Futo Journal Series (FUTOJNLS)
e-ISSN :2476-8456 p-ISSN : 2467-8325
Volume-2, Issue-1, pp- 218 - 226
www.futojnls.org

Research Paper July 2016

Impacts of Crude Oil Pollution on Body Water Balance, Derivable Energy and Metabolic Water from Freshwater Fishes

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Abstract

*The impacts of Bonny-Light crude oil (BLCO) pollution on body water balance and the amounts of energy and metabolic water that can be derived from the protein and glycogen contents of some freshwater fishes were assessed. The freshwater fishes studied were Channa obscura (*snakehead*), Hemichromise longatus (*tilapia*) and Clarias gariepinus (*catfish*) and Papynocranus afar (*knife fish*). Results suggested that the levels of energy and metabolic water that could be derived from the protein and glycogen contents of the BLCO-polluted freshwater fishes were significantly ($p < 0.05$) lower than those of their unpolluted counterparts. Though BLCO did not affect the moisture contents of the fishes, it had large effects on those of snakehead ($r = 0.65$, $p > 0.05$), tilapia ($r = 0.72$, $p > 0.05$) and catfish ($r = 0.65$, $p > 0.05$) and a small effect ($r = 0.22$, $p > 0.05$) on that of knife fish. In conclusion, Bonny-Light crude oil pollution did not impair water balance in the fishes but reduced the energy and metabolic water that could be derived from their protein and glycogen contents.*

Keywords: *Bonny-Light crude oil, derivable, energy, freshwater fishes, metabolic water, pollution*

1.0 Introduction

1.1 Crude Oil Pollution

Oil pollution elicits more public concern than any other waste or spilt material, even if the latter are potentially or actually far more hazardous (Wardley-Smith, 1979). Few coastlines in the world remain uncontaminated by oil and oil products. Oceanographers estimate that somewhere between three million and six million metric tons of oil is discharged into the world's oceans each year from both land- and sea-based operations; with most oil spills resulting not from catastrophic, headliner accidents but from routine open-sea bilge pumping and tank cleaning (Cunningham *et al*., 2005). The adverse effects of crude oil pollution are well known to many; especially on fishes where it causes death due primarily to asphyxiation.

1.2 Fishes, Crops and Crude Oil Pollution

Fishes undoubtedly add to the animal protein stock for humans (Wardlaw & Kessel, 2002). The ever increasing world population and improvements in some world economies makes fish consumption an

alternative to world protein (Cunninigham *et al*., 2005). This water resource is also used as an index for water pollution (Broeg *et al*., 1999). Crude oil pollution affects water quality. Mukherjee and Jana (2007) reported that water quality affects succinate dehydrogenase (SDH) activity, protein content and RNA/DNA ratios in fish raised in ponds of a sewage-fed fish farm. Hepatic protein synthesis, liver fatty acids, cholesterol and triacylglycerol were elevated by Prudhoe Bay crude oil (Khan *et al*., 1987). Crude oil pollution increased the ash and lipid contents of fishes and food crops (Khan *et al*., 1987; Ibegbulem *et al*., 2006; Ezeonu, 2010; Osam *et al*., 2011).

Germination and growth of guinea corn were inhibited by high concentrations of Bonny-Light crude oil (Akaninwor *et al*., 2007). Decreases in the protein, carbohydrate and energy values of food crops have also been reported (Akaninwor *et al*., 2007; Ezeonu, 2010; Osam *et al*., 2011). Serum alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymic activities were elevated in Wistar rats (Akaninwor *et al*., 2006); indicating hepaotoxicity. However, Sunmonu and Oloyede (2006) reported that lactate dehydrogenase (LDH) activity was increased in catfish liver, whereas those of AST and ALT were reduced. Water soluble fractions of crude oil reduced the weight of catfish (Nwabueze & Agbogidi, 2010); with that of Qua Iboe Light crude oil causing gill lamellae disintegration and erosion in juveniles of African freshwater catfish (George *et al*., 2014). Crude oil did not affect the survival of *Daphnia magna* below a concentration of 100 ppm, but concentrations above 100 ppm sharply decreased it; concentrations of 400 ppm and above caused the death of all cladoceran specimens of *D. magna* after 96 h (Lennuk *et al*., 2015).

1.3 Foods as Sources of Energy and Metabolic Water

Organisms derive energy from the foods they consume. They also derive water when such foods are metabolised. Metabolic water arises from metabolic activities in the body. It is a source of additional water in the body. When oxygen acts as the final recipient of electrons in the electron transfer chain, a molecule of water is produced per pair of electrons, two hydrogen ions and half molecule of oxygen consumed (Nelson & Cox, 2000; Wardlaw & Kessel, 2002; Beattie, 2006). Water is also produced in the reaction catalysed by enolase (an enzyme that reversibly dehydrates 2-phosphoglycerate to phosphoenolpyruvate in glycolysis) and some antioxidant enzymes like catalase (which decomposes hydrogen peroxide) and glutathione peroxidase (which decomposes hydrogen peroxide and other peroxides) (Nelson & Cox, 2000). Starch is a better substrate for the production of water than fat because more oxygen is consumed during the oxidation of fat (Takei *et al*., 2012). Metabolic water allows some animals to survive in xeric environments without drinking water for extended periods (Nelson & Cox, 2000; Takei *et al*., 2012).

1.4 Crude Oil Pollution and Clean-Up of Adanta Freshwater Stream

The Rumuekpe-Bomu Trans-Niger high pressure pipeline conveying Bonny-Light crude oil (BLCO) to its point of export failed on August 12, 1995, spilling its content for 18 hours into the Adanta freshwater stream in Isiokpo community, Ikwerre Local Government Area of Rivers State, Nigeria. The stream has its source at Ubima and is a tributary to the New Calabar River (Fig. 1). A physical clean-up of brushes along the water way commenced on January 5, 1996. Fish samples used in this study – *Channa obscura* (snakehead), *Hemichromise longatus* (tilapia), *Papynocranus afar* (knifefish) and *Clarias gariepinus* (catfish) - were caught on June 5, 1996, in the affected areas that still had tale-tell signs of the BLCO pollution after the physical cleanup exercise (Ibegbulem, 1997). Onyeike *et al*. (2000) reported that the nutritional values of BLCO-polluted fishes were not affected. Whereas the organ weights of rats that were placed on BLCO polluted fish-containing diets were not affected, their carcass lipid contents were increased (Ibegbulem *et al*., 2006). Fishes are very mobile animals. In the aquatic environment, partitioning between water and animal may be the most important avenue for both uptake and loss of hydrocarbons (Burns &

Teal, 1973). This seems to suggest that when fishes leave the polluted environment, they lose all polluting characteristics and when they move back to the polluted environment they re-acquire such characteristics (Ibegbulem, 1997).

1.5 Objectives of the Study

The present study aims at determining the impacts that Bonny-Light crude oil pollution have on body water balance and the amounts of energy and metabolic water derivable from the protein and glycogen contents of some BLCO-polluted freshwater fishes.

2.0 Materials and Methods

2.1 Water and Fish Samples

The BLCO polluted and unpolluted water samples were collected as described by APHA (1985). The polluted water was collected 1.5 km downstream (point C, Fig. 1; at points where the impact of the spillage were considered highest), while the unpolluted water was collected 4.0 km upstream (point A, Fig. 1) from the point of entry of the oil slick (point B, Fig. 1) into the Adanta freshwater stream, Isiokpo community, Ikwerre Local Government Area of Rivers State, Nigeria. The crude oil polluted and unpolluted fish samples were caught at the areas (spanning 5.0 m radius) where their water samples were collected. Fishing was carried out for 48 h. Water samples were collected in plastic containers, whereas the fishes were separated according to species, washed in double distilled water, packed into separated polythene bags and transferred to the laboratory for analyses.

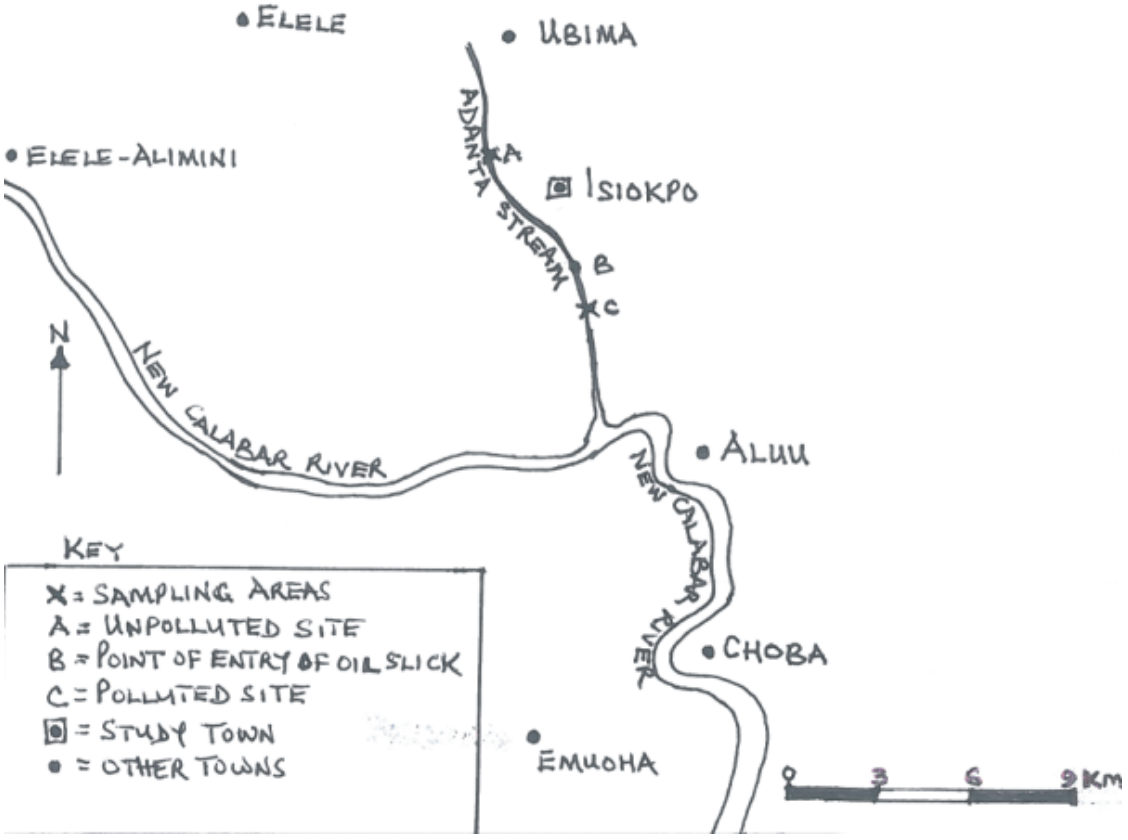

Figure 1: Map of the Adanta stream showing sampling areas

2.2 Determination of Hydrocarbon Content of Water Samples

Water samples were analysed for their hydrocarbon contents using the procedures of APHA (1985).

2.3 Determination of Body Water Content

Body water content was determined as described by AOAC (2006) by drying 10.0 g fish body tissue to constant weight at 85 ^{o}C.

2.4 Determination of Protein Content

Protein content was determined using the methods of Allen *et al*. (1996) and AOAC (2006). Fish body tissues were homogenised in a Waring blender. A quantity (1.0 g) of the homogenized body tissues was digested in 25.0 mL H_2S0_4 that contained 0.5 g sodium sulphate-copper (II) sulphate as digestion catalyst. The digest was diluted to 100.0 mL and an aliquot (10.0 mL) of the diluted digest was distilled into 10.0 mL boric acid solution that contained 2 drops of methyl red-methylene blue double indicator using a Kjeldahl distillation apparatus. The distillate was titrated to end point with 0.1 M HCl and the titre value used to calculate its nitrogen content. Tissue nitrogen contents were multiplied by 6.25 to obtain their percentage protein contents.

2.5 Determination of Glycogen Content

Tissue glycogen contents were determined as described by Plummer (1971). Fish body tissue protein and nucleic acid contents were precipitated when 10.0 g tissue was homogenized in 30.0 ml of 5 % ice cold trichloroacetic acid (TCA) and filtered through a Whatman 54 filter paper. The filtrate (collected in an ice cold flask) was mixed with twice its volume of 95 % C_2H_5OH, stirred and left to stand until the precipitate of glycogen flocculated. (1.0 g of NaCl was added and the filtrate warmed gently when there was difficulty in precipitating glycogen.) The filtrate was centrifuged at 2000 × rpm for 5 min and the supernatant discarded. The precipitate was re-dissolved in 5.0 mL of distilled water and the glycogen re-precipitated using 10.0 mL of 95 % C_2H_5OH. The precipitate (glycogen) was centrifuged and washed twice with 10.0 ml 95 % C_2H_5OH and once with 5.0 mL diethyl ether. The residue (glycogen) was spread on a watch glass and dried in desiccators. The glycogen was dissolved in 10.0 mL of distilled water and the solution made up to 100.0 mL using distilled water. Anthrone reagent (4.0 mL) was added to 1.0 mL of the protein-free glycogen solution, and to each of a series of 10-50 mg/100 mL standard glycogen solutions, and rapidly mixed. The tubes were placed in a boiling water bath for 10 min with a marble placed on top to prevent water loss by evaporation. The solutions were cooled and their absorbance readings taken at 620 nm against a reagent blank. The concentrations of the unknown glycogen solutions were then extrapolated from the standard curve prepared using the standard glycogen solutions.

2.6 Determination of Amounts of Derivable Energy and Metabolic Water

Derivable energy contents were estimated by multiplying the protein and glycogen contents, respectively, with the Atwater factor of 4 (Codex Alimentarius, 2001; FAO, 2003), whereas derivable metabolic water content was calculated on the basis of 41.3 g H_2O/100 g protein and 55.1 g H_2O/100 g carbohydrate (Mellanby, 1942; Klaassein, 1996).

2.7 Statistical analysis

Data were analysed using the Student's *t* test and mean was declared significantly different at $P \leq 0.05$. The effect size (a measure of the degree to which a parameter was impacted by treatment) was determined as the correlation coefficient (*r*) as described by Field (2005) by converting the *t*-statistics into *r*-

value at 95 %. The *r* was calculated as the square root of the ratio of the square of the *t*-value to the sum of the square of the *t*-value and the degree of freedom (*df*).

3.0 Results and Discussion

3.1 Crude Oil Content of Polluted Portion of Adanta Stream

Sampling of three portions of the BLCO polluted site of the Adanta stream showed that they contained 37.33 ± 23.28 mg/L of hydrocarbons. These areas were definitely polluted because their lowest hydrocarbon content of more than 14 mg/L exceeded the WHO/UNICEF (2010) limit of 10.0 mg/L. On the other hand, the unpolluted site did not contain any hydrocarbon.

3.2 Body water content of fishes

The BLCO did not affect ($p > 0.05$) the body water contents of the fishes (TABLE 1a). This suggests that the fishes did not lose their abilities to maintain water balance. The BLCO however had large effects on the body water contents of the snakehead, tilapia and catfish ($r = 0.65$, $r = 0.72$ and $r = 0.65$, respectively, $p > 0.05$) and a small effect ($r = 0.22$, $p > 0.05$) on the body water of the knifefish. The results contrasted with the report of Nwamba (2009) which posited that crude oil and its products altered the water chemistry of catfish juveniles.

Table 1a: Body water contents (g/100 g) of freshwater fishes*

Fish	Crude oil polluted Fresh water fishes	Unpolluted Fresh water fishes
Snakehead	78.77 ± 0.10^{a}	78.26 ± 0.50^{a}
Tilapia	78.85 ± 0.30^{b}	79.39 ± 0.34^{b}
Knifefish	78.23 ± 0.12^{c}	78.09 ± 0.52^{c}
Catfish	79.28 ± 0.30^{d}	78.89 ± 0.25^{d}

*Wet-weight; values are mean ± SD of triplicate determinations. Values on the same row bearing the same superscript letter are not significantly different ($p > 0.05$).

3.3 Protein Content of Fishes

The protein contents of the fishes did not generally increase or decrease (TABLE 1b). Whereas those of the snakehead and knifefish were reduced ($p < 0.05$) upon exposure to BLCO pollution, those of tilapia and catfish increased. This reinforces the concept of specie differences in the responses to the presence of foreign compounds in organisms. In this scenario, increase in the protein contents of these fishes could be due to growth (Wardlaw & Kessel, 2002) because of increase in the number of cells, tissues and lean muscles. It could also mean an increase in mixed function oxidase (MFO) activity (Payne and Penrose, 1975; Guengerich, 1991) in a bid to biotransform BLCO and/or its water soluble fractions. The reverse holds for a decrease in protein content. High concentrations of hydrocarbons can retard the growth of fish (Ghatak and Konar, 1991). Akaninwor *et al*. (2007), Ezeonu (2010), Osam *et al*. (2011), Gbadebo & Adenuga (2012) and Ordinioha & Brisibe (2013) also reported decreases in the protein content of foods due to failure of food crops to germinate and grow. The protein contents of the BLCO polluted tilapia and catfish increased relative to their unpolluted counterparts (TABLE 1b). Crude oil pollution has been reported to increase body nitrogen and plasma protein contents which indicated impaired water balance (Nwamba, 2009; Wegwu & Omeodu, 2010). However, the protein contents of the freshwater fishes used in this study did not indicate impaired water balance when juxtaposed with their body water contents (TABLE 1a); though the BLCO had

large effects on some of their body water contents. This seems to suggest that increase in body or plasma protein content does not necessarily indicate impaired water chemistry that may pre-dispose the fish to stress and disease.

Table 1b: Protein contents (g/100 g) of freshwater fishes*

Fish	Crude oil polluted Fresh water fishes	Unpolluted Fresh water fishes
Snakehead	12.58 ± 0.06^{a}	15.15 ± 0.11^{b}
Tilapia	11.72 ± 0.03^{c}	10.45 ± 0.01^{d}
Knifefish	13.26 ± 0.07^{e}	17.24 ± 0.02^{f}
Catfish	11.66 ± 0.02^{g}	11.24 ± 0.08^{h}

*Wet-weight; values are mean ± SD of triplicate determinations. Values on the same row bearing different superscript letters are significantly different ($p < 0.05$).

3.4 Glycogen Content of Fishes

The BLCO reduced the glycogen contents of snakehead, tilapia and catfish (Table 1c). Body glycogen reserves are normally depleted under stressful conditions. (Harris, 2006). Curiously, the glycogen contents of the knifefish were higher ($p < 0.05$) than their unpolluted counterparts. This seemed to suggest that though the protein content of the knifefish was low, it was not put under stress by the crude oil. The mostly lower glycogen contents of the BLCO affected fishes may be due to their proportionately higher lipid and ash contents as reported by Khan *et al*. (1987), Ibegbulem *et al*. (2006), Ezeonu (2010) and Osam *et al*. (2011).

Table 1c: Glycogen contents (g/100 g) of freshwater fishes*

Fish	Crude oil polluted Fresh water fishes	Unpolluted Fresh water fishes
Snakehead	3.01 ± 0.03^{a}	3.38 ± 0.05^{b}
Tilapia	3.96 ± 0.05^{c}	6.11 ± 0.07^{d}
Knifefish	4.56 ± 0.04^{m}	2.38 ± 0.07^{n}
Catfish	3.35 ± 0.10^{g}	5.76 ± 0.08^{h}

*Wet-weight; values are mean ± SD of triplicate determinations. Values on the same row bearing different superscript letters are significantly different ($p < 0.05$).

3.5 Derivable Energy from Protein and Glycogen Contents of Fishes

The derivable energy from the proteins and glycogens were lower ($p < 0.05$) in the BLCO polluted fishes (Table 2a). These seemed to suggest that the BLCO depleted the derivable energy stocks of fishes by depleting mainly their glycogen contents (Table 1c). Organisms that are under stress are known to expend much of the glycogen stocks as they mobilise such for energy purposes (Harris, 2006). The lipid contents of the fishes were left out while calculating the derivable metabolic water because of the difficulty in separating the hydrocarbon from BLCO from their total body hydrocarbon. The polluted fishes may have had higher lipid contents due to the increase in liver lipids (Khan *et al*., 1987), and/or induction MFO activity and synthesis of MFO cofactors, like phosphatidylcholine and phosphatidylethanolamine (Guengerich, 1991). The degree of induction reliably follows the concentration of the crude oil in water (Payne and Penrose,

1975; Walton *et al*., 1978). The impact of BLCO spillage has been reported to vary in four different species of fishes; concentrations followed the trend *Ictalurus* sp. (catfish) < *Pseudotolithu* ssp. (croakers) < *Clarias* sp. (mudfish) < *Cichlidae* sp. (tilapia) according to surface area covered with the crude oil (Olajide *et al*., 2009). Onyeike *et al*. (2000) and Ibegbulem *et al*. (2006) reported that fishes that are free-range in nature and live or presumably re-enter such crude oil polluted environment have their ash and lipid contents increased unlike their free-range counterparts that were fished from the unpolluted sections of the same stream.

Table 2a: Derivable energy (kcal/100 g) from protein and glycogen contents of freshwater fishes*

Fish	Crude oil polluted Fresh water fishes	Unpolluted Fresh water fishes
Snakehead	62.36 ± 0.09^{a}	74.12 ± 0.10^{b}
Tilapia	65.72 ± 0.02^{c}	66.24 ± 0.05^{d}
Knifefish	71.28 ± 0.03^{f}	78.48 ± 0.07^{g}
Catfish	60.04 ± 0.07^{m}	68.00 ± 0.10^{n}

*Calculated from Tables 1b and 1c; values are mean ± SD of triplicate determinations. Values on the same row bearing different superscript letters are significantly different ($p < 0.05$).

3.6 Derivable Metabolic Water from Protein and Glycogen Contents of Fishes

Higher metabolic water can be derived from the unpolluted fishes (Table 2b). The lipid contents of the fishes were not used in calculating the volume of metabolic water derivable from the fishes because of the reasons stated earlier in the preceding paragraph. We did not also include the 3.0 g H_2O/g glycogen released from storage site when glycogen is released for oxidation (Mellanby, 1942; Klaassein, 1996) because we did not intend to have the glycogen metabolised in the fishes but in their consumers. Even if the water molecules derived from the release of glycogen *in vivo* was added, the derivable metabolic water pattern would have remained the same.

Table 2b: Derivable metabolic water (g/100 g) from protein and glycogen contents of freshwater fishes*

Fish	Crude oil polluted Fresh water fishes	Unpolluted Fresh water fishes
Snakehead	6.86 ± 0.07^{a}	8.12 ± 0.05^{b}
Tilapia	7.02 ± 0.03^{a}	7.69 ± 0.01^{b}
Knifefish	7.99 ± 0.09^{a}	8.43 ± 0.03^{b}
Catfish	6.67 ± 0.05^{a}	7.81 ± 0.07^{b}

*Calculated from Tables 1b and 1c; values are mean ± SD of triplicate determinations. Values on the same row bearing different superscript letters are significantly different ($p < 0.05$).

4.0 Conclusion

Pollution of the Adanta-Isiokpo freshwater stream by Bonny-Light crude oil reduced the amounts of energy and metabolic water that can be derived from the protein and glycogen contents of the freshwater fishes. However, such pollution did not affect the total body water content of the fishes.

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