

Acute Exposure to Textile Waste Water Altered the Reproduction Biomarkers in *Clarias gariepinus* Broodstock

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ABSTRACT This study was prompted by an earlier study that revealed the absence of gonads in all fish species caught around the Itoku tributary of the Ogun River, an area known for active textile activities. Thus, this study investigated the effect of textile wastewater on reproduction biomarkers in African catfish broodstock. Male and female broodstock were exposed to varying pre-determined concentrations (0ppb-T0, 0.5ppb-T1, 0.35ppb-T2, 2ppb-T3) of the textile wastewater for a period of 96 hours. Water quality parameters, seminal/ovarian hormones, metabolites, ions, oxidative, enzymes, and sperm viability were assayed. The water quality parameters of the wastewater indicated varying degrees of physical and chemical pollution. The values of hormones were significantly different ($p < 0.05$) although a trend was not observed. Prolactin, however, showed a marked difference statistically across the treatments in the male broodstock while a reverse trend was observed in the female broodstock. The values recorded for seminal metabolites increased significantly compared to the control except for T3 for cholesterol and T1 glucose which were significantly lower than the control. In the female broodstock, the values for glucose and creatinine significantly increased compared to the control. Seminal ions evaluated showed significant differences across treatments. Except for T1, there was a significant reduction observed in the values of sodium, chloride, and calcium compared to the control. For ovarian ions, a significant increase was recorded across the treatments compared to the control. However, sodium recorded a significant decrease compared to the control except for T3 where the value was higher than the control. In the male broodstock, aspartate aminotransferase and alanine aminotransferase values were significantly lower compared to control while an opposite trend was observed in the female broodstock. The concentration of sperm and life- to-death ratio significantly reduced across treatments compared to the control. These results indicate that the textile wastewater altered the reproduction biomarkers in the male and female broodstock. The implication of this is that reproduction in fish might become a challenge in textile wastewater polluted Itoku tributary of the Ogun River.

Keywords: Aquatic health; pollution; reproduction biomarkers

INTRODUCTION

Water pollution occurs when unwanted materials with potential to threaten humans, fish, and other natural systems find their way into different aquatic ecosystems. Globally, aquatic environments are exposed to a few pollutants particularly from anthropogenic activities (Hughes *et al.*, 2013; Malaj *et al.*, 2014). These pollutants are discharged into the water bodies and cause various harmful effects on the aquatic biota (Ekubo & Abowel, 2011; Sharma, 2012; Ghani, 2015; Schmeller *et al.*, 2018). Industries are among the most important point source of pollutants that discharge a huge amounts of waste substances into aquatic ecosystems. They can generate both organic and inorganic wastes, which could alter all or parts of the biological, physical, and chemical characteristics of the receiving water bodies (Gomez *et al.*, 2008). Depending on the dose and exposure time, some of these pollutants are toxic to living systems and cause serious impairment to aquatic life (Ogundiran *et al.*, 2010; Hampel *et al.*, 2015). This is because pollutants in the receiving water bodies could accumulate in water, sediment, and living systems. They also accumulate in the food chain and thus cause adverse effects in aquatic systems (Dabrowska *et al.*, 2013). Hence, it is important to periodically measure the effects of these

pollutants on aquatic biota.

The textile industry is one of the major sources of pollutants to the receiving water bodies since it requires high volume of water that eventually results in high volume of wastewater (Roy *et al.*, 2010; Olisah *et al.*, 2021). Depending on the types of raw materials and daily products, textile industry employs variety of chemicals such as detergents and dyes (Roy *et al.*, 2010; Singha *et al.*, 2021). Furthermore, the dyeing process contributes high concentration of chromium, copper, mercury, and zinc, and could have a high levels of colour, toxicity, and turbidity (Roy *et al.*, 2010; Bashir *et al.*, 2020). Thus, the quantities and characteristics of discharged textile wastewater vary depending on the amount of water consumed, and the types and amounts of raw materials used as reported by Kassaye (2013). Effluent from the textile industry commonly contains high concentrations of organic and inorganic substances and is characterized by high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and pH values but low Dissolved Oxygen (DO) with strong colour (Ghaly *et al.*, 2014; Berradi *et al.*, 2019). As mentioned by Singha *et al.* (2021), up to 50% of dyes are lost as a waste substance in the wastewater, and has a serious negative impact on aquatic

biota, and changes the aesthetic value of the environment (Mudhoo *et al.*, 2020; Patil *et al.*, 2022). Abnormal high or low dissolved solids disturb the osmotic balance of native species. High salt content may cause an increase in suspended solids. Higher doses of salt are toxic to aquatic organisms as they expose the organisms to changes in osmotic pressure causing swelling or dehydration. In addition, due to chemical pollution, the normal functioning of the cell is disturbed and this in turn may cause alteration in the physiological and biochemical mechanisms of animals as reported by Khalaj *et al.* (2018), and Elgarahy *et al.* (2021) resulting in impairment of important functions like respiration, osmoregulation, hormonal regulation, reproduction and may even cause mortality (Al-Busaidi *et al.*, 2011; Rahman *et al.*, 2012; Ghaedi *et al.*, 2015). Although, there are guidelines and policies for industrial discharge standards in several countries, however, these strategies are not applicable in the case of developing and underdeveloped countries, Nigeria inclusive, thus making the vulnerability of aquatic ecosystems beyond the boundaries of nations. Abeokuta in Ogun State is known for its local textile industries (Adire and Kampala) which are very active along some points of the Ogun river (Oloyede *et al.*, 2014). With the increased global demand for adire/kampala and the comparative increase in their production, the use of synthetic dyes has contributed to dye wastewater becoming one of the significant sources of severe pollution problems (Ogugbue & Sawidis, 2011; Olisah *et al.*, 2021), especially along Itoku tributary of the Ogun River. The toxicity of textile wastewaters pose threat to fish biota, both directly and indirectly. The direct accumulation of pollutants is a considerable issue, however, water pollution-induced toxicity also forms a matter of concern. Fish can serve as bio-indicators of environmental pollution and therefore can be used for the assessment of the quality of the aquatic environment (Zhao *et al.*, 2012; Authman *et al.*, 2015; Al-Ghanim *et al.*, 2016). Primarily, sub-lethal effects of stress as biochemical changes at sub-cellular levels may induce a sequence of structural and functional alterations at higher levels of the organization. Thus, biochemical parameters are good tools for detecting the effect of any environmental stress on well-being of fish (Parrino *et al.*, 2018; Fazio, 2019).

One of the most sensitive endpoints for evaluating the reproductive fitness of a fish population in any environment is the measure of gamete quality, described as the ability of sperm to fertilize an egg and produce a viable larva (Wang *et al.*, 2014). Common measures of gamete quality include milt volume, the viability of spermatozoa, egg morphology and chemical profile of gametes and seminal fluid, hatchability of eggs and malformation rate of embryos and fry survival (Hajirezaee *et al.*, 2010; Wang *et al.*, 2012), which are critical for fry integrity and larval survival in a number of species. Indices of reproductive failure such as decreased sperm count, acrosome integrity, delayed hatching of fish eggs, and yolk-sac abnormalities have been attributed to contaminant exposure and uptake (Mathur *et al.*, 2010; Zhao *et al.*, 2014). Pollutants can also change sex hormone levels via stress-related mechanisms or through effects on other hormonal pathways (Brüning *et al.*, 2016). Therefore, biomarker profiles of fish exposed to textile wastewater will give a clearer picture of the reproductive health of fish. This study was prompted by the findings of a preliminary

study where an absence of gonads was observed in some fish species caught in the Itoku tributary along Ogun River. Studies have been published on the environmental pollution on Ogun River which focused on water quality (Tufekci *et al.* 2007; Ojekunle *et al.*, 2014). Also, several studies on the effect of textile wastewaters on the physico-chemical properties of natural waters were reported by Tamburlini *et al.* (2002) and Das *et al.* (2011). Although, some authors have worked on pollution biomarkers using fish tissues (Olaganathan & Patterson 2013; Taiwo *et al.*, 2015), however, there are few studies on the effect of textile wastewater on fish reproductive health, and presently, no information on fish exposed to textile wastewater from Itoku tributary along Ogun River. Hence, the study assessed the reproductive health of *C. gariepinus* male and female broodstock exposed to textile waste water to give insight into what might have caused the absence of gonads in sampled fish from the region.

MATERIALS AND METHODS

Collection of textile wastewater sample

Textile wastewater for the experiment was collected at textile industry located at Itoku, Abeokuta, Ogun State, Nigeria. The untreated wastewater was collected and transported using 25L capacity rectangular plastic containers.

Physico-chemical analysis of textile wastewater

Tilapia A sample of the collected textile waste water was collected in sampling bottles and taken to the laboratory for analysis of total hardness, biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, total suspended solids, turbidity, alkalinity, chloride, carbonate, bicarbonate, nitrate, nitrite, phosphate, sodium, potassium, calcium, magnesium, copper, zinc, lead, iron, manganese and mercury. After digestion, heavy metals and trace elements were determined spectrophotometrically using atomic absorption spectrophotometer while other physico-chemical parameters were evaluated following standard methods for water and wastewater as described by APHA (1995).

Collection and acclimatization of fish sample

Fish samples were obtained from fish farm at Ayetoro, Ogun State. The fishes were acclimatized to laboratory conditions for 2 weeks before stocking into experimental tanks. The fish were fed commercial feeds once daily during this period.

Experimental setup

Certified healthy male (24) and female (16) broodstock catfish (*Clarias gariepinus*) were chosen and divided into 4 groups of 3 fishes (male) and 2 fishes (female). Each group was replicated. The average weight of the broodstock ranged from 450g-500g with each treatment having equal weights. After monitoring the concentration levels of textile effluent in the water along the Itoku tributary over the rainy and dry season, dilution rates were determined and used for this study. The fishes were grouped into the designated treatment as shown below;

Treatment 0 (T0)- Control fishes maintained in 35 litres of fresh water.

Treatment 1 (T1) - 35 litres of fresh water + 0.05ppb of textile waste water

Treatment 2 (T2) - 35 litres of fresh water + 0.35ppb of

textile waste water

Treatment 3 (T3) – 35 litres of fresh water + 2ppb of textile waste water

Fishes were a fed daily with pelleted diet (40% crude protein) at a rate of 3% body weight per day and water was changed twice daily completely in the morning and partially in the evening for a period of 96 hours. The water replacement was done to facilitate the removal of nitrogenous waste excreted by the fishes and the removal of unconsumed food. As indicated above, the water replaced for each treatment always contained its required concentration. At the end of the experiment, sedation of the fish was done using clove oil. The sedated fishes were dissected to collect gonads for semen and ovarian assessments. Blood was also drawn from the caudal peduncle for hormone assay.

Determination of hormones

Using the collected blood, testosterone, estradiol, and prolactin were evaluated using ELISA test kits following the manufacturer's instructions.

Evaluation of seminal/ ovarian metabolites, ions, and stress enzymes

The Milt and ovarian follicles obtained from the testes and ovarian tissue respectively, were used for the determination of total protein, cholesterol, glucose, and creatinine following the procedure described by Hajirezaee *et al.* (2009). Cations and anions were determined spectrophotometrically. For the evaluation of seminal enzymes, milt was centrifuged to obtain a clear portion which was used for the evaluation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using Randox diagnostic kit. Ovarian enzymes were also evaluated after careful extraction of the ovarian fluid following the method of Lahnsteiner *et al.* (1995).

Sperm count and motility

The sperm count was assessed using a hemocytometer. Also, the qualitative and quantitative morphology and motility of sperms were observed with a light microscope (Zeiss, 015447, Germany) and their vitality was studied with eosin dye. Then, the percentage of live sperms to the total sperms was evaluated with a magnification of 40. The dead sperms were observed in reddish colour.

Statistical analysis

Data collected from the investigated parameters were subjected to one-way analysis of variance and post-hoc test was done using Duncan Multiple Range Test to check the level of significance at $p < 0.05$.

RESULTS AND DISCUSSION

Physico-chemical parameters of textile wastewater

Table 1 represents the mean and standard deviation of the physicochemical parameters of the water. the values observed are as follow: total hardness, 765.81±22.36; Biochemical Oxygen Demand, 162.96±4.18; Chemical Oxygen Demand, 243.24±4.58; Dissolved Oxygen, 1.83±0.04; Total Suspended Solids, 378.33±4.71; turbidity, 2.13±0.04; alkalinity, 210.88±1.24; chloride, 203.76±1.07; bicarbonate, 236.08±2.94; nitrate, 0.43±0.04; nitrite, 0.31±0.01; phosphate, 0.21±0.01; sodium, 12.29±0.42; potassium, 60.71±1.01; calcium, 35.59±0.84; magnesium,

26.27±1.79; toppe, 0.06±0.01; zinc, 0.08±0.00; lead, 0.11±0.00; iron, 0.01±0.00; manganese, 0.05±0.01 and mercury, 0.01±0.00.

Table 1. Physico-chemical parameters of the textile wastewater.

| Parameter | Mean | Std. Deviation |
|-----------------------|--------|----------------|
| Total Hardness (mg/L) | 765.81 | 22.36 |
| BOD (mg/L) | 162.96 | 4.18 |
| COD (mg/L) | 243.24 | 4.58 |
| DO (mg/L) | 1.83 | 0.04 |
| TSS (mg/L) | 378.33 | 4.71 |
| Turbidity | 2.13 | 0.04 |
| Alkalinity (mg/L) | 210.88 | 1.24 |
| Chloride (mg/L) | 203.76 | 1.07 |
| Carbonate (mg/L) | 20.83 | 1.17 |
| Bicarbonate (mg/L) | 236.08 | 2.94 |
| Nitrate (mg/L) | 0.43 | 0.04 |
| Nitrite (mg/L) | 0.31 | 0.01 |
| Phosphate (mg/L) | 0.21 | 0.01 |
| Sodium (mg/L) | 12.29 | 0.42 |
| Potassium (mg/L) | 60.71 | 1.01 |
| Calcium (mg/L) | 35.59 | 0.84 |
| Magnesium (mg/L) | 26.27 | 1.79 |
| Copper (mg/L) | 0.06 | 0.01 |
| Zinc (mg/L) | 0.08 | 0.00 |
| Lead (mg/L) | 0.11 | 0.00 |
| Iron (mg/L) | 0.01 | 0.00 |
| Manganese (mg/L) | 0.05 | 0.01 |
| Mercury (mg/L) | 0.01 | 0.00 |

Seminal hormones

Table 2 shows the hormone levels of fish exposed to textile wastewater. All parameters evaluated were significantly different ($p < 0.05$) although a trend was not observed. For testosterone, values recorded for T0 and T3 were not significantly different ($p > 0.05$) with the control recording the highest value (0.53±0.03). The same was observed in T1 and T2. There was no significant difference ($p > 0.05$) between T1 and T3 for estradiol concentration. The highest value was observed in T2 (0.34±0.04), although this was not statistically different ($p > 0.05$) from T0. Prolactin however showed a marked difference statistically ($p < 0.05$) across the treatments with the highest value recorded in T1 (3.21±0.01) and lowest in T3 (2.91±0.01).

Ovarian hormones

Table 3 shows that all values measured were significantly different ($P < 0.05$) across the treatments. T0 recorded the highest value (1.31±0.01) for testosterone, however, this was not significantly different ($p > 0.05$) from T2 (1.28±0.03). T3 had the highest estradiol concentration; T2 had the lowest, however, it was not statistically different from T0. Prolactin values observed showed that the values obtained significantly increased compared to the control (T0) except for T1.

Table 2. Hormonal profile of *C. gariepinus* brood stock exposed to textile wastewater.

| Parameters | T0 | T1 | T2 | T3 |
|----------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| Testosterone ($\mu\text{g/L}$) | 0.53 \pm 0.03 ^a | 0.41 \pm 0.01 ^b | 0.34 \pm 0.04 ^b | 0.52 \pm 0.02 ^a |
| Estradiol ($\mu\text{g/L}$) | 0.33 \pm 0.03 ^a | 0.17 \pm 0.02 ^b | 0.34 \pm 0.04 ^a | 0.22 \pm 0.01 ^b |
| Prolactin ($\mu\text{g/L}$) | 3.13 \pm 0.03 ^b | 3.21 \pm 0.01 ^a | 3.02 \pm 0.02 ^c | 2.91 \pm 0.01 ^d |

*Means with a different superscript in the same row are significantly different ($p < 0.05$).

Table 3. Hormonal profile of *C. gariepinus* brood stock exposed to textile wastewater.

| Parameters | T0 | T1 | T2 | T3 |
|----------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| Testosterone ($\mu\text{g/L}$) | 1.31 \pm 0.01 ^a | 1.01 \pm 0.01 ^b | 1.28 \pm 0.03 ^a | 1.00 \pm 0.01 ^b |
| Estradiol ($\mu\text{g/L}$) | 0.31 \pm 0.01 ^c | 0.38 \pm 0.02 ^b | 0.30 \pm 0.01 ^c | 0.49 \pm 0.02 ^a |
| Prolactin ($\mu\text{g/L}$) | 4.21 \pm 0.01 ^c | 3.89 \pm 0.02 ^d | 5.41 \pm 0.01 ^a | 5.08 \pm 0.08 ^b |

*Means with a different superscript in the same row are significantly different ($p < 0.05$).

Table 4. Seminal metabolites of *C. gariepinus* brood stock exposed to textile waste water.

| Metabolite | T0 | T1 | T2 | T3 |
|---------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| T.P (g/dl) | 2.55 \pm 0.05 ^d | 3.15 \pm 0.05 ^c | 5.35 \pm 0.15 ^a | 4.20 \pm 0.10 ^b |
| Chol (mg/dl) | 155.20 \pm 0.20 ^c | 207.05 \pm 0.05 ^a | 183.20 \pm 0.20 ^b | 150.05 \pm 0.05 ^d |
| Gluc (mg/dl) | 26.25 \pm 0.25 ^c | 16.05 \pm 0.05 ^d | 64.05 \pm 0.05 ^a | 49.15 \pm 0.15 ^b |
| Creat (mg/dl) | 0.35 \pm 0.05 ^c | 1.15 \pm 0.05 ^b | 1.60 \pm 0.20 ^a | 0.75 \pm 0.05 ^{bw} |

*Means with different superscript in the same row are significantly different ($p < 0.05$) T.P = Total protein, Chol= Cholesterol, Glu= Glucose, Creat= Creatinine.

Table 5. Ovarian metabolites of *C. gariepinus* brood stock exposed to textile waste water.

| Metabolite | T0 | T1 | T2 | T3 |
|---------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| T.P (g/dl) | 7.45 \pm 0.45 | 7.25 \pm 0.25 | 7.35 \pm 0.35 | 7.25 \pm 0.15 |
| Chol (mg/dl) | 204.10 \pm 0.10 ^c | 205.35 \pm 0.35 ^b | 258.45 \pm 0.45 ^a | 142.25 \pm 0.25 ^d |
| Gluc (mg/dl) | 89.45 \pm 0.45 ^d | 136.30 \pm 0.30 ^c | 182.35 \pm 0.35 ^a | 155.15 \pm 0.15 ^b |
| Creat (mg/dl) | 0.55 \pm 0.15 ^b | 0.65 \pm 0.05 ^b | 1.05 \pm 0.05 ^a | 1.30 \pm 0.10 ^a |

*Means with different superscript in the same row are significantly different ($p < 0.05$) T.P = Total protein, Chol= Cholesterol, Glu= Glucose, Creat= Creatinine.

Table 6. Seminal ions profile of *C. gariepinus* brood stock exposed to textile waste water.

| Parameters | T0 | T1 | T2 | T3 |
|-------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Sodium (mg/L) | 40.02 \pm 0.14 ^a | 32.03 \pm 0.36 ^c | 35.08 \pm 0.10 ^b | 29.41 \pm 0.45 ^d |
| Potassium (mg/L) | 19.32 \pm 0.22 ^c | 17.61 \pm 0.31 ^d | 20.59 \pm 0.19 ^b | 24.39 \pm 0.29 ^a |
| Chloride (mEq/l) | 33.30 \pm 0.60 ^b | 39.15 \pm 0.35 ^a | 21.30 \pm 0.40 ^d | 29.10 \pm 0.30 ^c |
| Calcium (mg/dl) | 9.25 \pm 0.25 ^b | 8.50 \pm 0.40 ^b | 9.25 \pm 0.05 ^b | 10.30 \pm 0.20 ^a |
| Magnesium (mg/dl) | 2.55 \pm 0.05 ^c | 3.15 \pm 0.05 ^b | 3.70 \pm 0.20 ^a | 2.85 \pm 0.05 ^b |

*Means with a different superscript in the same row are significantly different ($p < 0.05$).

Table 7. Ovarian ions profile of *C. gariepinus* brood stock exposed to textile waste water.

| Parameters | T0 | T1 | T2 | T3 |
|-------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Sodium (mg/L) | 47.24 \pm 0.24 ^b | 41.12 \pm 0.12 ^c | 39.28 \pm 0.28 ^d | 52.09 \pm 0.09 ^a |
| Potassium (mg/L) | 18.47 \pm 0.47 ^c | 19.21 \pm 0.21 ^{bc} | 22.01 \pm 0.01 ^a | 20.11 \pm 0.11 ^b |
| Chloride (mEq/l) | 22.20 \pm 0.20 ^c | 38.10 \pm 0.10 ^a | 24.15 \pm 0.15 ^b | 21.30 \pm 0.20 ^d |
| Calcium (mg/dl) | 8.30 \pm 0.30 ^b | 7.35 \pm 0.25 ^c | 9.05 \pm 0.05 ^{ab} | 9.80 \pm 0.20 ^a |
| Magnesium (mg/dl) | 1.65 \pm 0.25 ^c | 3.15 \pm 0.15 ^a | 2.70 \pm 0.10 ^{ab} | 2.30 \pm 0.20 ^{bc} |

*Means with a different superscript in the same row are significantly different ($p < 0.05$).

Table 8. Seminal enzymes of *C. gariepinus* broodstock exposed to textile waste water.

| Parameters (U/L) | T0 | T1 | T2 | T3 |
|----------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| Aspartate Aminotransferase | 112.50±0.50 ^a | 65.50±0.50 ^c | 37.40±0.60 ^d | 86.20±0.80 ^b |
| Alanine Aminotransferase | 31.60±0.40 ^a | 23.70±0.30 ^b | 18.60±0.40 ^c | 22.55±0.45 ^b |
| Alkaline Phosphatase | 15.75±0.25 ^d | 18.70±0.30 ^c | 32.65±0.35 ^a | 25.80±0.20 ^b |

*Means with different superscript in the same row are significantly different (p<0.05).

Table 9. Ovarian enzymes of *C. gariepinus* broodstock exposed to textile wastewater.

| Parameters (U/L) | T0 | T1 | T2 | T3 |
|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Aspartate Aminotransferase | 73.10±0.10 ^c | 69.50±0.50 ^d | 95.20±0.20 ^a | 91.25±0.25 ^b |
| Alanine Aminotransferase | 13.20±0.20 ^d | 18.25±0.25 ^c | 19.15±0.15 ^b | 26.25±0.25 ^a |
| Alkaline Phosphatase | 34.25±0.25 ^a | 31.15±0.15 ^c | 32.25±0.25 ^b | 30.25±0.25 ^d |

*Means with different superscript in the same row are significantly different (p<0.05).

Table 10. Sperm count and motility of *C. gariepinus* broodstock exposed to textile waste water.

| Parameters | T0 | T1 | T2 | T3 |
|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Conc. (×10 ⁹) | 78.50±0.50 ^a | 68.50±0.50 ^b | 60.50±0.50 ^d | 65.50±0.50 ^c |
| Motility% | 71.50±0.50 ^a | 49.50±0.50 ^c | 56.50±0.50 ^b | 32.50±0.50 ^d |
| TD | 63.75±0.25 ^a | 42.65±0.35 ^d | 60.55±0.55 ^b | 59.10±0.10 ^c |
| DH | 54.65±0.35 ^b | 40.60±0.40 ^d | 48.45±0.45 ^c | 56.40±0.40 ^a |
| FT | 58.80±0.20 ^b | 66.55±0.55 ^a | 38.80±0.20 ^d | 46.60±0.40 ^c |
| BT | 43.80±0.20 ^b | 52.70±0.30 ^a | 45.80±0.80 ^b | 50.85±0.85 ^a |
| L/D RATIO | 42:58 ^a | 33:67 ^b | 36:64 ^b | 24:76 ^c |

*Means with different superscript in the same row are significantly different (p<0.05).

TD=Tail droplet, DH=Detached head, FT=Free tail, BT=Bent tail, L/D=Life/death ratio.

Seminal metabolites

The values recorded for seminal metabolites increased significantly (P<0.05) compared to the control except for T3 for cholesterol and T1 glucose which were significantly lower (P<0.05) than the control. T2 recorded the highest values for total protein (5.35±0.15), glucose (64.05±0.05), and creatinine (1.60±0.20) (Table 4).

Ovarian metabolites

Values for glucose and creatinine significantly increased (P<0.05) compared to the control with the highest concentration observed in T2 (182.35±0.35) and T3 (1.30±0.10) respectively (Table 5). Except for T3, the concentration of cholesterol significantly increased (P<0.05) compared to the control. There was a significant reduction (P<0.05) in the concentration of total protein compared to the control.

Seminal ions

All ions evaluated showed significant difference (p<0.05) across treatment. T0 (40.02±0.14) had the highest value for sodium and the lowest in T3 (29.41±0.45). A significant increase was observed in potassium concentration except for T1 (17.61±0.31) where the value observed was lower than the control (19.32±0.22). A reverse trend of potassium was observed for chlorine concentration. The value recorded for calcium in T0 and T2 were not statistically significant (p<0.05), however, a significant reduction and increase was observed in T1 and T3 respectively, compared to the control. For magnesium, the control (T0) was significantly lower

(p<0.05) than the other treatments (Table 6).

Ovarian ions

Potassium and magnesium concentrations in T0 were significantly lower (p<0.05) than the rest of the treatments. For sodium, T0 was significantly higher than the treatment group except for T3 (52.09±0.09) where the value observed was higher than T0 (47.24±0.24). For chlorine, T0 (22.20±0.20) was statistically higher (p<0.05) than T1 (38.10±0.10) and T2 (24.15±0.15) but lower than T3 (21.30±0.20). Calcium recorded a significant increase (p<0.05) compared to T0 (8.30±0.30), except for T1 (7.35±0.25) where there was a reduction in the concentration observed.

Seminal stress enzymes

In Table 8, all parameters evaluated were significantly different (p<0.05). T0 value (112.50±0.50) for AST was significantly higher (p<0.05) than the other treatments with the lowest concentration observed in T2 (37.40±0.60). The same trend was observed in the values recorded for ALT. ALP value for T0 was statistically lower (p<0.05) than the other treatments.

Ovarian stress enzymes

Except for T1, all other treatments were significantly higher (p<0.05) than the control, T0. T0 value for ALT was significantly lower (p<0.05) compared to other treatments while an opposite trend was observed for ALP values where T0 was significantly higher than the other treatments

(Table 9).

Sperm count and motility

The concentration of sperm ranged from 60.50 to 78.50 in T2 and T0 respectively with T0 significantly higher ($p < 0.05$) than the other treatments. Motility significantly reduced ($p < 0.05$) in all treatments except in the control, T0. The percentage of sperm with dropped tail significantly reduced ($p < 0.05$) compared to the control. The highest percentage of sperm with detached heads was recorded in T3 (56.40 ± 0.40) and T1 (40.60 ± 0.40) had the lowest percentage which is significantly lower than the control, T0 (54.65 ± 0.35). Free tailed sperm values were statistically different ($p < 0.05$) across the treatments with the highest occurrence in T1 (66.55 ± 0.55) and lowest in T2 (38.80 ± 0.20). Bent tail sperms values were significantly different ($p < 0.05$), however, T0/T2 and T1/T3 were not statistically different ($p > 0.05$). The highest life to death ratio was recorded in T0 (42:5) while the lowest was in T3 (24:76) (Table 10).

Discussion

A wide range of chemicals enter the aquatic ecosystem from various sources (including industrial wastewater) and elicit responses that impair reproduction in fishes. According to Hajirezaee *et al.* (2010), the survival of a species is highly dependent on the reproductive success of the species in question and for economically relevant species, water quality monitoring is necessary to aid evaluation of challenges posed by the incidence of pollution. The physico-chemical parameters recorded for the textile wastewater used in this study were way below what could ensure fish survival, even at low concentrations. The low level of dissolved oxygen in the wastewater sample and exposure concentrations may be due to the high level of organic material in the effluent that requires high levels of oxygen for chemical oxidation and decomposition. This may explain the high level of COD in wastewater samples and may have grave implications for the survival of aquatic organisms that require a DO range of 5.40-8.50 mgL^{-1} for survival (Fakayode, 2005). Furthermore, acidic pH coupled with low DO may have a negative impact on the ability of fish to feed resulting in starvation and subsequent weight loss in parent fish. In addition, sub-lethal acid stress affects reproduction in fish. Baumann *et al.* (2012), Frommel *et al.* (2012) and Kroeker *et al.* (2013) stated that the stress of acidification induced various physiological and ecological problems in fish. They reported that when mature rainbow trout were reared in pH 4.5 just prior to spawning, the eyeing rate (index indicating normal development) of embryos from females exposed to acidic waters decreased drastically even when embryos were cultured in neutral water after fertilization. Total hardness is known to be a mixture of divalent salts; however, calcium and magnesium are the most common sources of water hardness.

The value obtained in the present study for the hardness of the wastewater samples was higher than the acceptable limit of 100 mg/L reported by WHO (2003). When industrial, municipal, and domestic sewage enters a surface water body, microorganisms begin to decompose the organic materials by consuming the dissolved oxygen (DO) of water. This can quickly deplete the available oxygen and subsequently affect aquatic life forms (Ekubo & Abowel,

2011). BOD is the amount of oxygen required by the microorganisms to decompose the organic substances aerobically in water. The Biochemical Oxygen Demand (BOD) and chemical Oxygen Demand (COD) obtained in the study were higher than the recommended level (3-20 mg/L and 50 mg/L) by Boyd (2003). A high BOD and COD are an indications of water pollution. The value for total suspended solids, turbidity, and alkalinity were beyond the permissible limits of 80 mg/L , 1.2 NTU, and less than 54-107 mg/L respectively, for fish culture, which might turn the water unsuitable for domestic, agricultural, and fisheries utilization (De, 1989; DOE, 1997; WHO 1984; 2003). The values recorded in the present study for chemical properties such as chloride, carbonate, bicarbonate, nitrate, nitrite and phosphate were above the recommended value (0.04-0.2 mg/l for chloride, 0.09 mg/l for carbonate, 99.90 mg/l for bicarbonate, 0.04 mg/l for nitrate, 0.01 for nitrite, 3.13 mg/l for chloride and 0.06 mg/l for phosphate) as reported by Durborrow *et al.* (1997), Boyd & Tucker (1998) and Tucker (1991).

Boyd & Tucker (1998) stated that increased concentration of these chemical properties leads to bioaccumulation. Metal like sodium, potassium, calcium, magnesium, copper, zinc, lead, iron, manganese, and mercury which occur in water in large or small amounts are important in the primary productivity of a water body. However, the values obtained in the present study were greater than the limit value (1-50 mg/L for potassium, 31-34.8 mg/L for calcium, 4.0-6.4 mg/L , 0.001-0.01 mg/l for copper, 0.01-0.05 mg/l for zinc, 0.0003 mg/l for hg, 0.004-0.008 mg/l for lead) recommended for metal as reported by Hem (1970), Syobodova *et al.* (1993) and FAO (1993); except for the values for iron, manganese, and sodium which falls between recommended levels (0.1-0.2 mg/l for iron, 20-200 mg/l and 5-252 mg/l). Bohl (1989) stated that an increase in the concentration of metals can cause damage to some vital tissues and organs in fish and may also have a harmful effect on reproduction. At very low concentrations, they reduce the viability of spermatozoa, reduce egg production and affect the survival rate of developing eggs and fry. In addition, behavioural responses such as uncoordinated swimming movement, hyperventilation, gasping and occasional darting movements in *C. gariepinus* broodstock exposed to different concentrations of textile wastewater, is an indication of the toxic effect of the pollutant. This deviation from normal behavior in fish was further confirmed when such responses were not observed in the control setup with no textile wastewater. Consistent with these reports are temporary loss of equilibrium, hyperventilation and hyperactivity in juvenile members of *C. gariepinus* exposed to textile industry effluent (Adeogun & Chukwuka, 2011). Little *et al.* (1993) also observed that behavioural changes in fish occur 75% earlier than the onset of significant mortality in fish. Some other authors have reported similar observations in different fish species. For example, Srivastava *et al.* (2007) reported hyper-excitation, convulsions and rapid opercula movement in *Labeo rohita* and *Channa punctatus* exposed to paper mill effluent. Pathan *et al.* (2009) observed hyper-excitation, erratic swimming, convulsions and jerky movement in *Rasbora daniconius* exposed to paper mill effluent.

Ankley *et al.* (2001) reported that the reproductive output

of species is an integral endpoint in fish toxicology studies and an important aspect of risk assessment for aquatic ecosystems. The dynamics of synthesis and interrelationships of androgens and the control of their bioavailability are very essential to the regulation of pubertal development in male African catfish (Cavaco, 2005). Among other features of sperm physiology that are considered crucial to reproductive success, sperm motility and volume are two of the most important factors for determining embryonic hatching success. The significant decreases recorded in sperm motility and milt volume of male broodstock in all exposure concentrations compared with control exposure could be attributed to altered morphology and reduced energetic reserves and quality of spermatozoa. Alterations in reproductive function as shown by plasma androgen concentration and reduction in sperm motility were recorded by Runnals *et al.* (2007) on exposure of the fathead minnow fish *Pimephales promelas* to clofibrac acid. Hernandez-Ochoa *et al.* (2005) also reported that low levels of lead in spermatozoa and seminal fluid resulted in decreased semen quality in Mexican human male population. Other reports have shown that metals have been associated with reduced semen quality in rodents and humans (Kumar *et al.*, 2005; Wirth *et al.*, 2007). In freshwater fishes, Ofor & Udeh (2012) reported that sperm mortality caused low fertilization.

In this study, significant reduction in plasma levels of the hormones – testosterone, estradiol and prolactin was observed. This corroborates the study of other endocrine disruptors in African catfish (Akbarsha *et al.*, 2000; Aire, 2005; Sayed *et al.*, 2012; Ozegbe & Aina, 2012). The seminal total protein level, in the current study, increased in the different wastewater treatments compared to the control. Similar results were found in *C. gariepinus* where total protein level elevated with the presence of high concentrations of cadmium (Gaber *et al.*, 2013). However, seminal cholesterol level increased in the wastewater treatments compared to the control. This is not in agreement with the result obtained in *C. gariepinus* maintained in cadmium contaminated water for a period of 14 days (Samuel *et al.*, 2017). The significant increase between treatment groups in glucose concentrations may be considered to be the manifestation of stress condition probably due to the toxic nature of the textile waste water. The increase in the glucose and cholesterol level of the tissue in exposed fish clearly indicated that the glycogen reserves are being used to meet the strain caused or it may be the results of degradation of protein under stress. This agrees with Sepici-Dincel *et al.* (2009) and Cicik & Engin (2005), who reported increase in serum glucose levels in fish under stress. Das *et al.* (2004) reported decreased activities of AST and ALT in Indian major carps exposed to nitrite toxicity. They suggested that the decrease of transferases is as a result of diversion of alpha-amino acids in the tricarboxylic acid (TCA) cycle as keto-acids to augment energy production. The lowered activity of seminal AST and ALT in this study showed that inactive transamination and oxidative deamination occurred.

Reports have indicated that the quality of fish sperm may be as important as the quality of fish eggs to achieve viable progenies and subsequent larval survival (Ajavi *et al.*, 2008; Oguntuase & Adebayo, 2014). Results revealed in the present study agree with Delistraty and Stone (2007) who observed that low concentrations of lead negatively

impacted fish health and reproduction. Banaee *et al.* (2011) reported that increased activity of ALP and creatinine in the plasma of *Clarias buthupogon* and *Heterobranchius longifilis* indicate that long-term exposure to chemical pollutants cause tissue damage in fish and this has also been reported in *Oncorhynchus mykiss* and *Channa punctatus*, exposed to diazinon and monocrotophos respectively. In the present study, it revealed that chronic exposure of *C. gariepinus* to textile wastewater resulted in the elevation of the activities of ovarian AST and ALT which may indicate the degenerative changes and hypo-function of liver due to the effect of toxicants on the hepatocytes which result in tissue damage. This is in support of the findings of Natarajan (1985) and Tiwari & Singh (2004) who were of the opinion that stress is generally known to elevate aminotransferase in fish. They opined that exposing fish to contaminated water distorts enzymatic activities in the fish. The ovarian mineral constituents of *C. gariepinus* exposed to a varying proportion of textile wastewater revealed that potassium, calcium, and magnesium ion concentrations tends to increase in the T3 and T4. The increase tends to support the report of Booth *et al.* (1982) who stated that elevation in mineral constituents is attributed to the decline in the extracellular space. This suggests that the obstruction might have been a result of the high percentage of wastewater which is detrimental to the metabolic system of the fish. Also, Shaanmugam (1993) reported that elevation in the concentration of calcium and magnesium resulted in the impaired ability of fish to dynamically emit an excess of calcium ions through kidneys. The increment in the divalent cations like calcium and magnesium may be result in damage to the skin injury membrane permeability (Rafia & Foozia, 2013).

CONCLUSION

The short-term exposure to textile wastewater caused a varying degrees of damage to the reproductive health of fish. These findings justified to an appreciable extent, the absence of gonads in fish caught in Itoku tributary along the Ogun River. Textile production generates income, hence boosting the economy. However, wastes from this industry should be efficiently treated and disposed of in order to preserve the fishery and aesthetic value of the affected tributary. In addition, bio-accumulation of heavy metals in fish tissue can cause public health issues for consumers of fishery organisms from this water.

AUTHOR'S CONTRIBUTIONS

AFD is doing research, ideas, data generation, NAB is written manuscript and data analyze, MD manuscript preparation. ARM is written manuscript.

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