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Original article

Evaluation of the response of *Clibanarius africanus* (Decapoda: Paguridae) to crude oil in static bioassay

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ABSTRACT

The acute toxicity of Nigeria Bonny light crude oil against hermit crab, *Cliabanarius africanus* of a tidal creek, Eastern Obolo, Akwa Ibom State, Nigeria was investigated in the laboratory under static bioassay. The test crude oil was found to be poorly toxic to the test organism, resulting in delayed mortality and consequent extension of the bioassay to 8 days. Based on the LC_{50}, the toxicity of the test compound was more manifested on the 8 day than at 96 hour, with a toxicity factor showing that the test compound was approximately 12 times, more manifested against *C. africanus* on the 8 day than at 96 hour. Paired t-test showed that there was no significant difference between 96h LC_{50} (549.9 ml l^{-1}) and 8d LC_{50} (45.2 ml l^{-1}). The results of this study indicated that *C. africanus* is not a good early warning indicator for oil pollution but its response during spills could serve as a good indicator of adverse impact.

1. Introduction

Hydrocarbon pollution is a worldwide problem affecting the atmosphere, soil and water (Akpan and Frank, 2003). Petroleum oil constitutes a diverse range of hydrocarbons such as aliphatic, alicyclic and aromatic compounds (Atlas, 1988; Odokuma and Oliwe, 2003). Nigeria is a major oil producing country in the world with a

large number of on/off shore oil installations (Baker, 1981). Hydrocarbons can be quite destructive to marine ecosystems. They can directly poison marine life, disrupt ecosystems by destroying juvenile forms of life and other links in the food chain and interfere with the communication and information gathering systems of animals (Smith, 1977).

The effects of exploration and production activities of the oil industry on the Nigerian environment has been the cause of great concern in recent times. Since the early 1970s, studies have been carried out on the effects of crude oil with the initial ones concentrated on effects on land and aquatic environment with special emphasis on microorganisms and the degradation of oil by these organisms. Much of the existing literature concerned with the effects of petroleum hydrocarbons on marine species is based on the laboratory studies of acute toxicity (Morrow, 1973; Anderson *et al*., 1974). In Nigeria, relatively few studies have been conducted on toxicity of oil to local estuarine fauna. Because mortality may be delayed, short term toxicity test may be extended from 96h to 8 days (APHA *et al*., 1985).

This study therefore investigated the acute toxicity of Nigeria Bonny light crude oil against *Cliabanarius africanus* (hermit crab) through 96h and 8d in static bioassay, and compared the 96h LC_{50} value with the 8d LC_{50} value.

2. Materials and methods

The acute toxicity of Nigeria Bonny light crude against *C. africanus* was investigated to determine the 96h and 8d LC_{50} in static bioassay. Specimens of *C. africanus* were hand picked from Okoro Creek shoreline. Okoro Creek is located in Eastern Obolo Local Government Area, Akwa Ibom State, Nigeria along the Imo River Estuary between 7°30' and 6°00'E and 5°15' and 4°45'N (Fig.1). The water is brackish and the substratum is made of mud. The mangroves vegetation is dominated by the red mangrove, *Rhizophora racemosa*.

The test organisms were transported to the laboratory in plastic containers with water from the site and mud as substrate. In the laboratory, the crabs were kept in holding plastic containers (65 litres volume, 34 cm bottom diameter and 30cm depth) with water collected from where the organisms were picked and mud from the same site as substrate.

The hermit crabs fed with ground crayfish were kept in the containers for at least seven days, to allow them acclimate to laboratory conditions (28°C $\pm$ 2°C) before using them in bioassays. About 100 individuals were kept in each container. The water and substratum were changed every 48h to avoid accumulation of toxic waste metabolites from the specimens and remnants of food particles. The salinity of the water was 11‰. The total hydrocarbon content (THC) was 0 mg/l. Acclimation of test organisms to laboratory conditions and conduct of experiment were in accordance with guidelines for bioassay techniques (APHA *et al*., 1985).

The crude oil was obtained in 10 litres plastic from Department of Petroleum Resources, Eket, Akwa Ibom State, Nigeria. Water-soluble fraction (WSF) of the crude oil was prepared by rapidly stirring a 50:50 product-water mixture in a 1litre flask for three hours on a magnetic stirrer. The resultant solution was poured into a 1 litre separatory funnel and the system was allowed to stand for 24 hours to effect complete phase separation, after which the lower aqueous layer, containing the WSF, was collected for toxicity testing.

Plastic bowls (10 litres volume, 20cm surface diameter, 23cm bottom diameter and 10cm depth) were employed in all bioassays, as containers. Mud from the natural habitat of the hermit crab was provided at the bottom of each bioassay container to simulate its natural habitat. The mud was spread on a wooden board and dried for up to 7 days. The sun dried mud was then sieved using a 0.25mm mesh size sieve. A total of 500g of the prepared mud was weighed out and spread evenly on the bottom of the test media. A predetermined volume of the test compound was pipette into a measuring cylinder and made up to ½ litre by adding approximate units of filtered brackish water, to achieve the desired concentration of the test compound. 10 active specimens of about the same size (2.87 ± 0.12cm mean length and 1.97 ± 0.09g mean weight) were randomly introduced into each test medium, each concentration having a replicate including untreated control. The concentrations of the media were as follows: 70, 100, 150, 200, 250, 300 and 350 ml l^{-1}, and untreated control.

Mortality assessments were carried out every 24h over 8 days experimental period. *C. africanus* was taken to be dead if it failed to come out of its shell over observation period of 5 – 10 minutes or failed to withdraw feet outside shell when prodded with a glass rod. Due to delayed mortality, feeding of organisms in test media continued after 96h to the 8d. Feeding was done with 0.6g ground crayfish per test medium.

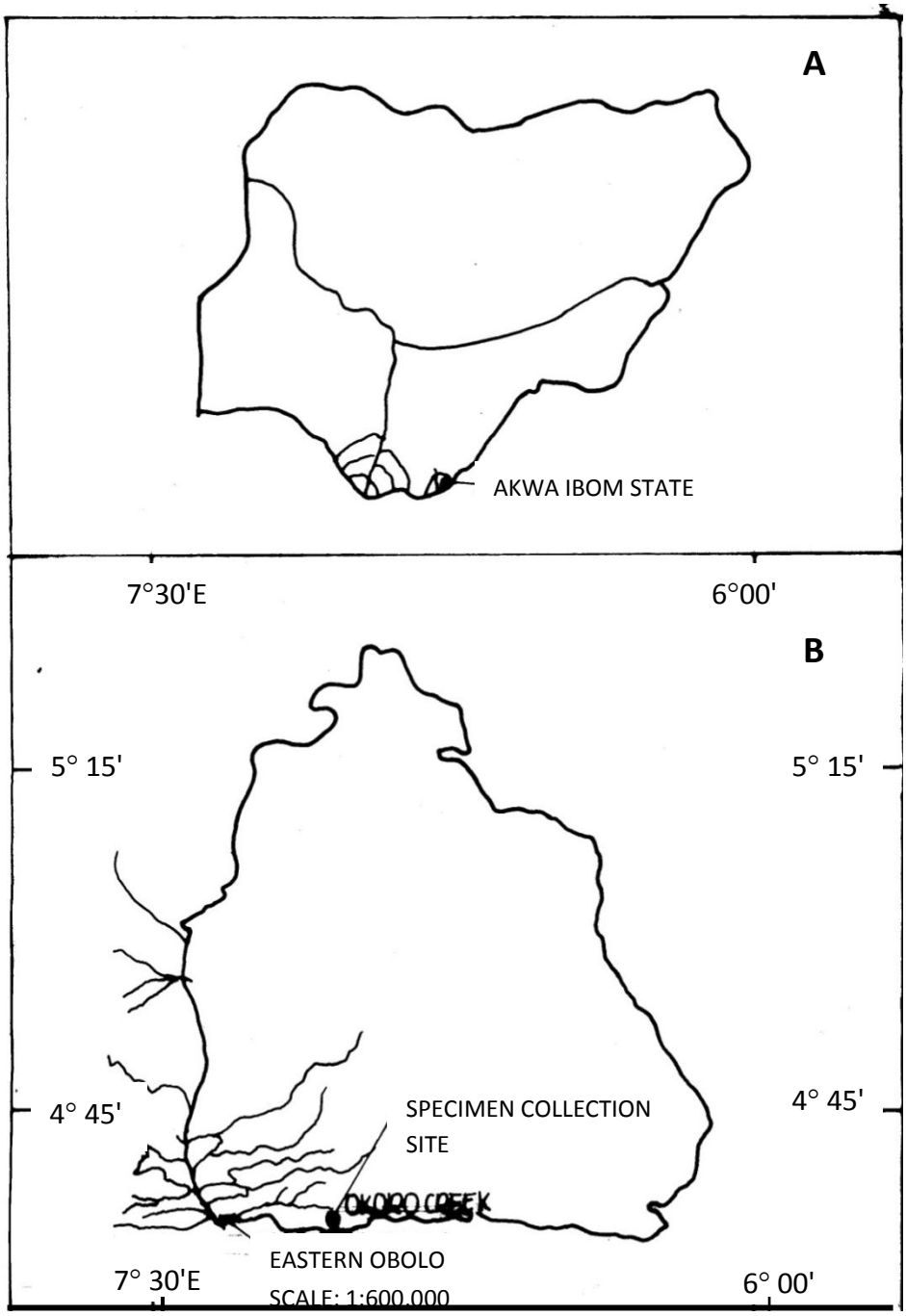

Fig. 1. Map of specimen collection site: A. Nigeria showing Alwa Ibom State, B . Akwa Ibom State showing specimen collection site in Okoro Creek.

Toxicological dose response (mortality) was analysed by probit analysis, after Finney (1982). The analysis including equation for probit line was achieved via a computer-run programme (SPSS 10 package) dependent on maximum likelihood weighted regression. The 96h LC_{50} values and their 95% confidence limits (CL) were derived from the analysis and used as indices for measuring the toxicity of the crude oil. Test of significance between the 96h LC_{50} and 8d LC_{50} was conducted using the paired t-test (Ogbeibu, 2005).

3. Results

When Boony light crude oil was tested against the test species, *C. africanus* under static bioassay conditions, it was found to be poorly toxic, resulting in delayed mortality and consequently the extension of the bioassay to 8 days. On the basis of LC_{50} values, toxicity of the crude oil was more manifested on the 8d than at 96h (Table 1).

Table 1
Toxicity of Bonny light crude oil against *C. africanus* in static bioassay

Time	LC50 (CL) ml l^{-1}	Regression equations (probit response)	T. F.
96h	549.9 (310.9 – 5898.6)	Y = 2.972 + 1.205X	1
8d	45.2 (7.9 – 76.5)	Y = 3.284 + 1.246X	12

LC = Lethal concentration, CL = 95% confidence limit, T. F. = Toxicity factor

Paired t-test showed that there was no significant difference (P>0.05) between the 96h LC_{50} and 8d LC_{50}, the computed values being 549.9 ml l^{-1} and 45.2 ml l^{-1} respectively. Computed toxicity factor/ratio based on the LC_{50} values showed that toxicity of the crude oil was approximately 12 times more manifested against *C. africanus* on the 8d than at 96h. Figure 2 depicts the log-dose probit graph illustrating the toxicity profile of the crude oil against the test species at 96h and 8d.

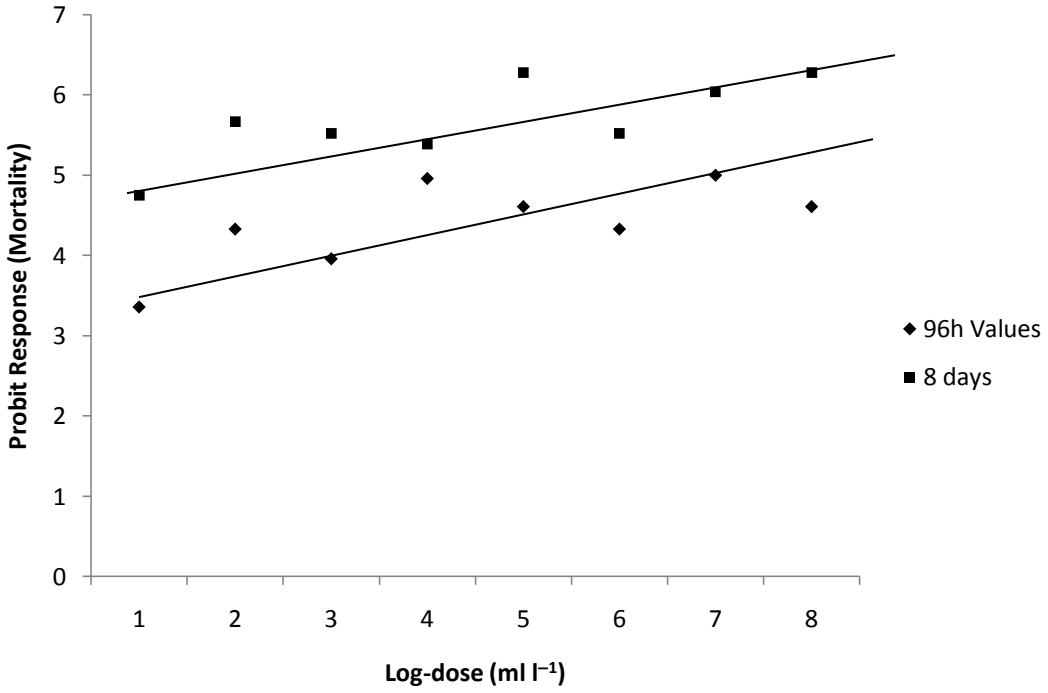

Fig.2. Log-dose probit graph depicting the relative toxicity of Bonny light crude oil against *C. africanus* based on 96 hours and 8 days values under static bioassay.

4. Discussion

Adverse effects resulting from exposure to crude oil can range from biochemical to organismal in scope (Singer *et al*., 1998). Generally, it has been well established that one of the mechanism of action of petroleum

products against exposed animals is that it limits gaseous exchange by coating the respiratory surfaces, such as spiracles, skin and gills of exposed organisms (Chukwu and Odunzeh, 2006). Determining toxic effects on decapods is complicated by three factors, namely, initial paralysis, delayed response, and much greater sensitivity at molting periods (APHA *et al*., 1985). This could be attributed to the delayed mortality of *C. africanus* to Bonny light crude oil in this study.

Comparisms of toxicological effects of crude oil WSF are difficult, because hydrocarbon concentrations present in the petroleum are extremely variable according to its origin (Neff *et al*., 2006). Other factors can be described, like the different methodologies applied for WSF preparation (Singer *et al*., 2000), and distinct tolerance to crude oil presented by different species (Ramachandran *et al*., 2006). Some of these factors could account for the difference between toxicity of *C. africanus* against crude oil in this study and its toxicity against other oil products, or other crustaceans against crude oil. For instance, while LC_{50} of 10.01 ml l^{-1} was established for *C. africanus* spent lubricant oil in 96h by Chukwu and Odunzeh (2006), LC_{50} of 45.2 ml l^{-1} was established for *C. africanus* against Bonny light crude oil in 8d for this study. This value is also different from 96h LC_{50} values recorded for other crustaceans in previous studies. Ndimele *et al*. (2010) recorded 96h LC_{50} value of 126 mg l^{-1} for the shrimp *Desmocaris trispinosa* against Bonny light crude oil. Singer *et al*. (1999) used a flow through exposure system to access the effects of Prudhoe Bay oil to *Holmesimysis* and *Atherinops affinis* and got LC_{50} values ranging from 14.28 – 16.84 mg l^{-1} and 5.77 – 12.17 mg l^{-1} respectively. Fuller *et al*. (2004) recorded LC_{50} of 0.62 mg l^{-1} for water-soluble fraction of Arabian medium oil to shrimp, *Americamysis ahia*.

5. Conclusion

The results of this study have shown that *C. africanus* is not a good early warning indicator of crude oil pollution. However, impact of crude oil on *C. africanus* at spills could serve as good indicator of adverse impact. This is important because previous study by Snowden and Ekweozor (1987) on the impact of oil spillage in the Bonny Estuary, Niger Delta, Nigeria recorded a near total elimination of the littoral infaunal and a highly significant mortality.

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