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Effect of Depuration Using Two Media on Polycyclic Aromatic Hydrocarbon Levels in Periwinkle (*Tympanotonus fuscatus*)

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Abstract
The effect of depuration of polycyclic aromatic hydrocarbons in periwinkles (*Tympanotonus fuscatus*) from the Santa Barbara Estuary of the Niger Delta using two different media (Tap and seawater) was studied for 96 hours. Results from the study showed a marked reduction in the concentration of PAHs from the first day of depuration and the last day of depuration. The results revealed that the media of depuration determines the type of PAHs present in the tissue of *Tympanotonus fuscatus*. Tympanotonus fuscatus depurated with Tap water had a higher percentage of 2, 3, and 4 ring PAHs, while those depurated with Seawater had the heavier 5 and 6 ring PAHs. A PAH concentration of 2.219 x 10^{-2} was recorded after 24hours of depuration using Seawater, while at the end of 96 hours, a concentration of 2.044 x 10^{-2} was recorded. For tap water, the PAH concentration after the first day was 1.646 x 10^{-2} ppm, while a concentration of 1.141 x 10^{-2} ppm was reported after 96 hours of depuration. PAH compounds such as 2-methyl naphthalene, Acenaphthylene, Fluorene, Acenaphthene, and Phenanthrene were absent in the tissue of Tympanotonus fuscatus depurated with Seawater. Benzo(a)pyrene equivalent (µg-BaP/kg) for Tympanotonus fuscatus depurated with Tap water was 5.007 µg/kg, while for seawater it was 11.067 µg/kg.

Keywords: Depuration, Polycyclic Aromatic Hydrocarbons, Tympanotonus fuscatus, Oil pollution, Carcinogenicity

Introduction

The increase in the world's population and the dependence on petroleum hydrocarbons as a source of energy and revenue for many states have left indelible traces of contaminants in our ecosystem. Products of these hydrocarbons are bioaccumulated by organisms in the environment, and this may eventually reach humans, who are the final consumers of most of these species. There have been reports of the accumulation of heavy metals by Periwinkles in the Niger Delta. Some of these metals are: cadmium, nickel, lead, iron, and chromium (Moslen et al., 2017; Udiba et al., 2020; Don-Lawson et al., 2021). Abiaobo et al. (2020) also reported the accumulation of heavy metals in the tissue of *Tympanotonus fuscatus* harvested from the Niger Delta. In the report of Nwankwo and Nlemanya (2019), there was a higher microbial load and accumulation of heavy metals in periwinkles harvested from a contaminated environment compared to those harvested from a non-contaminated environment. The organism *Tympanotonus fuscatus* feeds on sediments, thereby ingesting contaminants and food particles from its environment (Okereke et al., 2017; Udiba et al., 2020). The depuration technique has been widely used in the study of other Molluscan classes, such as those belonging to the class Bivalvia. The depuration technique has been employed to mitigate the effects of pollutants on bivalves in aquatic environments. Depuration, also known as self-cleansing, involves removing and transferring the bivalve from a contaminated environment to one free from contamination to remove accumulated pollutants from the bivalve's body (Tanacredi & Cardenas, 1991; Colakolu et al., 2014).

Depuration rates differ among bivalve species; this difference may in part be due to differences in how organisms function physiologically (Anacleto et al., 2015). As suggested by Colakolu et al. (2014), depuration should be conducted in a controlled environment, ideally using disinfected seawater treated with UV light and

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ozonation. Martinez-Albores et al. (2020) also suggested that depuration should be done in purified seawater. Though depuration improves the economic value and microbial quality of bivalves, it causes changes or loss of nutritional quality due to the physiological and traumatic stress involved (Ruano et al., 2012). Stara et al. (2020) in their work revealed that after the transfer of the test organism to pollutant-free water, alterations persisted in the study organism, especially for the hemocyte parameters. Thus, depuration doesn't restore all damaged tissues in the organism to their original state.

Different research has shown bivalves' ability to depurate pollutants. For instance, Loh et al. (2017) in their field study revealed that there was an increase in the level of PAH in the bivalve during a spill, and its reduction in the organism after the spill. The reduction in PAH levels in the organism occurred during the period of cleanup. Farid et al. (2012) also showed the rapid release of accumulated petroleum hydrocarbons in *C. fluminalis*, although the hydrocarbon was incompletely released. Saad et al. (2011) reported that the complete depuration of the bivalve *C. fluminea* occurred 26 days after depuration. Also, Mason (1988) showed that contaminants in mussels were gradually lost when placed in seawater, but the rate of loss slowed with longer exposure. The results of depuration have not all been positive. This was made known in the work of Tanacredi and Cardenas (1991), where *M. mercenaria* did not depurate after being transferred to a depuration medium.

Factors such as environmental concentration, level and time of exposure, and species' ability to metabolize compounds, determine the concentration of pollutants in the tissues of aquatic organisms (Asagbra et al., 2015). The ability to assimilate hydrocarbons differs among organisms (Jack et al., 2005; Saunders et al., 2022). The age of the organism affects its ability to cope with pollution. The reason for this is that younger organisms have a greater physiological vibrancy than older ones, making them less susceptible to pollution (Daka & Ekweozor, 2004). The accumulation rate of contaminants is regulated by the lipid content of the organism and the concentration of pollutants in water (Stegeman & Teal, 1973).

Given their mostly sedentary nature and filter-feeding characteristics, *Tympanotonus fuscatus* are susceptible to pollution, and thus they can accumulate and concentrate pollutants from their environment (Stegeman & Teal, 1973; Colakolu et al., 2014; Martinez-Alborez et al., 2020). Findings from this study will enable us to understand the impact of depuration on the quantity of polycyclic aromatic hydrocarbons in *Tympanotonus fuscatus* and to determine the best duration and medium for the depuration of *Tympanotonus fuscatus*.

Materials and Methods

Periwinkle samples harvested from the Santa Barbara estuary and 40L of brackish water were collected in a sterile plastic container and conveyed to the laboratory, and placed in a basin with tap water, and others were placed with another basin containing filtered brackish water (Okereke et al., 2017). Each basin contained about 40 periwinkles. The depuration test was set for 96 hours, and periwinkle samples were analyzed for PAH at 24, 48, 72, and 96 hours. 7.5 liters of Brackish and Tap water were added to two sterile containers, which served as depuration tanks, and the water (both brackish and tap water) was changed intermittently every 24hours (Amadi, 2016). The analysis of PAHs was performed using a Gas Chromatograph-Flame Ionization Detector (GC-FID). The analysis was performed by injecting 1 µL of the organic extract from the periwinkle using a hypodermic syringe into the column of the Gas Chromatograph (HP 5890 Series II GC). As the vapor constituent partitions between the liquid and gas phases, separation takes place. Also, the compounds of PAHs are detected as they emerge from the column by the FID, which responds based on the composition of the vapor. Results of PAH analysis were managed using MS Excel and analyzed using SPSS 20 software, and means were separated at $p>0.05$ significance level.

Results

A test for depuration was conducted for periwinkle (*Tympanotonus fuscatus*) obtained from the Santa Barbara Estuary of the Niger Delta. The test compared the rate of depuration between two media, salt water and tap water, and the test lasted for 96hours. Results from the test indicated a decrease in PAHs concentration in the organism. A comparative analysis between the composition of PAH in the 24hours and 96hours of experimentation using seawater as a means for depuration showed a reduction in the concentration of polycyclic aromatic hydrocarbons found in the tissues of the organism. Whereas a concentration of 2.219×10^{-2} (ppm) was recorded on the first day of the depuration test, a concentration of 2.044×10^{-2} (ppm) was recorded on the fourth day of the experiment (Table 1).

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For the tap water, the concentration of polycyclic aromatic hydrocarbons recorded in the tissues was 1.646 x 10^{-2} (ppm) at 24hours, while 1.141 x 10^{-2} (ppm) was recorded at 96 hours of the study (Table 2).

Table 1: Depuration of *Tympanotonus fuscatus* using Sea Water

Compound	24 hours	48 hours	72 hours	96 hours	Mean
Naphthalene	3.937 x 10^{-4}	3.265 x 10^{-5}	0	0	0.00011
2-methyl naphthalene	0	0	0	0	0
Acenaphthylene	0	0	0	0	0
Fluorene	0	0	0	0	0
Acenaphthene	0	0	0	0	0
Phenanthrene	0	0	0	0	0
Anthracene	1.781 x 10^{-3}	2.838 x 10^{-4}	5.113 x 10^{-4}	3.321 x 10^{-4}	0.0007
Fluoranthene	2.523 x 10^{-4}	3.097 x 10^{-4}	1.914 x 10^{-3}	1.515 x 10^{-3}	0.0010
Pyrene	5.145 x 10^{-4}	7.982 x 10^{-4}	8.451 x 10^{-3}	6.683 x 10^{-3}	0.0041
Benzo (a) anthracene	3.027 x 10^{-3}	3.705 x 10^{-4}	4.557 x 10^{-4}	4.573 x 10^{-4}	0.0011
Chrysene	5.468 x 10^{-3}	1.120 x 10^{-3}	3.307 x 10^{-3}	7.222 x 10^{-4}	0.0027
Benzo (b) fluoranthene	4.236 x 10^{-4}	2.295 x 10^{-4}	1.175 x 10^{-4}	1.639 x 10^{-3}	0.0006
Benzo (k) fluoranthene	5.022 x 10^{-3}	3.579 x 10^{-4}	3.640 x 10^{-4}	1.086 x 10^{-3}	0.0017
Benzo (a) pyrene	1.753 x 10^{-4}	7.138 x 10^{-3}	1.176 x 10^{-3}	2.467 x 10^{-4}	0.0022
Dibenz (a,h) anthracene	6.254 x 10^{-4}	5.482 x 10^{-3}	6.131 x 10^{-3}	1.579 x 10^{-3}	0.0034
Indeno (1,2,3-cd) pyrene	1.018 x 10^{-3}	8.324 x 10^{-3}	1.376 x 10^{-3}	3.131 x 10^{-3}	0.0034
Benzo (g,h,i) perylene	3.496 x 10^{-3}	1.040 x 10^{-3}	3.740 x 10^{-4}	3.054 x 10^{-3}	0.0020
Total (PPM)	2.219 x 10^{-2}	2.568 x 10^{-2}	2.418 x 10^{-2}	2.044 x 10^{-2}	
Mean	0.001848	0.002123	0.002197	0.001858	

Table 2: Depuration of *Tympanotonus fuscatus* using tap water

Compound	24 hours	48 hours	72 hours	96 hours	Total
Naphthalene	0	1.038 x 10^{-3}	2.121 x 10^{-4}	1.228 x 10^{-3}	0.0006
2-methyl naphthalene	0	0	0	0	0
Acenaphthylene	0	4.038 x 10^{-7}	0	0	0.0000001
Fluorene	1.697 x 10^{-6}	0	0	0	0.0000004
Acenaphthene	2.278 x 10^{-6}	0	0	0	0.0000006
Phenanthrene	2.349 x 10^{-4}	0	0	0	0.000058
Anthracene	2.022 x 10^{-4}	2.879 x 10^{-4}	2.531 x 10^{-3}	2.654 x 10^{-4}	0.00082
Fluoranthene	2.250 x 10^{-4}	3.130 x 10^{-4}	4.716 x 10^{-4}	1.823 x 10^{-3}	0.0007
Pyrene	4.188 x 10^{-3}	7.332 x 10^{-3}	2.061 x 10^{-3}	3.813 x 10^{-4}	0.0034
Benzo (a) anthracene	9.135 x 10^{-5}	1.687 x 10^{-4}	0	1.601 x 10^{-3}	0.0005
Chrysene	6.423 x 10^{-3}	7.561 x 10^{-4}	2.116 x 10^{-3}	1.677 x 10^{-3}	0.0027
Benzo (b) fluoranthene	4.668 x 10^{-4}	1.447 x 10^{-3}	5.689 x 10^{-5}	8.041 x 10^{-5}	0.0005
Benzo (k) fluoranthene	2.604 x 10^{-3}	1.201 x 10^{-3}	1.091 x 10^{-3}	3.215 x 10^{-3}	0.0020
Benzo (a) pyrene	1.783 x 10^{-4}	8.076 x 10^{-4}	3.550 x 10^{-5}	5.869 x 10^{-5}	0.0003
Dibenz (a,h) anthracene	1.194 x 10^{-3}	1.376 x 10^{-3}	4.261 x 10^{-3}	3.683 x 10^{-4}	0.0018
Indeno (1,2,3-cd) pyrene	3.903 x 10^{-4}	6.351 x 10^{-4}	7.111 x 10^{-4}	6.289 x 10^{-4}	0.0006
Benzo (g,h, i) perylene	2.661 x 10^{-4}	1.485 x 10^{-4}	2.528 x 10^{-4}	8.656 x 10^{-5}	0.0002
Total (PPM)	1.646 x 10^{-2}	1.551 x 10^{-2}	1.440 x 10^{-2}	1.141 x 10^{-2}	
Mean	2.123 x 10^{-3}	2.215 x 10^{-3}	2.376 x 10^{-3}	1.7556 x 10^{-3}	

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A scrutiny of both depuration media indicated that organisms depurated in Tap Water had lower concentrations of PAH in their tissues as compared to those depurated in seawater. The difference in rates of depuration between the media can be attributed to the presence of contaminants in the seawater.Amongst the aromatic hydrocarbons with low weight, Naphthalene and Anthracene were found to be present, while 2-methyl naphthalene, Acenaphthylene, Fluorene, Acenaphthene, and Naphthalene were not detected. Naphthalene was present on Days 1 and 2 of the experiment using seawater, but was not present in the study samples on Days 3 and 4 of the depuration tests. The heavy molecular weight polycyclic aromatic hydrocarbons (Fluoranthene, Pyrene, Benzo (a) pyrene, Chrysene, Benzo (b) fluoranthene, Dibenz (a,h) anthracene, Indeno (1,2,3-cd) pyrene, and Benzo (g,h,i) perylene were present throughout the depuration test using seawater. Overall, the sum of low-weight molecular polycyclic aromatic hydrocarbons in periwinkle (*Tympanotonus fuscatus*) after the test was less than the sum of high-weight molecular compounds present during the depuration test.

On the first day of depuration using tap water, Naphthalene, 2-methyl naphthalene, and Acenaphthylene were not detected, while Fluorene, Acenaphthene, Phenanthrene, and Anthracene were present. Of all the detected LMW PAHs, only Anthracene was present from Day 2 to Day 4 of the test, while Acenaphthylene was detected in specimens from Day 2 but not detected in Day 3 and 4. Though not detected on Day 1, Naphthalene was present in specimens from Day 2 to Day 4. Acenaphthylene, Fluorene, Acenaphthene, and Phenanthrene, which were not detected in specimens depurated with seawater, were detected in specimens depurated with tap water. Like seawater, the sum of HMW PAHs present in the specimen was higher than the LMW PAHs present.

In contrast, the different media (tap and seawater) used for the depuration test during the study had different concentrations of both the low molecular weight and high molecular weight PAHs. The total concentration of LMW PAH recorded was 3.321×10^{-4} mg/l and 8.8983×10^{-3} for HMW PAH. Tap water faired differently as the total LMW PAH recorded was 5.5347×10^{-3} mg/l and 5.119×10^{-2} mg/l for HMW PAH. Thus, the amount of HMW PAH using tap water as a depuration medium is lower than that of seawater.

The result from the analysis of the variance test between tap and seawater used for depuration showed that no significant variation existed in the quantity of PAH depurated by periwinkles in either tap or seawater ($P > 0.05$).

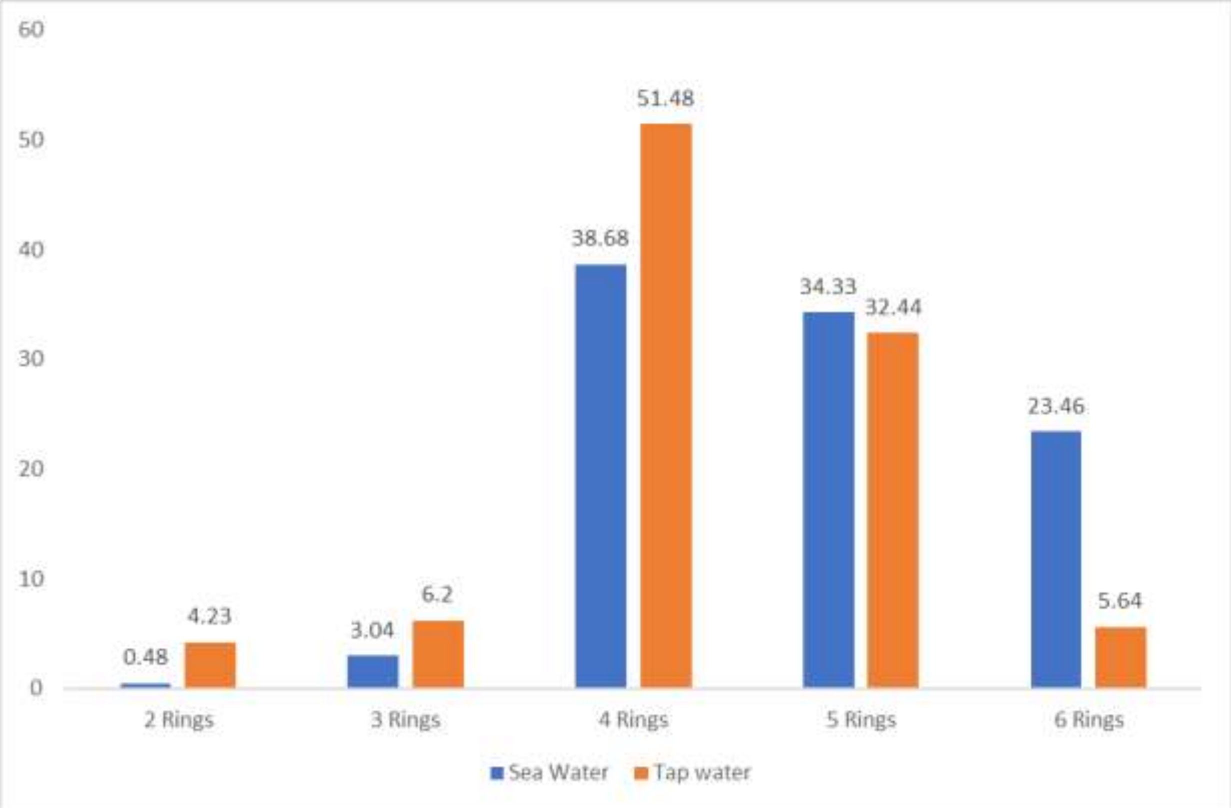

Figure 1: Percentage composition of PAH rings found in periwinkles from different media

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Table 3: Bapeq for *Tympanotonus fuscatus* depurated with tap and seawater

PAH	Sea Water	Tap Water	PEF	Sea Water BEC	Tap Water BEC
Benzo (a) anthracene	1.1	0.5	0.1	0.11	0.05
Chrysene	2.7	2.7	0.01	0.027	0.027
Benzo (b) fluoranthene	0.6	0.5	0.1	0.06	0.05
Benzo (k) fluoranthene	1.7	2	0.1	0.17	0.2
Benzo (a) pyrene	2.2	0.3	1	2.2	0.3
Dibenz (a,h) anthracene	3.4	1.8	2.4	8.16	4.32
Indeno (1,2,3-cd) pyrene	3.4	0.6	0.1	0.34	0.06
BaP equivalent (µg-BaP/kg)				11.067	5.007

Discussion

The presence of PAHs in the tissue of organisms indicates environmental pollution (Udofia et al., 2021). The abundance of PAHs in the tissues of periwinkle poses a health risk to consumers of this delicacy (Eduok & Ofonime, 2015).

The exposure of inhabitants to PAHs increases their chances of having cancer (Bostrom et al., 2002; Barbosa et al., 2023). However, HMW polycyclic aromatic hydrocarbons are less likely to be broken down when compared to LMW polycyclic aromatic hydrocarbons (Tarafhdar & Sinha, 2017). LMW PAHs have been reported to be associated with chronic acute effects (Souza et al., 2023). A comparative analysis of both depuration media showed that the samples depurated in tap water had a high percentage composition of PAH with 2, 3, and 4 rings; samples depurated with Seawater had higher percentages of heavier fractions of PAH with 5 and 6 rings.

The presence of both LMW and HMW polycyclic hydrocarbons from both depuration media may be the reason for the deterioration of the health of consumers in the long term. Okereke et al (2023) reported a decrease in the total fungal count and coliform count from periwinkles depurated for 96 hours; these findings are similar to those of this research, as there was a differential reduction in the quantity of PAHs in both media (Tap and seawater) between 24hours and 96hours after the depuration test.

Okereke et al. (2017) showed that time was essential in the depuration of heavy metals from *Tympanotonus fuscatus*. They reported a reduction in heavy metal concentration in *Tympanotus fuscatus* at 96 hours; they also reported a statistically significant difference at different depuration time intervals. The reductions as a result of depuration in Okereke et al. (2017) were not in line with the findings of this research, as the results of this research showed that the difference in depuration was not statistically significant ($p>0.05$) for both media. However, there were reductions in the concentration of PAHs from 24hours to 96hours. Also, Salama et al. (2022) in their 21-day study of depuration in *Procambarus clarkii* reported a decrease in heavy metals available in the organs of the organism.

The statistically insignificant result obtained from the ANOVA test between depuration time from both media used during the study could have an enormous implication on the health status of the inhabitants of this estuary and those who harvest *Tympanotus fuscatus* from this estuary for their consumption. The consumption of *Tympanotonus fuscatus* from this estuary may lead to chronic health implications. To determine the carcinogenicity of PAHs, the benzo(a)pyrene tool is a justifiable tool that can serve that purpose. To calculate carcinogenicity using BaP, individual PAH is multiplied by the PAH's potency equivalence factor (PEF) to produce a PAH-specific BaP equivalent concentration (BEC). The sum of individual BECs for the measured PAHs gives the BaP equivalent (ATSDR, 2022).

The total BaP for the two media used for depuration were 11.067 and 5.007 µg/kg for seawater and tap water, respectively, and are less than the cancer CV for benzo(a) pyrene of 65 µg/kg, thus the chances of causing cancer are minimal. However, there could be a possibility of toxicity due to chronic effects of these contaminants.

The BaP TEQ value of 0.12874 was reported for the periwinkle *Pachymelania fusca mutans* harvested from a crude oil-polluted coastal area in the Niger Delta by Odesa and Olannye (2024). This value is less than the TEQ

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value reported by this study. However, Dokubo and Igwe (2019) reported a TEQ value of 0.000403 for periwinkles (*Tympanotonus fuscatus*) harvested from Kula in the Niger Delta.

Conclusion

The presence of different polycyclic aromatic hydrocarbons (PAHs) identified as priority PAHs by the USEPA in the tissues of *Tympanotonus fuscatus* after depuration from the Santa Barbara Estuary highlights the severe environmental degradation in the study area, as with most parts of the Niger Delta. There is a tendency for the possible bioaccumulation of this contaminant in the consumers of *Tympanotonus fuscatus*, given the regularity with which it is harvested and used as a food source. Thus, depuration with seawater should be discouraged, and the use of pipe-borne water should be encouraged. *Tympanotonus fuscatus* should be depurated for at least 96 hours. This practice would help reduce the contaminant load in the organisms and, consequently, diminish the impact of such contaminants on human health.

Recommendation

The study recommends that the depuration of *Tympanotonus fuscatus* for at least 96hours before use, and also, there is a greater responsibility on the government and IOCs to fully implement modalities to curb the incessant release of crude oil into the environment.

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