

## Organo-somatic Index and Condition Factor of *Clarias gariepinus* (Burchell, 1822) Sub-Adult Exposed to Sub-Lethal Concentrations of Gold Crew Oil Spill Dispersant

### ABSTRACT

The organosomatic index and condition factor of *Clarias gariepinus* exposed to sub-lethal concentration of gold crew oil spill dispersant was conducted over a two-week period. During this period 250 live sub-adult *Clarias gariepinus* were acclimated for 14-days in a square 2000L tank at the fisheries and Aquatic Environment Aquacultural Centre, Rivers State University. A preliminary range finding test was conducted using nominal concentrations (1ml/L, 2ml/L, 4ml/L, 6ml/L and 8ml/L) of gold crew oil spill dispersant. The result revealed 2ml/L as the lowest concentration that triggered mortality within 24 hours after which an acute bioassay was conducted and the LC<sub>50</sub> determined using probit analysis revealed 1.16 as the LC<sub>50</sub> of *Clarias gariepinus*. This formed the basis for the concentration (0.0ml/L (control), 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L) used to test the sub-lethal effect of the gold crew oil dispersant on the organosomatic index and condition factor of the fish species. A group of 10 fishes (three replicates) were kept in a static renewal state for two weeks in a row and the liver, spleen, heart, gonad and viscera were excised on a weekly basis and weighed using a digital weighing scale, before the excision of the organs the fishes were first weighed and the length taken in cm. Physical and chemical parameters of the test medium was also conducted *in-situ* using a multi-parameter checker (Extech DO 700). Results for physicochemical variables revealed values for; temperature: week 1; 28.30°C (0.15ml/L) - 27.20°C (0.0ml/L), week 2; 26.00°C (0.0ml/L, 0.075ml/L and 0.3ml/L) - 25.67°C (0.15ml/L and 0.6ml/L), pH; week 1; 4.82 (0.15ml/L) - 4.06 (0.0ml/L), week 2; 7.38 (0.0ml/L) - 5.94 (0.6ml/L), DO; week 1; 6.12mg/l (0.0ml/L) - 5.00mg/l (0.15ml/L, 0.3ml/L and 0.6ml/L), week 2; 6.01mg/l (0.0ml/L) - 4.93mg/l (0.3ml/L), TDS: week 1 89.00mg/l (0.6ml/L) - 154.00mg/l (0.3ml/L), EC: week 1: 188.33 µs/cm (0.6ml/L) - 166.33µs/cm (0.0ml/L), week 2: 357.67µs/cm (0.0ml/L) - 238.67µs/cm (0.075ml/L). Results for organosomatic index revealed values for; HSI; week 1; 0.41 (0.0ml/L) - 2.52 (0.15ml/L), week 2; 0.93 (0.075ml/L) - 1.52 (0.6ml/L); GSI; week 1; 1.58 (0.6ml/L) - 3.83 (0.15ml/L), week 2; 1.11 (0.0ml/L) - 4.66 (0.6ml/L), VSI; week 1; 2.91 (0.6ml/L) - 3.70 (0.15ml/L), week 2; 1.50 (0.0ml/L) - 4.33 (0.6ml/L), SSI; week 1; 0.11 (0.0ml/L) - 0.24 (0.15ml/L), week 2; 0.06 (0.15ml/L) - 0.15 (0.3ml/L), CSI; 0.07 (0.3ml/L) - 0.16 (0.075ml/L and 0.15ml/L), week 2; 0.11 (0.0ml/L) - 0.19 (0.6ml/L). Condition factor values revealed results for; week 1; 0.57 (0.15ml/L) - 1.00 (0.0ml/L), week 2; 0.71 (0.3ml/L) - 1.05 (0.075ml/L) depicting poor physiological condition for fishes in the treatment group. There is therefore urgent need for public awareness campaigns to educate communities about the risks associated with the dispersant use and the importance of minimizing their environmental footprint.

**KEY WORDS:** *Clarias gariepinus*, Sub Lethal Exposure, Condition Factor, Organosomatic Index

### 1.0 INTRODUCTION

Organosomatic index are ratios of the weight of internal organs to the total body weight of an organism. They are used to assess the health condition of invertebrate and vertebrate species, including fish, and to monitor the influence of environmental factors on them (Gondimet *et al.*, 2020; Amachree and Idam 2022). The most used organosomatic indexes in stress-related studies include the hepatosomatic index (HSI), renatosomatic index (RSI), gills-somatic index (GSI), viscerosomatic index (VSI), spleenosomatic index (SSI) and cardiosomatic index (CSI) (Amachree and Idam, 2022). Organosomatic indexes can be linked to the effects of chemicals on target organs such as the gills, liver, and kidney, as well as used as indices of change in nutritional and energy status (Dekić *et al.*, 2016).

The condition factor, on the other hand, is a quantitative indicator of fitness that relates to weight and lengths and can provide insights into the physiological condition and energy reserves of the organisms (Gupta *et al.*, 2017). The effect of sub-lethal concentrations of

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goldcrew oil spill dispersants on the organosomaticindex of the African catfish (*Clarias gariepinus*) has been a topic of interest in environmental studies. The commercial catch of *Clarias gariepinus* by artisanal fishers in Nigeria, particularly in the Niger Delta region, which has been affected by the oil spills and the continuous use of dispersants has thus prompted this investigation into the organosomaticindex and condition factor of *C. gariepinus* exposed to sub-lethal concentration of gold crew oil spill dispersant. The dispersant, which is used to change the inherent properties of oil, has the potential to affect the health and well-being of aquatic organisms including fish (Ugbomehet *et al.*, 2019). Aquatic ecosystems are increasingly challenged by oil spills, which can have devastating consequences for fish population. While acute toxicity often dominates initial concerns, the sub-lethal effects of oil spill dispersants (OSDs) remain poorly understood, yet potentially pose long-term threats to fish health and fitness.

Understanding the effects of sub-lethal concentration of gold crew on the organosomaticindex and condition factor of *C. gariepinus* is crucial for the sustainable management of fisheries and the preservations of the aquatic ecosystems and provide insights into the potential health risk s associated with the use of dispersants on oil spill investigation.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

The research was carried out at the Rivers State University Aquacultural Centre, Department of Fisheries and Aquatic Environment, Faculty of Agriculture.

### 2.2 Procurement of Test Organism

250 live sub-adult *Clarias gariepinus* with weight ranging from 250 to 300g was purchased from Idi-Onyana farms on the Abua-Ahoada Road in Rivers State, Nigeria.

### 2.3 Acclimatization and Feeding of Test Fish

The purchased *Clarias gariepinus* was acclimated for 14 days in a square 2000L tank at the Fisheries and Aquatic Environment Aquacultural Centre. The tank was filled with borehole water and water exchanged daily, and the fishes were fed with 3mm blue crown feed to satiation.

### 2.4 Procurement of Gold Crew Oil Spill Dispersant

A 4 L plastic gallon of gold crew oil spill dispersant was acquired from a chemical shop in Port-Harcourt and stored for use in the production of the test solution.

### 2.5 Preliminary Range Finding Test

Five concentrations (1ml, 2ml, 4ml, 6ml, and 8ml) was generated by serial dilution of from each stock solution of the gold crew oil spill dispersant on a volume to volume V/V ratio.

A group of five test fishes was subjected to the nominal concentration (1ml, 2ml, 4ml, 6ml and 8ml) for 24 hours. The test fishes were monitored after an 8-hour exposure time (USEPA 2010) and a 4-hour interval and a control to observe the lowest concentration with evidence of behavioral anomaly (erratic swimming and hyperventilation).

### 2.6 Definitive Test (LC<sub>50</sub>)

The preliminary range finding test observations served as the foundation for the nominal concentration used in the definitive test. However, it included four different concentration treatments as well as a control (0.0ml/L, 1.0ml/L, 1.2ml/L, 1.4ml/L and 1.6ml/L).

The test solution was renewed every 24 hours, and the fishes were not fed for the whole 96-hour duration.

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## 2.7 Chronic testing

Following the observation of the LC50, four nominal concentrations (0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L) of the stock solution plus a control (0.0ml/L) was produced by serial dilution of the stock solution in 30 liters of water. The fishes were placed in the 50-litre plastic tanks containing the test solution and dilution water at random. The test solution was renewed every day. There were three replications of 10 fish for each treatment concentration, with no gender consideration.

## 2.8 Determination of some physical and chemical variables

The water quality of the test solution in the dilution water and control treatment were assessed *in-situ* to determine its suitability for fish survival based on specified quality parameters (APHA, 2005); consequently, in this study, pH, dissolved oxygen (DO), conductivity, and temperature were measured on a weekly basis.

## 2.9 Dissection of Fish

At the end of each week, the fish from each tank containing the test solution in dilution water and the control treatment was immobilized by cervical dislocation prior to dissection on a dissecting board; a surgical blade was used to dissect the fish, and the gills, liver, spleen, heart and gonad) were removed for analysis.

## 2.10 Organosomatic index

$$\text{Organosomatic index} = \frac{\text{Weight of the organ(g)}}{\text{Weight of the fish(g)}} \times 100$$

## 2.11 Condition Factor

$$K = \frac{\text{Weight of fish(g)}}{L^3} \times 100$$

## 2.12 Statistical Analyses

Microsoft excel (version 2016) was used to obtain the descriptive analysis, condition factor, organosomatic index of the fish species across the treatments. While variance analysis and mean separation across the various treatments for physical and chemical variables, condition factor and organosomatic index were obtained using Minitab version 19.

## 3.0 RESULTS

Fig. 1 shows the linear relationship between mean probit mortality and log concentration of *C. gariepinus* exposed to sub-lethal concentrations of gold crew oil spill dispersants. The 96-hr lethal concentration (LC<sub>50</sub>) of the dispersant obtained from graphical illustration was 1.16ml/L. The coefficient of determination ( $r^2$ ) between the Log concentration of the dispersant (Gold crew oil spill dispersant) and the probit mortality was 0.98.

In this study at week 1, it was observed that as the concentration of the gold crew oil spill dispersant increased from 0.075ml/L to 0.6ml/L, the temperature generally rose compared to the control. The temperature regime initially peaked at 0.15ml/L (28.30°C), then decreased at 0.3ml/L (26.77°C) before rising again at 0.6ml/L (27.70°C). In week 2, there was a consistent temperature maintained across the different concentrations including the control concentrations (0.0ml/L) except for slight variations at 0.15ml/L and 0.6ml/L which shared a decrease (Table 1).

The concentration of gold crew oil spill dispersant influenced the pH concentrations in week 1, the control had a pH of 4.06 while the dispersant concentrations (0.075ml/L, 0.15ml/L, 0.3ml/L, and 0.6ml/L) showed higher pH values with 0.15ml/L having the highest at 4.82. Moving to week 2, the control pH increased to 7.38 and dispersant concentrations resulted in

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lower pH values compared to the control. Notably 0.6ml/L had the lowest pH at 5.94(Table 1).

In week 1, the control had a higher dissolved oxygen level than the dispersant concentrations, showing a decrease in dissolved oxygen as the dispersant concentration increased. In week 2, the trend continued with marginal fluctuations in DO levels across dispersant concentrations compared to the control. Again, concentrations higher than 0.075ml/L demonstrated a consistent DO level of around 5.00mg/l, showing a similar pattern as observed in week 1(Table 1).

For Total dissolved solids (TDS) there is a noticeable variation in measurements between the control and the different concentrations of the dispersants in both weeks. In week 1, the dispersant concentration seemed to have a mixed effect on TDS compared to the control. In week 2, especially at higher concentrations (0.3ml/L and 0.6ml/L) the TDS levels showed a significant fluctuation compared to the control (Table 1).

In week 1, the electrical conductivity (EC) values for different concentrations of gold crew oil spill dispersant showed an increase from the control (166.33 $\mu$ s/cm) to the higher concentrations 0.6ml/L (188.33  $\mu$ s/cm). In week 2, there was a noticeable decrease in the electrical conductivity values across all concentrations in comparison to the control except in 0.3ml/L concentration (Table 1).

The results for hepatosomatic index in week 1 showed a noticeable variation across the different treatments and control with the treatment levels indicating a significantly higher value( $p<0.05$ ). Values (mean  $\pm$  SD) were  $0.41 \pm 0.11$ ,  $2.26 \pm 0.08$ ,  $2.52 \pm 0.3$ ,  $1.32 \pm 0.67$  and  $1.78 \pm 0.46$  for control, 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L respectively (Table 2).

In week 2, the reverse was the case, the control had a significantly higher levels compared to the treatments except 0.6ml/L that recorded a significantly higher values than the control. Values (mean  $\pm$  SD) were  $1.12 \pm 0.26$ ,  $0.93 \pm 0.11$ ,  $1.01 \pm 0.15$ ,  $1.09 \pm 0.17$  and  $1.52 \pm 0.37$  for control, 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L respectively (Table 3).

The results for gonadosomatic indices(GSI) of *C. gariepinus* exposed to various concentrations of gold crew oil spill dispersant in week 1, reveal significant changes in the gonadosomatic index in the treatment levels when compared to the control except for 0.6ml/L that had a significantly lower value, other treatment levels were higher than the control in week 1. The values (mean  $\pm$  SD) were  $1.93 \pm 0.14$ ,  $3.34 \pm 0.10$ ,  $3.83 \pm 0.11$ ,  $3.07 \pm 1.18$ , and  $1.58 \pm 0.08$  for control, 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L respectively (Table 2).

In week 2, gonadosomatic indexes for *C. gariepinus* recorded a significant variation in the treatment concentrations when compared to the control. The treatment levels recorded a significantly higher value (Table 3). Generally, the gonadosomatic index of *C. gariepinus* between period of exposure across treatment levels revealed a significant change between treatment levels in week 1 and 2 with week 1 recording higher values across all treatment levels except in 0.6ml/L concentration where week 2 recorded a higher value (Fig. 2).

In week 1, the results for viscerasomatic index (VSI) of *C. gariepinus* exposed to various concentration of gold crew oil spill dispersant revealed significant changes in the viscerasomaticindex in the treatment levels. Apart from 0.6ml/L that had lower viscerasomaticindex values other treatments had higher values compared to the control. In week 1. The values (mean  $\pm$  SD) were  $3.03 \pm 0.55$ ,  $3.45 \pm 0.53$ ,  $3.70 \pm 0.52$ ,  $3.16 \pm 1.00$  and  $2.91 \pm 0.50$  for control, 0.075ml/L, 0.15ml/L, 0.3ml/L, and 0.6ml/L respectively (Table 2).

In week 2, the treatment concentrations revealed a significant change in index. All the treatment levels recorded a significantly higher index compared to the control. The values (mean  $\pm$  SD) were  $1.50 \pm 0.28$ ,  $3.10 \pm 0.45$ ,  $2.99 \pm 0.39$ ,  $2.71 \pm 0.49$  and  $4.33 \pm 0.10$  for control(0.0ml/L), 0.075ml/L, 0.15ml/L, 0.3ml/L, and 0.6ml/L respectively Table 3). Generally, the results for viscerasomatic index between exposure periods and treatment levels

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followed the same pattern of the gonadosomatic index. Values were higher in all other treatments levels in week 1 except for 0.6ml/L concentration where week 2 recorded a significantly higher level (Fig. 3).

Conversely, the results for spleenosomatic index (SSI) of *C. gariepinus* exposed to different concentrations of gold crew oil spill dispersants in week 1, varied along treatment levels. However, the treatment levels recorded higher values compared to the control. The values (mean  $\pm$  SD) were  $0.11 \pm 0.01$ ,  $0.21 \pm 0.03$ ,  $0.24 \pm 0.03$ ,  $0.17 \pm 0.04$  and  $0.15 \pm 0.04$  for control(0.0ml/L), 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L (Table 2).

In week 2, the spleenosomatic index revealed variation between the treatment levels and the control with 0.3ml/L recording the highest value. The values (mean  $\pm$  SD) were  $0.09 \pm 0.03$ ,  $0.07 \pm 0.00$ ,  $0.06 \pm 0.00$ ,  $0.15 \pm 0.03$  and  $0.09 \pm 0.01$  for control(0.0ml/L), 0.075ml/L, 0.15ml/L, 0.3ml/L and 0.6ml/L respectively (Table 3). Generally, the results for spleenosomatic index between exposure periods and treatment levels revealed a significant variation between weeks. Values were higher in week 1 compared to week 2 across treatment levels (Fig. 4).

The results for cardiosomatic index of *C. gariepinus* exposed to different concentrations of gold crew oil spill dispersant in week 1 revealed significant changes in the cardiosomatic indices of the *Clarias gariepinus* when compared to the control. 0.075ml/L and 0.15ml/L had higher values than the control while the other treatments (0.3ml/L and 0.6ml/L) recorded lower values compared to the control (Table 2).

In week 2, all the treatment levels recorded significantly higher changes in the cardiosomatic index compared to the control (Table 3). Generally, the results for cardiosomatic index between exposure periods and treatment levels revealed a significant variation between weeks. Values were higher in week 1 for 0.0ml/L, 0.075ml/L, and 0.15ml/L while week 2, had higher values for 0.3ml/L and 0.6ml/L concentration (Fig. 5).

The condition factor values varied significantly( $p < 0.05$ ) in the treatment levels when compared to the control in week 1 (Table 4).

In week 2, a similar trend was noted for the other treatments levels except for 0.075ml/L concentration that had a relatively high condition factor in comparison with the control (Table 4).

**Table 1: Physico-Chemical Variables of Sub-Lethal Concentrations of Gold Crew Oil Spill Dispersant in Test Water**

Parameter	Exposure Period	Concentration (ml/L)				
		0.0	0.075	0.15	0.3	0.6
Temperature (°C)	Week 1	27.20 <sup>ab</sup> ± 0.70	27.53 <sup>ab</sup> ± 0.68	28.30 <sup>a</sup> ± 0.27	26.77 <sup>b</sup> ± 0.12	27.70 <sup>ab</sup> ± 0.52
	Week 2	26.00 <sup>a</sup> ± 0.00	26.00 <sup>a</sup> ± 0.00	25.67 <sup>a</sup> ± 0.78	26.00 <sup>a</sup> ± 0.00	25.67 <sup>a</sup> ± 0.58
pH	Week 1	4.06 <sup>b</sup> ± 0.10	4.75 <sup>ab</sup> ± 0.46	4.82 <sup>a</sup> ± 0.38	4.75 <sup>ab</sup> ± 0.05	4.78 <sup>ab</sup> ± 0.11
	Week 2	7.38 <sup>a</sup> ± 0.21	6.03 <sup>c</sup> ± 0.02	6.07 <sup>c</sup> ± 0.06	6.60 <sup>b</sup> ± 0.02	5.94 <sup>c</sup> ± 0.15
DO (mg/l)	Week 1	6.12 <sup>a</sup> ± 0.16	5.04 <sup>b</sup> ± 0.04	5.00 <sup>b</sup> ± 0.02	5.00 <sup>b</sup> ± 0.02	5.00 <sup>b</sup> ± 0.02
	Week 2	6.01 <sup>a</sup> ± 0.02	5.02 <sup>b</sup> ± 0.01	4.98 <sup>b</sup> ± 0.08	4.93 <sup>b</sup> ± 0.10	5.01 <sup>b</sup> ± 0.01
TDS (mg/l)	Week 1	78.67 <sup>c</sup> ± 1.16	76.67 <sup>c</sup> ± 2.08	82.67 <sup>b</sup> ± 0.58	83.00 <sup>b</sup> ± 1.73	89.00 <sup>a</sup> ± 0.00
	Week 2	247.30 <sup>a</sup> ± 169.60	215.30 <sup>b</sup> ± 93.00	223.70 <sup>b</sup> ± 103.50	154.00 <sup>c</sup> ± 25.90	211.70 <sup>b</sup> ± 113.00
EC (µs/cm)	Week 1	166.33 <sup>c</sup> ± 5.13	170.00 <sup>b</sup> ± 1.00	173.00 <sup>b</sup> ± 2.00	175.00 <sup>b</sup> ± 4.00	188.33 <sup>a</sup> ± 3.06
	Week 2	357.67 <sup>a</sup> ± 73.93	238.67 <sup>e</sup> ± 102.63	290.67 <sup>c</sup> ± 120.03	338.67 <sup>b</sup> ± 33.95	265.33 <sup>d</sup> ± 92.80

DO; Dissolved Oxygen, TDS; Total Dissolved Solids, EC; Electrical Conductivity

Means with different alphabets across rows indicates a significant effect (ANOVA,  $p < 0.05$ ) within concentrations with respect to exposure periods

**Table 2: Organosomatic Indices of *Clarias gariepinus* exposed to Sub-Chronic Levels of Gold Crew Oil Spill Dispersant for one week**

Treatments(ml/L)	Organosomatic Indices				
	HSI	GSI	VSI	SSI	CSI
0.0 (Control)	0.41 <sup>e</sup> ± 0.11	1.93 <sup>d</sup> ± 0.14	3.08 <sup>d</sup> ± 0.55	0.11 <sup>e</sup> ± 0.01	0.11 <sup>c</sup> ± 0.1
0.075	2.26 <sup>b</sup> ± 0.08	3.34 <sup>b</sup> ± 0.10	3.45 <sup>b</sup> ± 0.53	0.21 <sup>b</sup> ± 0.03	0.16 <sup>b</sup> ± 0.04
0.15	2.52 <sup>a</sup> ± 0.3	3.83 <sup>a</sup> ± 0.11	3.70 <sup>a</sup> ± 0.52	0.24 <sup>a</sup> ± 0.03	0.16 <sup>a</sup> ± 0.03
0.3	1.32 <sup>d</sup> ± 0.67	3.07 <sup>c</sup> ± 1.18	3.16 <sup>c</sup> ± 1.00	0.17 <sup>c</sup> ± 0.04	0.07 <sup>e</sup> ± 0.05
0.6	1.78 <sup>c</sup> ± 0.46	1.58 <sup>e</sup> ± 0.08	2.91 <sup>e</sup> ± 0.50	0.15 <sup>d</sup> ± 0.04	0.09 <sup>d</sup> ± 0.03

Means with different alphabets down the column indicates a significant effect (ANOVA, p<0.05) within concentrations.

**KEY**

HSI; Hepatomasomatic Index, GSI; Gonadosomatic Index, VSI; Viscerosomatic Index, SSI; Spleenosomatic Index, CSI; Cardiosomatic Index.

**Table 3: Organosomatic Indices of *Clarias gariepinus* exposed to Sub-Chronic Levels of Gold Crew Oil Spill Dispersant for Two Weeks**

Treatments(ml/L)	Organosomatic Indices				
	HSI	GSI	VSI	SSI	CSI
0.0 (Control)	1.12 <sup>b</sup> ± 0.26	1.11 <sup>e</sup> ± 1.55	1.50 <sup>e</sup> ± 0.28	0.09 <sup>b</sup> ± 0.03	0.11 <sup>d</sup> ± 0.03
0.075	0.93 <sup>d</sup> ± 0.11	1.55 <sup>d</sup> ± 0.78	3.10 <sup>b</sup> ± 0.45	0.07 <sup>c</sup> ± 0.00	0.13 <sup>c</sup> ± 0.00
0.15	1.01 <sup>d</sup> ± 0.15	2.27 <sup>c</sup> ± 0.25	2.99 <sup>c</sup> ± 0.39	0.06 <sup>d</sup> ± 0.00	0.13 <sup>c</sup> ± 0.00
0.3	1.09 <sup>c</sup> ± 0.17	3.33 <sup>b</sup> ± 0.98	2.71 <sup>d</sup> ± 0.49	0.15 <sup>a</sup> ± 0.03	0.15 <sup>b</sup> ± 0.09
0.6	1.52 <sup>a</sup> ± 0.37	4.66 <sup>a</sup> ± 0.55	4.33 <sup>a</sup> ± 0.10	0.09 <sup>b</sup> ± 0.01	0.19 <sup>a</sup> ± 0.02

Means with different alphabets down the column indicates a significant effect (ANOVA, p<0.05) within concentrations.

**KEY**

HSI; Hepatomasomatic Index, GSI; Gonadosomatic Index, VSI; Viscerosomatic Index, SSI; Spleenosomatic Index, CSI; Cardiosomatic Index.

**Table. 4: Mean condition Factor Values for *Clarias gariepinus* Exposed to Sub-Lethal Concentration of Gold Crew Oil Spill Dispersant**

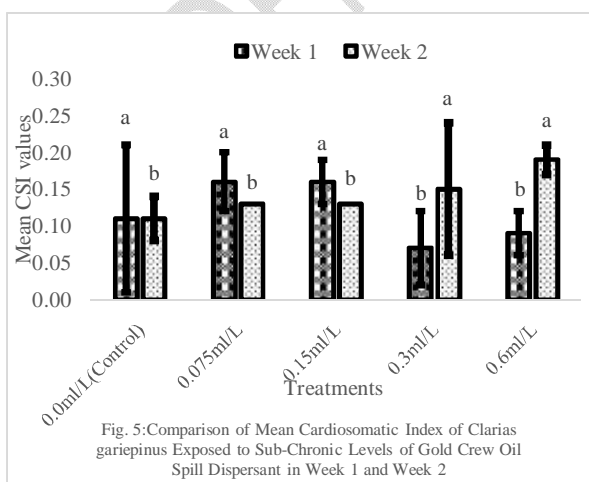
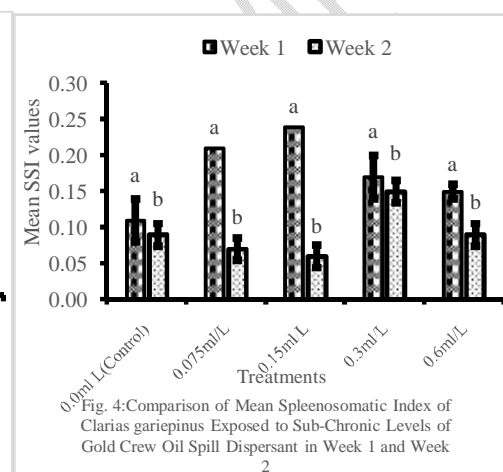
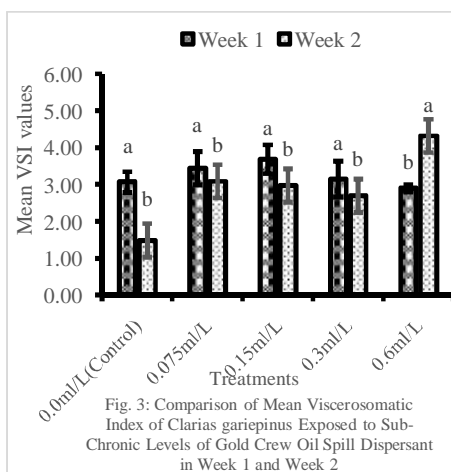
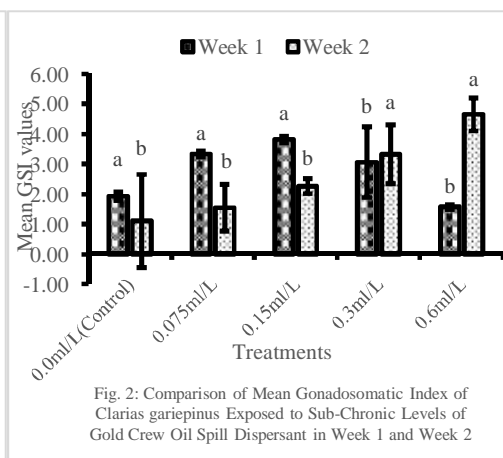
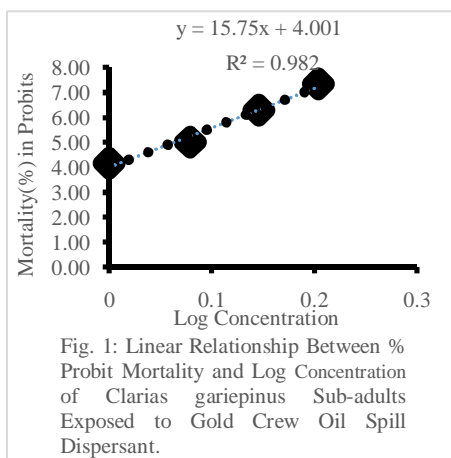
Exposure Period (Weeks)	Treatments(ml/L)				
	0.0(Control)	0.075	0.15	0.3	0.6
Week 1	1.00 <sup>a</sup> ± 0.30	0.88 <sup>b</sup> ± 0.11	0.57 <sup>e</sup> ± 0.08	0.68 <sup>c</sup> ± 0.60	0.59 <sup>d</sup> ± 0.01

Week 2	1.00 <sup>b</sup> ± 0.02	1.05 <sup>a</sup> ± 0.02	0.87 <sup>c</sup> ± 0.05	0.71 <sup>e</sup> ± 0.13	0.80 <sup>d</sup> ± 0.02
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Means with different alphabets across rows indicates a significant effect (ANOVA, p<0.05) within concentrations with respect to exposure periods

UNDER PEER REVIEW





#### 4.0 DISCUSSION

This study found out that the LC<sub>50</sub> of gold crew oil spill dispersant for *C. gariepinus* was 1.16mg/L. This value is similar to the LC<sub>50</sub> values reported for other oil spill dispersants, e.g., Corexit 9500; 1.2mg/L (NRC, 1989), Dispersit; 1.5mg/L (King *et al.*, 1995). Suggesting high toxicity even at lower concentration.

This study observed a positive correlation between gold crew oil spill dispersant concentration and temperature in week 1. This finding aligns with previous study by Ugbomah *et al.* (2019) which reported increased temperature with dispersant application. The initial peak at 0.15ml/L and subsequent decrease at 0.3ml/L suggest potential metabolic activity or chemical reaction influencing the temperature dynamics this assertion agrees with the reports of Atlas and Hazen (2011) that opined that dispersants can stimulate the growth of hydrocarbon-degrading bacteria, generating heat as a by-product of metabolism, it also corroborates the information provided by Liu *et al.* (2019) which reports that dispersants can affect the surface tension of water potentially influencing heat transfer and evaporation rates (Liu *et al.*, 2019). In week 2, a consistent temperature across all concentrations, except for minor variations, indicates a stabilizing effect. This suggests that the dispersant concentration might have reached an equilibrium or alternative processes might have become dominant.

The findings for pH in this present study revealed a significant effect of dispersant concentrations on pH particularly, in week 1.

In week 1, the control group exhibited a pH of 4.06, indicating an acidic environment. Conversely, the dispersant treated groups demonstrated higher pH values, suggesting a shift towards alkalinity. Notably, the 0.15ml/L concentration displayed the highest pH values of 4.82 indicating a less acidic environment compared to the control. These findings are consistent with previous studies that have reported the pH-raising effect of dispersant (Das and Chandrasekaran, 2011; Li *et al.*, 2012).

The observed increase in pH in dispersant-treated groups can be attributed to the presence of alkaline components in the dispersant formulation. These alkaline components, such as carbonates and bicarbonates neutralize the acidity of the surrounding environment, leading to a shift in pH (Sarkis *et al.*, 2011).

However, the trend reversed in week 2. The control pH increased substantially to 7.38, indicating a more neutral environment. Interestingly, the dispersant concentration led to lower pH values compared to the control. The 0.6ml/L concentration exhibited the lowest pH at 5.94, suggesting a shift back towards an acidic environment.

The observed decrease in pH in dispersant-treated groups in week 2 could be due to several factors. Firstly, the dispersant components might have been metabolized by micro-organisms in the environment, leading to the release of acidic byproducts. Secondly, the dispersants itself might have undergone chemical degradation, releasing acidic compounds into the environment. Additionally, the dispersant might have interfered with the natural buffering capacity of the water, making it more susceptible to changes in pH.

The findings in the dissolved oxygen concentration observed in this study revealed a significant effect of dispersant concentration on DO levels, particularly in week 1.

In week 1, the control group exhibited a higher DO level than the dispersant-treated groups. As the dispersant concentration increased, a decrease in DO was observed. This suggest that the dispersant may have interfered with oxygen exchange at the water-air interface, leading to a depletion of DO. This trend aligns with previous studies reporting the potential for oil spill dispersants to decrease DO levels in aquatic environments (Chen *et al.*, 2022; Lee *et al.*, 2023).

Several mechanisms could explain the observed decrease in DO in dispersant-treated groups Firstly, the dispersant itself might have consumed oxygen during its degradation process. Additionally, the dispersants might have enhanced the microbial degradation of organic

matter, thereby increasing oxygen demand in the water column (Lee *et al.*, 2021). Furthermore, the dispersant could have formed a film on the water surface, hindering oxygen diffusion from the atmosphere into the water.

In week 2, the trend of decreasing DO with increasing dispersant concentration continued, albeit with less pronounced differences compared to week 1. This suggests that a certain equilibrium might have been reached between oxygen consumption and production in the system.

The dispersant concentrations seemingly exerted a mixed effect on total dissolved solids compared to the control in Week 1. While some concentrations like 0.075ml/L and 0.15ml/L, displayed similar total dissolved solid levels to the control, others showed slight deviations. These mixed effects suggest that specific dispersant concentrations might influence the solubilisation and mobilisation of various dissolved solids in the water, leading to fluctuations in overall TDS levels.

The observed fluctuations in total dissolved solid levels at higher dispersant concentrations might be attributed to the dispersant's interaction with various organic and inorganic matter present in the water. This interaction could potentially lead to increased release of dissolved solids into the water column, thereby elevating total dissolved salts.

In week 1, the EC values increased with increasing dispersant concentration. The control group exhibited an EC value of 166.33 $\mu$ S/cm, whereas the highest concentration (0.6ml/L) showed a significantly higher value of 1.88 $\mu$ S/cm. This observed increase in EC can be attributed to the presence of electrolytes in the dispersant formulation. These electrolytes, such as sodium, and chloride ions, contribute to the overall conductivity of the solution (Sarkar *et al.*, 2006). This finding aligns with previous studies of Liu *et al.* (2015) and Wu *et al.* (2018) that have reported similar increase in electrical conductivity upon dispersant application.

Interestingly, a contrasting pattern emerged in week 2. The EC values across all dispersant concentrations, except 0.3ml/L, decreased significantly compared to the control group (207.33 $\mu$ S/cm). The 0.3ml/L concentration displayed a slight increase in EC, but it remained lower than the control value. This observed decrease in EC suggests complex interactions between the dispersant and the test medium overtime. This may be attributable to the fact that the dispersant components might have undergone biodegradation by micro-organisms in the test medium, leading to the depletion of electrolytes and a subsequent decrease in electrical conductivity (Sarkis *et al.*, 2011).

In week 1, results for hepatosomatic index showed that treatment levels recorded significantly higher HSI values compared to the control. This finding suggests that exposure to gold crew oil spill dispersant in week 1 caused a significant increase in liver size relative to body weight in treated fish compared to the control. This increase in HSI could be attributed to various factors, including hepatocellular hypertrophy; dispersant exposure might have induced hepatocyte enlargement due to increased metabolic activity in response to the dispersant's toxic components (Liu *et al.*, 2022). It could also be linked to bile stasis; dispersant-induced damage to bile ducts could lead to bile accumulation in the liver, resulting in increased HSI (Wang *et al.*, 2020). Additionally, it could also be attributable to lipid accumulation; dispersant exposure might have increased lipid deposition in the liver, contributing to the observed HSI increase (Yu *et al.*, 2019).

In week 2, the trend reversed with the control group exhibiting significantly higher HSI values compared to the treated group except for 0.6ml/L group, which showed a significantly higher HSI than the control. These results suggest that the initial response to the dispersant exposure in week 1 might have subsided in week 2, potentially due to detoxification and elimination of the dispersant from the fish's body over time (Barron *et al.*, 2018). It could also be that the fish have developed physiological adaptations to mitigate the dispersant

detrimental effects (Moller *et al.*, 2020). However, the sustained higher HSI in the 0.6ml/L group suggests potential long-term effects of dispersant exposure at higher concentrations.

In week 1, all treatment concentrations except 0.6ml/L caused a significant increase in GSI compared to the control. This initial increase could be attributed to stress response, as pollutants are known to trigger the release of hormones, including those involved in gonadal development (Aluru &Orunonye, 2016). Additionally, the dispersant might have inadvertently acted as an endocrine disruptor, interfering with the delicate hormonal balance necessary for normal reproductive function (Oliveira *et al.*, 2009). However, the GSI in fish exposed to 0.6ml/L of dispersant was significantly lower than the control in week 1. This suggests a possible inhibitory effect at this specific concentration, potentially due to direct damage to gonadal tissues or disruption of specific enzymatic pathways involved in steroidogenesis (Van der Oost *et al.*, 2003).

By week 2, a further increase in GSI was observed in all treatment groups compared to week 1. This continued elevation could be indicative of a compensatory mechanism by the fish to counteract the initial stress-induced hormonal imbalances (Shreck and Tort, 2016). Alternatively, the dispersant might have altered the metabolic pathways involved in energy allocation, leading to an increased investment in gonadal development, even in the presence of a stressful environment (Adams *et al.*, 2011).

In week 1, all the treatment levels except 0.6ml/L showed significantly higher VSI values compared to the control. This indicates that the dispersant caused an increase in the relative size of the visceral organs which could be attributable to increased metabolic activity; The dispersant may have induced stress in the fish, leading to increased metabolic activity and energy expenditure. This could result in the visceral organs working harder and becoming enlarged (Ogamba *et al.*, 2014). It may also be due to histopathological changes; The dispersant may have caused damage to the internal organs leading to inflammation and swelling. This could also contribute to an increase in VSI (Jiraungkoorskulet *et al.*, 2023). Interestingly, the VSI in the 0.6ml/L treatment group was lower than the control. This could be due to a hermetic effect, where exposure to a low concentration of dispersant stimulates a beneficial response in the fish.

In week 2, all treatment levels showed significantly higher VSI values compared to the control. This suggests that the effects of the dispersant persisted overtime and may even have become more pronounced. The continued increase in VSI could be due to cumulative effects of the dispersant on the fish's health. Overtime, the damage to the internal organs and the accumulation of contaminants could become more severe, leading to further enlargement of the visceral organs.

During week 1, spleenosomaticindex (SSI) values increased with increasing dispersant concentrations. This suggests a dose-dependent effect of gold crew on the spleen, potentially causing splenic hyperplasia or increased activity in response to the dispersant's toxic components. In week 2, while the overall trend of higher SSI in treated groups compared to the control remained, the values varied between treatment levels. Interestingly, the highest SSI was observed in the 0.3ml/L group. This suggests a non-linear dose-response relationship, where intermediate concentrations may elicit a stronger splenic response compared to higher or lower doses. These findings are consistent with previous studies on the effects of oil spill dispersants on fish health. For instance, Olukunle *et al.* (2002) reported an increase in spleen size in African catfish (*Clarias gariepinus*) exposed to crude oil, suggesting splenic involvement in detoxification and immune response to pollutants. Similarly, Ogamba *et al.* (2014) observed a slight increase in liver and spleen size in *Clarias gariepinus* exposed to sub-lethal concentrations of paraquat a commonly used herbicide. While the underlying mechanisms remain unclear, the observed splenic enlargement in *C. gariepinus* exposed to gold crew oil spill dispersant could be attributable to many factors

which include action of the immune system; the dispersant components might induce an immune response in the spleen, leading to an increased cell proliferation and organ size. It could also be hematopoiesis; the spleen plays a crucial role in blood cell production. Exposure to the dispersant might stimulate erythropoiesis or other blood cell production pathways, resulting in splenic enlargement.

In week 1, the observed increase in CSI at lower concentrations (0.075ml/L and 0.15ml/L) compared to the control suggests a possible compensatory response by the fish. This increase could be attributable to hypertrophy of the heart muscle, potentially driven by increased metabolic demands associated with stress and detoxification efforts (Ogamba *et al.*, 2014). Conversely, the decrease in CSI at higher concentrations (0.3ml/L and 0.6ml/L) may indicate cardiotoxicity or impaired cardiac function induced by the dispersant (Olakunle *et al.*, 2002). By week 2, a significant increase in CSI was observed across all treatment levels compared to the control. This consistent elevation suggests a prolonged stress response in fish, potentially leading to long-term consequences for the fish, such as reduced growth and reproductive capacity (Hinton *et al.*, 2000).

The results from this present study revealed that the condition factor varied significantly between the control and treatment groups, with the control group consistently exhibiting higher values than the treated groups. This indicates that exposure to gold crew oil spill dispersant, even at sub-lethal concentrations negatively impacts the overall health and well-being of *Clarias gariepinus*.

This finding is consistent with previous studies on the effects of dispersants on fish health. For instance, Okoye *et al.* (2016) reported a significant decrease in the condition factors of *C. gariepinus* exposed to sub-lethal concentration of Corexit 9500 dispersant. Adeyemo *et al.* (2019) observed a negative impact on the condition factor of *Oreochromis niloticus* exposed to sub-lethal concentrations of Finasol OSR dispersant.

The decrease in K observed in the treated groups can be attributed to several factors. Dispersants can alter the absorption and utilization of nutrients by fish, leading to reduced energy intake and decreased body weight (Okoye *et al.*, 2016). Additionally, dispersants can induce stress response in fish, which can further deplete energy reserves and contribute to weight loss (Adeyemo *et al.*, 2019).

Interestingly, the K values were generally higher in week 2 Compared to week 1. This suggests that the fish may have undergone some degree of adaptation to the dispersant over time. However, it's important to note that this adaptation may not be sufficient to fully counteract the negative effects of the dispersant on fish health [104].

Furthermore, the observation that K value in the 0.075ml/L (week 2) treatment group was greater than 1 suggests that this concentration may be less harmful than the other concentrations tested.

## CONCLUSION

This study demonstrated that gold crew oil spill dispersant has a detrimental effect on the fish, with a lethal concentration (Lc50) of 1.16mg/L.

Furthermore, the dispersant significantly altered the physico-chemical properties of the water, and sub-lethal concentrations caused pathological changes and affected their organosomatic index and condition factor. These findings indicate that gold crew oil spill dispersant, even at sub-lethal levels disrupts the physiological well-being of *C. gariepinus*.

## RECOMMENDATION

Based on these observations, the use of Gold Crew dispersant in the aquatic ecosystems should be discouraged. Alternative spill response strategies that prioritize oil containment and removal, with minimal dispersant use should be employed. Additionally, further research is



recommended to evaluate the long-term effects of gold crew dispersant on aquatic life and explore the potential for less-toxic dispersant formulations.

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