ANIMAL RESEARCH INTERNATIONAL

Volume 4 Number 3 (2007)

An International Journal Publishing Original Research Involving the Use of Animals and Animal Products

ISSN: 159-3115

Website: zoo-unn.org

AGE FACTOR AND PROXIMATE COMPOSITIONS OF THE MUSCLE OF *Heterobranchus bidorsalis* EXPOSED TO GRADED CONCENTRATIONS OF BONNY-LIGHT CRUDE OIL

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ABSTRACT

Variations in the proximate compositions of three age groups of Heterobranchus bidorsalis exposed to graded concentrations of Bonny-light crude oil (BLCO) were investigated in the laboratory. The fish were exposed to 1.00, 2.00, 4.00 and 8.00 ml L^{-1} concentrations of BLCO for 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 the juvenile (JV), the yearling (YRL) and the adult (AD) fish were BLCO-concentration dependent. Lower CP values in the adult fish than in the juveniles or the yearlings implies that the crude oil compounds might have depleted the quantity of protein faster in the adults than in the juveniles or the yearlings. Significant decreases ($P < 0.05$) in the EE content of the fish muscle could be attributed to the harmful effects of petroleum-related aromatic compound (ACs) on animals. These ACs might have caused decreases in the muscle triglycerides of the total lipid (EE) content of the three age groups of the fish. Significant increases ($P < 0.05$) in the nitrogen free extract (NFE) of the fish muscle might have been due to the high energy demand imposed on the fish as a positive survival value under the condition of crude oil stress.

Keywords: *Heterobranchus bidorsalis*, Age groups, Proximate composition, Bonny-light crude oil, Toxicity

INTRODUCTION

Most of the Nigerian aquatic environments have witnessed a number of oil spills. Over 6744 cases of oil spill accidents have occurred between 1976 and 2005 resulting in the release of more than 2.4 million barrels of crude oil on land and the coastal water environments (Nwilo and Badejo, 2005). Oil producing communities have, as a result, suffered various forms of environmental degradation, deprivation and spoilage. Akingbade (1991) recorded varying levels of petroleum hydrocarbons in the body organs of fishes, frogs and snails in areas where oil spills are prevalent. Previous works of Whipple (1979) and Brown *et al.* (1999) on rivers, lakes and estuaries with continuous input of oil pollutants have recorded the presence of monocyclic aromatic hydrocarbon including benzene in both water and fish tissues.

The degree of exposure of aquatic organisms to oils 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 mammals extensively metabolize most ACs in their livers and predominantly excrete them into bile (Varanasi *et al.*, 1989). The clariids, especially the large species are esteemed food fish throughout Nigeria (Awachie, 1973). They are estimated to contribute 40% of the fishes of the Anambra river system flood plain in Nigeria (Awachie and Ezenwaji, 1981). Since 1960, considerable progress has been made the world over in the culture of catfishes especially *Clarias gariepinus* (Huisman, 1985). Another clariid of the genus *Heterobranchus* was identified as a top priority specimen for aquaculture in Africa and the propagation of the hybrid (*C. gariepinus* x *H. bidorsalis*) is being intensified (Hecht and Lublinkhof, 1985). Certain physiological and behavioural activities such as breeding, migration and aestivation have been found to affect fish tissues proximate composition (Colman *et al.*, 1982; Hodgkiss and Man, 1997). Maximum accumulation of fat in *Sarotherodon mossambicus* occurred in June - July, a period that coincided with the breeding period in Plover Cone Reservoir, Hong Kong (Hodgkiss and Man, 1997). The muscle triglycerides of the total lipid content in *Oncorhychus masu* juveniles were noted to decrease at the early stages of sea water life (Ota, 1976). However, the protein content which forms 14 – 21% of the net weight of whole fish is of greater importance and a better indicator of fish quality than the less permanent fat (Lagler *et al.*, 1977). Sea fish generally contain more minerals than freshwater fish (Lagler *et al.*, 1977), since the former derive their minerals from food or water in which they live. The higher mineral content (calcium) in female Osteichthyes than in males especially during the breeding period has been attributed to increases in protein-bound calcium during the breeding period

(Urist and Schyeide, 1961). Detailed proximate analyses are needed to determine the effect of the infiltration of crude oil compounds into the muscle tissues of different age groups of *Heterobranchus bidorsalis* since this fish commands high market value in Nigeria. Crude oil exposures of adult marine fish have been reported to increase the mortality rate and changes in the haemoglobin content of blood (Tatem *et al*., 1979). In Nigeria, work has been done on the effect of different concentrations of Bonny-light crude oil on the mortality rate of *H. bidorsalis* (Nwamba *et al.,* 2001) and *C. gariepinus* (Ugwu *et al.,* 2003). This study presents the results of an experiment designed to consider age factor in the proximate composition of the muscle of *H. bidorsalis* exposed to graded concentrations of Bonny-light crude oil. The essence was to ascertain the extent to which the crude oil pollution affected the quality of fish flesh with respect to age of the fish.

MATERIALS AND METHODS

Nine hundred (900) fish specimens of three different age groups of *H. bidorsalis* comprising 300 juveniles (14.08 ± 0.12 g), 300 yearlings (24 ± 0.16 g) and 300 adults (420 ± 2.30 g) were transported from a private fish hatchery (Aquafish Nigeria Limited, higher than those exposed to 1.00 - 8.00 ml L^{-1} BLCO concentrations (Tables 2, 3, 4 and 5) during the toxicity and the recovery periods. Conversely, the values of the nitrogen-free extract (NFE) of the fish exposed to the various BLCO concentrations were higher than those of the control. The percent compositions of CP, EE, AS and DM decreased significantly ($P < 0.05$) in the three age groups (JV, YRL and AD) with increasing concentrations of BLCO (1.00 – 8.00 ml L^{-1}), while the NFE values increased. Crude fibre values were however not significantly different ($P < 0.05$) between the control and the BLCO-treated groups. This situation was evident during the toxicity and recovery periods (Table 2, 3, 4, and 5). The water temperature was 26 ± 0.26° C and the pH was 6.80 ± 0.12.

The values of the CP of juveniles exposed to 1.00 – 8.00 ml L^{-1} BLCO concentrations during the 4 days toxicity period (16.69 ± 11 – 10.25 ± 0.07 %) (Table 2) were higher than those of the yearlings (17.57 ± 1.46 – 9.17 ± 0.05 %) and the adults (14.91 ± 1.02 – 7.78 ± 0.05%). This state of affairs was also evident during the recovery period at day 14 (Table 3), day 28 (Table 4) and day 42 (Table 5). The values of EE, AS and DM followed the trend exhibited by the CP values both during the toxicity and the recovery periods. Conversely, the values of the NFE of the yearlings were higher than those of the juveniles and the adults at day 4 (Table 2), day 14 (Table 3), day 28 (Table 4) and day 42 (Table 5) irrespective of the BLCO concentrations to which the fishes were exposed. The values of the fish nutrients (CP, EE, and AS) in the JV, the YRL and the AD increased at certain percent magnitudes as the fish specimens recuperated from their exposures to the crude oil pollutant. From our results, there were 5% increases in the values of these nutrients in all the age groups between day 4 and day 14; while 15 % increases were recorded between day 14 and day 28. CP, EE and AS values, on the other hand, increased by a magnitude of 25 % between day 28 and day 42 of the recovery period. Although the computations of the NFE values of the fish exposed to the different concentrations of BLCO were done by difference between % CP + % EE + % AS + % CF + % moisture and 100 %, noticeable decreases in the values of this nutrient (NFE) were evident as the surviving fish specimens recuperated from exposures to the crude oil pollutant. For example, while the NFE value of the JV exposed to 1.00 ml L^{-1} BLCO concentration was 58.44 ± 2,31% at day 4 (Table 2), the corresponding NFE values at days 14, 28 and 42 recovery periods were 56.11 ± 1.31 % (Table 3), 50.52 ± 1.17 % (Table 4) and 48.05 ± 1.31% (Table 5) respectively.

Records of fish mortality and survivals during this study (Table 6) indicated that each of the age groups (JV, YRL or AD) under investigation 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 L^{-1} BLCO concentrations.

DISCUSSION

Fishes are exposed to a wide range of contamination in aquatic environments. Short term exposures of fish larvae to pollutants increased susceptibility to other environmental stresses and changes in the rates of growth and development (Rulfson, 1971). 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 protein synthesis, or on the rate of its degradation. Singh *et al*. (1996) stated that the quantity of protein may be affected by impaired incorporation of amino acids in the polypeptide chains.

The inhibition of protein deposition in the fish muscle tissue of our study, as the BLCO concentration increased (1.00 – 8.00 ml L^{-1}) (Table 2, 3, 4, and 5) agreed with the report of other workers. Reeta *et al*. (1993) reported inhibition in the total serum protein of an air-breathing fish *Heteropneustes fossilis* after exposure to different pesticides (DDT, YBHC and Malathion). Ravichandran *et al.* (1994) reported depletion of protein from 17 – 45 % due to proteolysis after exposing *Oreochromis mossambicus* to nominal concentrations of phenol. Ogueji and Auta (2007) also reported inhibition in the total serum protein of *Clarias gariepinus* exposed to acute concentrations of a pyrethroids insecticide (lambda-cyhalothrin). In this study, the CP values of the adult fish muscle were lower than those of the juveniles and yearlings: both at the toxicity (4 days) and at the recovery (14, 28 and 42 days) periods. This implies that the crude oil compounds might have depleted the quantity of protein faster in the adults than the juveniles and yearlings. Bradbury *et al.* (1987) pointed out 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 ether extract (crude fat) content of fish muscle in this study also agree with the reports of other workers. For example, the muscle triglycerides of the total lipid content in *Oncorhynchus masu* juveniles were noted to decrease at the early stages of sea water life (Ota, 1976). This study recorded significant decreases ($P < 0.05$) in the EE content of *H. bidorsalis* juveniles and yearlings as the concentrations of BLCO to which they were exposed increased from 1.00 to 8.00 ml L^{-1} (Table 2, 3, 4, and 5). Nevertheless our present results varied with the report of Ogueji and Auta (2007) who recorded significant ($P < 0.05$) dose-dependent elevations in the triglyceride levels in the blood serum of *Clarias gariepinus* (Teugels) subjected to acute exposure of lambda-cyhalothrin (a commonly used pyrethroids insecticide). Similarly, Krishna *et al*. (1994) reported increased levels of phospholipids and cholesterols contents in the tissues of *Oreochromis mossambicus* subjected to acclimation in sub-lethal acid water (pH, 4.00).

Table 2: Proximate composition of the muscle of three age groups of *Heterobranchus bidorsalis* exposed to graded concentration of Bonny-light crude oil (BLCO) during 4 days toxicity period[1]

Age of fish	Nutrient (%)	BLCO concentration (ml L^{-1}) 0.00 (control)	1.00	2.00	4.00	8.00
Juvenile (7 weeks)	CP[2]	19.64 ± 1.12^{a}	16.69 ± 1.11^{b}	14.19 ± 1.02^{c}	12.06 ± 0.06^{d}	10.25 ± 0.07^{e}
	EE[3]	8.66 ± 0.48^{a}	7.36 ± 0.34^{b}	6.26 ± 0.21^{c}	5.32 ± 0.17^{d}	4.52 ± 0.06^{e}
	AS[4]	3.12 ± 0.03^{a}	2.65 ± 0.34^{b}	2.25 ± 0.01^{b}	1.91 ± 0.01^{c}	1.62 ± 0.02^{c}
	DM[5]	17.48 ± 1.03^{a}	14.86 ± 1.03^{b}	12.63 ± 0.07^{c}	10.74 ± 0.04^{d}	9.13 ± 0.05^{e}
	NFE[6]	51.10 ± 2.01^{a}	58.44 ± 2.31^{b}	64.67 ± 2.17^{c}	69.97 ± 2.42d	74.48 ± 2.43^{e}
	CF[7]	1.02 ± 0.01^{a}	1.03 ± 0.00^{a}	1.01 ± 0.02^{a}	1.03 ± 0.0^{a}	1.02 ± 0.03^{a}
Yearling (11 months)	CP	17.57 ± 1.46^{a}	14.93 ± 1.10^{b}	12.69 ± 0.06^{c}	10.79 ± 0.05^{d}	9.17 ± 0.05^{e}
	EE	4.61 ± 0.04^{a}	3.92 ± 0.05^{b}	3.33 ± 0.04^{c}	2.83 ± 0.03^{d}	2.41 ± 0.03^{d}
	AS	2.23 ± 0.02^{a}	1.90 ± 0.03^{b}	1.62 ± 0.02^{b}	1.38 ± 0.01^{bc}	1.17 ± 0.01^{cd}
	DM	17.45 ± 1.04^{a}	14.83 ± 1.06^{b}	12.61 ± 1.01^{c}	10.72 ± 0.06^{d}	9.11 ± 0.06^{e}
	NFE	58.14 ± 2.24^{a}	64.42 ± 2.05^{b}	69.75 ± 2.15^{c}	74.28 ± 2.18^{d}	78.14 ± 2.44^{e}
	CF	1.02 ± 0.02^{a}	1.01 ± 0.02^{a}	1.01 ± 0.03^{a}	1.03 ± 0.00^{a}	1.02 ± 0.00^{a}
Adult (5 months)	CP	14.91 ± 1.02^{a}	12.67 ± 0.07^{b}	10.77 ± 0.06^{c}	9.15 ± 0.05^{d}	7.78 ± 0.05^{e}
	EE	8.77 ± 0.46^{a}	7.45 ± 0.32^{b}	6.33 ± 0.30^{c}	5.38 ± 0.24^{d}	4.57 ± 0.16^{e}
	AS	3.01 ± 0.028^{a}	2.56 ± 0.02^{b}	2.18 ± 0.01^{b}	1.85 ± 0.01^{c}	1.57 ± 0.01^{c}
	DM	26.91 ± 2.33^{a}	22.87 ± 1.24^{b}	19.44 ± 1.13^{c}	16.52 ± 1.03^{d}	14.04 ± 1.04^{e}
	NFE	46.40 ± 1.12^{a}	54.45 ± 1.19^{b}	61.28 ± 2.18^{c}	67.10 ± 2.32^{d}	72.07 ± 2.02^{e}
	CF	1.01 ± 0.0^{a}	1.01 ± 0.01^{a}	1.02 ± 0.0^{a}	1.01 ± 0.02^{a}	1.01 ± 0.0^{a}

[1]Values followed by the same superscripts in the same row are not significantly different (P > 0.05). [2]Crude protein, [3]ether extracted, [4]ash, [5]dry matter, [6]nitrogen free extract, [7]Crude fibre.

Table 3: Proximate composition of the muscle of three age groups of *Heterobranchus bidorsalis* exposed to graded concentrations of Bonny-light crude oil (BLCO) during 14-day toxicity period[1]

Age of Fish	Nutrient (%)	0.00 ml L^{-1} (Control)	BLCO Concentration (ml L^{-1}) 1.00	2.00	4.00	8.00
Juvenile (7 weeks)	CP[2]	20.23 ± 1.12^{a}	17.52 ± 1.01^{b}	14.90 ± 0.06^{c}	12.66±0.07^{d}	10.25 ± 0.07^{e}
	EE[3]	8.92 ± 0.04^{a}	7.37 ± 0.03^{b}	6.57 ± 0.04^{c}	5.59 ± 0.03^{d}	4.52 ± 0.06^{e}
	AS[4]	3.22 ± 0.01^{a}	2.78 ± 0.01^{b}	2.36 ± 0.01^{c}	2.01 ± 0.01^{c}	1.62 ± 0.02^{c}
	DM[5]	18.00 ± 1.01^{a}	15.86 ± 0.07^{b}	13.26 ± 0.08^{c}	11.28 ± 0.06^{d}	9.13 ± 0.05^{e}
	NFE[6]	49.63 ± 1.12^{a}	56.11 ± 1.13^{b}	62.91 ± 1.15^{c}	68.46 ± 1.16^{d}	74.48 ± 2.43^{e}
	CF[7]	1.02 ± 0.03^{a}	1.01 ± 0.04^{a}	1.00 ± 0.05^{a}	1.01 ± 0.02^{a}	1.01 ± 0.01^{a}
Yearling (11 months)	CP	17.92 ± 0.07^{a}	15.68 ± 0.09^{b}	13.32 ± 0.07^{c}	11.33 ± 0.07^{d}	9.63 ± 0.04^{e}
	EE	4.70 ± 0.02^{a}	4.12 ± 0.02^{b}	3.50 ± 0.02^{c}	2.97 ± 0.02^{d}	2.53 ± 0.02^{d}
	AS	2.27 ± 0.01^{a}	2.01 ± 0.01^{a}	1.70 ± 0.01^{b}	1.45 ± 0.01^{b}	1.23 ± 0.01^{b}
	DM	17.80 ± 0.08^{a}	15.57 ± 0.08^{b}	13.24 ± 0.06^{c}	11.26 ± 0.06^{d}	9.57 ± 0.05^{e}
	NFE	57.31 ± 1.14^{a}	62.62 ± 1.14^{b}	68.24 ± 1.16^{c}	72.99 ± 1.12^{d}	77.04 ± 1.14^{e}
	CF	1.03 ± 0.02^{a}	1.00 ± 0.00^{a}	1.02 ± 0.00^{a}	1.02 ± 0.02^{a}	1.01 ± 0.00^{a}

Adult (15 months)	**CP**	15.06 ± 0.07^{a}	13.30 ± 0.05^{b}	11.31 ± 0.04^{c}	9.61 ± 0.05^{d}	8.17 ± 0.03^{e}
	EE	8.87 ± 0.05^{a}	7.82 ± 0.03^{b}	6.65 ± 0.03^{c}	5.65 ± 0.03^{d}	4.80 ± 0.02^{e}
	AS	3.01 ± 0.02^{a}	2.69 ± 0.01^{b}	2.29 ± 0.01^{b}	1.94 ± 0.01^{c}	1.65 ± 0.01^{c}
	DM	27.18 ± 1.14^{a}	24.01 ± 1.14^{b}	20.41 ± 1.05^{c}	17.35 ± 1.01^{d}	14.74 ± 0.06^{e}
	NFE	45.89 ± 1.11^{a}	52.18 ± 1.06^{b}	59.34 ± 1.13^{c}	65.45 ± 1.03^{d}	70.64 ± 1.32^{e}
	CF	1.04 ± 0.00^{a}	1.02 ± 0.00^{a}	1.00 ± 0.00^{a}	1.04 ± 0.00^{a}	1.01 ± 0.02^{a}

[1]Values followed by the same superscripts in the same row are not significantly different (P > 0.05). [2]Crude protein, [3]ether extract, [4]ash, [5]dry matter, [6]nitrogen, free extract, [7]crude fibre.

Table 4: Proximate composition of the muscle of three age groups of *Heterobranchus bidorsalis* exposed to graded concentration of Bonny-light crude oil (BLCO) during 28-day recovery period[1]

Age of Fish	Nutrient (%)	0.00 ml L^{-1} (Control)	BLCO concentration (ml L^{-1})			
			1.00	**2.00**	**4.00**	**8.00**
Juvenile (7 weeks)	**CP[2]**	20.84 ± 1.13^{a}	18.40 ± 1.01^{b}	14.90 ± 0.07^{c}	12.66 ± 0.05^{d}	10.76 ± 0.04^{e}
	EE[3]	9.19 ± 0.05^{a}	8.12 ± 0.04^{b}	6.57 ± 0.03^{c}	5.59 ± 0.02^{d}	4.75 ± 0.02^{e}
	AS[4]	3.32 ± 0.01^{a}	3.49 ± 0.02^{a}	2.36 ± 0.01^{b}	2.01 ± 0.01^{b}	1.70 ± 0.01^{c}
	DM[5]	18.54 ± 1.12^{a}	19.47 ± 1.12^{b}	13.26 ± 0.06^{c}	11.28 ± 0.04^{d}	9.59 ± 0.04^{e}
	NFE[6]	48.11 ± 1.16^{a}	50.52 ± 1.17^{b}	62.91 ± 1.18^{c}	68.46 ± 1.16^{d}	73.20 ± 1.15^{e}
	CF[7]	1.02 ± 0.01^{a}	1.01 ± 0.00^{a}	1.03 ± 0.00^{a}	1.05 ± 0.04^{a}	1.01 ± 0.02^{a}
Yearling (11 months)	**CP**	18.28 ± 1.03^{a}	16.46 ± 0.06^{b}	13.32 ± 0.06^{c}	11.33 ± 0.06^{d}	9.63 ± 0.04^{e}
	EE	4.79 ± 0.02^{a}	4.33 ± 0.02^{a}	3.50 ± 0.02^{b}	2.97 ± 0.01^{c}	2.53 ± 0.02^{c}
	AS	2.23 ± 0.01^{a}	2.11 ± 0.01^{a}	1.70 ± 0.01^{b}	1.45 ± 0.01^{b}	1.23 ± 0.01^{b}
	DM	18.16 ± 1.10^{a}	16.35 ± 1.01^{b}	13.24 ± 0.07^{c}	11.26 ± 0.05^{d}	9.57 ± 0.06^{e}
	NFE	56.45 ± 1.13^{a}	60.75 ± 1.14^{b}	68.24 ± 1.15^{c}	72.99 ± 1.11^{d}	77.05 ± 1.10^{e}
	CF	1.00 ± 0.02^{a}	1.04 ± 0.03^{a}	1.01 ± 0.00^{a}	1.05 ± 0.05^{a}	1.01 ± 0.01^{a}
	CP	15.36 ± 0.05^{a}	13.97 ± 0.06^{b}	11.31 ± 0.05^{c}	9.61 ± 0.05^{d}	8.17 ± 0.04^{e}
Adult (15 months)	**EE**	9.04 ± 0.04^{a}	8.21 ± 0.04^{b}	6.65 ± 0.02^{c}	5.65 ± 0.02^{d}	4.80 ± 0.02^{e}
	AS	3.07 ± 0.02^{a}	2.82 ± 0.02^{b}	2.29 ± 0.01^{c}	1.94 ± 0.01^{d}	1.65 ± 0.01^{d}
	DM	27.72 ± 1.13^{a}	25.21 ± 1.11^{b}	20.41 ± 1.10^{c}	17.35 ± 1.01^{d}	14.74 ± 0.08^{e}
	NFE	44.81 ± 1.13^{a}	49.79 ± 1.12^{b}	59.34 ± 1.13^{c}	65.45 ± 1.15^{d}	70.64 ± 1.16^{e}
	CF	1.02 ± 0.00^{a}	1.01 ± 0.00^{a}	1.01 ± 0.01^{a}	1.01 ± 0.00^{a}	1.01 ± 0.02^{a}

[1]Values followed by the same superscripts in the same row are not significantly different (P > 0.05). [2]Crude protein, [3]Ether extract, [4]Ash, [5]Dry matter, [6]Nitrogen free extract. [7]Crude fibre.

Table 5: Proximate compositions of the muscle of three age groups of *H. bidorsalis* exposed to graded concentration of Bonny-light crude oil during 48 days of recovery period[1]

Age of Fish	Nutrient (%)	0.00 ml L^{-1} (Control)	BLCO Concentration (ml L^{-1})			
			1.00	**2.00**	**4.00**	**8.00**
Juvenile (7 weeks)	**CP[2]**	21.46 ± 1.11^{a}	19.32 ± 1.01^{b}	15.65 ± 0.05^{c}	13.29 ± 0.05^{d}	11.30 ± 0.04^{e}
	EE[3]	9.47 ± 0.05^{a}	8.53 ± 0.04^{b}	6.90 ± 0.03^{c}	5.87 ± 0.02^{d}	4.99 ± 0.02^{e}
	AS[4]	3.42 ± 0.02^{a}	3.66 ± 0.01^{a}	2.48 ± 0.02^{b}	2.11 ± 0.01^{b}	1.79 ± 0.01^{c}
	DM[5]	19.10 ± 1.01^{a}	20.44 ± 1.10^{b}	13.92 ± 0.04^{c}	11.84 ± 0.03^{d}	10.07 ± 0.05^{e}
	NFE[6]	46.55 ± 1.24^{a}	48.05 ± 1.31^{b}	61.05 ± 1.33^{c}	66.89 ± 1.31^{d}	71.85 ± 1.40^{e}
	CF[7]	1.03 ± 0.00^{a}	1.02 ± 0.01^{a}	1.03 ± 0.02^{a}	1.01 ± 0.02^{a}	1.04 ± 0.03^{a}

Yearling (11 months)	**CP**	18.65 ± 1.07[a]	19.19 ± 1.03[b]	13.99 ± 0.04[c]	11.90 ± 0.05[d]	10.11 ± 0.04[e]
	EE	4.86 ± 0.03[a]	5.03 ± 0.02[b]	3.66 ± 0.02[b]	3.12 ± 0.02[d]	2.66 ± 0.01[e]
	AS	2.37 ± 0.01[a]	2.44 ± 0.01[a]	1.79 ± 0.01[b]	1.52 ± 0.01[b]	1.29 ± 0.01[bc]
	DM	19.24 ± 1.13[a]	19.07 ± 1.02[a]	13.90 ± 0.04[b]	11.82 ± 0.06[c]	10.05 ± 0.05[d]
	NFE	54.88 ± 1.24[a]	54.27 ± 1.14[b]	66.66 ± 1.22[c]	71.64 ± 1.24[d]	75.89 ± 1.25[e]
	CF	1.01 ± 0.0[a]	1.02 ± 0.03[a]	1.03 ± 0.03[a]	1.01 ± 0.01[a]	1.02 ± 0.02[a]
Adult (15 months)	**CP**	15.51 ± 0.06[a]	16.13 ± 0.05[b]	11.88 ± 0.05[c]	10.09 ± 0.05[d]	8.58 ± 0.03[e]
	EE	9.22 ± 0.04[a]	9.49 ± 0.04[a]	6.98 ± 0.03[b]	5.93 ± 0.03[c]	5.04 ± 0.02[d]
	AS	3.10 ± 0.01[a]	3.16 ± 0.0[b]	2.40 ± 0.02[b]	2.04 ± 0.01[b]	1.73 ± 0.01[c]
	DM	27.99 ± 1.22[a]	29.39 ± 1.14[b]	21.42 ± 1.10[c]	18.22 ± 1.01[d]	15.48 ± 0.05[e]
	NFE	44.18 ± 1.24[a]	41.83 ± 1.21[b]	57.32 ± 1.31[c]	62.72 ± 1.32[d]	69.17 ± 1.23[e]
	CF	1.05 ± 0.04[a]	1.03 ± 0.02[a]	1.04 ± 0.02[a]	1.02 ± 0.02[a]	1.01 ± 0.00[a]

[1]Values followed by the same superscripts in the same row are not significantly different ($P > 0.05$). [2]Crude protein, [3]Ether extract, [4]Ash, [5]Dry matter, [6]Nitrogen free extract. [7]Crude fibre.

Table 6: Percent mortality and survival of three age groups of *Heterobranchus bidorsalis* during exposure to different concentrations of Bonny-light crude oil (BLCO): toxicity (4 days) and recovery (42 days)

Study Period	Duration (days)	Age of fish	% Mortality BLCO Concentration (ml L^{-1}) 0.0 (Control)	1.0	2.0	4.0	8.0	% Survival BLCO Concentration (ml L^{-1}) 0.0 (Control)	1.0	2.0	4.0	8.0
Toxicity phase	**4**		0.0	10.0	0.0	40.0	50.0	100.0	90.0	100.0	60.0	50.0
Recovery phase	**14**	**Juvenile (7 weeks)**	0.0	8.0	6.0	32.0	40.0	100.0	92.0	92.0	68.0	60.0
	28		0.0	2.0	1.0	24.0	36.0	100.0	98.0	99.0	76.0	64.0
	42		0.0	1.0	0.0	16.0	16.0	100.0	99.0	100.0	84.0	74.0
Toxicity phase	**4**		0.0	5.0	1.0	34.0	42.0	100.0	95.0	9.0	66.0	78.0
Recovery phase	**14**	**Yearlings (11 months)**	0.0	4.0	4.0	24.0	33.0	100.0	96.0	96.0	76.0	67.0
	28		0.0	1.0	1.0	18.0	25.0	100.0	99.0	99.0	82.0	75.0
	42		0.0	1.0	0.0	12.0	16.0	100.0	99.0	100.0	88.0	84.0
Toxicity phase	**4**		0.0	3.0	1.0	26.0	30.0	100.0	97.0	99.0	74.0	70.0
Recovery phase	**14**	**Adults (15 months)**	0.0	2.0	3.0	18.0	21.0	100.0	98.0	97.0	82.0	79.0
	28		0.0	1.0	0.0	14.0	17.0	100.0	99.0	100.0	86.0	83.0
	42		0.0	0.0	0.0	8.0	10.0	100.0	100.0	100.0	92.0	90.0

These workers argued that toxicants generally cause the accumulation of triglycerides in fatty livers and that this accumulation occurs as a result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cell in the systemic circulation (Gabriel, 1986). Decreases in the crude fat (EE) content of fish muscle in our study might be due to the harmful affect of petroleum-related aromatic compounds (ACs) to animals (NRC, 1985). Varanasi *et al.* (1989) stated that fish and marine mammals extensively metabolize most ACs in their livers and predominantly excrete these metabolites into bile. Therefore, the ACs of the BLCO in this study might have caused decreases in the muscle triglycerides of the total lipid content (EE) in *H. bidorsalis* juveniles and yearlings exposed to the oil pollutant.

The significant increases ($P < 0.05$) in the values of NFE (digestible carbohydrates) in the fish muscle of this study were BLCO concentration dependent. This might also be attributed to the stress induced by the crude oil pollutant. Glucose which constitutes one end-product of carbohydrate digestion might be increased as a general response of fish to acute or sub-lethal pollutant effects (Ceron *et al.,* 1997; Luskova et al., 2002). Wedemeyer and Mcleay (1981) stated that 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 catecholamine (Nakano and Tomilinson, 1976). Both of these groups of hormones produce hyperglycaemia (Ogueji and Auta, 2007). The trend of NFE increases in *H. bidorsalis* in this study is consistent with the reports of above-mentioned workers, although the variations in the NFE values were more pronounced in the yearlings than in the juveniles or the adults (Tables 2, 3, 4 and 5).

In this investigation, the BLCO concentration-dependent depression of crude protein and crude fat, and the consequent elevation of the digestible carbohydrate content of the muscle of *H. bidorsalis* age groups could be due to the high energy demands imposed on the fishes as a positive survival value under the crude oil stress. In this context, the depletion of the essential muscle nutrients (CP and EE) and possibly the occurrence of hyperglycaemia must have resulted in high percent mortality and low survivals of the fish as the BLCO concentrations increased (Table 6).

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