



Impact of Water Soluble Fractions of Crude Oil on Growth Performance of *Ptychadena mascariensis*

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ABSTRACT

Study on the impact of sub-lethal concentrations (0mg/L, 0.3mg/L, 0.75mg/L, 1.5mg/L, 2.25mg/L and 3.0mg/L) of water-soluble fractions (WSFs) of crude oil on the growth performance of the frog *Ptychadena mascariensis* tadpoles was carried out under laboratory conditions in the University of Port Harcourt, during a 12-week period of exposure in a renewal static bioassay system. Growth of *P. mascariensis* was observed to reduce significantly with increasing time of exposure and concentrations of WSFs of crude oil when compared with the controls. Weight gain and SGR were all significantly reduced in the test group compared to the control group ($p < 0.05$). However, the length gain was not significantly reduced ($P > 0.05$). The regression co-efficient obtained for the length-weight relationship for the frogs in the control was 3.005, while for frogs were exposed to crude oil of various concentrations ranged from 2.0952 – 2.4369. This suggested an isometric growth form in all specimens in the control and a negative allometric growth pattern for frogs exposed to crude oil. This study indicated that crude oil had a negative impact on the growth rates of and growth pattern *P. mascariensis*.

Keywords:

Amphibians, bio-indicator, growth rates, length-weight relationship

INTRODUCTION

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 animals (Ekweozor, 1989). 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).

Pollution may damage organisms directly by increasing their mortality, or interfering with the processes of food acquisition and uptake, and reducing their growth and reproduction rates. Growth represents the integration of feeding, assimilation and energy expenditure over a period of time. Poor growth means less energy is available for reproduction, which will in turn reduce the species fitness and lead to a decline in population. Growth rate of an organism therefore can serve as a time-integrated indicator of the general well being of the organism.

Frogs are excellent indicators of pollution due to the sensitivity of their skin and eggs to both aquatic and terrestrial agents as they can readily absorb substances from their environment, making them very sensitive to perturbations in ecosystems. For this reason, their aquatic larval stages are increasingly viewed as bio-indicators of health of aquatic systems (Hartwell and Olivier, 1998). In amphibian larvae no barriers are provided by scales, feathers or fur covering. The thinness of their skin makes them highly permeable to aquatic pollutants. In larval stages of amphibians, uptake of crude oil particles from the environment occurs principally through external and internal gills as well as cutaneous respiratory surfaces and gastro intestinal ducts. The primary routes of exposure of crude oil to larval amphibians are principally through direct uptake from water and diets (Grillitsch and Chorance, 1995).

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 environmental contamination and destruction (Nafagha, 2007), predation (Lefcort and Blaustein, 1995), competition from exotic non indigenous species (Jennings and Hayes, 1985), parasites (Sessions and Ruth, 1990), disease (Carey and Bryant, 1998), ultraviolet radiation (Blaustein *et al.*, 2003) and climate change (Corn *et al.*, 1989). Growth rates and body size are important intraspecific characteristics for adult amphibians. First, rapid growth immediately following metamorphosis reduces the time to sexual maturity for anurans, allowing them to reproduce earlier (Turner, 1960). Second, reproductive output is often correlated with body size. For example, larger Spotted Salamanders (*Ambystoma maculatum*) and Wood Frogs (*Rana sylvatica*) produce significantly larger clutches

than do smaller conspecifics (Woodward, 1982; Howard, 1988), and larger male Bullfrogs (*Rana catesbeiana*) mate more frequently each year than do smaller males (Howard, 1988). In some *Ambystoma*, larger individuals breed earlier in the season than do smaller individuals (Lowcock *et al.*, 1992), perhaps offering a time advantage to their offspring in a rapidly fluctuating environment. Finally, larger male body size may be important in species where individuals defend territories (Howard, 1988). Amphibians exhibit indeterminate growth continuing to grow beyond reproductive maturity (Perrin and Sibley, 1993). Therefore, factors that affect growth and body size is of utmost importance in ensuring the survival of amphibians.

Given the global amphibian population decline and paucity of information on frogs, using growth rate as a marker for health, this study monitored the growth of *P. mascariensis* over a period of 84 days to investigate the effect of WSF of crude oil on the frogs

MATERIALS AND METHOD

Tadpoles of the frogs *Ptychadena mascariensis* were collected from laboratory bred frogs at the Laboratory of the Animal and Environmental biology, University of Port Harcourt. A total of 180 tadpoles were used for the experiment and were maintained at 22-24°C with 12L: 12D fluorescent lighting. Clean water was maintained by pouring off old water every 2 days and replacing it. Tadpoles were pooled into a single tank and then randomly assigned to 18 20-L cubical plastic transparent tanks with 20 tadpoles per tank for each treatment including the controls filled with 5 cm of fluid. A static renewal bioassay was carried out with triplicates for 84days (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. Range finding tests were done to determine the threshold concentrations that will cause mortality (Reish and Oshida, 1986). The tadpoles were introduced into the crude oil contaminated water from where they were fed with diet of corn and cowpea seeds. The water samples were analyzed as described in standard method for examination of water and wastewater (APHA, 1985). At the end of the experiment, the limbs were formed and they were juvenile frogs at Gosner stages 44-47 (Gosner, 1960). The plastic tanks were covered with wire mesh to prevent them climbing out since they were very active animals.

Weight and length of the organisms 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 initial mean weight of frogs was 0.1 ± 0.03 g while the mean length was 1.6 ± 0.03 cm.

The mean growth rate of frogs at different concentrations was represented in a growth curve by plotting the mean weight of the frogs at different time intervals (in weeks). Measurement of the tadpoles began 7days post hatch and continued for 84days (12 weeks). The weight in grams was plotted against time in weeks to give the growth curve. Also the length in centimeters is plotted against time (in weeks) to get the growth in length.

The length-weight relationship (LWR) was estimated by using the equation by Pauly (1983):

$$W = aL^b$$

Where W= weight (g), L= total length (cm), a = constant, b= growth exponent.

A logarithmic transformation was used to make the relationship linear $\log_{10} W = \log_{10} a + b \log_{10} L$

Specific growth rate (SGR) = this relationship of difference in the weight of fish within the experiment.

$$SGR = \ln (W_2 - W_1) * 100 / T_2 - T_1$$

Where W2 = weight of fish at T2 and W1 = weight of fish at T1

Data was reported as means plus or minus ($\pm$) the standard deviation of the mean. Microsoft Excel (2010) was used for compilation of means and standard deviation. The length weight relationship was analyzed using the FiSAT (FAO-ICLAM stock assessment tools) software. Data on growth parameters were analyzed by one-way analysis of variance. T-test was used to determine levels of significant differences in the growth parameters. Analysis of variance was carried out on the physico-chemical of the water.

RESULTS

The values for Dissolved oxygen, pH, salinity, total alkalinity, and total hardness is given in Table 1 and showed consistence with increase in crude oil concentration and were within the recommended limits for aquatic organisms throughout the bioassay period. The results of the water parameters are given in Table 1. 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 of water parameters

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

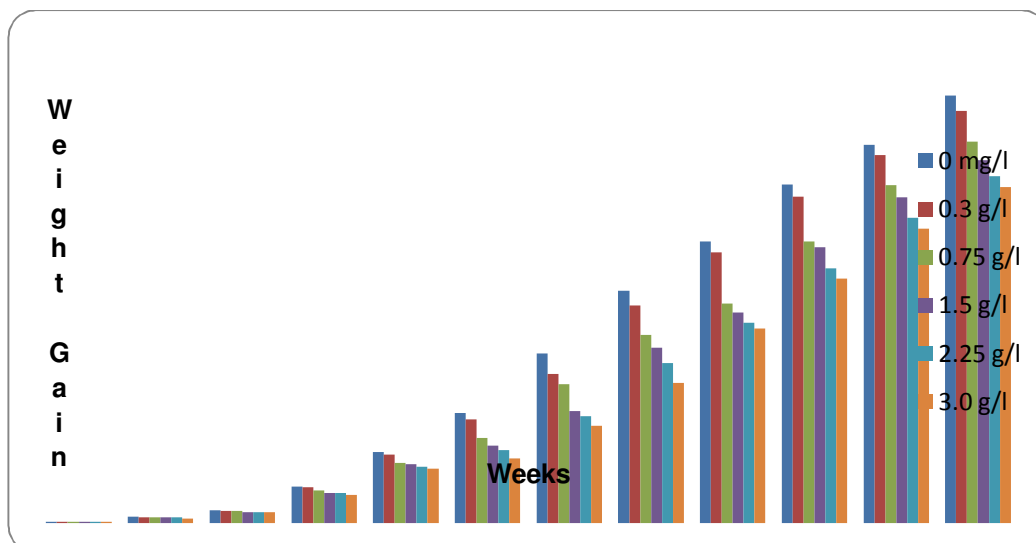
Mean values in the same row with the same superscript are not significantly different at $F \alpha = 0.05$.

Growth rate of *P. mascariensis* was observed to reduce with increasing time of exposure and concentration of WSFs of crude oil when compared with control frogs. See Fig 1 for weekly measurements of weight gain of frogs over a period of 12 weeks at different concentrations of WSFs of crude oil. The total weight gain, increase in length, specific growth rate and length-weight relationship of frogs are presented in Table 2. Fish weight was observed to significantly ($P < 0.05$) reduce with increasing concentration of WSFs with a total mean increase of 39.72g for frogs at concentration of 3mg/L frogs while frogs at the control had a mean weight gain of 31.17g. Length gain showed a total

increase of 12.3cm for frogs at the control and 12.1cm for frogs at concentration of 3mg/L. The ANOVA result indicated that there was no significant difference in the gain in length of frogs in the control and frogs exposed to other concentrations of crude oil ($p > 0.05$). For the specific growth rate (SGR), 4.42 was recorded for the control while the value of 4.14 was recorded for frogs exposed to concentration of crude oil of 3.0mg/L. ANOVA indicated that significant differences ($p < 0.05$) existed in the SGR of the frogs in the control and frogs of other concentrations. Also the length-length relationship had a regression coefficient (b – values) of 3.0050 for the control, and 2.0423 respectively for 3.0mg/L.

Table 2: Growth parameters of frog *P. mascariensis* after 84 days exposure to WSF of crude oil

Conc.	Initial weight (g)	Final weight (g)	Weight gain (g)	Initial length (cm)	Final length (cm)	Length gain (cm)	SGR	Regression coefficient (b)
0mg/L	0.1	39.72±.2	39.72 ± .04	1.6	13.9±.1	12.3±.2	4.42	3.0050
0.3mg/L	0.1	38.36±.2	38.36 ± .03	1.6	13.9±.1	12.3 ±.2	4.39	2.8369
0.75mg/L	0.1	35.43±.2	35.43 ± .02	1.6	13.9±.1	12.29±.2	4.29	2.0952
1.5mg/L	0.1	34.84±.1	34.84 ± .05	1.6	13.8±.1	12.18±.2	4.23	2.0760
2.25mg/L	0.1	32.21±.2	32.21 ± .03	1.6	13.7±.1	12.1±.2	4.18	2.0387
3.0mg/L	0.1	31.17±.0	31.17 ± .03	1.6	13.7±.1	12.1±.2	4.14	2.0423

**Fig 1: Growth rate of frogs at various concentrations over a period of 12 weeks**

DISCUSSION

These results showed that crude oil have a negative effect on the growth the frogs. Exposure of frogs to water-soluble fractions of crude oil may have resulted in reduced food intake and thus lower body weight. This finding simulates the work of Kori-Siakpere (2000) who noted that exposure of fish to WSFs of crude oil, can result in reduced feeding and lower body weight.

This reduced growth performance agrees with other works that have been carried out that indicated dose-dependent effect of toxicant on growth. This includes the works of Esenowo *et al.* (2010); Nwabueze and Agbogidi (2010); Afolabi *et al.* (1985). Similarly, Ofojekwu, *et al.* (2002) reported that fish are known to increase their metabolic rates to metabolize and excrete aromatic hydrocarbons and consequently allocate more energy to homeostatic maintenance than storage, hence a reduction in growth rate. Ofojekwu and Onah (2002) showed that the aromatic compounds from crude oil were responsible for retardation of growth in fishes. In the research by Peakall *et al.* (1982) in which tadpoles of southern leopard frog (*Rana sphencephala*) was exposed to TPH from diluents, the specific growth rate

was significantly reduced. Also the research by Little *et al.* (1998) in which of Pacific herring (*Clupea pallasii*) and pink salmon (*Oncorhynchus gorbuscha*) treated with petroleum-derived polycyclic aromatic hydrocarbons (PAHs) showed a significantly reduction in growth.

The value of the regression co-efficient obtained for the length-weight relationship for the combined frogs in the control was 3.0 (this indicated an isometric growth form) while that for the frogs exposed to crude oil at various concentrations ranged from 2.0423 - 2.8369. This suggested a negative allometric growth form in all the specimens exposed to crude oil. This showed that pollution by WSF of crude oil affected the growth pattern of the frogs. In a comparative study on L-W relationships of *Oreochromis niloticus* and *Oreochromis aureus* in which polluted and non-polluted parts of Lake Mariat, Egypt it was reported by Incardona and Scholz (2005), that there were highly significant variations of L-W relationships of both species in polluted and non-polluted parts of the lake. Similarly, Bakhoum (1994) reported differences in L-W relationships of *Oreochromis niloticus* in a polluted canal compared with those of other authors in different localities and times. These differences were

attributed to the effect of eutrophication and pollution on growth and other biological aspects of *O. niloticus*.

CONCLUSION

Many pollutants exist in the aquatic environment for short or long time periods at sub lethal levels. These levels are not noticed because they do not cause immediate mortality to the aquatic animals. However the consequences of such effects are morphological and physiological, causing illness and reducing fitness for life. This study indicated that crude oil contamination definitively affect the frogs negatively by causing alterations in the growth rate and growth pattern. Pollution may damage organisms directly by increasing their mortality rates, or interfering with the processes of food acquisition and uptake. Growth represents the integration of feeding, assimilation and energy expenditure over a period of time. Poor growth means less energy is available for reproduction, which will in turn reduce the species fitness and lead to a decline in population. The use of growth responses as important biomarkers for the possible effects of crude oil on amphibians is an important tool for monitoring the health of these tetrapods in areas where they have the potential to being exposed.

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