THE EXPOSURE OF *Heterobranchus bidorsalis* JUVENILES TO DIFFERENT CONCENTRATIONS OF BONNY-LIGHT CRUDE OIL AND THEIR EFFECTS ON AMYLASE AND CRETININE KINASE ACTIVITIES

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ABSTRACT

The effects of exposing Heterobranchus bidorsalis juveniles (14.08 ± 0.12 g) to different concentrations of Bonny-light crude oil (BLCO) on amylase and cretinine kinase activities were studied. The exposure of the fish to 1.00, 2.00, 4.00, 8.00 ml L^{-1} BLCO and a control for 4 days toxicity period indicated that the significant increases (P < 0.01) in the serum amylase (SRA) and the hepatic cytosolic amylase (HCA) activities in the fish were BLCO concentrations dependent. Reduced SRA and HCA activities noticed within the first 14 days of the recovery period implied that the removal of the oil pollutant from the ambient water chemistry probably lowered the pressure on the blood serum and liver amylase enzyme to catalyse the metabolism of the ingested carbohydrates. Significant increases (P < 0.05) in the serum cretinine kinase (SRCK) and the hepatic cytosolic cretinine kinase (HCCK) activities in the fish also followed the pattern shown by the SRA and the HCA activities. The increased SRA, HCA, SRCK and HCCK activities in the blood serum and liver of the fish were indications of a shift in the carbohydrate metabolism due to crude oil exposure.

Keywords: *Heterobranchus bidorsalis*, Bonny-light crude oil, Serum, Cytosolic, Amylase, Cretinine kinase.

INTRODUCTION

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 potential of using biomarkers for monitoring both environmental quality and the health of organisms inhabiting polluted ecosystems has received increasing attention in recent years (Lopes *et al.*, 2001; Samecka-Cymerman and Kempers, 2003; Gauthier *et al.* (2004).

The effect of xenobiotic contamination (including crude oil) in an ecosystem can be estimated through analysis of biochemical changes in organisms inhabiting that polluted environment (Tuvikene *et al.*, 1996; Norris *et al.*, 2000; Brewer *et al.*, 2001). The biochemical response of aquatic organism to pollution is given by changes several key enzymes, especially those of biotransformation systems. The value of tissue enzyme activities in the diagnosis of the effects of pollutant is an emerging area in aquatic toxicology and remediation programmes (Oluah *et al.*, 2005). Thirugnanam and Forgash (1977) studied the anticholinesterase effect of chlorpyrifos to *Fundulus heteroclistis* and reported that increased concentration of the mosquito pesticides in salt water mash habitat resulted in maximum anticholinesterase inhibition in the fish.

Increased glucose-6-phosphatase and glycogen phosphorylase activities were observed in *Cyprinus carpio* exposed to paraquat (Simon *et al.*, 1983). A herbicide (Basalin) in contact with a freshwater fish, *Nemachelinus* sp. affected the activities of lactate dehydrogenase, alkaline phosphatase and glutamic pyruvate transaminase in the fish (Verma et al. 1981). Oluah and Amalu (1998) reported increased activities of alanine and aspartate aminotransferases in *Clarias albopuntatus* exposed to copper. Certain pesticides were observed to inhibit alkaline phosphatase and glucose-6-phosphatase activities in the fish *Mytulus vittatus* (Werma, *et al.,* 1981).

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. This study presents the results of the exposure of *Heterobranchus bidorsalis* juveniles to different concentrations of Bonny-light crude oil and their effects on amylase and cretinine kinase activities. The essence was to determine the impact of the various concentrations of this crude oil pollutant on the energy metabolism of this highly priced food fish in Nigeria.

MATERIALS AND METHODS

Six hundred (600) juveniles of *Heterobranchus bidorsalis* (Geoffroy St. Hilaire, 1809) (14.08 ± 0.12g} were transported from a private fish hatchery at Otor-Oweh, Delta State to the Fisheries Laboratory of Enugu State University of Science and Technology, Enugu. At the Fisheries Laboratory in Enugu the fishes were acclimatized for 14 days and fed 38% crude protein diet at 3% body weight per day (bw d^{-1}).

Batches of twenty (20) juveniles of *H. bidorsalis* were randomly stocked in triplicates in 15 plastic containers with 24 litre dechlorinated tap water and which were previously contaminated with 5 ml of Bonny-light crude oil (BLCO) at 1.00, 2.00, 4.00 and 8.00 ml L^{-1} concentrations. Three (3) plastic containers not contaminated with BLCO were left as the controls. Mosquito-mesh nets were used to cover the containers to prevent fish escape.

Two experimental phases were adopted for the study. The toxicity phase lasted for 4 days (96h), while the recovery phase lasted for 42 days and was monitored at fortnightly (14 days) intervals. Fish were monitored each day in both phases for mortality and the surviving fish recorded. At the end of the toxicity period, the surviving fishes and plastic containers were washed and replenished with dechlorinated tap water. A 38% crude protein diet (Tables 1) was fed to fish at 3% bw d^{-1} during the toxicity period (4 days) and 5% bw d^{-1} during the recovery period (42 days). Records of the water temperature (26 ± 0.50° C) and pH (6.80 ± 0.02) were taken with the aid of a maximum and minimum mercury-in-glass Celsius thermometer and a pH meter (Model Ph-I-20-L) respectively.

The blood and liver tissues were sampled at day 4 (for the toxicity period) and at days 14, 28 and 42 (for the recovery period). The blood samples were collected by both the cardiac puncture method and the severance of the caudal peduncle using disposable hypodermic syringe (Oluah, 1999). The liver was excised and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized as described by Devi *et al.* (1993) and then placed in ice-cold 0.25M sucrose (Oluah et al., 2005). The liver homogenate was centrifuged at 5000 rpm for 15 minutes at 4° C and the supernatant was transferred into clean microfuge tubes. The samples were stored at -80° C until enzymatic assays were carried out (Ozmen *et al.*, 2005). The blood was similarly centrifuged for 15 minutes at 1000 rpm to obtain the serum. The serum was also stored at -80° C in clean microfuge tubes.

Total protein concentrations of the liver supernatants and blood serum were determined according to the method of Lowry *et al.* (1951) using BSA as the standard at 695 nm. Blood serum (Serum amylase (SRA) and Serum cretinine kinase (SRCK)), hepatic cytosolic amylase (HCA) and hepatic cytosolic cretinine kinase (HCCK) concentrations were determined. All enzymatic assays were conducted spectrophotometrically at appropriate wavelengths using a microplate reader system (VersaMax, Moleclar Devices Corp., USA) at 25° C. Samples were assayed in triplicates. Data collected were analysed using descriptive statistics and analysis of variance (ANOVA) to indicate statistical significance ($P < 0.05$). Differences were partitioned with the least significant difference.

RESULTS

The values of the serum amylase (SRA) and the hepatic cytosolic amylase (HCA) were lower in the control fish than in those exposed to BLCO concentrations (1.00 – 8.00 ml L^{-1}) (Table 2) for both of the toxicity and recovery periods of the study. Amylase enzyme concentration in the fish blood serum increased significantly ($P < 0.01$) during the toxicity period as the concentration of oil in the water increased from 1.00 ml L^{-1} BLCO (103.30 ± 1.01 µ L^{-1}) to 8.00 ml L^{-1} BLCO (283.35 ± 1.1 bµ L^{-1}). There was a corresponding increase in the HCA concentration as the BLCO concentrations increased from 1.00 to 8.00 ml L^{-1} (Table 2).

Table 1: Gross Composition of the Experimental Diet Fed to *Heterobranchus bidorsalis* Fingerlings Stocked in Crude Oil Polluted Water

Feed ingredient	% Composition
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free extract	34.46
Total	100.00

[1]*Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): thiamine, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3,000; niacin, 150; vitamin B_{12}, 0.06; retinyl acetate (500,000 IU/g), 6; menadione-Na-bisulphate 80; inositol, 400; biotin, 2; vitamin C, 200; alphatocopherol, 200; cholecalcipherol, 1,000,000 IU/g.*
[2]*Contained as g/kg of premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 0.50; $CuSO_4.5H_2O$, 0.50; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and Cellulose, 380.97.*

When the oil pollutant was removed during the 14 days recovery period, both the SRA and the HCA concentrations in the fish were reduced by a measure of 20 % (Table 2) irrespective of the BLCO concentration to which the fishes were exposed. In addition, there were obvious increases in the SRA and HCA concentrations in the fish as the recovery period extended from day 14 to day 42. Significant variations ($P < 0.01$) in the SRA and HCA.

Table 2: Serum and Hepatic Cytosolic Amylase Concentration in *Heterobranchus bidorsalis* Juveniles Exposed to Different Concentrations of Bonny-light Crude Oil (BLCO) for 4 Days (Toxicity) and 42 Days (Recovery) Periods[1]

Study Period	Duration (days)	BLCO concentration (ml L[1])									
		0.00 (Control)		1.00		2.00		4.00		8.00	
		SRA	HCA	SRA[2]	HCA[3]	SRA	HCA	SRA	HCA	SRA	HCA
Toxicity Phase	4	75.36 ±0.29^h	113.04 ±1.03^a	103 ±1.01^a	154.45 ±1.08^b	144.62 ±1.12^c	216.93 ±1.51^d	202.47 ±1.43^d	303.71 ±1.56^e	283.35 ±1.16^f	425.03 ±1.82^g
Recovery Phase	14	76.14 ±0.44^i	116.08 ±1.15^c	86.64 ±0.62^a	123.96 ±1.07^b	115.70 ±1.12^c	173.54 ±1.08^d	161.98 ±1.10^e	242.97 ±1.23^f	226.68 ±1.24^g	340.02 ±1.31^h
	28	78.04 ±0.56^h	119.12 ±1.13^i	86.77 ±0.71^a	130.16 ±1.06^b	121.49 ±1.04^c	182.22 ±1.12^d	170.82 ±1.11^e	255.12 ±1.32^f	238.01 ±1.16^g	357.02 ±1.23^h
	42	80.11 ±0.63^i	123.34 ±1.04^j	99.79 ±0.16^a	149.64 ±1.13^b	139.71 ±1.14^c	209.55 ±1.16^d	195.59 ±1.17^e	293.39 ±1.46^f	272.71 ±1.13^g	410.57 ±1.38^h

[1]Means in the same row followed by the same superscript differ significantly ($P < 0.05$). [2]Serum amylase concentration (μL^{-1}), [3]Hepatic cytosolic amylase (μg^{-1})

Table 3: Serum and Hepatic Cytosolic Cretinine Kinase Concentration in *Heterobranchus bidorsalis* Juveniles Exposed to Different Concentrations of Bonny-light Crude Oil (BLCO) for 4 Days (Toxicity) and 42 Days (Recovery) Periods[1]

Study Period	Duration (days)	BLCO concentration (ml L[1])									
		0.00 (Control)		1.00		2.00		4.00		8.00	
		SRCK	HCCK	SRCK[2]	HCCK[3]	SRCK	HCCK	SRCK	HCCK	SRCK	HCCK
Toxicity Phase	4	0.28 ±0.03^a	0.42 ±0.06^c	0.33 ±0.01^a	0.50 ±0.03^b	0.45 ±0.02^c	0.68 ±0.04^d	0.63 ±0.06^d	0.95 ±0.05^e	0.88 ±0.04^f	1.32 ±0.01^g
Recovery Phase	14	0.30 ±0.02^c	0.45 ±0.02^b	0.26 ±0.02^a	0.42 ±0.04^b	0.36 ±0.02^c	0.54 ±0.03^d	0.50 ±0.02^d	0.76 ±0.05^e	0.71 ±0.04^e	1.06 ±0.08^f
	28	0.36 ±0.02^c	0.51 ±0.03^d	0.27 ±0.02^a	0.42 ±0.04^b	0.38 ±0.01^c	0.57 ±0.04^d	0.53 ±0.03^d	0.80 ±0.06^e	0.75 ±0.05^f	1.11 ±0.09^g
	42	0.42 ±0.02^b	0.57 ±0.03^c	0.31 ±0.02^a	0.48 ±0.04^b	0.44 ±0.03^b	0.59 ±0.04^c	0.61 ±0.05^d	0.92 ±0.07^e	0.86 ±0.06^f	1.28 ±0.10^g

[1]Means in the same row followed by the same superscript differ significantly ($P < 0.05$). [2]Serum cretinine kinase concentration (μL^{-1}), [3]Hepatic cytosolic cretinine kinase (μmg^{-1})

Table 4: Percent mortality and Survival of *Heterobranchus bidorsalis* juveniles during exposure to Different Concentrations of Bonny-light Crude Oil (BLCO) (4 days) and recovery (42 days)

Study Period	Duration (days)	% Mortality BLCO concentration (ml L[1])					% Survival BLCO concentration (ml L[1])				
		0.00 (Control)	1.00	2.00	4.00	8.00	0.00 (Control)	1.00	2.00	4.00	8.00
Toxicity Phase	4	0.00	10.00	0.00	40.00	50.00	100.00	90.00	100.00	60.00	50.00
Recovery Phase	14	0.00	8.00	6.00	32.00	40.00	100.00	92.00	92.00	68.00	60.00
	28	0.00	2.00	1.00	24.00	36.00	100.00	98.00	99.00	76.00	64.00
	42	0.00	1.00	0.00	16.00	26.00	100.00	99.00	100.00	84.00	74.00

concentrations in the fish were also recorded as the fishes recuperated, from their exposures to the various BLCO concentrations and control (Table 2). As was the case with the amylase concentration, both the serum cretinine kinase (SRCK) concentration and the hepatic cytosolic cretinine kinase concentration were least in the control fish than in those exposed to BLCO concentrations (Table 3). The cretinine kinase concentration in fish blood serum also increased significantly ($P < 0.05$) during the toxicity period (Table 3) as the concentrations of BLCO increased from 1.00 ml L^{-1} (SRCK = 0.33 ± 0.01 µ L^{-1}) to 8.00 ml L^{-1} (SRCK = 0.88 ± 0.04 µ L^{-1}). The corresponding values of the HCCK concentration at this period were 1.00 ml L^{-1} (HCCK = 0.50 ± 0.03 µmg^{-1}) to 8.00 ml L^{-1} (HCCK = 1.32 ± 0.10 µmg^{-1}).

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

The percent mortality (PM) and survival (PS) of the fish during the toxicity and recovery periods of the study (Table 4) indicated that the fish exposed to 4.00 and 8.00 ml L^{-1} BLCO died more and survived less. 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 amylase and cretinine kinase, many of which present high activity at low concentrations. Fish digestive enzymes exhibit optimal activity at temperatures much higher than the ambient temperature of fish (Fereidoon and Janaka-Kamil, 2001). Changes in the activity of tissue glycogen and glucose modulating enzymes have been reported in common carp exposed to paraquet (Simon *et al.*, 1983). 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 our study indicated that the increases in SRA and HCA activity 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 increases in the 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 increases in LDH activity with the duration of exposure of *C. albopunctatus* to the agro-chemical pollutants, this study recorded reduced SRA and HCA

concentrations within 14 days (Table 2), as the fishes recuperated from the stress of exposing them to 1.00 - 8.00 ml L^{-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 hepatic (liver) amylase activity to metabolize the ingested carbohydrate (Table 1a) and release energy required for fish to respond to the infiltrating oil pollutant in the blood stream. The haemotological effects of starvation (Norman *et al.*, 1980), stress (Scott and Rogers, 1981), 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 disease which as a result, provoke quick responses in the physiology of the fish, especially haematological parameters.

The increases in the SRA and HCA (Table 2) and the SRCK and HCCK (Table 3) concentrations in the fish between days 14 and 42 of this study were consistent with the report of Oluah *et al.* (2005) mentioned above. Other workers who had earlier recorded similar results include: Christensen *et al.* (1977) and Devi *et al.* (1993) who reported increased muscular LDH activity in brook trout (*Salvelinus fontinalis*) and fiddler crab (*Uca pugilator*) exposed to cadmium respectively. Parathion was also found to elicit increased LDH activity in rat (Gallo and Lawryk, 1991); while lindane caused a 2-fold increase in liver myeloperoxidase activity in rat (Junge *et al.,* 2001).

While the amylase enzyme catalyses the biochemical conversion of the ingested carbohydrate in the fish intestinal tract to glucose, cretinine kinase is involved in the glycolytic pathway to energy metabolism of glucose/glycogen via the blood and the liver of the fish. Therefore, the increased activities of SRA, HCA, SRCK, HCCK in both the serum and the liver of *H. bidorsalis* juveniles of this study are indications of a shift in the carbohydrate metabolism arising from glucose and glycogen catabolism which eventually culminate in the release of energy needed for metabolic activities in the fish. Neff and Anderson (1987) enunciated some deleterious effects of exposing fish to crude oil contamination to include: alteration of the immune response metabolism, changes in liver metabolism and haemorrhage. The present results are in consonance with the report of these workers since the highest percent mortality and the lowest percent survival of *H. bidorsalis* juveniles (Table 4) were recorded during the 4 days toxicity period.

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