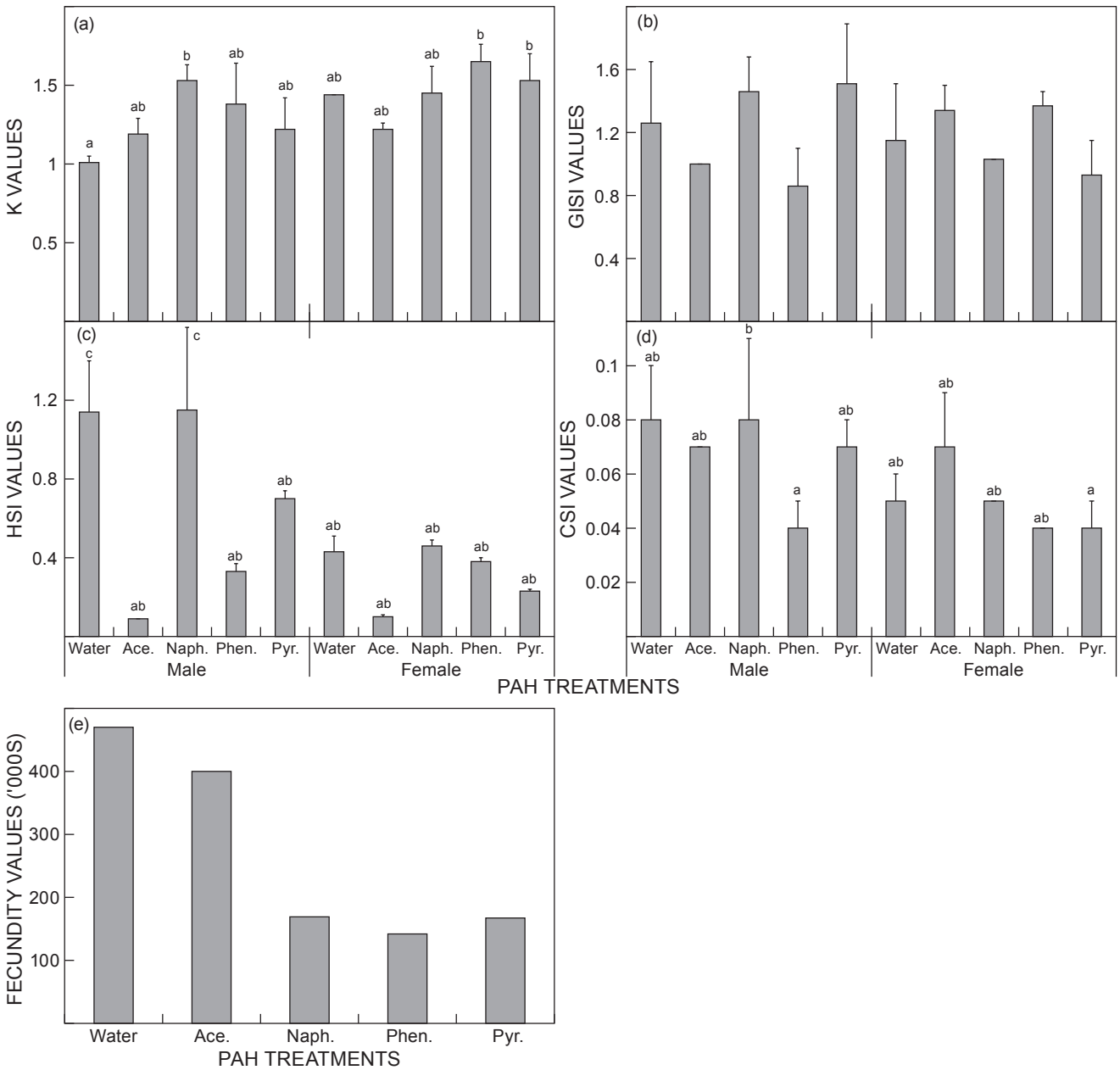

Figure 1: Physiological indices in *Clarias gariepinus* broodstock after eight weeks of exposure to sublethal concentrations of naphthalene, phenanthrene and pyrene. (a) Condition Factor (K), (b) Gill Somatic Index (GiSI), (c) Hepatosomatic Index (HSI), (d) Cardiosomatic Index (CSI), (e) Fecundity. Note: Ace (Acetone solvent control) - 0.72 µl l^{-1}; Naph. (Naphthalene) - 0.72 mg l^{-1}; Phen. (Phenanthrene) - 0.15 mg l^{-1}; Pyr. (Pyrene) - 0.14 mg l^{-1}; $n = 2$. Dissimilar letters in superscripts across columns represent significant difference ($p < 0.05$) between treatments

revealed that hepatosomatic index (HSI) values were significantly higher ($p < 0.05$) in naphthalene and pyrene-treated males (1.15 ± 0.42 and 0.70 ± 0.04) compared with solvent control (0.09 ± 0.00) (Figures 1a to d). There were no significant differences ($p > 0.05$) in the condition factor, gill somatic index and cardiosomatic index of PAH-exposed broodstock and acetone control. Fecundity was lower by factors of 2.4 ×, 2.8 × and 2.4 × respectively in the naphthalene, phenanthrene and pyrene treatments compared with acetone control (Figure 1e).

Clarias gariepinus broodstock biochemical indices

The evaluation of gill and liver biochemical indices in *C. gariepinus* broodstock exposed to sublethal PAH concentrations showed that there were no statistically significant ($p > 0.05$) differences between the exposed and control groups in the values of MDA, SOD, CAT, GSH, and GST (Figures 2a to e). However, there was a significant ($p < 0.05$) reduction in liver catalase levels of phenanthrene-treated females (6.80 ± 0.95 U mg l^{-1} protein) compared with the solvent control (16.03 ± 1.97) (Figure 2c).

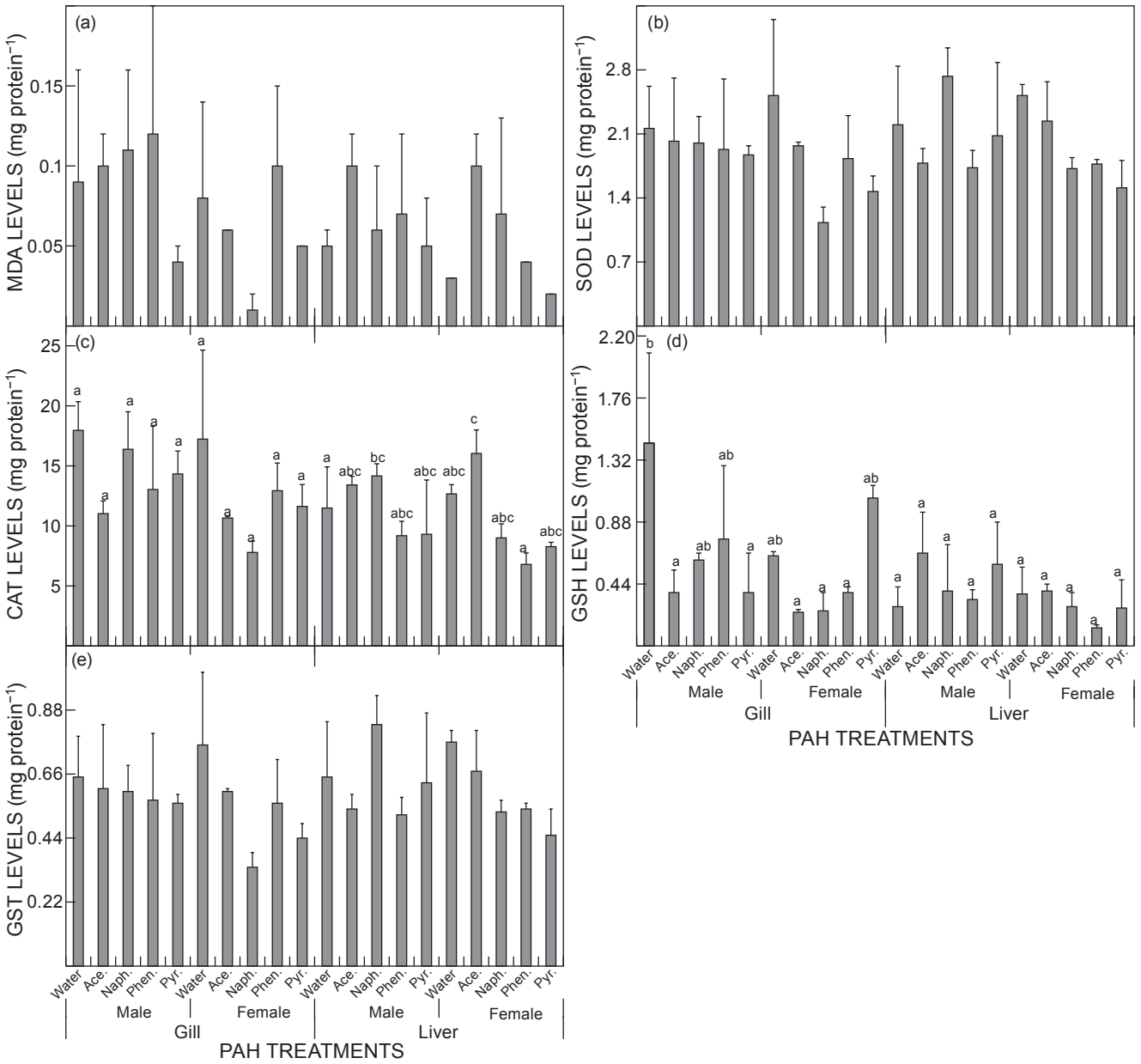

Figure 2: Biochemical Indices in gills and liver of *Clarias gariepinus* broodstock after eight weeks of exposure to sublethal concentrations of naphthalene, phenanthrene and pyrene. (a) Malondialdehyde (MDA), (b) Superoxide dismutase (SOD), (c) Catalase (CAT), (d) Reduced Gluthathione (GSH), (e) Gluthathione-S-Transferase (GST). Note: Ace (Acetone solvent control) - 0.72 µl l^{-1}; Naph. (Naphthalene) - 0.72 mg l^{-1}; Phen. (Phenanthrene) - 0.15 mg l^{-1}; Pyr. (Pyrene) - 0.14 mg l^{-1}; $n = 2$. Dissimilar letters in superscripts across columns represent significant difference ($p < 0.05$) between treatments.

Clarias gariepinus broodstock histological indices

The results of the histological evaluations in the gills, liver and ovaries of *C. gariepinus* exposed to sublethal PAH concentrations revealed various histological alterations (gill histology (Figures 3a to j); liver histology (Figures 4a to j); ovary histology (Figures 5a to e). Gill alterations observed include epithelial hyperplasia and lifting in phenanthrene-treated males (Figure 3g), oedema in phenanthrene-treated

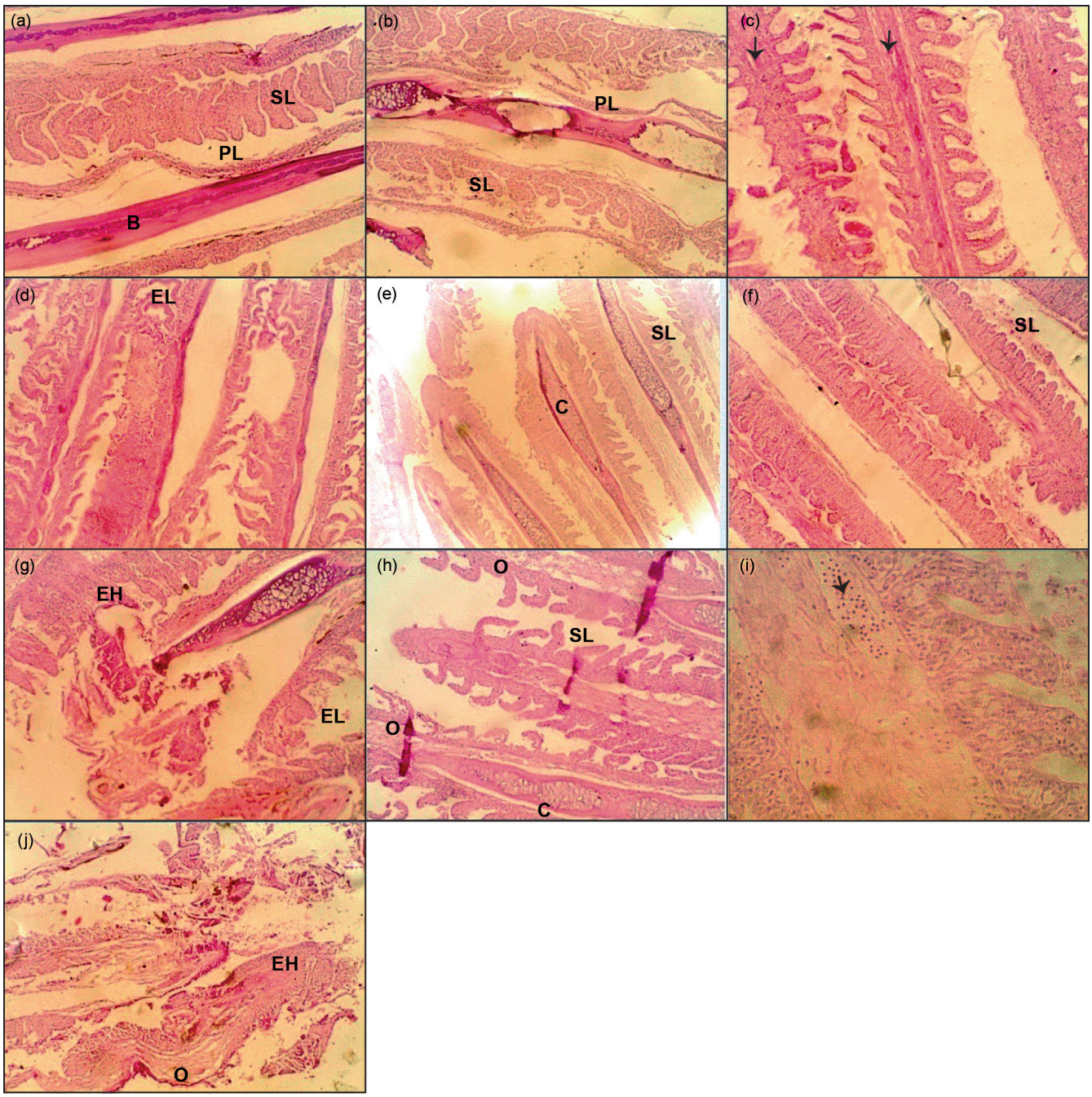

Figure 3: Photomicrographs of longitudinal histological sections through the gills of broodstock *Clarias gariepinus* after eight weeks of exposure to sublethal concentrations of naphthalene, phenanthrene, pyrene and controls. (a) Untreated male - normal appearing secondary lamellae SL and primary lamellae PL. Bone B is also normal (mag. - × 100). (b) Untreated female - normal appearing gills with primary lamellae PL and secondary lamellae SL. (mag. - × 100). (c) Acetone (7.2 µl l^{-1}) Male - arrows pointing toward congestion (mag. - × 100). (d) Acetone (7.2 µl l^{-1}) Female - epithelial lifting EL (mag. - × 100). (e) Naphthalene (0.72 mg l^{-1}) Male - normal cartilage C and secondary lamellae SL (mag. - × 40). (f) Naphthalene (0.72 mg l^{-1}) Female - normal appearing secondary lamellae SL. (mag. - × 100). (g) Phenanthrene (0.14 mg l^{-1}) Male - epithelial hyperplasia EH, epithelial lifting EL (mag. - × 100). (h) Phenanthrene (0.14 mg l^{-1}) Female - normal cartilage C and secondary lamellae SL. Oedema O (mag. - × 100). (i) Pyrene (0.15 mg l^{-1}) Male - Inflammatory cells (arrow) are seen (mag. - × 400). (j) Pyrene (0.15 mg l^{-1}) Female - distorted gill architecture with epithelial hyperplasia EH, Oedema O (mag. - × 100)

females (Figure 3h), inflammatory cells in pyrene-treated males (Figure 3i) and oedema, epithelial hyperplasia and lifting in pyrene-treated females (Figure 3j). Liver histological alterations were mild to moderate vacuolation in male naphthalene-treated catfish (Figure 4e), mild vacuolation and haemosiderin pigment in female naphthalene-treated catfish (Figure 4f), mild to moderate vacuolation in male phenanthrene-treated catfish (Figure 4g), vacuolation in female phenanthrene-treated catfish (Figure 4h), severe vacuolation in male pyrene-treated catfish (Figure 4i) and sinusoidal

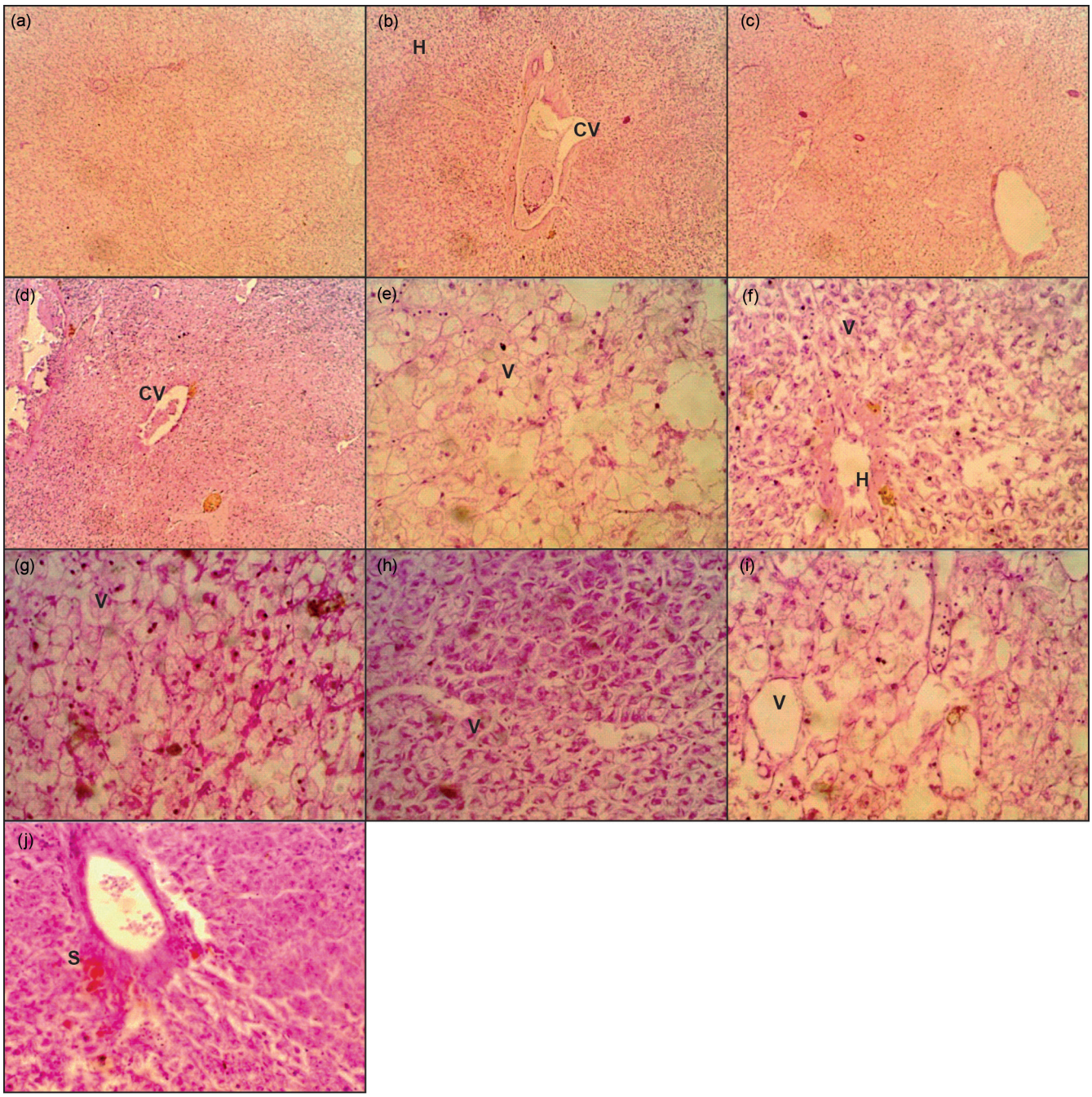

Figure 4: Photomicrographs of histological longitudinal sections through the liver of broodstock *Clarias gariepinus* after eight weeks of exposure to sublethal concentrations of naphthalene, phenanthrene, pyrene and controls. (a) Untreated male - normal liver architecture (mag. - × 100). (b) Untreated female - normal liver architecture with radiating hepatocytes HC towards the central vein CV, which appears congested (mag. - × 100). (c) Acetone (7.2 µl l^{-1}) male - preserved liver architecture (mag. - × 100). (d) Acetone (7.2 µl l^{-1}) female - dilated central vein CV (mag. - × 100). (e) Naphthalene (0.72 mg l^{-1}) male - mild to moderate vacuolation V (mag. - × 400). (f) Naphthalene (0.72 mg l^{-1}) female - mild vacuolation V, Haemosederin pigment H (mag. - × 400). (g) Phenanthrene (0.14 mg l^{-1}) male - mild to moderate vacuolation V (mag. - × 400). (h) Phenanthrene (0.14 mg l^{-1}) female - vacuolation V (mag. - × 400). (i) Pyrene (0.15 mg l^{-1}) male - severe vacuolation, V (mag. - × 400). (j) Pyrene (0.15 mg l^{-1}) female - sinusoidal congestion S (mag. - × 400)

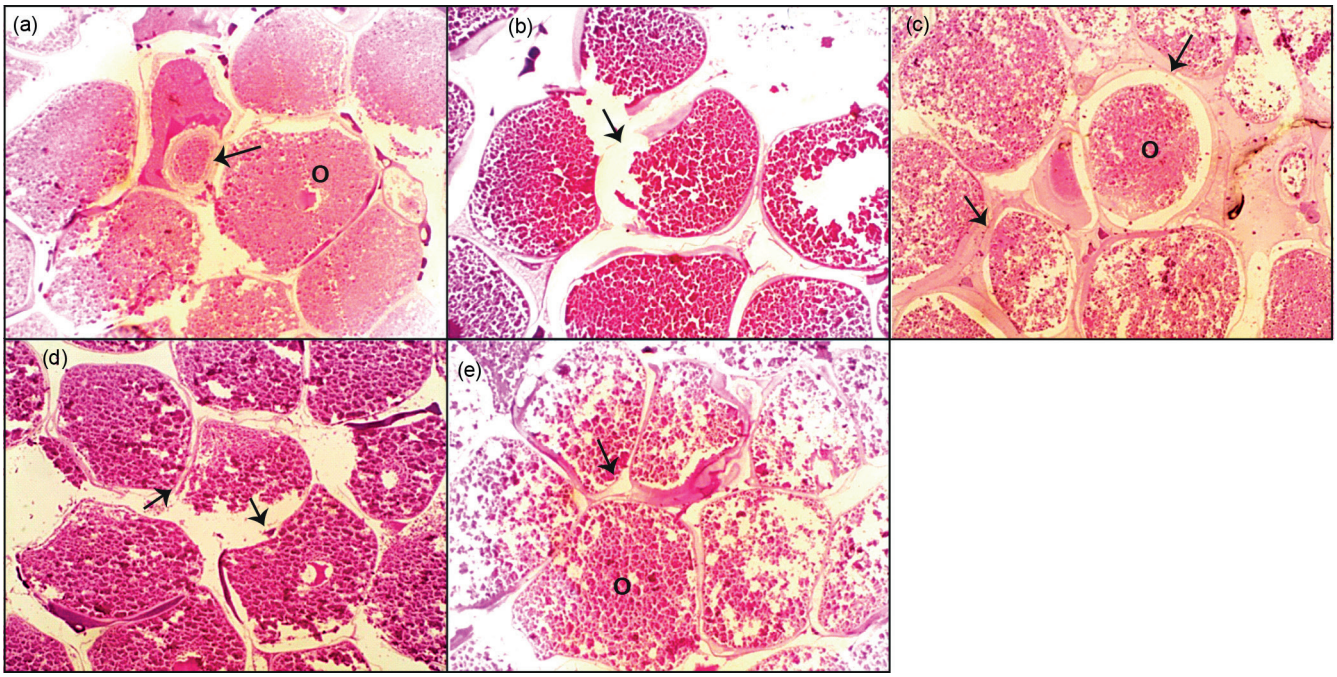

Figure 5: Photomicrographs of histological longitudinal sections through the ovary of female *Clarias gariepinus* broodstock after eight weeks of exposure to sublethal concentrations of naphthalene, phenanthrene, pyrene and controls. (a) Untreated - normal oocyte O and zona radiata (arrow pointing towards) (mag. - × 40). (b) Acetone (7.2 µl l^{-1}) - distorted zona radiata (mag. - × 40). (c) Naphthalene (0.72 mg l^{-1}) - normal oocyte O and zona radiata (arrowed) (mag. - × 40). (d) Phenanthrene (0.14 mg l^{-1}) - degenerated zona radiata (arrowed) (mag. - × 40). (e) Pyrene (0.15 mg l^{-1}) - normal oocyte O, the zona radiata is somewhat preserved (arrowed) (mag. - × 40)

congestion in female pyrene-treated catfish (Figure 4j). Ovary histological alteration was degenerated zona radiata in the phenanthrene-treated *C. gariepinus* only (Figure 5d).

Teratogenic (histological) indices in embryos spawned from PAHs-treated Clarias gariepinus

The assessment of histological alterations in embryos spawned from *C. gariepinus* broodstock pre-exposed to sublethal concentrations of naphthalene, phenanthrene and pyrene revealed no histological alterations (Figure 6a to e).

Discussion

In this study, the 3– and 4–ringed PAHs, phenanthrene and pyrene respectively were more toxic compared with the 2–ringed PAH, naphthalene. Similar results were reported for *Grandidierella japonica* (benthic amphipod) and *Takifugu obscurus* (puffer fish) after 96 h exposure to naphthalene (5.3 and 8.69 mg l^{-1}), phenanthrene (0.35 and 0.43 mg l^{-1}) and pyrene (0.06 and 0.07 mg l^{-1}), respectively (Lee et al. 2004, 2005). Cheevaporn (2010) and Oliveira et al. (2012) also reported 96 h LC_{50} values of 0.80 and 0.87 mg l^{-1}, respectively, for Nile tilapia *Oreochromis niloticus* and common goby *Pomatoschistus microps* exposed to Pyrene. The lower toxicity of naphthalene compared with the other PAHs could be attributed to its high volatility, which reduces the concentration available for uptake and metabolism by the exposed fish. Also, the toxicity of PAHs increases with their lipohilicity (Barta et al. 2005, Saiz et al. 2009).

Hepatosomatic index (HSI) is usually indicative of toxicant effects (Sinaei and Maschinchian 2014). The higher HSI values observed in the naphthalene and pyrene-treated male catfish compared with the solvent control (acetone) indicates PAH toxicity in the fish, because the liver is a target organ for detoxification of xenobiotics. Increases in liver size are commonly seen in fish that have been exposed to toxicants, because of the induction of hepatic microsomal P-450 for detoxification of the pollutants (Huuskonen and Lindstrom-Seppa 1995, Kumari 2014). However, the differential responses between the males and females show that the females might be more resistant to the toxic effects of the PAHs compared with the males (Anderson et al. 2003). Also, these differential responses can serve as sensitive sex-specific biomarkers to these PAHs (Chen et al. 2016). The reduction in the fecundity of the exposed female *C. gariepinus* in this study is similar to the observations of Perrichon et al. (2015) who reported a significant reduction in egg production in zebrafish after dietary exposure to PAHs.

According to Ji et al. (2012), there is a balance between the generation of reactive oxygen species (ROS) (leading to oxidative stress) and their elimination by different antioxidant enzymes under normal physiological conditions. The similarities in MDA and antioxidant enzyme levels observed in the exposed and control groups might result form the counteraction of the ROS produced by the antioxidant enzymes bringing about a balance in the biochemical indices. This result disagrees with that of Otitoloju and Olagoke (2011), who reported significant increases in MDA in the liver and gills of *Clarias gariepinus* exposed to sublethal

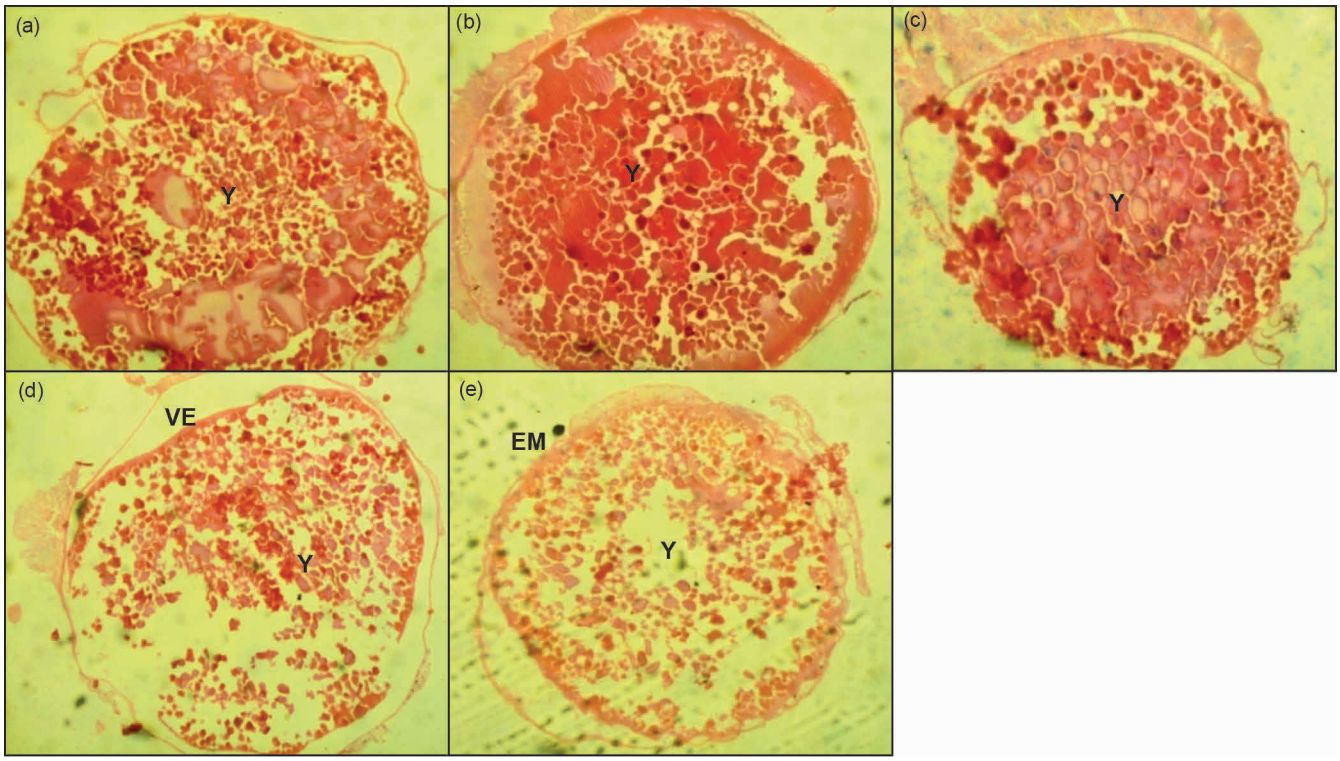

Figure 6: Photomicrographs of histological sections through embryos (within 1 h post-fertilisation) of *Clarias gariepinus* spawned from broodstock after eight weeks of exposure to naphthalene, phenanthrene, pyrene and controls. (a) Untreated - normal appearing embryo. mag. - × 100. (b) Acetone (7.2 µl l^{-1}) - normal appearing embryo. mag. - × 100. (c) Naphthalene (0.72 mg l^{-1}) - normal appearing embryo. mag. - × 100. (d) Phenanthrene (0.14 mg l^{-1}) - normal appearing embryo. Mag. - × 100. (e) Pyrene (0.15 mg l^{-1}) - normal appearing embryo. Mag. - × 100. Y - Yolk, EM - egg membrane, VE - vitelline membrane

concentrations of petroleum hydrocarbons (toluene, xylene and benzene). The life stage of fish used in this study could affect the uptake and metabolism of the sublethal concentrations of PAHs used in this study, because of its small body surface area compared with those of earlier life stages (Rhind 2009). However, because the antioxidant enzymes are especially high in the liver, a major organ for xenobiotic uptake and enzymatic transformation of ROS, the lower CAT levels observed in the liver of the phenanthrene-treated female fish could be a result of the antioxidant's response in combating the effects of lipid peroxidation.

Gills are the primary target of waterborne contaminants, because they are the first port of entry into the fish (Poleksic and Mitrovic-Tutundzic 1994). The gill alterations observed are consistent with those reported in the gills of *Mugil cephalus* and *Lutjanus russellii* from the Koggala lagoon, Sri Lanka, polluted by PAHs, amongst other anthropogenic pollutants (Ranansingha and Pathiratne 2015). Similarly, gill hyperplasia has been observed in several fish species exposed to oil under laboratory and field conditions (Akaishi et al. 2004; Simonato et al. 2008). Because the fish gills perform vital functions, such as respiration, osmoregulation and waste excretion (Poleksic and Mitrovic-Tutundzic 1994), the alterations observed could impair the normal functioning of the gills leading to physiological distress (Ranansingha and Pathiratne 2015).

According to Leonardi et al. (2009), liver histopathology is a biomarker of environmental stress, because it provides a definite biological end-point of historical exposure. The liver alterations observed, such as sinusoidal congestion indicative of circulatory disturbances and hepatocyte vacuolation (regressive disturbances) have also been observed in fishes captured from the PAHs-polluted Koggala Lagoon, Sri Lanka (Ranansingha and Pathiratne 2015). Also, extensive vacuolisation in the liver of *Clarias gariepinus* exposed to phenanthrene concentrations between 6.2 and 72 µg l^{-1} has been reported (Karami et al. 2016).

Furthermore, the results of the histological examination of the ovaries revealed little evidence of reproductive impairment, except for phenanthrene-treated catfish. This agrees with Love and Goldberg (2009), who reported a lack of pronounced atresia in Pacific sanddab *Citharichthys sordidus* captured at oil platforms in the southern California Bight. Oocyte atresia is characterised by the degeneration of the oocyte at any stage in its development (Love and Goldberg 2009; Gaber 2013) and could be pathologic as a result of exposure to xenobiotic compounds despite its normal occurrence in the ovaries of all fish species (Hinton et al. 1992).

Several studies have documented the effect of PAHs and their mixtures on early life stages of fish (Carls et al. 2008; Incardona et al. 2012; Sogbanmu et al. 2016). However, prenatal or parental PAH exposure, as demonstrated in this study, did result in histological alterations; though,

multigenerational phenotypic impacts have been observed in zebrafish exposed to benzo(a)pyrene (Corrales et al. 2014). The reason for the lack of histological alterations in the embryos of *C. gariepinus* in this study could result from lack of conspicuous reproductive impairment in the parent fish. Other reasons could be the route of entry of the toxicants, early depuration of the PAHs, lack of transmission of the PAHs or the exposure duration of the parent generation was not long enough to allow for transfer of the PAHs (Perrichon et al. 2015).

Conclusions

Multiple biomarkers, including physiological, biochemical, histological and teratogenic indicators were used to evaluate the impact of PAH contamination in a commercially important fish species. Sex-specific responses (differential responses to biomarkers assessed in male and female fish) and the utility of biomarkers at the cellular and individual levels of organisation were demonstrated for holistic evaluations of PAHs in ecotoxicological studies. Because of the ubiquity of PAH contamination in urban Nigerian water bodies, ecotoxicological monitoring programmes using multiple biomarkers, such as those we examined, might be useful for assessing effects of priority pollutants, for instance PAHs, on commercially important fisheries.

Declaration of interest

The authors report no conflict of interest.

Ethics Statement

All applicable international guidelines for the care and use of animals were followed. This study followed the principles in the AVMA Guidelines for the euthanasia of animals (AVMA 2013).

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