EFFECT OF DIFFERENT CONCENTRATIONS OF BONNY-LIGHT CRUDE OIL ON ALANINE KINASE AND ALANINE TRANSAMINASE ENZYME ACTIVITIES IN *HETEROBRANCHUS BIDORSALIS* JUVENILES.

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Abstract

The effect of exposing juveniles of *Heterobranchus bidorsalis* (Geoffroy St. Hilaire, 809) (mean weight: 15.02±0.14g) to different concentrations of Bonny-light crude oil (BLCO) on alanine kinase and alanine transaminase activities were studied. The exposure of the fish to 1.00, 2.00, 4.00, 8.001L^{-1} BLCO and a control (0.00m1L^{-1}) within 4 days toxicity and 42 days recovery periods indicated that the significant increases ($P<0.05$; $P<0.01$) in the serum alanine transaminase (HCAT) activities were dependent on the BLCO concentrations. The reduced SAK and (HCAK) activities noticed within the first 14 days recovery period implied that the removal of the oil pollutant probably lowered the pressure on the SAK and HCAK to participate in the metabolism of ingested carbohydrate. The significant increases in the serum alanine transminase (SAT) and the hepatic cytosolic alanine transminase (HCAT) activities in the fish corresponded with the trend shown by the SAK and HCAK activities. Generally, the increased activities of SAK, HCAK, SAT and HCAT in *H. bodorsalis* juveniles in this study might be due to a shift in the carbohydrate metabolism of the fish caused probably by the crude oil exposure.

Keywords: *Heterobranchus bidorsalis*, Bonny-light crude oil, Alanine kinase, Alanine transaminase, serum, cytosolic.

Introduction

The degree of exposure of marine organisms to crude oil and its fractions is often assessed by measuring their body burden of petroleum-related aromatic compounds (ACs) because ACs are potentially harmful to animals (NRC, 1985). Fish and marine animals extensively metabolise most ACs in their livers and predominantly excrete them into bile (Varanasi *et al.*, 1989). The pollution of water sources due to xenobiotics may play a major role in the decline of aquatic animals. Increasing awareness of the adverse effects of anthropogenic activities and pollution on aquatic environment has focused interest on health of fish populations and possibilities to utilize these health parameters for assessment of the quality of aquatic environment (Henry *et al.*, 2004).

The response of aquatic organisms to pollution is given by changes through expressions of several key enzymes, especially those of biotransformation systems (Ozmen *et al.*, 2005). The value of tissue enzyme activities in the diagnosis of the effects of pollutants is one of the emerging areas of interest in aquatic toxicology, monitoring and remediation programmes (Oluah *et al.*, 2005). The enzyme, esterases has been used as a biomarker for fish exposed to the random use of insecticides in aquatic systems (Ozmen *et al.*, 1999; Das and Mukherjee, 2000; Brewer *et al.*, 2001). Other biochemical markers such as carboxyl esterase (CE), lactate dehydrogenase (LDH), alanine and aspartate aminotransferase (ALT, AST) are also considered useful to determine the pollution level of water system (Basaglia, 2000). Some of these enzymes are perceived good bioindicators for animals chronically exposed to contaminants such as heavy metals and crude oil (Almeida *et al.*, 2001; den Besten *et al.*, 2001; Mazorra *et al*, 2002).

Thirugnam and Forgash (1997) studies the anti- cholinesterase effect of chloropyrifes to fish, *Fundulus heteroclistis*, while Simon *et al.*, (1985) recorded the effect of the exposure of *Cyprinus capio* to *paraquot* on glucose -6- phosphatase and glycogen phosphorylase activities. Research also showed that a herbicide (Basalin) in contact with fresh water fish, *Nemachelinus sp.* affected the activities of lactate dehydrogenase, alkaline phosphastastase and glutaminpyruvate transaminase in the fish (Rashawar and Lives 1983). Increased alanine aminotransferases activities were observed in *Clarias albopunctatus* exposed to copper (Oluah and Amalu, 1988). Moreover, certain pesticides were observed to inhibit alkaline phosphatase and glucose -6- phosphatase activities in *Mytuius vittatus* (Verma *et al.*, 1981).

There is a dearth of information on the effect of crude oil on metabolic enzymes of fresh water fishes in Nigeria, Omoregie *et al.* (1997), however, reported that the exposure of fish to crude oil fractions caused changes in the oxygen consumption, tissue glycogen and glucose levels of the fish. Against this background, this study was designed to investigate the effect of exposing *Heterobranchus bidorsalis* juveniles to Bonny-light crude oil on the activities of alanine kinase and alanine transaminase enzymes in the fish.

Materials and Methods

Fifteen (15) aerator-equipped, transparent, plastic aquaria ($55\times30\times30cm^3$) were randomly stocked with 300 juveniles of *Heterobranchus bidorsalis* (mean weight + standard error of mean (s.e.m.), 15.02±0.14g) at 20 fish/aquarium. The experiment was designed to have 12 aquaria with 25l dechlorinated tap water each and which were contaminated with 5ml of Bonny-light crude oil (BLCO) at 1.00, 2.00, 4.00mlL^{-1} and 8mlL^{-1} concentrations. Three (3) aquaria were not contaminated with BLCO and were left as the controls. Mosquito-mesh nets were used to cover the aquaria to prevent fish escape.

Two experimental periods were adopted for the study. The toxicity period lasted for 4 days (96h), while the recovery period (42 days) was monitored forthnightly. At the end of the toxicity period, the surviving fish and aquaria were washed and replenished with fresh dechlorinated tap water. A 38% crude protein diet (Tables 1a and 1b) was formulated and fed to the fish at 3% body weight per day (bw.d^{-1}) during the 4 toxicity period and 5% bw.d^{-1} during the 42 days recovery period. The filtration systems of the aquaria helped in the collection of faeces and other residues. Proximate analysis of the experimental diet (Table 1b) was carried out using Windham (1996) method. Records of the aquarium water temperature (26+0.5°C) and pH (6.60+0.40) were taken with the aid of a maximum and minimum mercury-in-glass thermometer and a Ph meter (model Ph-L-2- L) respectively.

The blood samples were collected by both the cardiac puncture method and the severance of the caudal peduncle, using disposable hypodermic syringes (Oluah, 1999). The liver was excised and washed in distilled water to recover traces of blood. The liver sample was macerated and homogenized as described by Devi *et al.* (1993). The liver homogenate was centrifuged at 5000rpm for 15minutes at 4C and the supernatant transferred into clean microfuge tubes. The samples were stored at-8°C until enzymatic assays were carried out (Ozemen *et al.*, 2005). The blood samples were similarly centrifuged for 15 minutes at 1000 rmp to obtain the serum. The serum was also stored at-8°C in clean microfuge tubes.

Total protein concentrations of the liver supernatants and blood serum were determined according to the method described by Lowry *et al.* (1951), using BSA as the standard at 695mm. Serum Alanine Kinase (SAK), Serum Alanine Transaminase (HCAT) concentrations were determined through enzymatic assays. All enzymatic assays were conducted spectrophotometrically at appropriate wave lengths, using a microplate reader system (VersaMax, molecular Devices Corp,. USA) at 25°C at the Bronilla Diagnostic Laboratory Enugu, Nigeria. Samples were assayed in triplicates and averaged; and the mean + s.e.m. presented.

Records of the percentage mortality (PM) and survival (PS) of the fish were taken during the 4 days toxicity and 42 days recovery periods thus. The analysis of variance (ANOVA) were used to analyze the data for statistical significance ($P<0.05$).

Results

Tables 1a and 1b show the gross and proximate compositions of the diet administered to *H. bidorsalis* juveniles during the 4 day toxicity and the 42 days recovery periods of the study. Table 2 summarizes the blood serum and hepatic (liver) cytosolic alanine kinase concentrations in the experimental fish. Table 3 also summarises the blood serum and hepatic cytosolic alanine transaminase concentrations. Table 4 shows the percent mortality and survival of the fish owing to their exposure of different concentrations of BLCO and the control. The control fish recorded lower concentrations of serum alanine kinase (SAK) during the toxicity and recovery periods than those exposed to BLCO concentrations (Tables 3). The SAK concentrations in the fish blood increased significantly ($P<0.05$; $P<0.01$) during the toxicity period as the BLCO concentrations in water increased from 1.00m1L^{-1} (3.032±0.15m.L^{-1}) to 8.00mlL^{-1} (83.20±0.18mlL^{-1}) (Table 2). There were corresponding increases in the hepatic alanine kinase (HCAK) concentrations in the fish as the BLCO concentrations increased from 1.00 to 8.00mlL^{-1}(Table 2).

When the oil pollutant was removed during the 14 days recovery period, both the SAK and HCAK concentrations in the fish were reduced by a measure of 20% (Table 2) irrespective of the BLCO concentrations to which the fishes were exposed. Increase in the concentrations of SAK and HCAK were subsequently recorded as the recovery period extended from day 14 to days 42. Significant variations ($P<0.05$; $P<0.01$) in the SAK and HCAK concentrations in the fish were also recorded as the fishes recuperated from their exposures to the various BLCO concentrations and the control (Table 2).

Both the serum alanine transaminase (SAT) concentrations and the hepatic cytosolic alanine transaminase (HCAT) concentrations were least in the control fish than in those exposed to BLCO concentrations (Table 3). The SAT concentration in the fish blood also increased significantly ($P<0.05$) during the toxicity period (Table 3) as the concentrations of BLCO increased from 1.00mlL^{-1} (SAT = 4.44±0.02mlL^{-1}) to 8.00m1L^{-1} (SAT 12.19±0.11mlL^{-1}). The corresponding values of the HCAT concentrations at this period were 1.00m1L^{-1} BLCO (HCAT = 6.66±0.04 m.g^{-1}) to 8.00mlL^{-1} BLCO (HCAT = 19.35±0.14mg^{-1}) (Table 3).

Twenty percent (20%) reductions in the values of SAT and HCAT concentration in the fish were also recorded within the first forthnight (14 days) of the recovery period, irrespective of the BLCO concentrations applied (Table 3). The concentration of alanine transaminase enzyme, however, increased as the recovery period extended from day 28 to day 42. Generally, there were significant variations ($P<0.05$) in the SAT and HCAT concentrations in the fish as they recuperated from their exposures to the various concentrations of BLCO.

The percent mortality (PM) and percent survival (PS) of the fish (Table 4) indicated that the fishes exposed to 4.00 and 8.00mlL^{-1} BLCO concentrations died more and survived less during the toxicity and the recovery periods of the study. The control fish, however, recorded zero percent (0.00%) mortality and a hundred percent (100.00%) survival during both study periods.

Discussion

Fish viscera are known to be a rich source of enzymes, including alanine kinase and alanine transaminase, many of which present high activity at low concentrations. Uys and Heunt (1987) characterized pancreatic enzymes, including trypsin, from the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822). Trypesin displayed optimal activity at pH 8.20 and at temperatures ranging from 30°C to 40°C. The activities of the alanine kinase and alanine transminase enzymes in *H. bidorsalis* juveniles of the present study were monitored at pH 6.60±0.40 and at temperature 26±0.50C. Fish digestive enzymes exhibit optimal activity at temperatures much higher than the ambient temperature of fish (Freidon and Janak-Kamil, 2001). Changes in the activity of tissue glycogen and glucose modulating enzymes have been reported in common carp exposed to paraquot (Simon *et al.*, 1985). Omoregie *et al.* (1997) reported that the exposure of fish to crude oil fractions caused changes in the oxygen consumption, tissue glycogen and glucose levels of the fish.

The result of this study indicates that the increase in SAK and HCAK activities of *H. bidorsalis* juveniles were dependent on the BLCO concentrations to which the fishes were exposed (Table 2). This result is consistent with the report of Oluah *et al.* (2005) who obtained increase in serum and liver lactate dehydrogenase (LDH) activity in *Clarias albopunctatus* exposed to increasing concentrations of sublethal gammalin 20 and Acetellic 25EC. Although Oluah *et al.* (2005) recorded increase in LDH activity with the duration of exposure of *C. albopunctatus* to the agrochemical pollutants, this study recorded reduced SAK and HCAK concentrations within 14 days (Table 2) as the fishes recuperated from the stress of exposing them to 1.00-8.00mlL^{-1} BLCO concentrations.

The present result implies that the removal of the oil pollutant from the ambient water chemistry must have reduced the pressure on the serum and the hepatic (Liver) alanine kinase activity to participate in the metabolism of the ingested carbohydrate to release energy. This energy was required by the fish to respond to the infiltrating oil pollutant into the blood stream. The haematological effects of stress (Scott and Rogers, 1981), starvation (Norman *et al.*, 1980) and health condition of the fish (Munkittrick and Leatherhead, 1983) consequent upon altered water chemistry have been studied. Oluah (2001) stated that the alterations of water quality usually predispose the fish to stress and diseases which as a result, provoke quick responses in the physiology of the fish, especially the haematological parameters.

The increases in SAK and HCAK (Table 2) and SAT and HCAT (Table 3) concentrations in the fish between days 14 and 42 of this study are consistent with the report of Oluah *et al.*, (2005). Other workers who recorded similar results include: Christensen et al. (1997) and Devi *et al.* (1993) who reported increased muscular LDH activity in brook trout *(Salvelinus frontinalis)* and Fiddler crabs (Uca pugilator) exposed to cadmium respectively. Parathion was also found to elicit increased LDH activity in rat (Gallo and Lawrky, 1991); while lindane caused a 2-fold increase in liver myeloperxidase activity in rat (Junge *et al.*, 2001).

Both the alanine kinase and alanine transaminase enzymes must have played useful roles in the glycolytic pathway of energy metabolism of glucose/glycogen via the blood and the liver of the fish. Therefore, the increased activities of SAK, HCAK, SAT and HCAT in both the serum and the liver of *H. bodorsalis* juveniles of this study are probably indications of a shift in the metabolism of carbohydrate. This shift must have emanated from the catabolism of glucose and glycogen, culminating in the release of energy needed for metabolic activities in the fish. Neff and Anderson (1987) listed some deleterious effects of exposing fish to crude oil contamination to include: alteration of the immune response mechanism, changes in liver metabolism, haemoragge and even death. The present results are consistent with the report of these workers since the highest percentage mortality and the lowest percentage survival of *H. bidorsalis* juveniles were recorded when the effect of the oil pollutant on the ambient water chemistry was most pronounced. i.e. the toxicity period.

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Table 1a. Gross composition of Experimental Diet	
Ingredients %	**Composition**
Yellow maize 9	. 2 6
Soyabean meal 5	4.84
Fish meal 1 .	6 5
Blood meal 1	0.97
Palm oil 5	. 0 0
Salt	0.25
Vitamin mix[1]0 .	6 0
Mineral mix[2]	2. 40
Total	**100.00**

1. Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): thiamin, 10, riboflaim 20. pyridoxin, 10; folacin, 5; panothenic acid, 40; cholin chloride, 200; niacin, 150; vitamin B_{12} 0.06; retinyl acetate (500,000lu/g); 6; menadione N-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alpha tocopherol, 50; chlorecalcipherol (1,000,000lu/g).
2. Contained as g/kg of premix: $FeSO_4.7H_20.5$; $MgSo_47H_20.133$; K_2So_4, 329.90; K1.0.15;$MnSo_4.H_20,0.7$; and cellulose 380.97.

Table 1b. Proximate Composition of Experimental Diet.	
Nutrient %	Composition
Crude protein	37.58
Ether extract	5.18
Ash	0.48
Dry matter	11.80
Nitrogen free extract	34.46
Total	**100.00**

Table 2. Serum and Hepatic cytosolic Alanine Kinase Concentrations of Bonny light crude oil for 4 days (Toxicity) and 42 days (Recovery) Periods.

		BLCO [1] Concentration (M1L								Control (M1L - 1)	
Study Period	Duration Days	1.00		2.00		4.00		8.00		0.00	
		SAK[2]	HCAK[2]	SAK	HCAK	SAK	HCAK	SAK	HCAK	SAK	HCAK
Toxicity Period	4	3.032[a] 0.15	45.48[b] 0.16	42.45[b] 0.16	63.69 0.	63.68[d] 0.16	59.43[e] 0.16	83.20[e] 0.32	124.80[g] 32	19.51[f] 0.14	29.27[a] 0.08
	14	24.26[a] 0.11	36. 38[b] 0.12	33.96[b] 0.11	50.95[c] 0.15	47.54[d] 0.12	71.32[e] 0.15	66.56[f] 0.15	99.84[g] 0.21	19.62[h] 0.11	29.38[a] 0.09
Recovery Period	28	25.47[a] 0.10	38.20[b] + 0.13	35.66[b] 0.12	53.50[c] 0.14	49.92[d] 0.13	74.89[e] 0.14	69.89[f] 0.14	104.83[g] 0.23	19.71[h] 0.12	29.56[a] 0.11
	42	29.29[a] 0.13	43.93[b] 0.16	41.01[b] 0.14	61.53[c] 0.16	57.14[d] 0.14	86.12[e] 0.18	80.37[f] 0.17	120.55[g] 0.28	19.87[h] 0.13	29.68[a] 0.10

[1] Bonny-light Crude oil, [2] Serum alanine kinase concentration ($u.L^{-1}$), [3] Hepatic cytosolic alanine kinase concentration (mg^{-1}), Numbers in the same row with similar superscripts are not significantly different ($P>0.05$), Numbers in the same with different superscript differ significantly ($P<0.05$).

Table 3. Serum and Hepatic cytosolic Alanine Transaminase Concentrations in *H. bidorsalis* Juveniles Exposed to Different concentrations of Bonny-light crude oil for 4 days (Toxicity) and 42 days (Recovery) Periods.

		BLCO [1] Concentration (M1L								Control (M1L - 1)	
Study Period	Duration Days	1.00		2.00		4.00		8.00		0.00	
		SAK[2]	HCAK[3]	SAK	HCAK	SAK	HCAK	SAK	HCAK	SAK	HCAK
Toxicity Period	4	4.44[a] 0.02	6.66[b] 0.04	6.22 [b] 0.03	9 33[C] 0.05	8 .71[d] 0.04	13.07[e] 0.12	12.19[F] 0.11	19.35[g] 0.14	3 .02[H] 0.02	4.53 [a] 0.03
	14	3 .73[a] 0. 02	5.33[b] 0.04	4.98[c] 0.03	7 .46[d] 0.04	6.97 [e] 0.03	10.46[f] 0.07	9.75[g] 0.10	15.48[h] 0.13	3 .52[a] 0.11	4 .62[e] 0.02
Recovery Period	28	3.73[a] 0.03	5 .60[b] + 0.03	5 .23[b] 0.02	7 .83[c] 0.03	7 .32[d] 0.04	10.24[e] 0.06	16.25[f] 0.14	104.83[g] 0.23	19.71[h] 0.12	29.56[a] 0.11
	42	29.29[a] 0.13	43.93[b] 0.16	41.01[b] 0.14	61.53[c] 0.16	57.14[d] 0.14	86.12[e] 0.18	80.37[f] 0.17	120.55[g] 0.28	19.87[h] 0.13	29.68[a] 0.10

Recovery Period	3.73[a] 0.03	5.60[b] 0.03	5.23[b] 0.02	7.83[c] 0.03	7.32[c] 0.04		10.24[d] 0.06	16.25[e] 0.14	3.64[a] 0.02	4.83 0.03
	4.29[a] 0.03	6.44[b] 0.04	6.01[b] 0.04	9.01[c] 0.06	8.42[d] 0.05	12.63[e] 0.12	11.78[f] 0.12	18.69[g] 0.15	3.86[h] 0.04	4.92 0.02