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Factors on Proximate Composition of Juveniles and Yearlings of *Heterobranhcus bidorsalis* Exposed to Graded Concentrations of Bonny-Light Crude Oil

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Abstract: Studies were carried out to assess the proximate compositions of male and female Juveniles (JVs) and Yearlings (YRLs) of *Heterobranchus bidorsalis* exposed to graded concentrations (1.00-8.00 mL L^{-1}) of Bonny-Light Crude Oil (BLCO). The experiment was monitored at 4 days (toxicity) and 42 days (recovery) periods. Significant decreases ($p<0.05$) in the Crude Protein (CP) Ether Extract (EE) Ash (AS) and Dry Matter (DM) contents of both the JVs and YRLs were BLCO concentration dependent. Significant increases ($p<0.01$) in the Nitrogen-Free-Extract (NFE) in the sex and age groups also depended on the concentrations of the oil pollutant. The lower CP, EE, AS and DM values of the male JVs and YRLs than those of their female counterparts implies that the crude oil compounds depleted these nutrients faster than in the males. This result is consistent with the reports of other workers who indicated that petroleum-related Aromatic Compounds (ACs) impacted harmful effects on fish species. These ACs might have caused higher decreases in the tissue triglyceride of the total lipid content (EE) of the male JVs and YRLs than those of their female counterparts. Significant increase in the NFE of the fish tissues might also have impacted high energy demand on the fish as a positive survival strategy against the crude oil stress.

Key words: *Heterobranchus bidorsalis*, juveniles, yearling, crude oil, toxicity, proximate composition

INTRODUCTION

Many aquatic organisms have rarely been used to assess the toxicities of crude oil on aquatic environment. Freshwater fishes have been used as 96 h bioassay test organisms (Kopperdaul, 1976) for the determination of crude oil toxicity. Owing to this reliance, freshwater quality near oil spills is suitable for estimating the survival and growth of sensitive stages of aquatic organisms.

The effect of crude oil on adult fish of *Clarias gariepinus* has been reported to increase mortality rate and changes in the haemoglobin concentration of fish blood (Ponat, 1975; Gyllenberg and Lindguist 1976; Tatem *et al*., 1979). Generally, concentrations of crude oil higher than 1 μg^{-1} are required to change the mortality rate, the heart beat and the haemoglobin count of fish. There are no reports in the literature on the effects of crude oil concentrations on the excretion rates of fish although changes in excretion as indications of physiological changes in a fish community are important in the recycling of nutrients for the primary producers. Detailed proximate analyses are needed to determine the effect of crude oil compounds on the body tissues of different sexes of *Heterobranchus bidorsalis* juveniles and yearlings owing to the commercial importance of this fish species in Nigeria.

Proximate composition is the nutrient status of any natural substance determined chemically in the laboratory. Protein is the basic component of animal tissue and is an essential nutrient for maintenance and growth (Steffens, 1989). Kaushik *et al*. (1995) stated that protein in the diet has an obligatory role to replacing lost body protein as well as losses due to amino acid oxidation and utilization for purposes other than systhesis and protein turn-over. Fat content is used to determine the well-being of fish (Tesch, 1971). The muscle triglycerides of the total lipids in the juveniles *Oncorhynchus masu* were noted to decrease at the early stages of sea water life (Ota, 1976). Fat content of herring caught during the spawning season at the coast of Rumoi in Hokkaido, Japan was

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found to be higher than non-spawning herring fish *Clupea* species and the African lungfish, *Protopterus annectens* (Tesch, 1971).

Minerals perform a wide variety of structural, biochemical and physiological functions in fish (Silva and Anderson, 1995). Six major elements (P, Ca, K, Na, Mg and S) and trace elements (Fe, Zn, Mn, Ni, I, Mb and Co) have been identified as essential for animal life (Underwood, 1977). Although most of these elements might be required by fish, only 6 dietary minerals have been shown to be required or utilized by salmonids (Silva and Anderson, 1995). Most fish species derive their minerals from food or the water in which they live. Sea fish therefore generally contain more minerals than freshwater fish (Lagler *et al*., 1977). The higher mineral content (calcium) in female osteichthyes than in males especially during the breeding period has been suggested as due to increase in protein bound calcium during the breeding period (Urist and Schyeide, 1961).

Much of the work on the effect of petroleum hydrocarbons on aquatic organisms have been restricted to studies and testing of single compounds (Anderson, 1971) probably due to difficulties in testing complex mixture of compounds associated with crude oil and petroleum fractions. The effect of Crude oil on a fisheries population must be considered in relation to many other interacting variables before one can estimate the relative contributions to the decline of a fishery (Whipple, 1979; Whipple *et al*., 1979). In Nigeria, Mwamba *et al*. (2001) reported that the activities of amylase and cretinine kinase enzymes in *H. bidorsalis* juveniles exposed to crude oil were dependent on the concentration of the oil pollutant.

With the incessant oil spills in Nigeria and the varying levels of petroleum hydrocarbons recorded in the body organs of fishes, frogs and snails (Akingbade, 1991) it is imperative that the quality of fish flesh exposed to different concentrations of crude oil be succinctly analyzed. Oil producing communities in Nigeria have suffered various forms of environmental degradation, deprivation and spoilage owing to this crude oil menace. This study therefore presents the results of exposing juvenile and yearling *H. bidorsalis* to graded concentrations of Bonny-light crude oil and the concomitant effect on the proximate compositions of the fish.

MATERIALS AND METHODS

One thousand two hundred fish specimens of two sexes of different age groups of *Heterobranchus bidorsalis*, comprising 600 female and male juveniles (14.04+0.24g) at 1:1 ratio and 600 female and male yearling (24.03+0.14g) also at 1:1 ratio were purchased from Aquafish Nigeria Limited, Ihiala, Anambra State, Nigeria. The fishes were transported to the Fisheries Laboratory of Ebonyi State University, Abakaliki, Nigeria and acclimatized for 14 days on a 38% crude protein diet fed at 3% body weight per day (bw d^{-1}).

Batches of twenty *H. bidorsalis* of each age group and sex were randomly stocked in triplicates in 60 plastic containers (25 L capacity) with 24 L dechlorinated tap water. Fourty-eight of these containers were previously contaminated with 5 mL BLCO at 1.00, 2.00, 4.00 and 8.00 mL L^{-1} concentrations. Twelve containers were uncontaminated with BLCO and 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 (96 h) while the recovery phase which lasted for 42 days 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 fish and plastic containers were washed and replenished with dechlorinated tap water. A 38% crude protein diet (Table 1) was fed to fish at 3% bwd^{-1} during the toxicity period (4 days) and 5% bwd^{-1} during the recovery period (42 days). Fish were weighed fortnightly (14 days) during the recovery period with the aid of a top-loading electronic Mettler balance (Model PT 600) and the diet to be administered adjusted in accordance with the body

Table 1: Gross and proximate composition of the experimental diet fed to two sex groups of juvenile sand yearlings of *Heterobranchus bidorsalis* 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 fibre	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free-extract	36.46
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose (mg kg^{-1} of diet): thiamine, 10; riboflavin, 0; pyridoxine; 10; folacin; 5; pantothenic acid, 40; choline chloride,3,000; niacin, 150; menadione-Na-bisulphate, 80; inositol, 400; biotin, ; vitamin C,; 200 alphatocophrol, 200; colecalcipherol, 1,000,000 iu g^{-1}, [2]Contained as g kg^{-1} of premix: $FeSO4.7H_2O$, 5; $MgSO4.7H_2O$, 132; K2SO4; 329.90; Kl, 0.15; NaCl, 45; Na_2SO4, 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

weight of fish. The proximate compositions of the fish were determined at days 4, 14, 28 and 42 of the study period; while that of the diet was determined at the beginning of the study.

Windham (1996) method of proximate analysis was adopted. The percent nitrogen content was determined by the micro-kjeldahl method and converted to total protein equivalent by multiplying by 6.25 (Windham, 1996). The crude fat was measured in a soxhlet apparatus of lipid by petroleum ether (b.pt. 40-60°C) extraction. The dry matter content was determined by drying 2.00g triplicate samples at 105°C to constant weight and calculating the percentage dry matter using the formula:

$$\frac{X - Y}{X} \times 100$$

Where X = weight of wet sample; Y = dry weight sample. Ash was determined by combusting 2.00 g of sample in a muffle furnace at 600°C for 12 h. The digestible carbohydrate content was computed by obtaining the difference between the % crude protein + % crude fat+%ash+% dry matter and 100%. Data collected were analyzed using descriptive statistics and Analysis of Variance (ANOVA) to indicate statistical significance ($p<0.05$) (Steel and Torrie, 1990). Differences were partitioned by the Duncan's (1955) Multiple Range Test method.

RESULTS

The values of the Crude Protein (CP) Ether Extract (EE) Ash (AS) and Dry Matter (DM) contents of both sexes (male and female) of the Juvenile (JV) and Yearling (YRL) *H. bidorsalis* were significantly higher ($p<0.05$) than those exposed to 1.00-8.00 mL L^{-1} BLCO concentrations (Table 2 and 3). These results were recorded both at the toxicity (4 days) and recovery (42 days) period of the study. Conversely, the values of the Nitrogen-Free-Extract (NFE) of both sexes and age groups of fish exposed to the various BLCO concentrations were significantly higher ($p<0.05$) than those of the control. The percent compositions of CP, EE, AS and DM decreased significantly ($p<0.05$) in the two age groups (JV and YRL) of both sexes (male and female) of the fish as the BLCO concentrations increased from 1.00 to 8.00 mL L^{-1} (Table 2 and 3); while the NFE values increased. Significantly, the recorded values of CP, EE, AS and DM of the male JVs and YRLs exposed to 1.000-8.00 BLCO concentrations at the toxicity and recovery periods (Table 2) were significantly lower than the corresponding values of the nutrients for the female JVs and YRLs (Table 3). This trend was the reverse for the NFE values where the male JVs and YRLs recorded higher values (Table 2) than the female JVs and YRLs (Table 3).

The range values of the CP of the male JVs (10.-16.64%) and YRLs (9.15-14.90%) during the 4 days

Table 2: Proximate compositions of male juveniles[1] and yearlings[2] of *Heterobranchus bidorsalis* exposed to graded interactions of bonny-light crude oil for 4 days (toxicity) and 42 days (recovery) periods

			BLCO[3] Concentration (mL L^{-1})				
			Control 0.00 mL L^{-1}	1.00		2.00	
Study period	Duration (days)	Nutrient	JV	YRL	JV	YRL	JV
Toxicity Period	4	CP[6]	19.22+1.04[a]	17.02±1.0[b]	16.64±0.06[c]	14.90±0.06[d]	14.16±0.07e
		EE[7]	8.47±0.04[a]	4.47±0.02[b]	7.34±0. 03[c]	3.91±0.02[d]	6.4±0.03[e]
		AS[8]	3.06±0.01[a]	2.16±0.01[b]	2.64±0.02[b]	1.10±0.01[c]	3.19±0.02[a]
		DM[9]	17.10±1.01[a]	16.91±0.06[b]	15.07±0.06[c]	14.79±0.07[d]	12.60±0.06[e]
		NFE[10]	52.15±1.30[a]	59.44±1.21[b]	58.31±1.22[c]	65.30±1.30[d]	63.81±1.31[e]
	14	CP	20.23±1.12[a]	17.92±0.07[b]	17.52±0.01[b]	15.69±0.09[c]	14.90±0.06[d]
		EE	8.92±0.04[a]	4.70±0.02[b]	7.73±0.03[b]	4.12±0.01[b]	6.57±0.04[d]
		AS	3.22±0.01[a]	2.27±0.01[b]	2.78±0.01[c]	2.01±0.01[b]	2.36±0.01[b]
		DM	18.00±0.01[a]	17.80±0.08[b]	15.86±0.07[c]	15.57±0.08[c]	13.26±0.08[d]
		NFE	49.63±1.12	57.31±1.14[b]	56.11±1.13[c]	62.62±1.14	62.91±1.15
	28	CP	20.40±1.11[a]	18.65±0.07[b]	18.10±1.01[c]	15.85±0.05[d]	15.05±0.04[e]
		EE	9.01±0.03[a]	4.75±0.02[b]	7.81±0.03[c]	4.16±0.02[d]	6.64±0.03[e]
		AS	3.25±0.01[a]	2.29±0.01[b]	2.81±0.01[c]	2.03±0.01[b]	2.38±0.01[b]
		DM	18.18±1.02[a]	17.98±0.08[b]	16.02±0.06[c]	15.73±0.05[d]	13 .39±0.04[e]
		NFE	49.16±1.11[a]	56.97±1.40[b]	55.67±1.32[c]	62.23±1.44[d]	62.54±1.43[e]
	42	CP	21.46±1.11[a]	18.65±0.07[b]	19.32±1.01[c]	19.19±1.03[c]	15.65±0.05[d]
		EE	9.47±0.05[a]	4.86±0.03[b]	8.53±0.04[c]	5.03±0.02[d]	6.90±0.03[e]
		AS	3.42±0.02[a]	2.37±0.01[b]	3.66±0.01[a]	2.44±0.01[b]	2.48±0.02[b]
		DM	19.10±1.01[a]	19.24±1.134[a]	20.44±1.10[b]	19.07±1.02[a]	13.92±0.04[c]
		NFE	46.55±1.24[a]	54.88±1.23[b]	48.05±1.31[c]	54.27±1.14[d]	61.05±1.33[e]

Table 2: Continue

Study period	Duration (days)	Nutrient	BLCO[3] Concentration (mL L^{-1}) Control 0.00 mL L^{-1} JV[4]	4.00 YRL[5]	4.00 JV	8.00 YRL	8.00 JV
Toxicity Period	4	CP[6]	12.65±0.06^{f}	12.03±0.07^{f}	10.76±0.04^{g}	10.22±0.05	9.15±0.03^{h}
		EE[7]	3.33±0.02^{F}	5.31±0.02^{g}	2.82±0.01^{h}	4.51±0.02^{i}	2.40±0.01^{j}
		AS[8]	1.62±0.01^{c}	1.91±0.01^{c}	1.38±0.01^{cd}	1.6±0.02^{c}	1.17±0.01^{c}
		DM[9]	12.58±0.05^{e}	10.7±0.05^{f}	10.70±0.04^{f}	9.11±0.03^{g}	9.09±0.04^{g}
		NFE[10]	69.82±1.23^{f}	70.03±1.32^{g}	74.34±1.26^{h}	74.54±1.25^{i}	78.19±1.32^{j}
	14	CP	13.32±0.07^{e}	12.66±0.07^{f}	11.33±0.07^{g}	10.76±0.07^{h}	9.63±0.04^{i}
		EE	3.50±0.02^{e}	5.59±0.03^{f}	2.97±0.02^{g}	4.75±0.03^{b}	2.53±0.02^{h}
		AS	1.70±0.01^{d}	2.01±0.01^{b}	1.45±0.01^{d}	1.70±0.01^{d}	1.23±0.01^{d}
		DM	13.24±0.06^{d}	11.28±0.06	11.26±0.06^{e}	9.59±0.05^{f}	9.57±0.05^{f}
		NFE	68.24±1.16	68.46±1.16	72.99±1.12	73.20±1.13	77.04±1.14
	28	CP	13.45±0.05^{f}	12.79±0.04^{g}	11.44±0.04^{h}	10.87±0.03^{i}	9.73±0.04^{k}
		EE	3.54±0.01^{f}	5.65±0.02^{g}	3.00±0.01^{h}	4.80±0.02^{i}	.56±0.01^{j}
		AS	1.72±0.01^{d}	2.03±0.01^{b}	1.46±0.01^{d}	1.72±0.01^{d}	1.24±0.01^{de}
		DM	13.37±0.03^{e}	11.39±0.02^{f}	11.37±0.02^{f}	9.69±0.04^{g}	9.67±0.05$_{g}$
		NFE	67.92±1.50^{f}	68.14±1.51^{g}	72.73±1.46^{h}	72.92±1.51^{h}	76.80±1.43^{i}
	42	CP	13. 99±0.04^{f}	13.29±0.05^{f}	11.90±0.05^{g}	11.30±0.04^{h}	10.11±0.04^{i}
		EE	3.66 +0.02^{f}	5.87±0.02^{g}	3.12±0.02^{h}	4.99±0.02^{i}	2.66±0.01^{j}
		AS	1.79±0.01^{c}	2.11±0.01^{c}	1.52±0.01^{c}	1.79±0.01^{c}	1.29±0.01^{cd}
		DM	13.90±0.04^{c}	11.84±0.03^{d}	11.82±0.06^{d}	10.07±0.05^{e}	10.05±0.05^{e}
		NFE	66.66±1.22^{f}	66.89±1.34^{f}	71.64±1.26^{g}	71.85±1.40^{g}	75.89±1.25^{h}

[1]Swven weeks old, [2]Eleven months old, [3]Bonny-light crude oil, [4]Juvenile, [5]Yearling, [6]Crude protein, [7]Ether extract, [8]Ash, [9] Dry matter, [10]Nitrogen free extract. Values in the same row followed by the same superscripts are not significantly different ($p>0.05$); values in the same row followed by different superscripts differ significantly ($p<0.05$)

toxicity period were lower than the range of CP values recorded during the recovery period at days 14, 28 and 42 (Table 2). Similarly, the range values of CP of the female JVs (11.24-18.30%) and YRLs (10.07-16.39%) at toxicity were lower than the values recorded when the fishes recuperated from oil exposure between days 14 an 42 (Table 3). The values of EE, AS and DM followed the trend exhibited by the CP values between the toxicity and recovery periods for both sexes and age groups of the fish. The range of NFE values of the fish, nonetheless, were significantly higher ($p<0.01$) in both sexes and age groups of the fish during the toxicity period (4 days) than during the recovery period (days 14, 28 and 42 (Table 2 and 3).

Two observations were obvious from the results of this study namely: Whereas the CP, AS, EE and DM values of the JVs of both sexes (male and female) of the fish were significantly higher ($p<0.05$) than the corresponding values for the YRLs at both the toxicity and recovery periods, The nutrient values of the female JVs and YRLs were generally higher than those of the male JVs and YRLs when exposed to the various concentrations of BLCO (Table 2 and 3).

Both the male and the female JVs and YRLs recorded increase in the values of CP EE, AS and DM to certain percent magnitudes as the fishes recuperated from their exposures to the crude oil pollutant. From our results, there were 15% increases in the values of those nutrients in both sexes and age groups between day 4 and day 14; while 20% I ncreases were recorded between day 14 and day 28 as well as between day 28 and day 42 (Table 2 and 3). Although the computations for the NFE values of the fish were done through estimation of the difference between %CP + %EE + %AS + %DM and 100%, noticeable decreases in the values of this Nutrient (NFE) (Table 2 and 3) were obtained as the surviving fish recuperated from their exposures to the crude oil pollutant.

Records of fish mortality and survivals during this study (Table 4) indicated that each of the sexes (male and female) and age groups (JV and YRL) recorded higher mortality and lower survivals when exposed to 4.00-8.00 mL L^{-1} BLCO concentrations than when exposed to 1.00-2.00 mL BLCO concentrations.

DISCUSSION

Calamari and Naeve (1994) stated the need for an assessment of the level of heavy metal contamination of the African aquatic environment. This has led to the initiation of several pollution monitoring programmes such as the Mediterranean Pollution Monitoring Programme (MEDPOL), covering North, West and Central African Marine Pollution and Research Programme (WECAF 2) and the Eastern African Marine Pollution and Research Programme (EAF/6). These authors noted that for effective water pollution, control and management,

Table 3: Proximate compositions of female juveniles[1] and yearlings[2] of *Heterobranchus bidorsalis* exposed to graded interactions of bonny-light crude oil for 4 days (toxicity) and 42 days (recovery) periods

			BLCO[3] Concentration (mL L^{-1})				
			Control 0.00 mL L^{-1}	1.00		2.00	
Study period	Duration (days)	Nutrient	JV[4]	YRL[5]	JV	YRL	JV
Toxicity period	4	CP[6]	21.14±1.12[a]	18.72±0.08[b]	18.30±0.08[c]	16.39±0.09[d]	15.58±0.06[e]
		EE[7]	9.32±0.06[a]	4.92±0.02[b]	8.07±0.04[c]	4.30±0.0[b]	6.86±0.03[d]
		AS[8]	3.37±0.02[a]	2.38±0.01[b]	2.90±0.01[b]	1.21±0.01[c]	3.51+ 0.02[a]
		DM[9]	18.81±1.01[a]	18.60±0.08[a]	16.58±0.05[b]	16.27±0.16[b]	13.86±0.04[c]
		NFE[10]	47.36±1.14[a]	53.38±1.21[b]	54.15±1.23[c]	61.83±1.24[d]	60.19±1.19[e]
Recovery period	14	CP	23.26±1.13[a]	20.61+1.11[b]	20.15±1.03[b]	18.03±0.07[c]	17.14±0.06[d]
		EE	10.26±0.07[a]	5.41±0.02[b]	8.89±0.04[c]	4.74±0.02[d]	7.56±0.03[e]
		AS	3.70±0.01[a]	2.61+ 0.01[b]	3.20±0.01[a]	2.31±0.01[b]	2.71+ 0.01[b]
		DM	20.70 +1.10[a]	20.47±1.11[a]	18.24±1.01[b]	17.91+ 0.05[c]	15.36±0.05[d]
		NFE	42.08±1.20[a]	50.90±1.21[b]	49.51+ 1.32[c]	57.01+ 1.31[d]	57.23±1.32[d]
	28	CP	24.48±1.13[a]	21.72±1.10[b]	21.23±1.11[c]	19.02±0.09[d]	18.06±0.08[e]
		EE	10.81+ 0.07[a]	5.70±0.02[b]	9.37±0.05[c]	4.99±0.02[d]	7.79±0.04[e]
		AS	3.90±0.01[a]	2.75±0.01[b]	3.37±0.02[a]	2.44±0.01[b]	2.62±0.01[b]
		DM	21.82±1.03[a]	21.58±1.04[a]	19.22±0.07[b]	18.88±0.06[c]	16.07±0.06[d]
		NFE	38.99±1.14[a]	48.25±1.23[b]	46.81±1.24[c]	54.67±1.22[d]	55.28±1.25[e]
	42	CP	25.75±1.12[a]	22.38±1.10[b]	21.23±1.11[c]	23.03±1.13[d]	18.78±0.07[e]
		EE	11.36±0.08[a]	5.83±0.03[b]	10.24±0.06[c]	6.04±0.04[d]	8.28±0.05[e]
		AS	4.10±0.02	2.84±0.01	4.39±0.01	2.93±0.01	2.98±0.01
		DM	22.92±1.10[a]	23.09±1.12[b]	24.53±1.13[c]	22.88±1.11[a]	16.70±0.07[d]
		NFE	35.87±1.13[a]	45.86±1.21[b]	39.61±1.22[c]	45.12±1.25[b]	52.76±1.26[d]

Tamle 3: Counitnue

			BLCO[3] Concentration (mL L^{-1})				
			Control 0.00 mL L^{-1}	4.00		8.00	
Study period	Duration (days)	Nutrient	YRL	JV	YRL	JV	YRL
Toxicity period	4	CP[6]	13.92±0.06[f]	13.23±0.05[f]	11.84±0.04[g]	11.24±0.04[g]	10.07±0.03[h]
		EE[7]	3.66±0.0[e]	5.84±0.0[f]	3.10±0.0[e]	4.96±0.03[b]	2.64±0.01[g]
		AS[8]	1.78±0.01[c]	2.10±0.01[b]	1.52±0.01e	1.78±0.01[c]	1.29±0.01[e]
		DM[9]	11.79±0.03[d]	11.79±0.05[d]	11.77±0.04[d]	10.02±0.03[e]	10.00±0.03[e]
		NFE[10]	68.85±1.18[f]	67.04±1.23[g]	71.77±1.31[h]	72.27±1.32[i]	76.00±1.46[j]
Recovery period	14	CP	15.32±0.05[e]	14.56±0.05[f]	13.03±0.04[g]	12.37±0.04[h]	11.07±0.04[i]
		EE	4.03±0.02[d]	6.43±0.03[f]	3.42±0.01[f]	5.56±0.02[b]	2.91±0.01[g]
		AS	1.96±0.01[c]	2.31±0.01[b]	1.67±0.01[c]	11.96±0.01[c]	1.41±0.01[c]
		DM	15.23±0.04[d]	12.97±0.05[e]	12.95±0.04[e]	11.03±0.05[f]	11.01±0.05[f]
		NFE	63.46±1.36[e]	63.73±1.32[e]	68.93±1.24[f]	69.18±1.26[g]	73.60±1.26[h]
	28	CP	16.14±0.05[f]	15.35±0.05[g]	13.73±0.04[h]	13.04±0.03[h]	11.68±0.04[i]
		EE	4.25±0.02[d]	6.78±0.03[f]	3.60±0.01[g]	5.76±0.02[b]	3.07±0.01[g]
		AS	2.06±0.02[b]	2.44±0.02[b]	1.75±0.01[c]	2.06±0.01[b]	1.49±0.01[c]
		DM	16.04±0.05[d]	13.67±0.04[e]	13.64±0.05[e]	11.63±0.04[f]	11.60±0.04[f]
		NFE	61.51±1.26[f]	[61.76]±1.38f	67.28±1.35[g]	69.51±1.32[g]	72.16±1.38[h]
	42	CP	16.79±0.06[f]	15.95±0.07[g]	14.28±0.05[h]	13.56±0.06[i]	12.13±0.04[j]
		EE	4.39±0.02[f]	7.04±0.03[g]	3.74±0.01[h]	5.99±0.02[b]	3.19±0.02[h]
		AS	2.15±0.02	2.53±0.02	1.82±0.01	2.15±0.01	1.55±0.01
		DM	16.68±0.05[d]	14.21±0.05[e]	14.18±0.06[e]	12.18±0.06[f]	12.06±0.05[f]
		NFE	59.99±1.22[e]	60.27±1.23[f]	65.98±1.24[g]	66.22±1.26[f]	71.07±1.23

[1]Seven weeks old, [2]Eleven months old, [3]Bonny-light crude oil, [4]Juveniles, [5]Yearlings, [6]Crude protein, [7]Ether extract, [8]Ash, [9]Dry matter, [10]Nitrogen free extract. Values in the same row followed by the same superscripts are not significantly different ($p>0.05$). Values in the same row followed by different superscripts differ significantly ($p<0.05$)

there is need for a clear understanding of the principles of metal contamination. Fatty acid degradation of the liver of *Oreochromis niloticus*, oedema of secondary gill lamella and respiratory impairment of this fish have been reported by Omoregie and Ufodike (1991). Increase in blood glucose level is a general response of fish to acute pollutant effects including organophosphates and xenobiotics (Luskova *et al*., 2002). The quantity of protein in fish tissues is dependent on the rate of protein synthesis or on the rate of its degradation. Sigh *et al*. (1996) stated that the quantity of protein may be affected by impaired incorporation of amino acids in the polypeptide chain.

The inhibition of protein deposition in both the male and female juveniles and yearlings of *H. bidorsalis* of this study was BLCO concentration dependent (Table 2 and 3). Comparatively, the trend of protein inhibition by the oil pollutant was much pronounced in

Table 4: Percentage mortality and survival of two sex group of juvenile[1] and yearlings[2] of *Heterobranchus bidorsalis* exposure to different concentrations of Bonny-light Crude Oil (BLCO) (4 days) and recovery (42 days) period

		Mortality (%)									
		BLCO Concentration (mL L^{-1})									
		Control		1.00		2.00		4.00		8.00	
Study	Duration	0.00 mL L^{-1}									
period	(days)	JV[3]	YRL[4]	JV	YRL	JV	YRL	JV	YRL	JV	YRL
Toxicity	4	0.00	0.00	10.00	6.00	0.00	1.00	40.00	36.00	50.00	48.00
Recovery	14	0.00	0.00	8.00	4.00	6.00	5.00	32.00	26.00	40.00	36.00
	28	0.00	0.00	2.00	1.00	1.00	1.00	24.00	22.00	36.00	30.00
	42	0.00	0.00	1.00	0.00	0.00	0.00	16.00	12.00	26.00	20.00

[1]Seven weeks old, [2]Eleven months, [3]Juvenile, Yearling

Table 4: Continued

		Survival (%)									
		BLCO Concentration (mL L^{-1})									
		Control		1.00		2.00		4.00		8.00	
Study	Duration	0.00 mL L^{-1}									
period	(days)	JV	YRL	JV	YRL	JV	YRL	JV	YRL	JV	YRL
Toxicity	4	100.00	100.00	90.00	94.00	100.00	99.00	60.00	64.00	50.00	52.00
Recovery	14	100.00	100.00	92.00	96.00	94.00	95.00	68.00	74.00	60.00	64.00
	28	100.00	100.00	98.00	99.00	99.00	99.00	76.00	78.00	64.00	70.00
	42	100.00	100.00	99.00	100.00	100.00	100.00	84.00	88.00	74.00	80.00

[1]Seven weeks old, [2]Eleven months, [3]Juvenile, Yearling

the males (Table 2) than in the females (Table 3). However, the result of this study is consistent with the reports of other workers. For example, Reeta *et al.* (1993) reported inhibition in the total serum protein of *Heteropneustes fossilis* exposed to different pesticides (DDT, YBHC and Malathion). Ravichandran *et al.* (1994) reported depletion of protein from 79-45% owing to proteolysis after *Oreochromis mossambicus* was exposed to nominal concentrations of phenol. Ogueji and Auta (2007) also reported inhibition in the total serum protein of *Clarias gariepinus* (Teugels) exposed to acute concentrations of lambda-cyhalothrin (a pyrethroids insecticide). The implication of the results of our study is that since the CP values of female juveniles and yearlings of *H. bidorsalis* were higher than those of their male counterparts when exposed to oil pollution (4 days) and up to recovery (42 days), the physiological adjustments of the females to survive were bound to vary from the males. Since protein is the basic component of animal tissue and is an essential nutrient for maintenance and growth, the female juveniles and yearlings of *H. bidorsalis* are more likely to maintain life and grow than their male counterparts. In addition, the depletion of protein in the body tissues of fish implies that the crude oil compounds might have reduced this nutrient faster in the male JVs and YRLs than in their female counterparts. Bradbury *et al.* (1987) posited that the decreased protein content of fish exposed to pollutants might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The significant decreases in the crude fat content of the body tissues of male and female JVs and YRLs in this study (Table 2 and 3) are also consistent with the report of Ota (1976). This worker recorded decreases in the muscle triglycerides of the total lipid content in *Oncorhynchus masu* juveniles at their early stages of sea water life. However, the significant dose dependent elevations in the triglyceride levels in the blood serum of *Clarias gariepinus* Teugels exposed to acute concentrations of lambda-cyhalothrin (a pyrethriods insecticide) (Ogueji and Auta, 2007) were at variance with the results of this study. Similarly, Krishna *et al.* (1994) recorded increased levels of phospholipids and cholesterol in the tissues of *Oreochromis niloticus* subjected to acclimation in sub-lethal concentration of acid water (pH, 4.00) and argued that the accumulation of triglycerides in fatty livers of the fish was as a results of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchyma cells in the systemic circulation (Gabriel, 1995). Nevertheless, decreases in the crude fat content of male and female Jvs and YRLs in this study might be due to the harmful effects of petroleum-related Aromatic hydrocarbons (ACs) to animals (NRC, 1985). Fish and marine mammals extensively metabolize most ACs in their livers and predominantly excrete these metabolites into bile (Varanasi *et al.*, 1989). Therefore, the ACs of the BLCO in this study might have caused decreases in the tissue triglycerides of the total lipid content (EE) in the male and female JVs and YRLs of *H. bidorsalis* exposed to the oil pollutant.

The significant increases ($p<0.01$) in the values of NFE (digestible carbohydrates) in the fish tissue of this study were also BLCO concentration dependent. This might be attributed to the stress induced by the crude oil pollutant. Glucose as an end product of carbohydrate digestion increases as a general response of fish to acute or sub-lethal pollutant effects (Verma *et al*., 1983; Ghazaly, 1995; Ceron *et al*., 1997; Luskova *et al*., 2002). High levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids (Honstela *et al*., 1996) and catecholamines (Nakano and Tomlimson, 1967). Both of these groups of hormones produce hyperglycaemia (Ogueji and Auta, 2007). The trend of NFE increases in the age and sex groups of *H. bidorsalis* of this study is consistent with the reports of previous workers; although variations in the NFE values were more produced in the male JVs and YRLs than in their female counterparts (Table 2and 3).

In this experiment, the BLCO concentration dependent depressions in the values of CP, EE, AS and DM and the concomitant elevation of the NFE (digestible carbohydrate) values of the tissues of age and sex groups of *H. bidorsalis* could be due to the high energy demands imposed on the fishes as a positive survival strategy under the crude oil stress. In this contest, the depletion of the essential tissue nutrients (CP and EE) of the fish and possibly the occurrence of hyperglycaemia due to elevations in the NFE values must have resulted in the high percent mortality and low survivals of the fish as the BLCO concentration increased (Table 4).

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