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Condition Factor and Hepatosomatic Index of *Ptychadena mascariensis* Exposed to Crude Oil

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Abstract:

Amphibian population declines have been reported and many causes have been implicated which include habitat alteration, environmental change and pollution. Unfortunately, not much is known of the effects of crude oil pollution on the species. This study therefore investigated the haematological changes associated with exposure of the frog, *Ptychadena mascariensis* to sub-lethal concentrations of the water-soluble fractions of crude oil using static renewal bioassay system for 12 weeks. Water parameters were also monitored throughout the duration of the experiment. Water quality parameters remained within the ranges for water bodies in the Niger Delta with no significant differences between the control and treatment groups. The results revealed that crude oil had a negative impact on the frogs and showed dose-dependent reductions in K and HSI values. However, the values of K were not significantly reduced in the test groups compared to the control groups ($p>0.05$). HSI values were significantly reduced in the test groups compared to the control groups ($p<0.05$). Given the frequent cases of oil spills in the Niger Delta which expose frogs to persistent levels of crude oil in the environment, there are possible long-term effects on the physiological well-being of the frogs as demonstrated by the results of this study.

Introduction

Oil pollution, one of the environmental consequences of crude oil exploration and exploitation activities produces aqua-toxicological effects, which are deleterious to aquatic life (Kori-Siakpere, 2000). A variety of pollutants including crude oil and its products are known to induce stress conditions, which impair the health of fish (FEPA, 1999). Ekweozor, (1989) reported that frequent spillages of crude oil and its products in creeks and rivers of the Niger Delta have resulted in a marked reduction in the number of both freshwater and marine creatures. Amphibian populations are in decline in many areas of the world. Numerous physical and chemical causes have been postulated and, in some instances, interaction of multiple causes has been implicated. They include habitat alteration and habitat destruction (Nafagha, 1999) predation (Sessions and Ruth, 1990), competition from exotic non-indigenous species (Jennings and Hayes, 1985) parasites (Carey and Bryant, 2003), disease (Lefcort and Blaustein, 1995), ultraviolet radiation (Blaustein *et al*., 2003), climate change (Corn *et al*., 1989) and environmental contamination (Nafagha, 2007).

The condition of the liver and of the whole body, as measured with the hepatosomatic index (HSI) and condition factor (K) can provide information on potential pollution impacts. In fisheries science, the condition factor is used to evaluate the "condition", "fatness" or wellbeing of fish. Condition factor compares the wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition (Bagenal and Tesch, 1978). A high condition factor reflects good environmental quality. Hepatosomatic Index (HSI) is defined as the ratio of liver weight to body weight. It provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver).

Nebeker *et al.*, (1995) reported that amphibians are being used as bio-indicators of contaminants in fresh water. Amphibians, especially some particular frogs, are increasingly used as bioindicators of accumulation of pollutants (Loumbourdis *et al*., 2002). They are near the top of the food chain and invertebrates, which are their main food source, are an important pathway through which pollutants can enter the body of amphibians. Amphibians typically absorb water through the ventral skin (Papadimitriou *et al*., 2003) and because they spend a long time under water, they may accumulate various contaminants that are discharged into the water they inhabit; hence, they are sensitive to perturbations in ecosystems.

Given the global amphibian population decline, the current investigation was undertaken to understand the toxicity of sublethal concentrations of WSF of crude oil in the frog *P. mascariensis*, by determining its effects on the condition factor and hepatosomatic indices of this species.

Materials and Methods

Frogs collection, acclimatization and feeding were carried out following the method of Nafagha (2014). A static renewal bioassay was carried out with triplicates for 12 weeks. Treatments consisted of six different concentrations (0mg/L, 0.3mg/L, 0.75mg/L, 1.5mg/L, 2.25mg/L and 3.0mg/L) of water-soluble fractions of crude oil. The toxicant used was Bonny light crude oil and the water-soluble fractions was prepared following the method of (Anderson, 1977) by adding 1 part of crude oil to 9 parts of fresh water. The water samples were analyzed as described by APHA (1988).

Weight and length of the organisms (frog samples) were determined using a weighing balance (Mettler PN163) and measuring board respectively. Total length (TL) was measured from the tip of the snout to the extended tip of the tail. At post bud formation, the legs were extended before the measurements were taken. The lengths were taken with measuring board to the nearest 0.1 cm. Body weight of individual frog was measured to the nearest 0.1 g after removing the adhered water and other particles from the surface of body.

The condition factor was calculated according to the following formula by Pauly (1984) given as:

$$K = \frac{BW\ X100}{L^3}$$

BW is the body weight in grams; L is its length in cm.

The frogs were sacrificed and the liver dissected out, drained with filter paper and weighed. The Hepatosomatic Index (HSI) was calculated using the formula of Htun-hun (1978) as:

$$HSI = \frac{LW\ X100}{BW}$$

LW is the liver weight in grams

Data was reported as means plus or minus (±) the standard deviation of the mean. Microsoft Excel (2010) was used for compilation of means and standard deviation. Data on K and HIS were analysed by one-way analysis of variance (ANOVA) and their correlation coefficient values obtained. Analysis of variance was carried out on the Physico-chemical of the water.

Results and Discussion

Dissolved oxygen, pH, salinity, total alkalinity, and total hardness showed consistent variation with increase in crude oil concentration and time, however, these water quality parameters were within the recommended limits for aquatic organisms. The results of the water parameters are shown in Table 1. These variations remained within tolerable ranges throughout the bioassay period. The ANOVA carried out showed that there were no significant differences in the water samples in the control and that of treatment groups.

Table 1: Result of Physico-chemical parameters with standard errors

Conc.	Temp.	DO	pH	Alkalinity	Salinity	Total Hardness
0 mg/L	27.6 ± 0.3^{a}	4.48 ± 0.37^{a}	7.33 ± 0.07^{a}	54.24 ± 0.85^{a}	6.20 ± 0.49^{a}	1657 ± 13.87^{a}
0.3mg/L	27.6 ± 0.3^{a}	4.48 ± 0.42^{a}	7.33 ± 0.07^{a}	54.18 ± 0.90^{a}	6.20 ± 0.37^{a}	1670 ± 20.00^{a}
0.75mg/L	27.6 ± 0.3^{a}	4.48 ± 0.45^{a}	$7.33 \pm 0.\ 07^{a}$	54.18 ± 0.90^{a}	6.20 ± 0.60^{a}	1670 ± 21.37^{a}
1.5mg/L	27.6 ± 0.3^{a}	4.48 ± 0.43^{a}	7.33 ± 0.07^{a}	54.10 ± 0.90^{a}	6.20 ± 0.50^{a}	1675 ± 22.84^{a}
2.25mg/L	27.6 ± 0.3^{a}	4.40 ± 0.44^{a}	$7.30 \pm 0.\ 07^{a}$	54.10 ± 0.90^{a}	6.20 ± 0.37^{a}	1680 ± 20.82^{a}
3.0mg/L	27.6 ± 0.13^{a}	4.20 ± 0.40^{a}	7.30 ± 0.07^{a}	53.78 ± 0.90^{a}	6.00 ± 0.44^{a}	1680 ± 26.48^{a}

Mean with different superscript in the same column are not significantly different (P>0.05).

The results for condition factor (K) and hepatosomatic index (HIS) are as presented in Table 2. The highest value of K (1.482 ±0.008) was obtained for frogs in the control while the least K of 1.216 ± 0.001 was for the frogs exposed to 3.0mg/l of WSF of crude oil. The result indicated that there was a significant difference (p<0.05) between the condition factors of control treatment and that of the frogs exposed to other concentration.

Table 2: K and HSI of frog *P. mascariensis* after 12 weeks exposure to WSF of crude oil

Parameters	N	**Concentrations**					
		0mg/L	0.3mg/L	0.75mg/L	1.5mg/L	2.25mg/L	3.0mg/L
Body weight at WK 1 (g)	20	0.1 ± 0.01	0.1± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Body weight at WK 12 (g)	20	39.72 ± 0.01	38.36 ± 0.01	35.43±0.01	34.84±0.01	32.21 ± 0.01	31.17± 0.01
Weight Gain (g)	20	39.72 ± .04	38.36 ± .03	35.43± .02	34.84± .05	32.21 ± .03	31.17± .03
Length at WK 1	20	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.02	1.6 ± 0.02
Length at WK 12	20	13.9 ± 0.3	13.9 ± 0.3	13.89 ± 0.3	13.7 ± 0.3	13.7 ± 0.3	13.7 ± 0.3
Length gain (cm)	20	12.3 ± 0.2	12.3 ± 0.2	12.29 ± 0.2	12.18 0.2	12.1 ± 0.1	12.1± 0.2
Liver weight at WK 12 (g)	20	0.83±0.02	0.49±0.02	0.38±0.02	0.33±0.02	0.22±0.02	0.22±0.01
K at W2 12	20	1.482± .001	1.431 ±.030	1.325±.028	1.28 ±.058	1.257 ±.031	1.216 ±.028
HIS at WK 12	20	2.084±.0012	1.289 ±.001	1.055±.002	0.976±.001	0.680 ±.001	0.640 ±.001

Also, the HSI for the *P. mascariensis* exposed to different concentrations of WSF of crude oil showed the same trend as the values for K. HIS values ranged from 2.084 to 0.639. The result indicated that there was a significant difference (p<0.05) between the HSI of control treatment and that of the frogs exposed to other concentrations. Table 2 shows the Summary for mean, standard deviation and standard error for the condition factor (K) and hepatosomatic index (HSI) for each concentration.

Assessing morphological parameters is one of the most straightforward methods to study the effects of water contamination on fish because of the ease of recognition and examination when compared with other types of biomarkers (Sun *et al*., 2009). According to Bagenel, (1978), the condition factor indicates the well-being of the fish. Exposure of the frogs *P. mascariensis*, to different levels of WSF of crude oil concentration showed a gradual decrease in the value of K and HSI as the concentration of crude oil increased, which were significant. However, nearly all the specimens in the study were observed to be in good condition, as the values were higher than one (Charles-Barnham and

Baxter, 2003). A decrease in either the condition factor or HSI can also signal the deleterious effect of a stressor.

In the research of Ariweriokuma et *al.*, (2011), the sublethal effects of cypermethrin exposure to *Clarias gariepinus* were studied. The results obtained from that study indicated that no significant differences were recorded for the condition factor in the values of the control group and the treatment groups. However, HSI was significantly reduced when compared to the controls. These results are in agreement with the results obtained in this study. These findings were similar to those of Kicheniuk and Khan (1987) and Kori-Siakpere (2000), who noted that exposure of fish to WSFs of crude oil, resulted in reduced feeding and lower body weights which were reflected in a change in the condition factor. Exposure of the frogs to water-soluble fractions of crude oil may have resulted in reduced food intake and thus lower body weight and a corresponding reduced liver weight.

The findings of this study is also in agreement with those of Sindhe and Kulkarni (2004) in which the HSI values reduced on exposure of freshwater fish *Notopterus notopterus* to heavy metals at sublethal concentrations.. HSI values have also been reported to have decreased in the fish exposed to high concentrations of cadmium and zinc (Egunjobi, 2010). The reduction of HSI values suggested the mobilization of the liver reserves toward the metabolic requirements of the fish exposed. Hepatosomatic index is the main indicator of metabolic activity in animals. Reduction in HSI in the presence of pollutants has been suggested to be caused by the mobilization of the liver reserves toward the metabolic requirements of the exposure (Barton *et al*., 2002); hence a logical link then exists between this depletion of energy reserves and potential health problems for fish. A decrease in condition factor and HSI is considered a reflection of depletion in energy reserves (Goede and Barton, 1990; Barton *et al*., 2002) because these indices are positively related to total muscle and liver energy content (Stolyar *et al*., 2008).

Çiftçi, *et al*., (2015) studied the effects of heavy metals on K and HSI of *Oreochromis niloticus*, after exposing the fish at sublethal levels. A significant decrease in K and HSI was observed in the treatment groups when compared with control at the end of experiment. Barton *et al.*, (2002), in their study observed a significant and negative correlation between total mercury in whole yellow perch and condition. Those fish with higher mercury concentrations tended to have lower condition scores. This observed body condition changes according to Barton *et al*., (2002) was in rapid response to environmental perturbation and toxic chemical exposure.

This study affirmed that there is a variation in condition factor and hepatosomatic index of frogs exposed to crude oil.

Conclusion

The indices measured in the present study are useful for monitoring the long-term effects of frogs. The current study indicates that crude oil contamination affected the frogs negatively. Many pollutants exist in the aquatic environment at sublethal levels for short or long-time periods, and may not noticed because they do not cause immediate frogs mortality. However, the consequences of such effects are morphological and physiological, causing illness and reducing fitness for life. Therefore, the simple fact that sublethal concentration is considered safe because it does not kill any frogs does not mean that it can be used indiscriminately because contaminant effects can weaken frogs rendering them more susceptible to mortality from other causes. It is therefore plausible that the observed effects are contributing to the decline of frog populations in the area.

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