Continental J. Fisheries and Aquatic Science 5 (1): 24 - 30, 2011 ISSN: 2141 - 4181

http://www.wiloludjournal.com
Printed in Nigeria

EFFECT OF CRUDE OIL AND SOME PETROLEUM PRODUCTS ON *CLARIAS GARIEPINUS* FINGERLINGS (CATFISH: CLARIDAE)

[1]Ugwu, L.L.C., [1]Ude E.F., [2]Nwamba, H.O. and [2]Chima, I.N.
[1]Department of Fisheries and Aquaculture, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria, 480001.
[2]Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Nigeria.

ABSTRACT
Ninety (90) hatchery bred fingerlings of *Clarias gariepinus* (mean weight: 0.96 ± 0.1g) were randomly placed in 15 plastic baths (25 litres each) at the Research laboratory and were exposed to different concentrations of oil products to determine their effects on the fish, to facilitate inferential deductions that will enhance effective aquatic environmental management. Three (3) replicate basins of 5 experimental treatments (crude oil, petrol oil, kerosene oil, engine oil and control) were used at a concentration of 1.25ml. L^{-1}. The control experiment was devoid of oil treatment. Six (6) fingerlings were placed in each replicate basin, flooded with 20 litres of clean tap water and fed with nutrafin cichilid food, 2 times daily at 3% body weight. The results showed that the feeding behaviour and swimming performances of fish were reduced after 24 hours of the addition of the various oil pollutants. Mortality of fingerlings in the oiled basins increased as the hours of exposure increased (i.e. 24, 48, 72 and 96 hours). Recovery was not immediate in the treated basin while surviving fingerlings in the control basins grew up to post-fingerlings after 90 days (3 months). There were significant differences ($P<0.01$ and $P<0.05$) in the effect of crude oil and the petroleum products on the mortality rate of *C. gariepinus* when exposed to oil pollutants at 1.25ml. L^{-1} concentration.

KEYWORDS: *Clarias gariepinus,* mortality, pollutant, fingerlings.

INTRODUCTION

The Africa catfishes of the genus *Clarias* are a highly esteemed group of fishes in tropical Africa and they command high market value. Their hardy nature and possession of accessory air-breathing organs enable them tolerate adverse aquatic conditions (Reed *et al* 1967). Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil.

The concentration of crude oil toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved (Ikegwuani, 1980). Oil and oil products concentrations of 0.01ml.L^{-1} accelerated the death of fingerlings in aquatic environments (Lee, 1975). Adult fishes are more resistant to oil pollution since their bodies, mouth and gill chambers are coated with slimy mucus that resist wetting by oil. Quite unlike adult fish, which swim away from oil spill, many fish eggs and larvae float with the plankton at the water surface.

The concentrations of crude oil and fuel (0.05 – 10mL.L^{-1}) toxic to fish eggs and fingerlings have been estimated (Lonning, 1977). Freshwater fish are used as 96 hour bioassay organisms (Kopperdaul, 1976) for the determination of crude oil toxicity. Because of this reliance, freshwater quality near the spill is suitable for survival and growth of sensitive stages of aquatic organisms. Cardwell (1979), Stobber *et al.* (1978) and Kobayashi (1973) have demonstrated that such toxicity can occur in the aquatic environment near industrial oil spills and their results suggested that fish larvae, fingerlings and eggs are quite sensitive bioassay test organisms.

Large oil spills from Nigeria's oil industries are the most obvious form of oil pollution in recent times. Nearly 3000 cases of oil spill accidents that occurred between 1976 and 1990 resulted in the release of about 2.4 million barrels of crude oil, resulting in various forms of environmental degradation, deprivation and spoilage (Akingbade 1991).

An understanding of the effect of crude oil on development, growth, feeding, energetic and swimming activity of fingerlings is needed to assess the impact of oil pollution on fish populations (Anderson *et al*, 1974).

Although the uptake of crude oil and compounds from water is very rapid and bioaccumulation do occur, much is not known about what happens to these compounds within the fish. And although fishes have oxidative enzymes for metabolic detoxication of xenobiotics including aromatic petroleum hydrocarbons (Payne and Penrose, 1975), little is known about the metabolism of crude oil compounds in the fingerlings of *C. gariepinus*. However, most studies indicated a basic similarity of aromatic detoxication in mammals and fishes including evidence that mixed function oxidases (MFO) were induced in fish exposed to petroleum (Stageman and Sabo, 1976; Payne and Penrose, 1975).

The degree of exposure of marine organisms to oil is often assessed by measuring their body burden of petroleum related aromatic compounds (ACs) because ACs are potentially harmful to animals which extensively metabolize most ACs in their livers and predominantly excrete them into bile (Varanasi *et al*, 1989). A rapid screening method for bile, which determines the metabolites as fluorescent ACs (FACs) has proven useful in estimating the exposure of fish and marine mammals to petroleum (Varanasi *et al*, 1989). Dibenzo-thiophenols has been proposed by Krahn *et al* (1992) as promising marker compounds for identifying the exposure of fish to certain crude oils.

The uptake and translocation of crude oil compounds in fish may be through the gills, the guts or the intestinal wall (Roubal, *et al.*, 1977). The parent compounds readily solublize in cell membranes and are probably carried via the erythrocytes to the general circulation of the blood. Some of the compounds may be carried by lipoproteins and Leukocytes in the blood to the liver. Lee (1976) reported that the major route of excretion of petroleum metabolites is through the bile; into the intestine and out with faeces: while some are excreted through the gills and kidney.

The scarcity of *C. gariepinus* fingerlings to stock existing fishponds in Nigeria has been attributed to cannibalism and nutritional problems (Faturoti *et al.,* 1986) among other factors. The incessant occurrence of oil spill accidents from the Nigerian oil industry has constantly degraded the aquatic environments where fingerlings abound thereby depleting the already poor sources of supply. This study was undertaken to assess the effect of some petroleum products on the mortality rate of *C. gariepinus* and determine the comparative toxic level of the fractions of crude oil and petroleum products on the fish.

MATERIALS AND METHOD

Collection and Acclimation of Experimental Fingerlings

Ninety (90) fingerlings of *Clarias gariepinus* (Buchell) (mean weight: 0.96 ± 0.1g) were collected from Aquafish Nigeria Limited located at Ihiala, Anambra State of Nigeria and transported in 25 litre plastic containers to the Applied Biology laboratory of Enugu State University of Science and Technology. Water temperature was maintained at 27^{o}C during transportation by introducing ice cubes preserved in a portable cooler. The fingerlings were starved overnight to eliminate pre-consumed food; after which they were fed nutrafin cichilid food 2 times daily at 3% body weight for 96 h (4 days). The dissolved oxygen (D.O) content of water was maintained by aeration pumps connected to the experimental basins.

Experimental Design

Fifteen (15) plastic basins (25 litres each) were placed into five (5) groups according to experimental treatments (Crude oil, Petrol, Kerosene, Engine oil and Control) and replicated 3 times. Six (6) fingerlings (0.96 ± 0.1) were randomly allotted to the basins and flooded with 20 litres of tap water. Before the commencement of the experiment, the tap water sample was allowed to stand for 7 days to reduce the concentration of ammonia and chlorine. Water temperature in degree Celsius (^{o}C) and hydrogen ion concentration (pH) were also checked and recorded. The amount of free carbon dioxide was determined using Lind (1979) method.

The experiment which lasted for 94 days involved three phases. 1. Monitoring and recording of the mortality rate of fingerlings (*C. gariepinus*) under crude oil and petroleum fractions concentrations; 2. Observing the feeding behaviour and swimming performances of fingerlings; and 3. Checking the survival of fingerlings within 90 days after exposure to water – oil mixture for 96 h (4 days).

Determination of Physico-Chemical Parameters:

Water temperature (^{o}C) in the treatment basins were taken daily with the aid of mercury-in-tube thermometer; hydrogen ion concentration (pH) was determined with a litmus paper – dipped into the water-oil mixture and allowed to stand for 5 minutes before noting the corresponding colours of the pH indicator. Free carbon dioxide

(CO_2) was determined using the method described by Lind (1979): 250ml of water sample was measured into a 250ml Erlenmeyer flask. 10 drops of phenolphthalein indicator was added and titrated against sodium hydroxide (NaOH) solution until a weak pink colour was attained. The concentration of carbondioxide was calculated and expressed in $mg.L^{-1}$ thus:

Titre Value (ml) of N140 NaOH X 40 ($mg.L^{-1}$).
For the experimental treatments, increases in carbondioxide concentration were synonymous with decreases in dissolved oxygen concentration. This probably explained the reasons for the mortality of fingerlings recorded during the experiment (Table 4).

Exposure of Fingerlings to different Crude Oil and Petroleum fractions Concentrations:
The six (6) fingerlings of *C. geriepinus* (0.96 ± 0.1g) allotted per experimental treatment with $1.25mg.L^{-1}$ concentration of Crude oil, Petrol, Kerosene, Engine oil respectively and the control were maintained on a diet of nutrafin cichilid food and fed 2 times daily at 2% body weight. Uneaten food and fish excrements were periodically eliminated with a 5mm diameter plastic siphon and fresh water replenished to 20 litres mark.

The $1.25mg.L^{-1}$ concentration was adopted for the four oil treatments based on earlier findings (Nwamba *et al*, 2001) that the least concentration of crude oil that can probably kill half the population of catfish (*Heterobranchus bidorsalis*) fingerlings with 96 h (LC_{50}) was $1.25ml.L^{-1}$.

The *C. gariepinus* fingerlings in this experiment were monitored within 96 h of oil exposure for mortality, feeding behaviour and swimming performances. After 96 h exposure period, surviving fingerlings were transferred into fresh basins containing clean tap water and maintained for 90 days with their experimental feed to assess the success of their survival and degree of recovery from oil exposure.

Statistical analysis
The experiment was completely randomized and data obtained subjected to analysis of variance (ANOVA) Steel and Torrie, 1980).

RESULTS
The total numbers of dead fingerlings (*C. gariepinus*) per day in $1.25ml.L^{-1}$ concentration of crude oil, Kerosene, Petrol and Engine oil are shown in Table 1. The first record of mortality was obtained at 4.16pm on the second day of the experiment (24hours) when one fingerling in R_1 engine oil concentration died after struggling and gasping for air. Few seconds after this observation, one fingerling in R_1 crude oil concentration stood with its head pointing vertically upward, struggled for air and died (Table 1). In all, mortality of fingerlings in the various oiled basins increased as the hours of exposure to oil pollutants increased: (i.e., from 24, 48, 72 and96h).

Table 1: Number of dead fingerlings (*C. gariepinus*) per day in 1.25ml-l crude oil kerosene, petrol and engine oil.

TIME (HOURS)	REPLICATES	OIL CONCENTRATIONS (ml^{-1})				
		Crude oil	Petrol oil	Kerosene oil	Engine oil	Control
	R_1	1.00	0.00	0.00	1.00	0.00
24 h	R_2	0.00	0.00	0.00	0.00	0.00
	R_3	0.00	0.00	0.00	1.00	0.00
	Mean Value	0.33	0.00	0.00	0.67	0.00
	R_1	1.00	0.00	1.00	1.00	0.00
48 h	R_2	0.00	0.00	0.00	0.00	0.00
	R_3	0.00	0.00	0.00	1.00	0.00
	Mean Value	0.33	0.00	0.33	0.33	0.00
	R_1	1.00	0.00	1.00	1.00	0.00
72 h	R_2	0.00	0.00	0.00	0.00	0.00
	R_3	2.00	0.00	0.00	0.00	0.00
	Mean Value	1.00	0.00	0.33	0.00	0.00
	R_1	1.00	0.00	2.00	1.00	0.00
96 h	R_2	0.00	0.00	1.00	0.00	0.00
	R_3	1.00	0.00	0.00	0.00	0.00
	Mean Value	0.67	0.00	1.00	0.33	0.00

From the mortality records obtained, it was evident that the highest number of fingerlings died in the crude oil treatment (Table 2) followed by those in kerosene and engine oils respectively. Fingerlings in the petrol and the control treatments gave the same results before 96 h (Table 1). After 96 hours, fingerlings in the petrol oil treatment started dying. As indicated below, all the surviving fingerlings after 96 h were transferred into fresh basin with clean tap water and monitored for survival up to 90 days. *C. gariepinus* in the petrol oil treatment started dying after 98 hours while all the surviving fingerlings treated with crude oil and kerosene oil died within 14 and 21 days experimental period. Only fingerlings in the control survived without any record of mortality and grew up to post-fingerlings within 90 days (3 months).

TABLE 2: Percentage mortality rate of fingerlings (*C. gariepinus*) within 96 h (4 days).

Treatment	No. of fingerlings	Mean weight of fingerlings (g)	Number of dead fingerlings	Percentage mortality (%)
Crude Oil 1.25mL.L^{-1}	18	1.00	7.00	38.88
Petrol Oil 1.25mL.L^{-1}	18	0.70	0.00	0.00
Kerosene Oil 1.25mL.L^{-1}	18	0.90	5.00	27.78
Engine Oil 1.25mL.L^{-1}	18	0.80	4.00	22.23
Control	18	1.40	0.00	0.00

Table 3 shows the effect of oil treatments (Crude Oil, Petrol oil, Kerosene oil and engine oil) on the fingerlings of *C. gariepinus*. Apart from the control experiment, which recorded no fingerling mortality, the mean mortality within 96 h was highest in crude oil treatment (1.75) and least in petrol oil treatment (0.00). Statistically the effect of crude oil and some petroleum products on the mortality rate of *C. gariepinus* was significant at both 1% and 5% probabilities (Table 3).

Table 3: Effect of 1.25ml.L^{-1} Concentrations of Crude Oil and some Petroleum products on *C. gariepinus* Fingerlings.

Treatment	Number of dead fingerlings (mortality)			Total Mortality	Mean mortality
	*R_1	*R_2	*R_3		
1.25mL.L^{-1} Crude Oil	4.00	0.00	3.00	7.00	1.75
1.25mL.L^{-1} Petrol Oil	0.00	0.00	0.00	0.00	0.00
1.25mL.L^{-1} Kerosene Oil	3.00	2.00	0.00	5.00	1.25
1.25mL.L^{-1} Engine Oil	3.00	0.00	1.00	4.00	1.00
Control	0.00	0.00	0.00	0.00	0.00
Grand Total				16.00	4.00

F-value = 18.55; ($P>0.01$) and ($P<0.05$).
F-Tabulated = 3.48 (5%); 5.99 (1%). *R_1, R_2, R_3 = Replications

Oil was clearly observed on food particles in the treatment basins. No obvious dumping of food particles and food was layered in a similar manner in both control and oiled basins.
Feeding behaviour in all the oiled basins showed that the approach and browsing of food by fish was fast before the addition of crude oil and its fractions, and this was greatly reduced after 24 h. Feeding speed was observed to be normal in the control basin throughout the experiment. No special instrument was applied to measure fish speed and reaction to food availability. Swimming performances were normally fast throughout the experiment.

After 96 h (4 days) exposure and subsequent maintenance in tap water, surviving fingerlings were observed to have improved on their feeding behaviour while their swimming performance was the same as to when they were still in the oil mixture basins. There was increases in the free carbondioxide concentration (Table 4) as the exposure period increased.

TABLE 4
Free carbon dioxide (CO_2) mgl, records in oiled basins treated with crude oil and petroleum fractions:

PERIOD (HOURS)	REPLICATES	OIL CONCENTRATIONS ($ml.L^{-1}$)				
		Control	Crude	Kerosene	Engine	Petrol
	R_1	0.15	0.20	0.20	0.10	0.20
24	R_2	0.15	0.15	0.20	0.20	0.10
	R_3	0.15	0.50	0.50	0.10	0.10
	Mean Value	0.15	0.28	0.30	0.13	0.13
	R_1	0.10	0.30	0.15	0.15	0.10
48	R_2	0.30	0.20	0.15	0.20	0.10
	R_3	0.20	0.30	0.15	0.15	0.10
	Mean Value	0.20	0.29	0.15	0.17	0.10
	R_1	0.16	0.30	0.20	0.10	0.20
72	R_2	0.16	0.30	0.30	0.20	0.15
	R_3	0.16	0.30	0.20	0.10	0.10
	Mean Value	0.16	0.30	0.23	0.13	0.15
	R_1	0.20	0.40	0.30	0.50	0.20
96	R_2	0.20	0.40	0.30	0.50	0.20
	R_3	0.20	0.30	0.50	0.50	0.20
	Mean Value	0.20	0.37	1.00	0.50	0.20

DISCUSSION

Crude oil and petroleum fractions (Kerosene, Engine oil and petrol) cause the blockage of atmospheric oxygen from dissolving in water thereby limiting the supply of oxygen to fish fingerlings resulting to incidence of excretory waste products (carbondioxide, ammonia) in the ambient water environment. Increases in the free carbondioxide concentration (Table 4) as the exposure period increases could be synonymous with decreases in dissolved oxygen (D.O) concentration and this probably explained the mortality of *C. gariepinus* fingerlings recorded during the experiment (Tables 1 and 2). From the mean mortality of fingerlings (Table 1), the $1.25ml.L^{-1}$ crude oil treatment could probably be adjudged as the treatment that obliterated nearly half the population of fingerlings (7) from an original number of eighteen (18) within 96 h (LC_{50}). The percentage mortality derived from this estimation was 38.8% (Table 3). This study agreed with Nwamba *et al* (2001) experiment which recorded 56.0% mortality for *Heterobranchus bidorsalis* fingerlings treated with $1.25ml.L^{-1}$ crude oil. The mortality experienced in the crude oil and petroleum fractions treatments ($1.25ml.L^{-1}$) was in consonance with Lee's (1975) view that oil and oil products concentration of $0.01ml.L^{-1}$ accelerated the death of fingerlings in aquatic environments.

Comparing the percentage mortality rates of *C. gariepinus* (Table 2) in the four oil treatments, it was obvious that crude oil adversely affected the survival of fingerlings than any of its fractions (petrol, kerosene and engine oil). This observation provided useful insights to the menace of crude oil to aquatic organisms during regular oil spillage in Nigeria. The percentage mortality (38.8%) of *C. gariepinus* was, however, less than that recorded by Nwamba *et al* (2001) (56.0%) when *H. bidorsalis* fingerlings were exposed to crude oil ($1.25ml.L^{-1}$) for 96 hours. This revealed that *C. gariepinus* was more resistant and had a higher survival propensity than *H. bidorsalis* when exposed to crude oil pollution.

Recovery was not immediate for fingerlings in the treated basins while surviving fingerlings in the control basin grew up to post-fingerlings after 90 days (3 months). This agrees with Kopperdaul's (1976) report that freshwater quality near the spill is suitable for survival and growth of sensitive stages of aquatic organisms.

CONCLUSION

The responses (survival and mortality) of *C. gariepinus* in this study provided some basis for predicting the overall impact of Nigerian oil spills on fingerling populations. Hydrocarbons constitute a minor but ubiquitous component of all aquatic organisms. The chemical stability and wide structural differences make these hydrocarbons a good environment; especially when release is increasingly important to differentiate between biogenic and fossil fuel hydrocarbons in order to accurately assess the extent of oil pollution. Knowledge of

natural hydrocarbon background in Nigerian aquatic environments is limited and need to be expanded to include indicator species where possible.

REFERENCES

Akingbade, T. (1991). Nigeria: On the trial of environment. Triple 'E' Systems Associates Ltd. Lagos, pp 35-36.

Anderson, J.W., Neff, J.M., Cox, B.A. Tatem, H.E. and Hightower, G.M. (1974). Characteristics of dispersions and water solute extracts of crude oil and refined oils and their toxicity to aquatic crustaceans and fisheries. Mar. Biol. 27, 75-88.

Cardwell, R.D. (1979). Toxic substances and water quality effect of larval aquatic organisms. Washington Dept. of Fisheries Tech. Paper No 45; pp 71 – 79.

Faturoti, E.O., Balogun, A.M. and Ugwu, L.L.C. (1986). Nutrient Utilization and growth responses of Clarias (*Clarias Lazera*) fed different dietary protein levels. Nigerian Journal of Applied Fisheries and Hydrobiology 1:41-45.

Ikeaguani, F.D. (1980). Trends of petroleum exploration in Nigeria. NNPC Report Lagos.

Kopperdaul, F.R. (1976). Guidelines for performing static acute toxicity fish bioassays in municipal and industrial waste waters. California State Water Resources control board report. 65pp.

Krahn, M.M., D.G. Burrows, G.M. Yitalo, D.W. Brown, C.A. Wigren, Collier, T.K. Sin-Lan C. and Varanasi, U. (1992). Mass spectrometer analysis of aromatic compounds in bite of fish sampled after Exxon Valdes oil spill Environ Sci. Technol. 26, 116-126.

Lee, R.F. (1975). Fate of petroleum hydrocarbons in marine zooplankton. In: Proc. on prevention and control of oil pollution. American Petroleum Institute, Washington D.C. 549-553.

Lee, R.F. (1976). Metabolism of petroleum hydrocarbons in marine sediments. In: Sources, effects and sinks of hydrocarbons in aquatic environment. 333-344pp. American Institute Biological Sciences.

Lind, O.T. (1979). Handbook of common method in Limnology. The C.V. Mosby Publisher, Missouri, USA 2nd Edition. 199pp.

Lonning, S. (1977). The effects of crude Ekofi Sk oil and oil products on marine fish larvae. Astarte 10: 37-47.

Nwamba, H.O., Ugwu, L.L.C. and Aghamuo, H.U. (2001). Effect of Nigerian Bonny light crude oil on mortality rate of *Heterobranchus bidorsalis* fingerlings. In: proceedings of 16th Annual Conference and International Workshop of the Nigerian Association of Aquatic Science 3rd – 6th October 2001.

Payne, J.F. and Penrose, W.R. (1975). Induction of akyl hydrocarbon (benzo (a) Pyrene) hydroxylase in fish by petroleum. Bull Environ. Contam. Toxicol., 14: 112-226.

Reed, W.J. Burchard, Hopson, A.J., Jonathan, J. and Ibraham, Y. (1967). Fish and fisheries of Northern Nigeria. Govt. Press, London, 226pp.

Roubal, W.T., Collier, T.K. and Malins, D.C. (1977). Accumulation and metabolism of carbon – 14 labelled benzene, naphthalene and Anthracene by young cohosalmon (*Onchorynchus Kisutch*) Arch. Environ. Contam. Toxicol., 5: 515-529.

Stagemen, J.J. and Sabo, D.J. (1976). Aspects of the effects of petroleum hydrocarbons in intermediary metabolisms in marine fish. In: Sources, effects and sinks of hydrocarbons in aquatic environments pp 508, American Institute of Biological Sciences.

Steel R.G.D. and Torrie, J. H. (1980). Principles and procedures in statistics. McGraw-Hill, New York, NY, USA, Toronto and London, 451pp.

Stobber, Q.J., Dannel, P.A., Wert. M.A. and Nakatani, R.E. (1977). Toxicity of West Point effluent to marine indicator organisms part II. Univeristy of Washington College of Fisheries, Final Report, Fri-UN-7937.

Varanasi, U., S.L. Chan, MacLeod, W.D., Stein, J.E., Brown, D.W., Burrows, D.G., Tilbury, K.L., Landahl, J.J., Wigren, C.A., Hom, T. and Pierce, S.M. (1989). Surrey of subsistence fish and shellfish for exposure to oil spilled from the Exxon Valdez. NOAA Tech. Memo NMFD 1990, F/NWC-191.

Received for Publication: 02/05/2011
Accepted for Publication: 04/06/2011

Corresponding author:
Ude E.F.
Department of Fisheries and Aquaculture, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria, 480001
Email: **emm_ude@yahoo.co.uk**