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Assessment of the response of *Goniopsis pelii* (Decapoda, Graspidae) to crude oil in static bioassay

Alice Ekwu, Arit I. Utong, and Blessing J. Oribhabor

Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Uyo, P. M. B. 1017, Uyo, Akwa Ibom State, Nigeria. Corresponding author: B. J. Oribhabor, oribhaborblessjuls@yahoo.com

Abstract. The assessment of acute toxicity of Nigeria Bonny light crude oil against purple mangrove crab, *Goniopsis pelii* of a tidal creek, Oron, Akwa Ibom State, Nigeria was conducted in the University of Uyo, Uyo, Nigeria Fisheries laboratory under static bioassay in November, 2009. The crude oil was poorly toxic to the test organism, causing delayed mortality and consequent extension of the bioassay to 8 days. The LC_{50} indicated that the toxicity of the test compound was higher on the 8 day than at 96 hour, with a toxicity factor indicating that the crude oil was 3 times more toxic against *G. pelii* on the 8 day than at 96 hour. Paired t-test showed that there was no significant difference ($p>0.05$) between 96h LC_{50} (791.9 ml L^{-1}) and 8d LC_{50} (275.8ml L^{-1}). This study showed that *G. pelii* is not an early warning indicator for oil toxicity but its response during spills could serve as a good indicator of adverse impact.
Key Words: bonny light crude oil, *Goniopsis pelii*, static bioassay, tidal creek, Nigeria.

Introduction. Accidents involving petroleum hydrocarbon spills occur frequently around the world and the annual worldwide estimate of petroleum input to the sea exceed 1,300,000 metric tones (NAP 2003; Rodrigues et al 2010). Nigeria is a major crude oil producing country in the world with a large number of on-shore, off-shore oil installations (Baker 1981). The country has a total network of 5001 km of oil pipelines, consisting of 4315 km of multi-product pipelines and 66 km of crude oil pipelines (Renner et al 2008). Corrosion and leakages from these pipelines and vandalism from saboteurs bring about oil spills that cause harm to aquatic organisms.

Most assays on the toxicity of oil and oil dispersants all over the world involve the use of aquatic animals (Akintonwa & Ebere 1990; Chukwu & Odunzeh 2006). These tests are characterized by highly variable results that are dependent on the type of test organism and life stage as well as the origin and solubility of the oil and dispersant being assayed. Because mortality may be delayed, short term toxicity test may be extended from 96h to 8 days (APHA et al 1985).

Researches related to the toxic water-soluble fraction (WSF) of petroleum and derivates to brackishwater fauna are scarce. This study therefore evaluated the acute toxicity of Nigeria Bonny light crude oil against *Goniopsis pelii* (purple mangrove crab) through 96h and 8 days in static bioassay, and compared the 96h LC_{50} value with the 8 days LC_{50} value.

Materials and Method. Evaluation of the acute toxicity of Nigeria Bonny light crude oil against *G. pelii* was conducted to determine the 96h and 8 day LC_{50} in static bioassay in November, 2009. Specimens of *G. pelii* were hand picked into baskets from the inter-tidal mudflats of Uya Oron Creek with the aid of the local fishermen (Figure 1). Uya Oron Creek is located in Oron Local Government Area, Akwa Ibom State, Nigeria, between 7°30' and 8°15' E, and 5°00' and 5°30' N (Figure 2). The water is brackish and the substratum is made of mud. The mangroves vegetation is dominated by the red mangrove, *Rhizophora racemosa*.

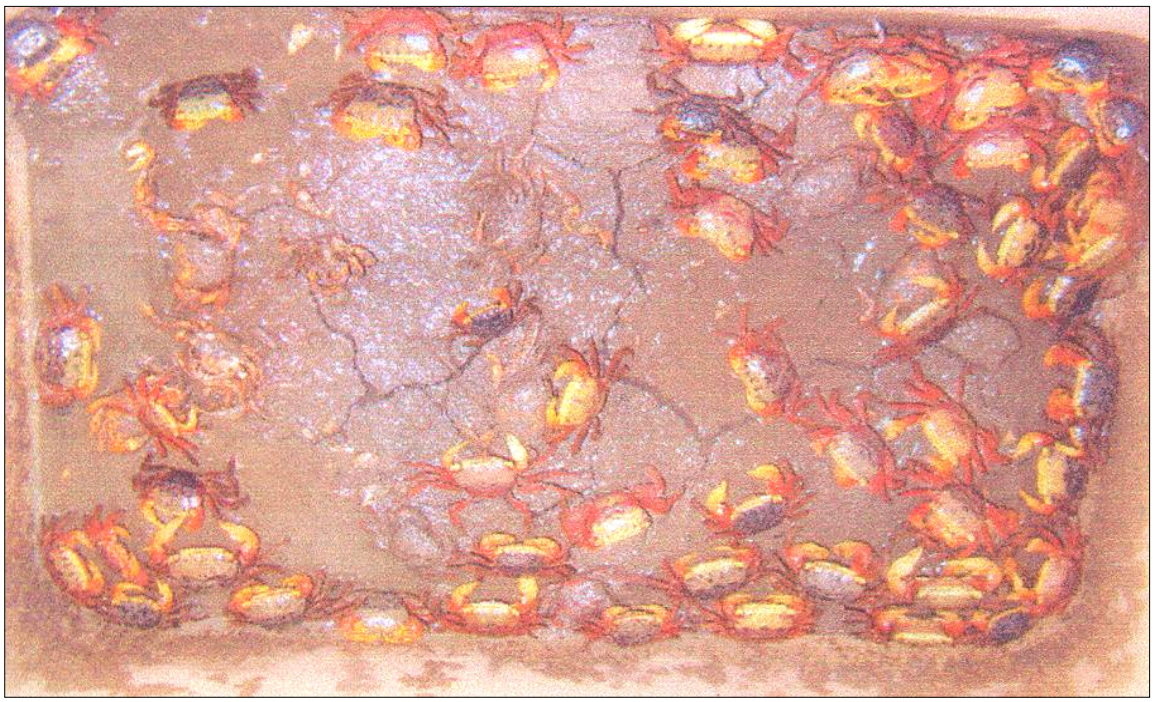

Figure 1. Crabs burrowing in mud at collection site.

The test organisms were transported to the University of Uyo, Uyo, Nigeria Fisheries 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 volume, 34 cm depth) with water collected from where the organisms were picked, and mud from the same site as substrate.

The purple mangrove crabs fed with ground crayfish were kept in the containers for at least seven days, to allow them acclimate to laboratory conditions (28°C ± 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 crabs and remnants of food. Acclimation of the test crabs to laboratory conditions and conduct of experiment followed the 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 1 L 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, 30 cm surface diameter, 23 cm bottom diameter and 10cm depth) were employed in all bioassays, as containers. Mud from the natural habitat of the purple mangrove 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 0.25 mm mesh size sieve. A total of 500 g 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 L by adding appropriate units of filtered water from the environment of the test organism, to achieve the desired concentration of the test compound.

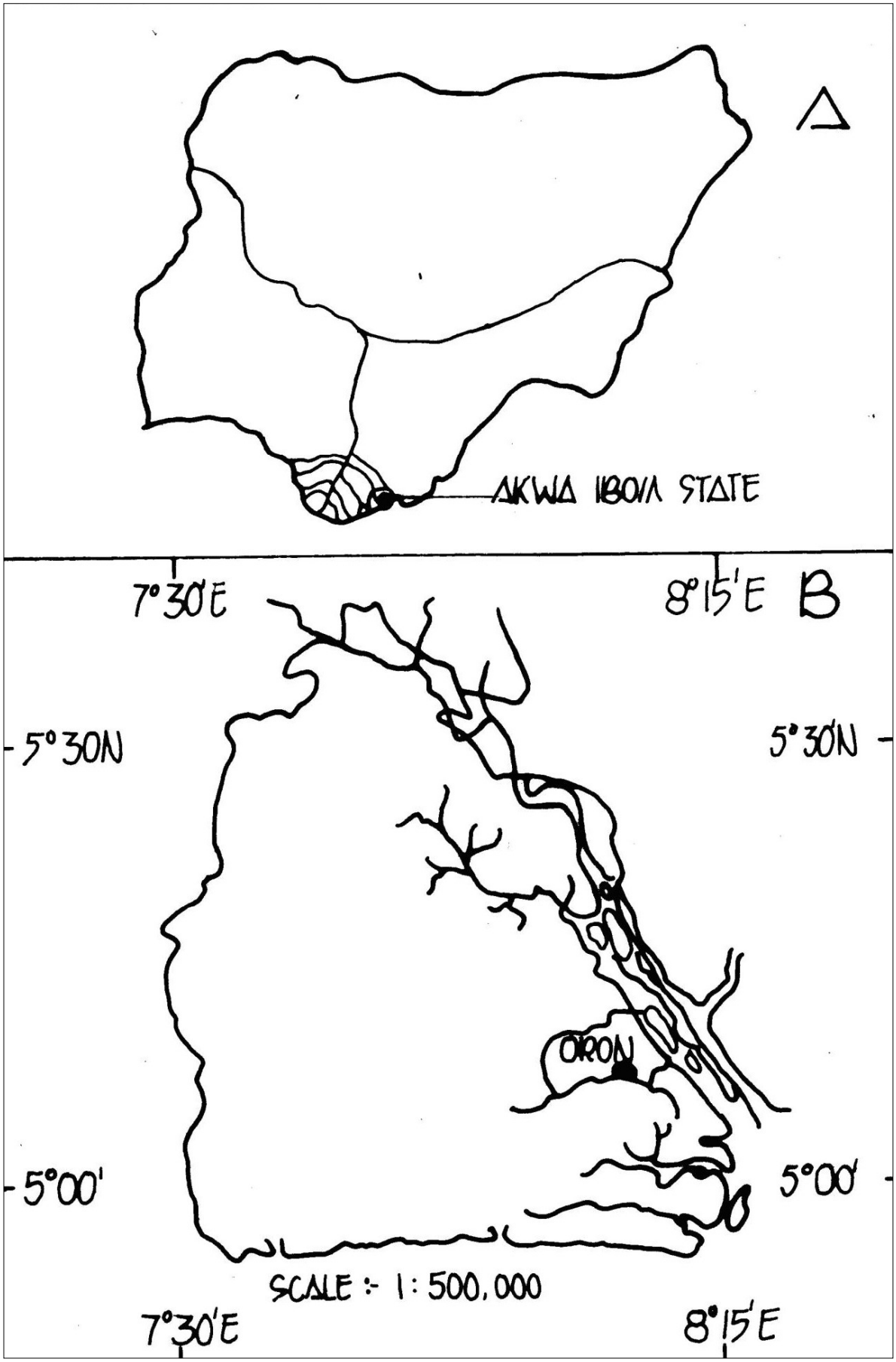

Figure 2. Map of specimen collection site: A. Nigeria showing Akwa Ibom State; B. Akwa Ibom State showing specimen collection site in Uya Oron Creek.

Ten active specimens of about the same size (3.25 ± 0.17 cm mean length and 2.89 ± 0.12 g mean weight) were randomly introduced into each test medium, each concentration having a replicate including untreated control (Figure 3). The concentrations of the media were as follows: 50, 70, 100, 150, 200, 250 and 350ml L^{-1}, respectively, and untreated control.

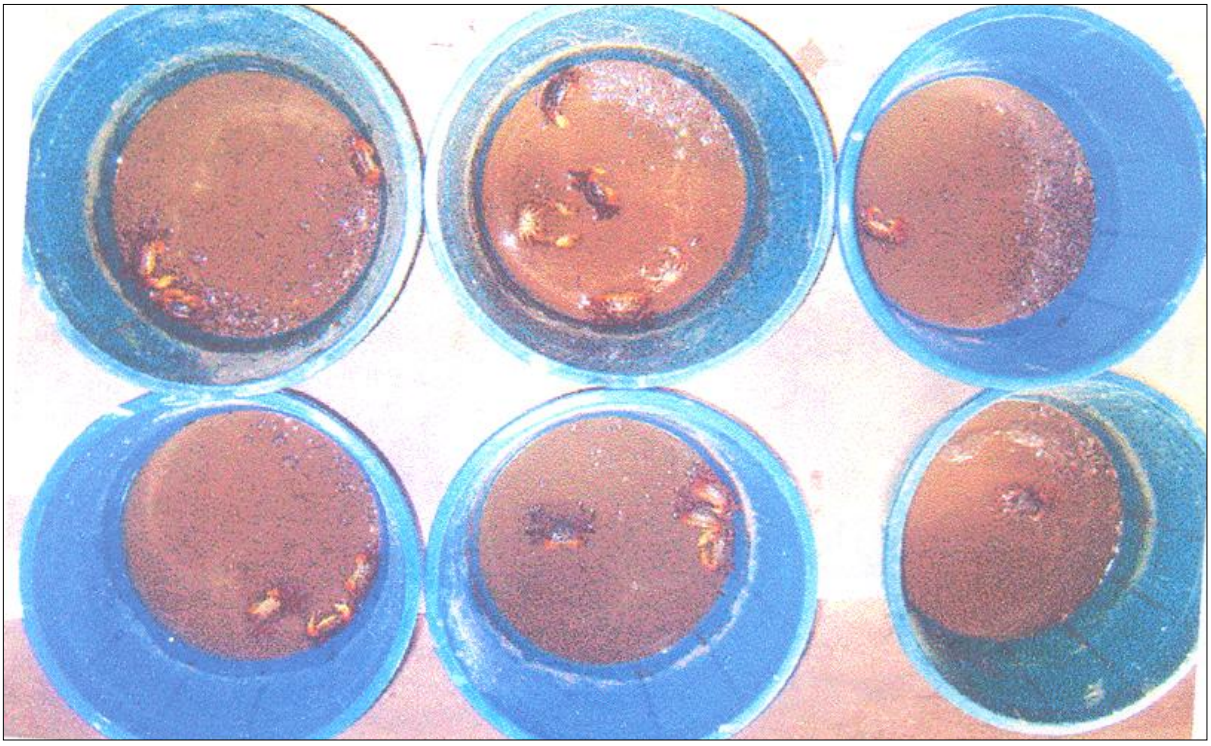

Figure 3. Bioassay containers with organisms in toxicants.

Mortality assessment was carried out every 24h over 8 days experimental period. *G. pelii* was taken to be dead if it failed to move when prodded with a glass rod. Due to delayed mortality, feeding of organisms in the test media continued after 96h to the day 8. Feeding was done with 1.4 g ground crayfish per test medium.

Toxicological dose response (mortality) was analysed by probit analysis, after Finney (1971). The analysis including equation for probit line was achieved via a computer-run program (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 8 day LC_{50} was conducted using the paired t-test (Ogbeibu 2005).

Results. Bonny light crude oil was found to be poorly toxic, when it was tested against *G. pelii*, resulting in delayed mortality, and consequently the extension of the bioassay to 8 days. On the basis of LC_{50} values, the crude oil was more toxic on the 8 day than at 96h (Table 1).

Table 1

Toxicity of Bonny light crude oil against *G. pelii*

Time	*LC_{50} (CL) ml L^{-1}*	*Regression equations (probit response)*	*T.F.*
96h	791.974 (407.225–15929.092)	Y = 2.945+1.363X	1
8 days	275.806 (195.179–630.613)	Y = 5.267+2.462	3

LC = lethal concentration, CL = 95% confidence limit, T.F. = toxicity factor

Paired t-test indicated that there was no significant difference ($p>0.05$) between the 96h LC_{50} and 8 days LC_{50} values, the computed values being 791.974 ml L^{-1} and 275.806 ml

L^{-1}, respectively. The computed toxicity factor/ratio based on the LC_{50} values indicated that Bonny light crude oil was 3 times more toxic against *G. pelii* on the 8 day than at 96h. The log-dose probit graph depicting the toxicity profile of the crude oil against the test organism at 96h and 8d were non-parallel (Figure 4).

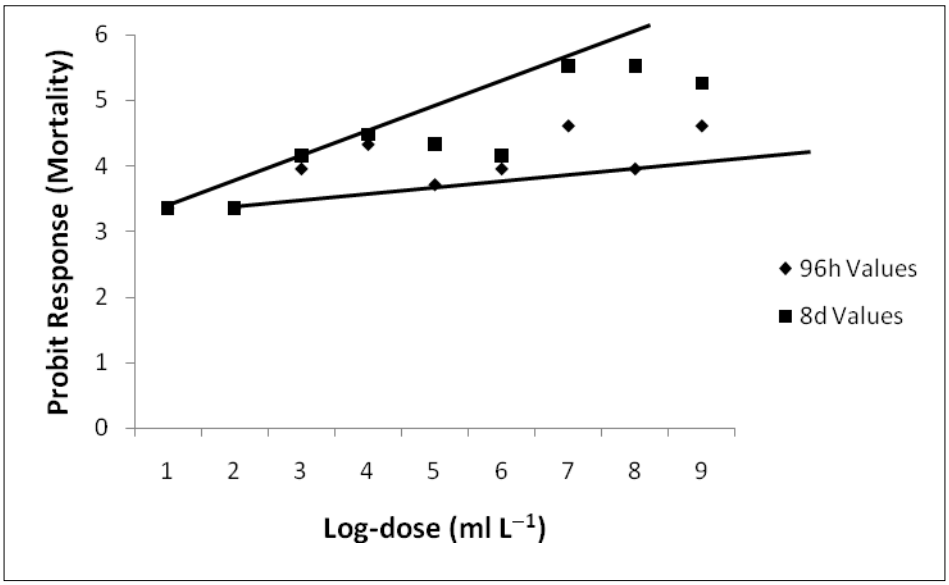

Figure 4. Log-dose probit graph depicting the relative toxicity of Bonny light crud oil against *G. pelii* under static bioassay.

Discussion. In short-term acute testing, the toxicity of water-accommodated, or water soluble fraction of untreated oil can be surmised to stem principally from dissolved hydrocarbons, because these solutions are generally free of significant amounts of particulate material (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 & 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 responsible for the delayed mortality of *G. pelii* in this study.

The concentrations of hydrocarbons present in the water soluble fractions (WSF) of different crude oils found in the literature are variable (Rodrigues et al 2010). This factor is responsible for the difficulty in comparison of toxicological effects of crude oil WSF.

The extremely low toxicity based on LC_{50} of Bonny light crude oil to *G. pelii* in the present study when compared to results of earlier studies in literature, is due to the delayed response which resulted in the extension of the bioassay beyond 96 hours. Chukwu & Odunzeh (2006) reported LC_{50} of 10.01 ml L^{-1} for *C. africanus* against spent lubricant oil in 96h. Ndimele et al (2010) reported 96h LC_{50} value of 126 mg L^{-1} for the shrimp, *Desmocaris trispinosa* against Bonny light oil. Singer et al (1999) used a flow through exposure system to access the effects of Prudhoe Bay oil to the kelp forest mysid, *Holmesimysis costata* and *Atherinops affinis* and got LC_{50} values ranging between 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 the shrimp, *Americamysis bahia*.

Conclusions. This study indicated that *G. pelii* is not a good early warning indicator of crude oil toxicity. However, impact of crude oil on *G. pelii* at spills could serve as good indicator of adverse impact. This is significant because earlier study on the impact of oil spillage in the Bonny Estuary, Niger Delta, Nigeria recorded a near total decimation of the littoral infaunal and a highly significant mortality.

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Authors:
Alice Ekwu, Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria, e-mail: ekwualice@yahoo.com
Arit I. Utong, Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria, e-mail: desarit4real@yahoo.com
Blessing J. Oribhabor, Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria, e-mail: oribhaborblessjuls@yahoo.com

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