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Growth performance and condition factor of *Oreochromis niloticus* (Linneaus, 1758) after exposure to chemically dispersed Bonny Light crude oil

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Abstract

Dispersants are employed in oil spill situations to emulsify the oil into the water column thereby, facilitating weathering; this is without consideration for aquatic life and productivity. A renewal static bioassay was used to assess the recovery of *Oreochromis niloticus* after exposure to water accommodated fraction of dispersed Bonny Light crude oil (WAFDCO) and water accommodated fraction of Bonny Light crude oil (WAFCO) at sub-lethal concentrations of 0.2, 0.4, 0.8 and 1.6ml/l, based on the growth performance and condition factor after 49-days exposure and 54-days grow-out phases. The results showed that, the physicochemical parameters of the test media were affected by the concentrations of WAFDCO and WAFCO. The mean weight gain of *O. niloticus* increased with time in the exposure regimes/concentrations. Growth performance was concentration dependent, the highest mean weight of fish (15.67±0.78g) was from 1.6ml/l concentration of the WAFCO, which was higher than the control. The mean relative growth rate was higher (10.40±0.69%) in the fish from WAFCO while the highest mean specific growth rate (0.85±0.05%) was from WAFDCO. The highest value (3.42) of condition factor (K), was obtained in the 1.6ml/l of the WAFDCO. The K values of the fish from the WAFDCO were slightly higher than those from the WAFCO, which were lower than the control. Condition factor was greater than three which implied that, the fish were in good state, thus, their recovery was good after removal from the contaminated environment; hence, all mitigative actions should be prompt during and after oil spills.

Introduction

The growth of fish is related to the health of fish, which depends on the quality and availability of food, and the environmental conditions in which it survives (Budy *et al* 2011; Nehemia *et al* 2012; Hamre *et al* 2014). The health and growth of fish are directly affected by the quality of the environment, which includes the physical, chemical, biological and biochemical factors that can be altered by extraneous influences such as oil spill, 'a bane' on environmental integrity. Fish poisoning occurs with exposure to petroleum products with behavioural changes like air gulping, increased opercular movement, loss of balance, dyspnoea; developmental abnormalities; reduced survival, even mortalities (Omoregie and Ufodike 1999; Lelei 2007; Olajide *et al* 2009; Udofia 2010; Sharaf and Abdel-Tawwab 2011; Olaifa 2012; Kazempoor *et al* 2017; Eriegha *et al* 2019; Offor *et al* 2020). Fish exposed to crude oil have also been observed to show subtle changes in heart shape and reduction in swimming performance, indicative of reduced cardiac output (Hicken *et al* 2011, Mager *et al* 2014; Stieglitz *et al* 2016). Crude oil-derived Polycyclic Aromatic Hydrocarbons (PAHs) have been shown to adversely impact early life stages of fish (Hicken *et al* 2011; Incardona *et al* 2011; Bornstein *et al* 2014). The phenotypes of cardiotoxicity range from bradycardia, arrhythmias, contractility defects, atrium-to-ventricle conduction blockade, and eventually heart failure (Incardona *et al* 2013; Jung *et al* 2013). Defects induced by PAHs have effects on cardiac conduction, which have their effects on the late stages of kidney development, neural tube structure and formation of the craniofacial skeleton of the eye and jaw (Incardona *et al* 2004; Huang *et al* 2013). The mutagenic and adverse whole-organismal effects of crude oil on fish development have been well reported (Sharaf and Abdel-Tawwab 2011; Huang *et al* 2013; Xu *et al* 2016; Anwar *et al* 2022).

The incidences of spills and the use of dispersants in spill situations have been shown to alter the components of the crude oil by increasing their functional water solubility

resulting in increased bioavailability and altered interactions between oil, dispersants and biological membranes (Pollino and Holdway 2002; Barron *et al* 2003; Koyama and Kakuno 2004; Couillard *et al* 2005; Lelei 2013). Dispersants are used to minimize the environmental impacts of oil spills but not eliminate the problems caused by oil spills. Oil Spill Dispersants (OSD) are composed of the surfactants, solvents (hydrocarbon and water-based) and stabilizing agents applied as quickly as possible after a spill since there is limited 'window of opportunity', primarily due to the changing properties of the oil and weathering of the oil, which may last from a few hours to several days depending on the type/volume of spilled oil (International Tanker Owners Pollution Federation Limited 2005; Marine Environment Protection 2006). The use of OSDs in spill situations are quick, may need no supervision and is cost effective when compared to mechanical means, but the cumulative effects are more deleterious and long-term. The possible toxic effects of dispersed oil in the water column on exposed organisms are of primary concern. Chemically dispersed crude oils are known to result in about a 20-fold increase of PAHs readily made bioavailable for uptake by exposed fish with deleterious effects (Ramachandran *et al* 2004; Fingas 2011; Lelei and Sikoki 2013; Mauduit *et al* 2016; Incardona 2017; Bejarano 2018; Chen *et al* 2018; Esteban-Sánchez *et al* 2021).

Dispersants are employed to mitigate spill situations despite their synergistic and deleterious effects (Lelei and Sikoki 2013; Chen *et al* 2018; Esteban-Sánchez *et al* 2021), to allow for recovery of the affected environments, as well as, the 'survivor-organisms.' It is pertinent to know how well the 'recovery-response' of exposed fish is. How well in this case, refers to the condition factor, an index, which indicates the well-being of the fish, reflecting interaction between biotic and abiotic factors in the physiological conditions of fishes. This study therefore, determined the growth performance and condition factor of *Oreochromis niloticus* (Linnaeus, 1758) after exposure to the water accommodated fraction (WAF) of the mixture of a dispersant, Goldcrew (SW) and Bonny Light crude oil as employed in a spill situation.

Materials and Methods

Fish sample collection

Three hundred and fifty fry of *Oreochromis niloticus* (Nile tilapia, Family: Cichlidae, Order: Perciformes, Class: Actinopterygii) of average length of 1.5cm and weight of 3.75g were procured from the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State and kept in 25L plastic tanks for fourteen (14) days to acclimate to the test medium before being used for the bioassay (Reish and Oshida 1986). They were fed twice daily with Coppens® feed of 0.8-1.2mm pellet size at 5% body weight during the test period.

Test solution

Bonny Light crude oil and Goldcrew (SW) dispersant were procured in I.5l air tight plastic bottles from an oil facility and stored at 28ºC for use in the preparation of the test solution. The water accommodated fraction (WAF) of the mixture of Bonny Light crude oil and Goldcrew (SW) dispersant (WAFDCO) and the WAF of the Bonny Light crude oil alone (WAFCO) were prepared by spinning 100ml of the crude oil and 40ml of Goldcrew (SW) in 1200ml of distilled water for 20hrs at 700rpm in a tightly covered 2000ml Pyrex conical flask using a magnetic stirrer (Kamag Keo) and the mixture allowed to stand for 1hour. The light, clear liquid below was siphoned into glass bottle with stopper, which served as the test solution for the WAFDCO regime. For WAFCO test solution, the mix was 100ml of the crude oil in 900ml of distilled water using the same procedure (Reish and Oshida 1986; Khan and Payne 2005; Lelei 2007). The application ratio of crude oil to Goldcrew (SW) was determined to be 1:30, with a 1:30 dilution ratio of dispersant to distilled water based on field application (Khan and Payne 2005). Range finding tests were done to determine the threshold concentrations based on previous findings (Lelei 2013).

Exposure regimes/experimental design

For the static renewal bioassay, test solutions of the exposure regimes (WAFDCO and WAFCO) of four sub-lethal concentrations: 0.2, 0.4 0.8 and 1.6ml/l (having determined their thresholds during the range finding tests for long-term bioassay) were made up to 12l in triplicates set up in 25l plastic tanks. Ten (10) *O. niloticus* were randomly introduced into each tank and the experiment maintained for 49-days at 12hr light and dark time. A modification of the methods of Ramachandran *et al* (2004) and Khan and Payne (2005). The test medium was renewed at 48hr interval to prevent sequestration/aging, by which, chemicals tend to become less available with time for uptake by organisms for partitioning into the aqueous phase (Khan and Payne 2005). During these exposure phases, the test organisms were fed twice daily with Coppens® feed of 0.8-1.2mm pellet size at 5% body weight.

During the phases, water quality parameters were determined according to APHA (1995). The physicochemical variables: dissolved oxygen (DO), temperature, total dissolved solids (TDS), redox potential, pH, conductivity and salinity were measured *in-situ* using Hanna HI 9828 pH/ORP/EC/DO water analysing device.

Post exposure/grow-out phase of *O. niloticus*

After the 49-day exposure period, the surviving fish were transferred (based on concentrations/exposure regimes' respectively) to clean water (the average lengths and weights of the test fish were determined at this point) for depuration/recovery for 54-days (modified after Khan and Payne, 2005). During this period, the feeding of fish was adjusted using Coppens® feed of 1.2-1.5mm pellet size fed at 3% body weight twice daily. The lengths and weights of

the fish from the exposure concentrations/regimes were taken bi-weekly (to reduce extrinsic stress) during the 54-day depuration/recovery and grow-out phase.

The total length (TL) of fish in centimetres was measured from the tip of the snout to the end of the caudal fin using a metre rule. Body weight was measured in grammes using an electronic digital balance (Kenex® Magno-1000).

Data collection

The bi-weekly measured lengths and weights of *O. niloticus* in the exposure concentrations/regimes were used to determine the growth performance based on; the mean weight gain (WG), Specific growth rate (SGR), which is the measure of the daily growth rate, and relative growth rate (RGR), which is the increase in growth of the fish during the grow-out phase. The survival rate (SR), which is the ratio of the number of the survivors to the total number of fish stocked per concentration, and the condition factor (Fulton's condition factor, K), which is the well-being of the fish (Ricker 1975) from the different treatments during the eight weeks grow-out phase were also determined.

The RGR and SGR were determined using the formulae by Bagenal and Tesch (1978) and Blackwell *et al* (2000) as follows:

$$\text{RGR (\%)} = \frac{(W_2 - W_1)}{W_1} \times 100$$

Where; RGR = Relative Growth Rate, W_1 = Initial weight of fish (g), W_2 = Final weight of fish (g)

$$\text{SGR (\%)} = \frac{Log_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

Where; SGR = Specific Growth Rate, Log_e= Natural Logarithm of base e, W_1 = Initial weight of fish (g) in T_1 days; W_2 = Final weight of fish (g) in T_2 days

Survival rate (SR), survival of the fish during the period was determined using the formula:

$$\text{SR (\%)} = \frac{Number\ of\ individual\ fish\ at\ the\ end\ of\ the\ experiment}{Number\ of\ individual\ fish\ at\ the\ start\ of\ the\ experiment} \times 100$$

Fulton's Condition Factor (K) was determined using the formula of Pauly (1993):

$$K = \frac{W \times 100}{L^3}$$

Where; W= Weight of fish (g); L= Total length of fish

Data analysis

The data obtained were expressed as means ± standard error (±SE). Data generated were subjected to One-way Analysis of Variance (ANOVA) to test for differences between and amongst the test concentrations/regimes at $p<0.05$.

Results

The results of the physicochemical parameters of the WAFDCO and WAFCO regimes are presented as means and standard errors (Table 1).

The mean temperature values from the concentrations of the WAFDCO exposure regime ranged from 25.58±0.14 to 25.85±0.14°C, which was narrow and lower than the 26.16±0.14°C mean value of the control. The mean DO values ranged from 2.65±0.27 to 2.87±0.27mg/l, which was lower than the 3.87±0.27mg/l of the control. The mean pH values ranged from 5.70±0.30 to 6.38±0.30, which was lower than the 5.80±0.30 of the control. The TDS and conductivity values showed slight reduction with increase in concentration compared to the control. Conversely, the values of the redox potential increased with increase in concentration though, lower than the control while values for salinity were the same. For the WAFCO regime, the mean temperature values ranged from 25.85±0.13 to 26.16±0.13°C, compared with 26.16±0.14°C mean value of the control. The mean DO values ranged from 2.43±0.21 to 2.53±0.21mg/l and were lower than the control. The TDS, conductivity, and redox potential values were affected by increase in concentration and lower than the control while the salinity was also not affected. Except for salinity, the physicochemical parameters were concentration dependent for the WAFDCO and WAFCO regimes.

The results of the growth performance of *O. niloticus* with respect to mean weight gain during the 54-day period is shown in Figure 1. The mean weights after the 49-day exposure phase gave the initial measurements for the grow-out phase. During the 54-day grow-out phase, the mean weights taken bi-weekly increased with time in the different exposure concentrations/regimes. From the results (Table 2), the highest mean weight of 15.67±0.78g was for fish from the 1.6ml/l concentration of the WAFCO exposure regime, which was followed by 10.87±0.15g of fish from the 0.2ml/l concentration of the WAFDCO exposure regime compared with the 5.03±0.09g of fish from the control. While the least mean weights of 4.90±0.59g and 7.63±0.23g were from the 1.6ml/l and 0.2ml/l concentrations of the WAFDCO and WAFCO regimes, respectively. The mean weight gain increased with concentration for the WAFCO exposure regime (except for the 0.8ml/l concentration at week 4) but was the reverse for the WAFDCO exposure regime; both regimes had higher values than the control.

The results of the relative growth rate (Table 2), showed that, fish from the 1.6ml/l concentration of the WAFCO exposure regime had the highest RGR (14.67±0.78%). The WAFDCO regime had its highest RGR of 9.87±0.15% from the 0.2ml/l concentration. The control had RGR of 4.03±0.09%.

The specific growth rate (SGR) results showed that, fish from the 0.2ml/l concentrations had the highest mean value of 0.82±0.08% for the WAFDCO and 0.85±0.05% for the WAFCO regimes; these values were higher than the control (0.51±0.08%). Survival rate during the growth-out phase was 100% for the exposure regimes and concentrations except for 0.8ml/l and 1.6ml/l of the WAFDCO regime, which were 97% and 87% respectively.

Table 1: Means and standard errors of the physicochemical parameters of the different concentrations of the WAFDCO and WAFCO test media during the 49-day exposure period

Conc. (ml/l)	Temperature (°C)	pH	TDS (ppm)	Conductivity (µS/cm)	Salinity (ppt)	DO (mg/L)	Redox Potential (eV)
0	26.16±0.14	5.80±0.30	63±3.15	126±6.20	0.06±0.003	3.87±0.27	38.6±3.40
0.2[A]	25.58±0.14	6.38±0.30	54±3.15	108±6.20	0.05±0.003	2.87±0.27	28.7±3.40
0.4[A]	25.69±0.14	6.35±0.30	53±3.15	107±6.20	0.05±0.003	2.84±0.27	29.5±3.40
0.8[A]	25.65±0.14	5.70±0.30	44±3.15	87±6.20	0.04±0.003	2.65±0.27	33.3±3.40
1.6[A]	25.85±0.14	6.14±0.30	48±3.15	97±6.20	0.05±0.003	2.70±0.27	31.3±3.40
0.2[B]	26.16±0.13	6.24±0.30	64±2.03	129±4.94	0.06±0.003	2.43±0.21	28.3±4.06
0.4[B]	26.01±0.13	6.21±0.30	55±2.03	130±4.94	0.06±0.003	2.45±0.21	25.2±4.06
0.8[B]	26.01±0.13	6.21±0.30	59±2.03	118±4.94	0.06±0.003	2.53±0.21	21.7±4.06
1.6[B]	25.85±0.13	5.95±0.30	60±2.03	120± 4.94	0.06±0.003	2.48±0.21	22.0±4.06

A= Concentrations of WAFDCO; B= Concentrations of WAFCO; Number of fish per concentration (N) = 30

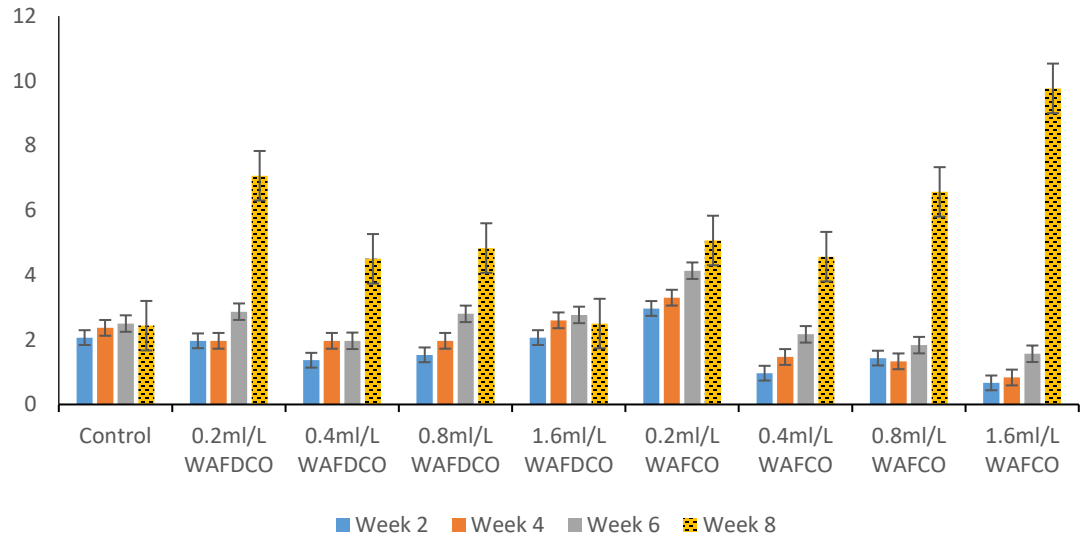

Figure 1. Bi-weekly mean weight gain (gm) of *Oreochromis niloticus* during the 54-day growth-out phase in the different concentrations of the WAFDCO and WAFCO regimes (Number of fish per concentration = 30)

Table 2: Growth performance of *Oreochromis niloticus* in 54 days after exposure to different concentrations of WAFDCO and WAFCO regimes

Conc. (ml/l)	Post Exposure Phase		Grow-Out Phase		RGR (%)	SGR (%)	SR (%)	Condition Factor (K)
	Length (cm)	**Weight (g)**	**Length (cm)**	**Weight (g)**				
0 (Control)	4.27±0.07	2.60±0.21	5.30±0.00	5.03±0.09	4.03±0.09	0.51±0.08	100	3.38
0.2 WAFDCO	4.57±0.33	3.80±0.36	6.97±0.03	10.87±0.15	9.87±0.15	0.82±0.08	100	3.21
0.4 WAFDCO	4.90±0.06	4.20±0.21	6.37±0.09	8.70±0.29	7.70±0.29	0.57±0.03	100	3.37
0.8 WAFDCO	4.40±0.10	3.17±0.12	6.37±0.09	8.00±0.35	7.00±0.35	0.72±0.06	97	3.10
1.6 WAFDCO	3.97±0.15	2.40±0.15	5.23±0.20	4.90±0.59	3.90±0.59	0.55±0.11	87	3.42
0.2 WAFCO	4.23±0.12	2.57±0.12	6.17±0.09	7.63±0.23	6.63±0.23	0.85±0.05	100	3.26
0.4 WAFCO	5.00±0.10	4.10±0.36	6.47±0.20	8.67±0.49	7.67±0.49	0.58±0.03	100	3.21
0.8 WAFCO	5.33±0.17	4.83±0.43	7.10±0.12	11.4±0.69	10.40±0.69	0.67±0.05	100	3.19
1.6 WAFCO	5.60±0.10	5.90±0.31	7.80±0.17	15.67±0.78	14.67±0.78	0.76±0.01	100	3.30

WAFDCO = Water Accommodated Fraction of Dispersed Bonny Light Crude Oil; WAFCO = Water Accommodated Fraction of Bonny Light Crude Oil; Post-Exposure Phase (Initial measurements); Grow-out Phase (Final measurements); RGR= Relative Growth Rate; SGR= Specific Growth Rate; SR= Survival Rate; K= Fulton's condition factor; Number of fish per concentration (N) = 30

With respect to the Fulton's condition factor (K), fish from the exposure regimes had K values greater than three. The highest K value of 3.42 was in fish from the 1.6ml/l concentration of the WAFDCO exposure regime. The K values of the fish from the WAFDCO regime were slightly higher than those obtained from the WAFCO regime, which were lower than the K value of the control.

Discussion

The values of the physicochemical variables obtained from the various concentrations of the WAFDCO and WAFCO exposure regimes showed slight differences in their values (especially for pH, TDS, conductivity and redox potential), which were concentration dependent, and different from the control. These values implied that, except for salinity, the physicochemical parameters were affected by the different concentrations and exposures regimes. Temperature values were lower while DO and pH values were higher in the test media of the WAFDCO regime than the WAFCO. This may be indicative of better physiological activities. The TDS values reflected the presence of organic matter as a result of activities of the fish, and were higher in the WAFCO. This was also observed in the conductivity values, which reflected the proportional relationship between TDS and conductivity. These values implied that, the fish from the WAFCO regime showed increased physiological processes than fish in the WAFDCO regime, since there is interrelationship between these parameters and fish physiology.

The deteriorating effects of the exposure regimes (WAFDCO and WAFCO) on the water quality parameters of the test media in this study were in agreement with the findings of Nwabueze and Agbogidi (2010), Eriegha *et al*. (2019) and Anwar *et al*. (2022). These implied adverse effects on the well-being of the test fish with possible impairment of their physiological activities. The effects on the exposed fish were concentration dependent reflected in the values of the physicochemical parameters. This was in line with the findings of Lelei and Sikoki (2013) and Fakuloju *et al*. (2018).

The survival rate, which was high during the grow-out phase in this study could be attributable to intrinsic conditions. The rates were similar to the findings of Olaifa (2012). This implied that, survival of exposed fish was higher when 'adverse environmental conditions' were eliminated.

The mean weight gain increased with concentration for the WAFCO exposure regime but was the reverse for the WAFDCO exposure regime; both regimes had higher values than the control. This implied that, the weights of the exposed fish were more affected by the WAFDCO exposure regime when compared with the WAFCO but these weights were better than the control. The values of the relative growth rate (RGR) implied that, RGR was better in the fish from the WAFCO exposure regime than the WAFDCO, which in turn was better than the fish from the control (WAFCO > WAFDCO > Control). With respect to the concentrations, the fish from the WAFDCO exposure regime showed reduced RGR with increase in concentration while, fish from the WAFCO regime exhibited increase in RGR with increase in concentration. The values of the specific growth rate (SGR) of fish exhibited fluctuations with the exposure regimes and concentrations, which were higher than the values of the fish from the control. Based on the growth parameters, fish performance was better in the fish exposed to the WAFCO than the WAFDCO and control. These implied that, adverse effects were less and recovery was better in the former.

The adverse effects of crude oil on growth performance and feed utilization in species of fish like Nile tilapia and African catfish have been widely reported (Omoregie and Ufodike 1999; Nwabueze and Agbogidi 2010; Udofia 2010: Sharaf and Abdel-Tawwab 2011; Abdel-Tawwab 2012; Olaifa 2012; George *et al* 2014; Fakolujo *et al* 2018; Ajah and Ukutt 2018; Anwar *et al* 2022). Most of such studies have been on the effects of the exposure. Munzurul Hasan *et al* (2022), in their studies showed that, recovery of exposed fish was quicker and profound at lower concentrations, which agreed with the findings of this study with respect to the mean weight gain, RGR and SGR of fish from the WAFDCO regime but was the reverse for the WAFCO regime for which the mean weight gain, RGR and SGR increased with increase in concentration; the values were better than the fish from the control. These gave credence to the notion of the 'phenomenon of sufficient challenge' put forth by Ottoboni (1991) and Frank and Ottoboni (2011), which postulated that, "very small doses of chemicals fed to animals produce healthier, long-lived animals than the control animals. Perhaps, due to the body's defence mechanisms that are stimulated after exposure to small amounts of foreign chemicals, which in turn, makes the body stronger". This was the observation in this study, that, low exposure concentrations resulted in 'survivor fish' which performed better (mean weight gain, RGR and SGR) than those in the control.

The values of condition factor (K) from this study, which were greater than 3 for the fish from the grow-out phase of the exposure regimes (WAFDCO and WAFCO), as well as, the control indicated a state of well-being and good recovery after exposure. The K value, which was highest (3.42) in the 1.6ml/l concentration of the WAFDCO regime, was of particular interest as it indicated the 'best recovery'. These values were comparable to the condition factor of *O. niloticus* from the wild from different studies: 1.9 on the average from Ebonyi River (Uneke 2015); 3.15 and 4.71 in the males and 3.16±4.37 in the females (Oso *et al* 2017); 1.809 from Wudil River, Kano State (Getso *et al* 2017); 1.48±0.13 for female and 1.32±0.09 for male in the lower River Benue (Laurat *et al* 2019); 2.05±2.04 for male and 2.74±3.09 for female in Calabar River (Joseph *et al* 2019); 2.33-3.83 for Oguta Lake (Aguora *et al* 2020) and

1.89±0.43 from the lower Cross Rivers (Ugbomeh *et al* 2021).

Bagenal and Tesch (1978) recommended K value range of 2.9-4.8 as suitable for mature freshwater fish. The K values from this study were within this recommended range. According to Maguire and Mace (1993), from a nutritional point of view, increase in K values indicates the accumulation of fat and sometimes gonadal development. This could be said to be the case with the fish exposed to 1.6ml/l concentration of the WAFDCO regime, which had the highest K value (3.42). Condition factor may vary among fish species in different locations (Blackwell *et al* 2000). It is used to compare the "condition", that is, fatness or wellbeing of fish (Seher and Suleyman 2012) and gives information on the physiological condition of fish in relation to its welfare. Perry *et al* (1996), reported that, fishes with a low condition index are presumably believed to have experienced adverse physical environment or insufficient nutrition. The K values >3 of the fish from this study have shown that, despite the exposure to 'adverse physical environment', having eliminated the 'contaminants', the fish recovered.

Conclusion

The findings of this study showed that, after exposure to WADFCO and WAFCO regimes, *O. niloticus* recovered on their removal from the regimes. This was reflected in the values of the mean weight gain, RGR, SRG and condition factor; with K values (greater than three), which implied good health state and indicated recovery of the fish. This buttresses the need to eliminate as soon as possible, environmental contaminants to facilitate the recovery of 'survivor organisms' present in such environments. Hence, environmental policies in this regard should be given the 'force of immediacy' by the policy makers and implementers.

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