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Morphometric Status of the Digestive Tract of *Heterobranchus bdorsalis* Juveniles Exposed to Different Concentrations of Bonny-Light Crude Oil

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Abstract: The morphometric status of the digestive tract of *Heterobranchus bidorsalis* juveniles exposed to graded concentrations of Bonny-Light Crude Oil (BLCO) was studied. The exposure of the fish to 1.00, 2.00, 4.00, 8.00 m L^{-1} and a control for 4 days toxicity period indicated that significant decreases ($p<0.05$) in the widths and lengths of the Oesophagus (OES) Stomach (ST) and Intestine (INT) of the fish were BLCO concentrations dependent. Increased OES, ST and INT values noticed within the first 14 days of the recovery period implies that the removal of the oil pollutant from the ambient water chemistry probably rescucitated the component parts of the digestive tract of the fish both in length and in width. The magnitude of rescucitation of these component parts improved between day 14 (5%) and day 42 (15%). This implies that the ability of *H. bidorsalis* juveniles to recover their full and inherent capacity to utilize ingested food after water pollution is time dependent.

Key words: *Heterobranchus bidorsalis*, bonny-light crude oil, morphometric status, digestive tract, toxicity

INTRODUCTION

The giant African catfish, *Heterobranchus bidorsalis* is one of the easiest and commonest fish to grow in ponds with a remarkable fast growth. Its ability to adapt to pond conditions, tolerate crowded conditions, accept artificial feeds and possess high quality flesh have enabled it gain tremendous population as a culture fish (Reed *et al*., 1967; Bard *et al*., 1976; Olatunde, 1983).

Oil spills constitute one of the most important sources of environmental problems in Nigeria's petroleum industry. The degree of exposure of aquatic organisms to oil 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 capacities of juvenile fish to utilize artificial diets in the digestive tract (especially the mucosal epithelia) have been studied (Kjorskik *et al*., 1991; Verreth *et al*., 1992). Larval fish are characterized by significant uptake of nutrients by the hind gut epithelial cells and intracellular digestion in the supranuclear vacuoles of those cells (Iwai and Tanaka, 1968; Watanabe, 1984).

Once food has been ingested by fish larvae, the digestion, absorption and assimilation of food can be studied using fluorescence, radio-labelling and/or histological methods (Rice *et al*., 1994). Walsh *et al*. (1987) and Walford *et al*. (1991) used fluorescence methods to follow particles passing through larval fish digestive tract, particularly noting time of passage and bottlenecks in passage. The determination of assimilation efficiency with radio labeled carbon has long been practiced with larval fish fed live food (Boehlert and Yoklavich, 1984). Recently, this practice has been used to compare uptake of artificial and live diets (Tandler and Kolkosky, 1991). Assimilation efficiency data, when compared with data on rates of ingestion of live vs. artificial diets can provide valuable insight into artificial diets deficiencies. Whereas fluorescence and radio-labelling studies are most useful in estimating process rates for whole organisms, histological studies are most useful in identifying specific digestion and absorption sites within the digestive tract (Rice *et al*., 1994). Bengston (1993) and Bengston *et al*. (1993) have studied uptake of live vs. artificial food by sampling larvae at time intervals after a single feeding and examining histological sections.

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Detailed anatomical studies are needed to determine the effect of the infiltration of crude oil compounds into the body organs and tissues of *Heterobranchus bidorsalis* juveniles. It has been suggested that the uptake and translocation of crude oil compounds in fish may be through the gills, the gut or the intestinal walls (Roubal *et al*., 1977). The workers maintained that the parent compounds of crude oil solubilize in the cell membranes and are carried via the erythrocytes to the general circulation of the blood. Crude oil exposure of the adult marine fish species have been reported to increase the mortality rate and changes in the haemoglobin content of blood (Tatem *et al*., 1979). Work has been carried out in Nigeria on the effect of different concentrations of Bonny-light crude oil on the mortality rate of *Clarias gariepinus* and *Heterobranchus bidorsalis* (Ugwu *et al*., 2006; Nwamba *et al*., 2001). This study was therefore designed to assess the morphometric status of the digestive tract of *Heterobranchus bidorsalis* juveniles exposed to different concentrations of Bonny-light crude oil. The essence was to portray from an empirical perspective any distortions in the size of the digestive tract consequent upon the exposure of the fish to crude oil pollution.

MATERIALS AND METHODS

Six hundred juveniles of *Heterobranchus bidorsalis* (14.02±0.24g) were transported from a private fish hatchery at Otor Oweh, Delta State to the Fisheries Laboratory of Ebonyi State University, Abakalaki. At the Fisheries Laboratory in Abakaliki the fishes were acclimatized for 14 days on a chick starter diet fed at 3% body weight per day (bw d^{-1}).

Batches of twenty juveniles of *H. bidorsalis* were randomly stocked in triplicates in 15 plastic containers with 24 L 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 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 periods were adopted for the study. The toxicity period lasted up to 4 days (i.e., 24, 48 and 96 h), while the recovery period lasted for 42 days and was monitored at forthnightly (14 days) intervals. Fish were monitored each day in both periods for mortality and the surviving fish records. At the end of the toxicity period, the surviving fishes and plastic containers were washed and replenished with dechlorinated tap water. A 30% crude protein diet (Table 1) was fed 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 (27±0.30°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-1-20-L), respectively.

Table 1: Gross and proximate compositions of the experimental diet fed to *Heterobranchus bidorsalis* juveniles 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^{-1} 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^{-1}), 6; menadione- Na-bisulphate, 80; inosilol, 400; biotin, 2; vitamin C, 200; alphatocopherol, 200; cholecalcipherol, 100,000 IU g^{-1}, [2]Cotaminated as g kg^{-} of premix:$FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; Nacc, 45; Na_2SO_4, 88; $ACCl_3$ 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

Dissection of fish from each triplicate treatment with BLCO was carried out in the laboratory at 24, 48 and 96 h during the toxicity period and at 14, 28 and 42 days during the recovery period. On each occasion, fishes were dissected with the aid of sharp surgical scissors and blades to display the digestive tract on a wooded board. The entire tract, starting from the oesophagus to the anus was carefully excised from the fish viscera and rinsed in clean distilled water and placed in 0.90% physiological saline solution. Subsequently, sections of the digestive tract namely: The Oesophagus (OES) the Stomach (ST) and the Intestine (INT) were cut for the necessary length and width measurements. All measurements were taken with a metal metre rule to the nearest 0.01cm. The Analysis of Variance (ANOVA) was used to test level significance at 5% probability while the Duncan (1955) Multiple Range Test was employed to partition differences between treatment means.

RESULTS

Width measurements (cm) of the Oesophagus (OES) the Stomach (ST) and the Intestine (INT) of *H. bidorsalis* juveniles exposed to different concentrations of BLCO

Table 2: Width measurements (cm) of the digestive tract of *H. bidorsalis* juveniles exposed to different concentrations of Bonny-light Crude Oil (BLCO) for 4 days (Toxicity) and 42 days (Recovery) periods

		BLCO Concentration mL L^{-1}														
		0.00 (Control)			1.00			2.00			4.00			8.00		
Study period	Duration	OES[2]	ST[2]	INT[3]	OES	ST	INT	OES	ST	INT	OES	ST	INT	OES	ST	INT
Toxicity period	24 h	1.14 ±0.13^{a}	0.54 ±0.02^{b}	0.15 ±0.01^{c}	1.72 ±0.14^{d}	0.63 ±0.03^{e}	0.19 ±0.02^{c}	0.98 ±0.04^{f}	0.61 ±0.03	0.21 ±0.01^{c}	0.95 ±0.06^{f}	0.53 ±0.02^{b}	1.02 ±0.10^{a}	0.95 ±0.05^{f}	0.52 ±0.3^{b}	1.21 ±0.12^{a}
	48 h	1.15 ±0.14^{a}	0.55 ±0.03^{b}	0.15 ±0.01^{c}	1.65 ±0.13^{d}	0.55 ±0.02^{b}	0.15 ±0.01^{c}	0.95 ±0.04^{e}	0.52 ±0.02^{b}	0.17 ±0.01^{c}	0.85 ±0.05^{e}	0.45 ±0.02^{f}	0.96 ±0.06^{g}	0.80 ±0.03^{e}	0.40 ±0.02^{e}	1.01 ±0.11^{a}
	96 h	1.04 ±0.12^{a}	0.45 ±0.01^{b}	0.15 ±0.01^{c}	1.53 ±0.12^{d}	0.43 ±0.02^{b}	0.12 ±0.01^{c}	0.81 ±0.03^{e}	0.43 ±0.02^{b}	0.12 ±0.01^{c}	0.75 ±0.04^{f}	0.35 ±0.01^{g}	0.92 ±0.05^{h}	0.74 ±0.04^{f}	0.31 ±0.02^{g}	0.82 ±0.04^{e}
Recovery period	14 days	1.09 ±0.13^{a}	0.47 ±0.01^{b}	0.16 ±0.02^{c}	1.16 ±0.12^{d}	0.45 ±0.01^{b}	0.13 ±0.01^{c}	0.85 ±0.04^{e}	0.45 ±0.04^{b}	0.13 ±0.01^{c}	0.79 ±0.05^{f}	0.37 ±0.02^{g}	0.97 ±0.06^{h}	0.77 ±0.04^{f}	0.33 ±0.01^{g}	0.86 ±0.05^{e}
	28 days	1.20 ±0.11^{a}	0.52 ±0.02^{b}	0.18 ±0.02^{c}	1.77 ±0.14^{d}	0.50 ±0.03^{b}	0.14 ±0.01^{c}	0.94 ±0.04^{e}	0.50 ±0.02^{b}	0.14 ±0.02^{c}	0.87 ±0.04^{f}	0.41 ±0.03^{g}	1.07 ±0.10^{a}	0.85 ±0.04^{f}	0.36 ±0.01^{g}	0.95 ±0.06^{e}
	42 days	1.38 ±0.12^{a}	0.60 ±0.03^{b}	0.21 ±0.01^{c}	2.04 ±0.15^{d}	0.62 ±0.02^{b}	0.16 ±0.02^{e}	1.08 ±0.12^{f}	0.58 ±0.02^{b}	0.15 ±0.01^{e}	1.00 ±0.10^{f}	0.47 ±0.03^{g}	1.23 ±0.11^{a}	0.98 ±0.02^{h}	0.14 ±0.02^{g}	1.09 ±0.11^{f}

[1]Oesophagus, [2] Stomach, [3] Intestine. Values followed by the same superscripts in the same row are not significantly different ($p>0.05$). Values followed by different superscripts in the same row differ significantly ($p<0.05$)

Table 3: Length measurements (cm) of the digestive tract of *H. bidorsalis* Juveniles exposed to different concentrations of Bonny-light crude Oil (BLCO) for 4 days (Toxicity) and 42 days (Recovery) periods

		BLCO Concentration mL L^{-1}														
		0.00 (Control)			1.00			2.00			4.00			8.00		
Study period	Duration	OES[2]	ST[2]	INT[3]	OES	ST	INT	OES	ST	INT	OES	ST	INT	OES	ST	INT
Toxicity period	24 h	1.10 ±0.10^{a}	0.54 ±0.03^{b}	4.50 ±0.13^{c}	1.15 ±0.10^{a}	0.76 ±0.04^{d}	3.64 ±0.12^{e}	1.01 ±0.03^{f}	0.67 ±0.03^{f}	0.15 ±0.11^{g}	0.96 ±0.05^{h}	1.63 ±0.03^{f}	0.94 ±0.12^{i}	0.92 ±0.04^{h}	1.56 ±0.02^{b}	2.87 ±0.12^{i}
	48 h	1.10 ±0.00^{a}	0.55 ±0.03^{b}	0.52 ±0.12^{c}	1.02 ±0.10^{a}	0.65 ±0.03^{d}	3.53 ±0.1^{e}	0.90 ±0.10^{a}	0.55 ±0.03^{b}	3.00 ±0.10^{f}	0.85 ±0.04^{g}	0.52 ±0.03^{b}	2.82 ±0.11^{h}	0.80 ±0.03^{g}	0.45 ±0.03^{i}	2.75 ±0.12^{ah}
	96 h	1.13 ±0.12^{a}	0.55 ±0.02^{b}	4.53 ±0.13^{c}	0.92 ±0.05^{d}	0.50 ±0.02^{a}	3.22 ±0.12^{e}	0.91 ±0.10^{d}	0.45 ±0.02^{f}	3.11 ±0.10^{e}	0.83 ±0.05^{g}	0.40 ±0.02^{f}	3.00 ±0.12^{e}	0.72 ±0.03^{h}	0.35 ±0.01^{i}	2.91 ±0.11^{e}
Recovery period	14 days	1.19 ±0.12^{a}	0.58 ±0.03^{b}	4.76 ±0.14^{c}	0.97 ±0.06^{d}	0.53 ±0.02^{b}	3.38 ±0.11^{e}	0.96 ±0.12^{d}	0.47 ±0.02^{f}	3.27 ±0.12^{e}	0.87 ±0.04^{g}	0.42 ±0.03^{f}	3.15 ±0.13^{e}	0.76 ±0.04^{h}	0.37 ±0.02^{i}	0.06 ±0.13^{e}
	28 days	1.31 ±0.01^{a}	0.64 ±0.04^{b}	5,24 ±0.14^{c}	1.07 ±0.14^{d}	0.58 ±0.03^{b}	3.72 ±0.01^{c}	1.06 ±0.04^{e}	0.52 ±0.02^{b}	3.60 ±0.02^{c}	0.96 ±0.04^{f}	0.46 ±0.03^{g}	3.47 ±0.10^{a}	0.84 ±0.04^{f}	0.78 ±0.01^{g}	3.37 ±0.06^{e}
	42 days	1.51 ±0.12^{a}	0.74 ±0.04^{b}	6.03 ±0.13^{c}	1.23 ±0.11^{d}	0.67 ±0.03^{e}	4.28 ±0.14^{f}	1.22 ±0.14^{d}	0.60 ±0.04^{e}	4.14 ±0.13^{f}	1.10 ±0.11^{d}	0.53 ±0.03^{g}	3.99 ±0.14^{h}	0.97 ±0.05^{i}	0.90 ±0.05^{d}	3.88 ±0.12^{h}

[1]Oesphagus, [2]Stomach, [3]Intestine, Values followed by the same superscripts in the same row are not significantly different ($p>0.05$); Value followed by different superscripts in the same row are significantly different ($p<0.05$)

(1.00-8.00 mL L^{-1}) and the control for 4 days (toxicity) and 42 days (recovery) periods are shown in Table 2. The Values of OES, ST and INT of the fish decreased with the increasing concentrations of BLCO during the toxicity period (Table 2). Additionally, OES, ST and INT values decreased as the period of fish exposure increased from 24 to 96 h irrespective of the BLCO concentration.

Sections of the fish digestive tract showed some degree of improvement during the recovery period (14-42 days) after the stress imposed by the oil pollutant was removed Values of OES, ST and INT of the fish increased by a magnitude of 5% between day 4 to day 14, 10% between day 14 to day 28 and 15% between day 28 and day 42. Statistical analysis of the values of OES, ST and INT obtained during this study indicate that the width of the component structures of the digestive tract of the fish varied significantly ($p<0.05$) with the increasing concentrations of BLCO to which the fishes were exposed (Table 2).

Table 3 shows the length measurements for OES, ST and INT of the fish. These values also decreased significantly with the increasing concentrations of BLCO (1.00-8.00 mL L^{-1}) in water during the 4 days toxicity period (Table 3). As was the case with the width measurements, the lengths of the OES, the ST and the INT of the fish decreased as the period of fish exposure to the oil pollutant increased from 24 to 96 h irrespective of the BLCO concentrations. Also recorded was the response of the fish digestive tract with respect to the adjustments in length when the oil pollutant was removed from the ambient water chemistry. The improvement in length of the intestinal tract followed the pattern shown by the width measurements above: i.e. the lengths of the OES, the ST and the INT improved at the rate of 5% on day 14, 10% on 28 and 15% on day 42 (Table 3). The cumulative mortality rate of the fish after the 96 h (4 days) toxicity period (Table 4), indicated that mortality records showed tremendous improvements during the recovery period (days 14-42).

Table 4: Mortality rate of *H. bidorsalis* jeveniles exposed to different concentrations of bonny-light crude oil within 4 days (Toxicity) and 42 days (recovery) periods

			% Mortality					% Survival				
			BLCO Concentration (mL L^{-1})					BLCO Concentration (mL L^{-1})				
	Duration	Initial no. of fish/treatment	0.00 (Control)	1.00	2.00	4.00	8.00	0.00 (Control)	1.00	2.00	4.00	8.00
	24 h	20	0.00	0.00	0.00	10.00	20.00	100.00	100.00	100.00	90.00	80.00
Toxicity period	48 h	-	0.00	2.00	0.00	10.00	10.00	100.00	98.00	100.00	90.00	90.00
	96 h	-	0.00	8.00	0.00	20.00	20.00	100.00	92.00	100.00	80.00	80.00
	Total (4 days)		0.00	10.00	0.00	50.00	50.00					
Recovery period	14 days	-	0.00	8.00	6.00	32.00	40.00	100.00	92.00	94.00	68.00	60.00
	28 days	-	0.00	2.00	1.00	24.00	36.00	100.00	98.00	99.00	76.00	34.00
	42 days	-	0.00	1.00	0.00	16.00	26.00	100.00	99.00	100.00	84.00	74.00

DISCUSSION

Kjorskik *et al.* (1991) and Verreth *et al.* (1992) provided an insight into the utilization of artificial diets in the digestive tract of juvenile fish and are of the opinion that the mucosal epithelial cells play conspicuous roles during this process. Other researchers including (Iwai and Tanaka, 1962; Watanabe, 1984) had previously suggested that the hind gut epithelial cells of juvenile fish play a leading role in the uptake of nutrients; while intracellular digestion occurs in the supranuclear vacuoles of these cells. Advanced studies on the digestion, absorption and assimilation of food by larval fish using fluorescence, radiolabelling and/or histological methods (Rice *et al.*, 1994). Research conclusions made by these workers were based on circumstances devoid of any infiltration of extraneous substances or crude oil compounds into the digestive tracts of the fish.

This study was anchored on the premise that the uptake and translocation of crude oil compounds in the fish, as stated by Roubal *et al.* (1977) could be through the gills, the gut or intestinal walls. These workers even suggested that the parent compounds of crude oil solubilize in the cell membranes and are trans-located to the general circulation of the blood via the erythrocytes. Working on *Clarias albopunctatus* (Oluah *et al.*, 2005) and *Heterobranchus bidorsalis* juveniles (Nwamba *et al.*, 2006) these workers stated that increased activities of fish digestive enzymes were dependent on the concentration of either crude oil or Gammalin 20 and Acetellic 25EC to which the fishes were exposed.

The results of our study indicated that alterations in the morphometric parameters (width and length) (Table 2 and 3) of the digestive tract of *H. bidorsalis* juveniles were also dependent on the concentration of the BLCO to which the fishes were exposed. The decreases in both the OES, the ST and the INT of the fish as the BLCO concentration increased from 1.00 to 8.00 mL L^{-1} were however at variance with report of Nwamba *et al.* (2006). These workers reported increased activities of amylase and cretinine kinase enzymes in H. bidorsalis juveniles as the concemtration of BLCO increased from 1.00 to 8.00 mL L^{-1}. Similarly, Oluah *et al.* (2005) recorded increased in the activity of serum and Liver Dehydrogenase (LDH) enzyme in *C. albopunctatus* exposed to increasing concentrations of sublethal Gammalin 20 and Acetellic 25EC. The result of the present study implies that increased concentration of the oil pollutant in the ambient water chemistry probably impacted negatively on the cells of the intestinal walls resulting in shrinkage and consequent reduction in the width and the length of the digestive tract.

The removal of the oil pollutant during the recovery period (14-42 days) obviously demonstrated the rescucitation of the digestive structures (OES, ST and INT) of the fish both in width (Table 2) and in length (Table 3). The magnitude of structural restoration of these component parts of fish digestive apparatus improved between day 14 (5%) and day 42 (15%). This implies that the ability of *H. bidorsalis* juveniles to be rescucitated to their full and inherent capacity to utilize ingested food after being subjected to crude oil pollution is time dependent.

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