Detection of Polynuclear aromatic hydrocarbons in mud skippers (*Gobiformes oxudecidae*) from Jones Creek, Delta State, Southern Nigeria

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Abstract

The measurement of polynuclear aromatic hydrocarbons (PAHs) was conducted with respect to Gobiformes oxudecidae (mudskipper) specimens collected from Jones creek, Delta State. They were analyzed for 16 priority PAHs spanning through a total duration of eighteen months which adequately captured both wet and dry seasons. The ΣPAHs varied from 0.000 to 0.106 mg/kg for mud skipper samples within the aforesaid duration of the research. The most dominant PAH observed were chrysene and benzo(a)anthracene with amounts of 0.106 ± 0.095 mg/kg and 0.042 ± 0.030 mg/kg. The most abundant PAH in terms of its detection is chrysene accounting for 20.63% of the 16 PAHs while the least was dibenzo(a,h) anthracene with relative abundance of 0.37%. The individual component ratio revealed that refining operations, domestic and industrial discharges and possible storm run-off could

likely be the source of contamination. The total amounts of PAHs in the rainy season were comparatively higher than values obtained in the dry season. Distribution patterns revealed that PAHs possessing 3 and 4 rings were dominant which confirmed the pyrogenic source of the detected PAH. The dominance of the low molecular weight PAHs across the sampling stations was indicative of their bioavailability for uptake by biota. Diagnostic ratio among others employed included phenanthrene/anthracene and benzo(a)anthracene/chrysene. Calculation based on these ratios to determine the PAHs emission origins suggested pyrolytic origin of the sediment associated PAHs coupled with a pyrolytic or pyrogenic source of PAHs present in the mudskipper specimens.

Key word: diagnostic ratio, Jones creek, mudskipper, PAHs, sediment

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) exist as chemical contaminants engendered *via* the pyrolysis or inadequate combustion of organic-carrying substance, remains from fossil fuels such as coal and other carbon substance comprising degradable (food) at high temperatures below oxygen-deficient environments and also classified as organic lipophilic moieties composed of two or more benzene rings (Gutiérrez *et al.*, 2015; Kılıç *et al.*, 2017). PAHs are known to encompass over 200 organic moieties comprising two or more fused aromatic rings (Domingo and Nadal 2015). Environmental PAHs can emanate from non - anthropogenic origins, which include; forest fires and volcanic emissions, and from man – made sources exemplified by coal combustion, emissions *via* vehicles exhaust, and oil lubricants of engine and cigarette smoke (Amirdivani *et al.*, 2019).

PAHs can be grouped with reference to the aromatic rings content, broadly tagged as light (2–3 rings) or heavy (4–6 rings) compounds (Purcaro *et al.*, 2013). PAHs have carcinogenic and mutagenic effects owing to their chemical structure. Pollution of soil, sediment, water and air environments can lead to contamination of food (flora and fauna) derived from these matrices with PAHs and substantial levels of these moieties have also been known to be at elevated temperatures during food processing (Chung *et al*, 2011). Recently, the detection of PAHs have been documented with respect to a variety of environmental media, such as; air, sediments, soils, dust (Hussain *et al.*, 2015; Kamal *et al.*, 2016; Hamid *et al.*, 2018) and even in pristine niches (e.g. Norwegian Arctic) (Hermanson *et al.*, 2005; Macdonald *et al.*, 2005). The United States Environmental Protection Agency (US EPA) acknowledged sixteen PAHs as "consent decree" primacy contaminants (Suman *et al*, 2016) of which seven PAHs have been tagged as budding anthropogenic carcinogens: Benz(a)anthracene (BaA), Benzo(a)pyrene (BaP), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Chrysene (Chry), Dibenz(ah)anthracene (DahA), and Indeno(1,2,3-cd) pyrene (IP) (Yu *et al.*, 2014).

The aforementioned group of contaminants (PAHs) gets in whole or parts of the environment: atmosphere, sediment, waters and soils. By extension, the risk of contamination of plants and animal products cannot be overemphasized. The unswerving ingestion of soiled food and forage by livestock is the primary route of entrance of organic pollutants into body of animals. Consequently, consumables ingested by humans, such as meat, fish, snails, milk are constantly being contaminated. It has been confirmed that 88–98% of cases of exposure to PAHs are foodborne (Bechtel *et al.*, 2009; Gutiérrez *et al.*, 2015).

Jones Creek is among the creeks located within the Niger Delta region of Southern Nigeria. Generally, the Niger delta area is known for myriads of oil exploration and exploitation activities (both legal and illegal). Numerous oil spills from these nefarious operations has had devastating impact on the environment (Raji and Abejude, 2013). Several authors have

observed that the anthropogenic activities have had potential adverse impacts on several components such as coastal and marine habitats, wildlife and fisheries sections of this fragile ecosystem (Fattal *et al.*, 2010; Allan *et al.,* 2012). On a daily basis, the principal diet of residents living in the Jones Creek are directly sourced from what the marine ecosystem provides consequently creating high potential for human exposure to these carcinogenic pollutants.

The primary objective of this research was to evaluate the amounts of PAHs in *Gobiformes oxudecidae* (mud skipper) specimens with respect to their toxicity potential and distribution comprehension. This work would provide a robust database for the PAHs levels in mudskipper within Jones creek.

MATERIALS AND METHODS

Description of sampling locations

Jones Creek which traverses some major oil producing areas of the Niger Delta region was studied. The creek is within the confines of Nigeria's Oil Mining Lease (OML) 42, Western Niger Delta of Nigeria (N05°40′40.6″ and E005°27′06.22″). The inhabitants are majorly fishermen and subsistence farmers. The sampling locations are adequately represented in the map depicted below (Fig. 1). Samples were collected monthly from two sites namely Jones Creek and Ibedi River which served as control site from March 2015 to August 2016.
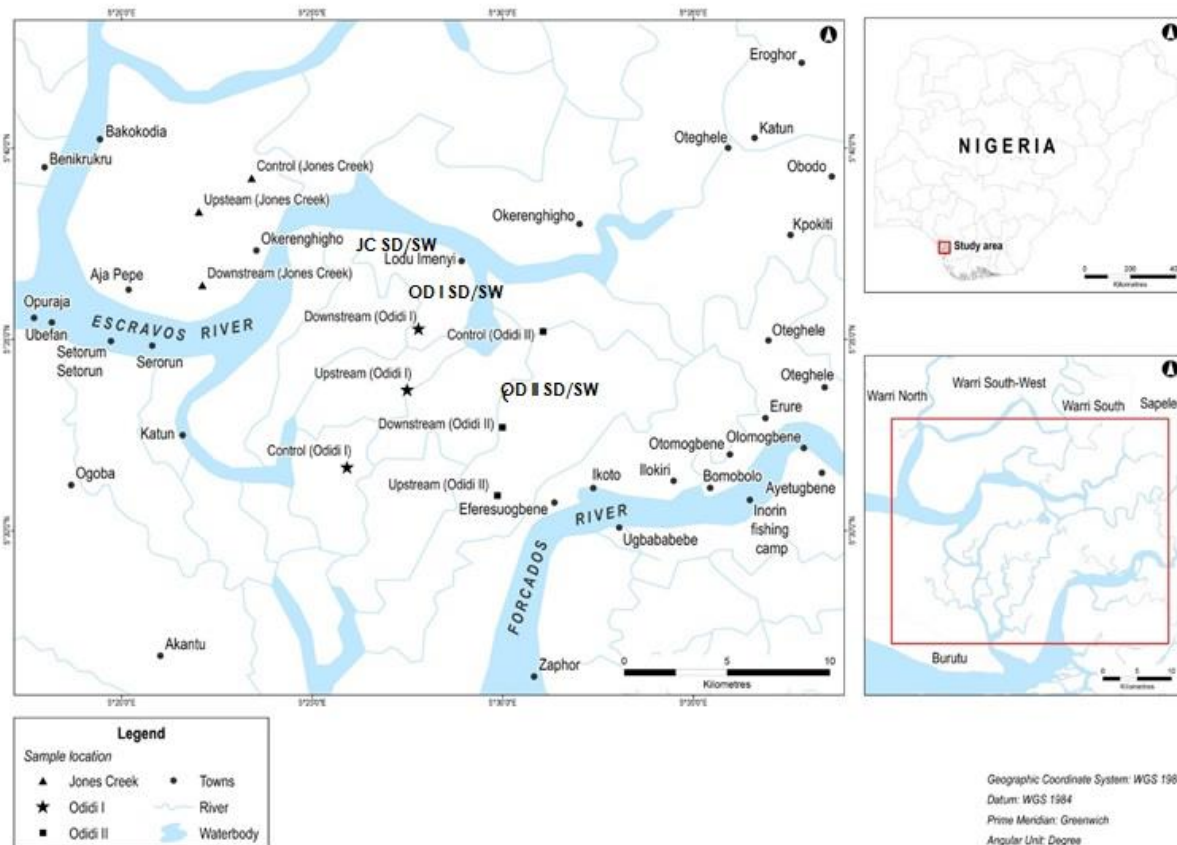

Fig.1: Map Showing sampling location

Chemicals

All the chemicals used were of either analytical or chromatographic grade. Methylene chloride was sourced from Sigma Aldrich (UK), hexane was purchased from Fisher Scientific. A standard solution with the following sixteen PAHs Naphthalene (NAP), acenaphthalene (ACL), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), Chrysene (CHR),

benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF) benzo[a]pyrene (BaP), indeno[1,2,3]pyrene (IP), dibenzo[a,h]anthracene (DbA), benzo[g,h,i]perylene (BPer) was obtained from Accustandards (USA). All the glassware, prior to usage were thoroughly washed using detergent and water, then rinsed with acetone and dried at 90°C.

Sample Collection and Clean-up
Mudskipper specimens were collected monthly for a sampling duration of eighteen months. Mudskipper samples were appropriately labeled and placed in an icebox before being transported to the laboratory. Samples were the stored at < 4°C until further studies.

Sample extraction and clean-up
The mudskipper specimens were pulverized and then ten grams (10g) portion was weighed and mixed with 5 g of Na_2SO_4. The sample was then immersed in an ultrasonic bath with 100 mL freshly prepared acetone and dichloromethane solution (1:1 v/v). The solution was sonicated for about 45 mins and the extracts concentrated to about 5mL using a rotary evaporator followed by blowing down under a gentle nitrogen stream to a volume of about 2 mL.

Prior to analysis, sample clean-up was conducted with the aid of Gas chormatography with flame ionization detection (GC-FID). The extracts were passed through a gel permeation chromatography (GPC) column and eluted with 50:50 dichloromethane: hexane solution. The eluent was then evaporated under a gentle stream of nitrogen (> 99% purity) to a final volume of 1ml for injection into the GC/FID.

Gas Chromatography Operating Conditions
Separation of the analytes was done on a HP-5, 30 m x 0.25 mm column and analyzed using a GC-FID (HP 6890 series) in splitless mode. Nitrogen (10.2 psi) was used as carrier gas at 1.5 mL/min. Both injector and detector temperatures were 250°C and 320°C respectively. Separation was conducted using the following oven temperature programme: start temperature at 80°C, held for 1 min, then ramped at the rate of 20°C/ min to 280°C, and further ramped at a rate of 2.5°C/min to 300°C and held for 10 mins. Peak detection and integration were attained using the ChemStation software. Identification and quantification were conducted with the aid of a 7 point calibration standard of known amounts using the internal standard.

The amount of each PAH in the analytes was calculated using the equation below:

$$Conc.\,of\,analyte\left(\frac{up}{kp}\right) = \frac{Ax.\,vt - D}{wd - CF} \ldots\ldots\ldots\ldots(1)$$

Where:

A_x	=	Area of the analyte in area count
V_t	=	Total volume of extract in µL
D	=	Dilution factor, dimensionless
W_d	=	Dry weight of samples extracted in grams (g)
CF	=	Average calibration factor for target PAH analyte

Quality control and data analysis
Blank samples were run intermittently during analysis, and the method blanks were treated in an identical manner as the actual samples. They were run to investigate the possibility of contamination peaks. Any PAH detected in the blanks were subtracted from the sample

extract. A ±0.05 min window retention time was employed for peak detection in the calibration standard. The accuracy of the analytical method employed was assessed through matrix spike techniques using surrogate standards. The average recovery ranged from 88-115% for all the recovery standards.

$$\% \text{ Recovery} = \frac{concentration\ extracted}{concentration\ spiked}\ x\ 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots. (2)$$

Samples were also analyzed in triplicates. Data analysis (mean, standard deviation, ANOVA) was carried out using Excel and SigmaPlot®. Total toxicity equivalent concentration (TTEC) was determined using the equation,

TTEC = ΣCn TEFn

Where Cn is the amount of the particular congener n in the PAH mixture, TEFn is the toxicity equivalency factor for the individual congener n.

RESULTS AND DISCUSSION

Concentrations of PAHs detected in mudskipper in Jones Creek and Control location throughout the sampling cycle is depicted in Table 1. In descending order, benzo (k) fluoranthene gave the highest PAHs concentration with an meant amount of 0.068±0.017 mg/kg closely followed by chrysene with mean concentration of 0.062 ± 0.011 mg/kg while dibenzo (a,h) anthracene and benzo(g,h,i)perylene were the least with each showing the same mean concentrations of 0.001 ± 0.0001 mg/kg. In terms of relative abundance, chrysene was found to be the most detected PAH in the mudskipper samples accounting for 26.36% of the total PAH. The next PAH was acenaphthene which accounted for about 16.83% of the PAHs obtained. The PAH that showed the least abundance was dibenzo(a,h)anthracene with 0.02%. Relative distribution of other PAHs in terms of abundance include naphthalene (6.81%), acenaphthalene (6.53%), fluorene (8.02%), phenanthrene (8.41%), fluoranthene (7.73%), pyrene (3.03%), benzo(a)anthracene (3.07%), benzo(b)fluoranthrene (1.11%), benzo(a)pyrene (2.56%), benzo(k)fluoranthrene (3.49%), indeno(1,2,3-cd)pyrene (0.75%), and benzo(g,h,i) perylene (0.50%). The percentage abundance of 2 and 3 membered rings Polycyclic Aromatic Hydrocarbons depicted higher relative percentage abundance of 51.39% (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene and anthracene), on the other hand, the 4 membered rings PAHs (fluoranthene, pyrene, benzo(a)anthracene and chrysene) gave 40.19%, while the remaining 5 and 6 membered rings PAHs comprising of benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)perylene and benzo(g,h,i)perylene) percentage abundance was found to be 8.42%. Comparatively, cumulative amounts of PAHs obtained during the wet months (April to October) – 0.891 mg/kg were found to be higher than in the dry months (October to March) – 0.184 mg/kg (Fig. 2). Maximal amounts of the PAHs was obtained in the month of April with values of 0.444 mg/kg for acenaphthene and 0.152 mg/kg for phenanthrene. Maximal amounts of PAHs was obtained in the month of January 2016 with fluoranthene as the topmost. Apart from the month of November, 2015, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)perylene, and benzo(g,h,i)perylene) were below the detection limit of the equipment.

The concentrations of chemical toxicants like PAHs has been brought to public domain recently because it provide a valuable tool for assessment of the exposure of human beings to toxic chemicals which bioaccumulate in aquatic organisms garnered from contaminated waters and sediments. It further helps to correlate this exposure by human beings with potential risk assessments. Total PAH concentration detected in the mudskipper samples was 0.235mg/kg. This trend contrasted with PAH concentrations reported by several authors; Sinaei and Mashinchian (2014) and Saunders *et al.* (2022) with respect to specimens of

Bolephthalmus dussumieri collected from coastal areas of the Persian Gulf and tiger prawns as well as estuarine shrimps collected from Bodo, Southern Nigeria. From the results , low molecular weight PAHs (152-178 g/mol) – acenaphthene, acenaphthylene, anthracene, fluorene and phenanthrene accounted for about 46.60% of the PAHs detected, while medium molecular weight compound (202 g/mol) – anthracene, fluoranthene and pyrene represent 15.56% of the PAHs detected in the mudskipper samples. High molecular weight compound (228 – 278g/mol) – benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene were detected in 37.84% of the samples collected. The total PAH levels recorded for the mudskipper samples contrasted with PAH values previously recorded for *Penaeus monodon* (shrimps) sampled from the same area as reported by Ukpebor and Ejeomo (2017). The most prevalent PAHs in this study was chrysene, a high molecular weight PAH which varied from below detection limit to 0.311 mg/kg. The mean levels of chrysene in this study (0.062 ± 0.011 mg/kg) is more than the levels detected by Alomirah *et al.* (2009) but less than the concentrations (47.2 mg/kg) reported by Saeed *et al.* (1995). Elevated amounts of fluorene and acenapthene suggested that the possibility of diesel emission, incineration or the combustion of oil. The low amounts of fluorine was indicative of the possibility that oil combustion may not be a dominant origin whilst relatively high levels of phenanthrene would indicate that diesel emission or incineration as a possible source of the PAH.

Seasonal trends were also observed in the data recorded with an increased concentration of PAHs obtained during the wet months in comparison with the dry months. A similar observation was reported by Jaward *et al.* (2012) and this observed trend could be as a result of storm-water run-off and wet deposition. Maximal ΣPAH amounts were obtained in the month of April. Low molecular weight PAHs (MW < 200 g mol^{-1}) present the maximal amounts and more numerous moieties was found to be fluorene (0.071 mg/kg). Lower amounts were documented for PAHs with 5 and 6 rings. These observations were consistent with the earlier researches (Carricchia *et al.*, 1999).

Table 1: Mean concentration (mg/kg) and percentage of mudskipper borne PAHs

PAHs	Concentration (mg/kg)	Control site (mg/kg)	% within ΣPAHs	*TEF	Toxicity Equivalent Concentration (mg/kg)
Naphthalene	0.016 ± 0.039	0.001 ± 0.002	6.81	0.001	1.6E-05
Acenaphthalene	0.015 ± 0.023	0.009 ± 0.025	6.53	0.001	1.53E-05
Acenaphthene	0.040 ± 0.012	0.001 ± 0.002	16.83	0.001	3.95E-05
Florene	0.019 ± 0.029	BDL	8.02	0.001	1.88E-05
Phenathrene	0.020 ± 0.042	BDL	8.41	0.001	1.97E-05
Anthracene	0.011 ± 0.019	0.001 ± 0.002	4.79	0.01	0.000113
Fluoranthene	0.018 ± 0.028	BDL	7.73	0.001	1.82E-05
Pyrene	0.007 ± 0.010	BDL	3.03	0.001	7.12E-06
Benzo(a)anthracene	0.007 ± 0.010	0.001 ± 0.001	3.07	0.1	0.00072
Crysene	0.062 ± 0.011	BDL	26.36	0.01	0.000619
Benzo(b)fluoranthrene	0.003 ± 0.003	BDL	1.11	0.1	0.00026
Benzo(a)pyrene	0.006 ± 0.003	BDL	2.56	1	0.006005
Benzo(k)fluoranthene	0.068 ± 0.017	BDL	3.49	0.1	0.000819
Indeno(1,2,3) perylene	0.002 ± 0.005	BDL	0.75	0.1	0.000176
Dibenzo(a,h)anthracene	0.001 ± 0.001	BDL	0.02	1	4.05E-05
Benzo(g,h,i) perylene	0.001 ± 0.001	BDL	0.50	0.1	0.000117
ΣPAHs	0.235	0.012	100.00		0.0090

Concentration = mean ±SD; * Toxicity Equivalency Factor, BDL – below detection limit

The result of analysis showed that the Total Toxicity Equivalent Concentration (TTEC) of PAHs was obtained as 0.0090 mg/kg as represented in Table 1 above. This value was found

to be lower than the amount (0.0229 mg/kg) obtained by Olayinka *et al.* (2019) in *Drepane africana* (fillet) samples collected from the Atlas Cove jetty in Lagos. The highest toxicity equivalent concentration obtained was found to be 0.01 mg/kg for benzo(a)pyrene. The second maximal amount of 0.02 mg/kg was obtained for benzo(k) fluoranthrene, indeno (1,2,3-cd) pyrene and dibenzo(a,h) anthracene. The least concentration value of 8.00 E-06mg/kg was obtained for benzo(g,h,i) perylene. Different ratios were used to predict the likely origins of hydrocarbons. The phenanthrene/anthracene ratios (Fig. 3) indicated different ratios but majorly most months ratio gave values less than 10 which suggested hydrocarbons might be of pyrolytic origins. An/178 ratio [Anthracene/(Anthracene + Phenanthrene)] have also been utilized to characterize the nature of the PAHs genesis in the environment. From the Fig. 3, below, Ratio An/178 > 0.1 which indicated the dominance of combustion processes for PAHs formation. The ratio of An/178 ranged between 0.07 to 1.0, with the highest value of 1 obtained in the month of September and October 2015 during this study. The BaA/228 ratio (Benzo(a)Anthracene/ Benzo(a)Anthracene+Chrysene) 0.35 indicates pollution by polyarenes formed as a result of pyrolytic processes (Yunker *et al.*, 2002). In the current research, the values of BaA/228 ranged between 0.07 to 1.0, with maximum values recorded in the months of February and August 2016. Other ratios used include IP/(IP + BPer), (Indeno(1,2,3-cd)pyrene/[Indeno(1,2,3-cd)pyrene) + Benzo(g,h,i)perylene] values of 0.5 suggested biomass (grass, wood and coal combustion). The aforementioned was indicative of a pyrolytic origin.
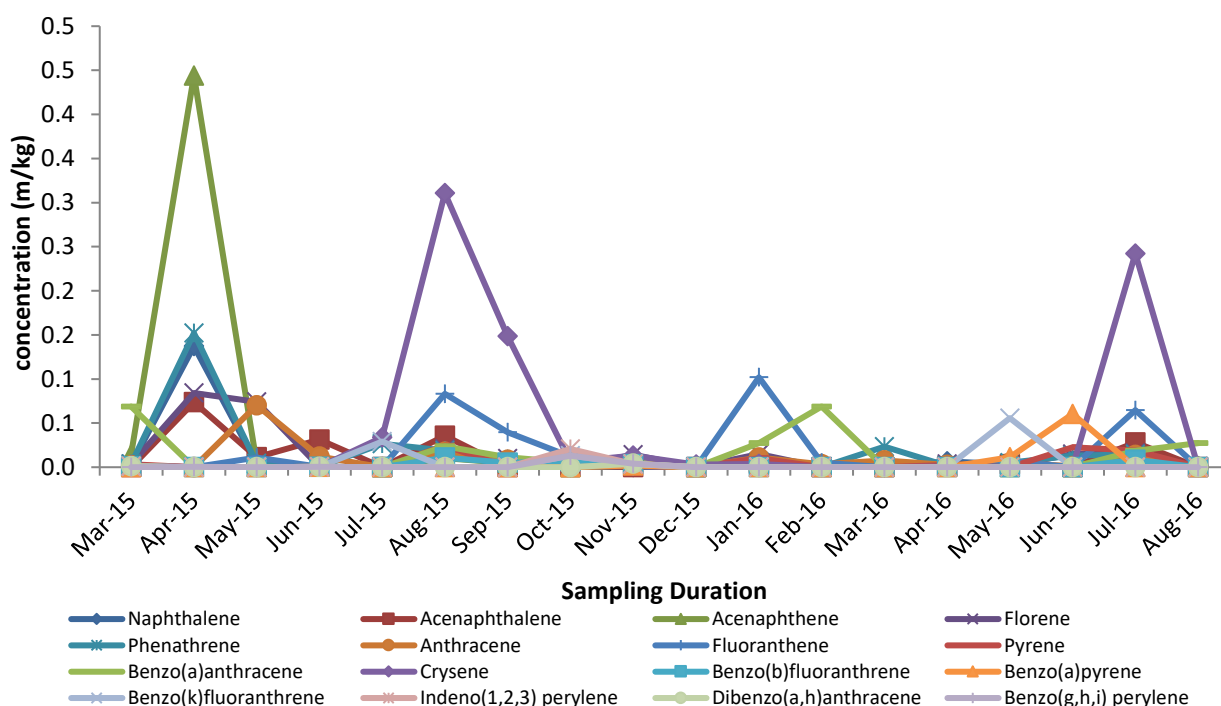

Fig. 2: Concentrations of each PAH detected in the mudskipper specimens for the duration of the sampling period (eighteen months)
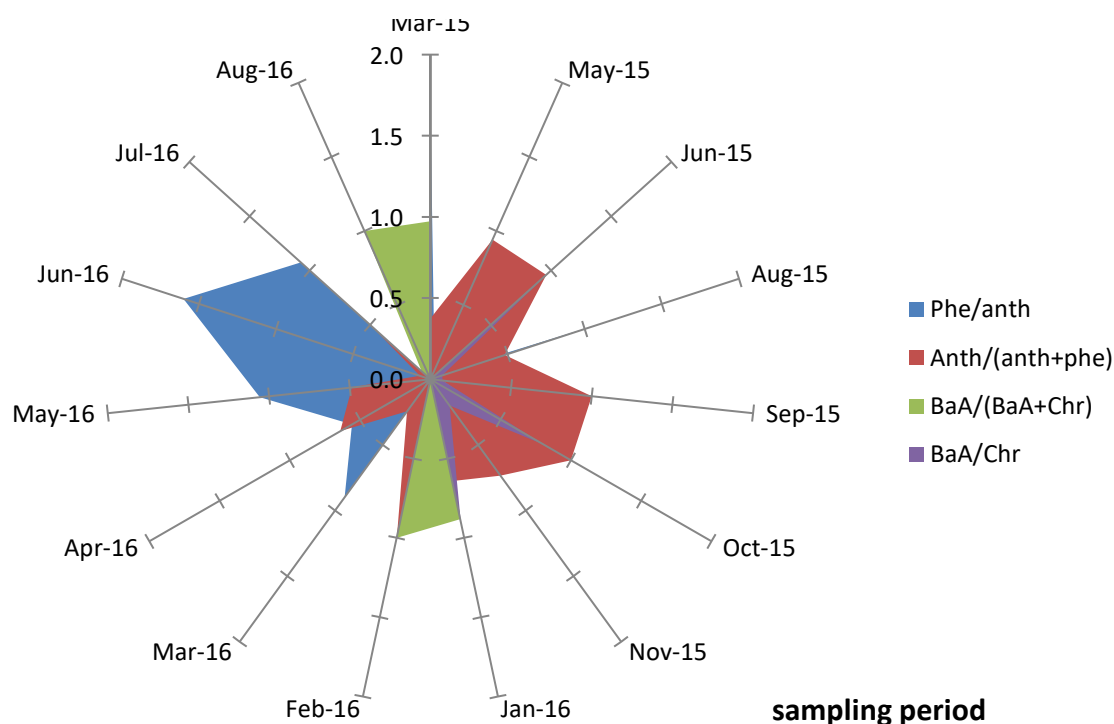
Fig. 3: Diagnostic ratios obtained for the sampling duration

Health Risk Assessment

Concentrations of PAHs obtained comprised of moieties that are suspected carcinogens. Benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene are likely carcinogens. USEPA had derived toxicity equivalency factors (TEFs) for some PAH congeners to ascertain their relative carcinogenicity in comparison to benzo(a)pyrene as a reference chemical (Hussein *et al.*, 2016). Generally, the total amount of PAHs in collected biota indicated that PAH contamination in mudskipper specimens from Jones Creek were relatively high when compared with control site samples wherein concentration ranges were from below the detection limit of the instrument to 0.003 ± 0.001 mg/kg. The results indicated that the levels of these PAHs were higher in the wet months than the dry months, which is likely due to increased input *via* runoff from sources around the estuary. Another contribution to elevated PAH amounts found in Jones Creek could be likely due to illegal refining activities within that environment.

CONCLUSION

Detection of PAHs can be regarded as a likely public health risk as some of these compounds have been described as carcinogens and mutagens (for both aquatic biota and humans). Data from this study provided useful information with respect to bio-concentrated PAHs levels in mudskipper specimens collected from Jones Creek, Delta State. The diagnostic ratios between the PAHs indicated pyrolysis as the most probable source of the contamination.

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