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Effects of Toluene on Some Physicochemical Parameters of The Test Water, Reproductive, Hatching Success and Growth Performance of *Clarias gariepinus* (Burshell, 1822)

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ABSTRACT: The effects of toluene on the reproductive, hatching success and growth performance of *Clarias gariepinus* was investigated. The study lasted for 12 weeks with 6 varied concentrations including the control. The concentrations were 0.001%, 0.0015%, 0.002%, 0.0025%, 0.003% and 0.00 which served as the control. During the study, some physicochemical parameters (pH, Temperature, dissolved oxygen and conductivity) were monitored. The results showed that there were variation in these parameters but the differences in mean physicochemical parameters were not statistically significant ($P > 0.05$) apart from conductivity which had a significant differences in mean values ($P < 0.05$). The control (0.00%) recorded the least mean conductivity value of 8.0±0.00 while the 0.001% concentration had the highest with 18.66±0.00 uS/cm. Retarded growth was observed in the study when comparing the specific growth rate of the control (SGR = 6.86%) with those of the exposed groups (4.56, 4.48, 4.47, 4.36 and 4.36) being values for 0.001%. 0.0015%, 0.002%, 0.0025% and 0.003% respectively. The difference between the SGR of the control when compared with the treated groups was statistically significant ($P < 0.05$). Reproductive success analysis showed a decrease in hatching success across the concentrations and high mortality of eggs with increased concentration. The control had 36.6% hatching success, while 0.001% and 0.003% concentrations had 36.1% and 5.0% respectively. It can be concluded that toluene has an adverse effects on the reproductive, hatchability success and growth performance of *C. gariepinus*. Therefore, it should be used with restraint and discharges containing toluene and other hydrocarbon compound should be properly treated before released into the environment.

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In Nigeria, exploration and exploitation of crude oil has been on the increase due to the Country's dependence on oil to boast and sustain her economy (Balogun and Karem, 2013). This scenario has resulted in oil spills which have caused much harm to aquatic organisms, decline in the type of exotic fish species and reduced yield from aqua cultural efforts (UNDP, 2010).

More so, large volumes of petroleum products such as gasoline, engine oil, naphthalene, benzene, toluene and other industrial effluents are either directly or indirectly discharged into the aquatic environment thereby causing ecological imbalance in the ecosystem (Kori-Siakpere, 2000).

Many aquatic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and their halogenated forms are chemically quite unstable. Owing to their lipophilic nature they can easily penetrate biological membranes and accumulate in organisms. PAHs are the most toxic among the hydrocarbon families. The United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) have identified 16 PAHs as priority pollutants such as toluene, while some of these, e.g. benzo (a) anthracene, chrysene, benzo (a) pyrene are considered to be potential human carcinogens (Catoggio, 1991).

Toluene (methylbenzene, toluol, phenylmethane) is an aromatic hydrocarbon (C_7H_8) commonly used as an industrial solvent for the manufacturing of paints, chemicals, pharmaceuticals, and rubber. Toluene is found in gasoline, acrylic paints, varnishes, lacquers, paint thinners, adhesives, glues, rubber cement, airplane glue, and shoe polish. At room temperature, toluene is a colorless, sweet smelling and volatile liquid (McKeown, 2015).

Oil pollution is one of the environmental constraints that have produced adverse effects on the wellbeing of aquatic lives and their reproductive capacities (Kori-Siakpere 2000). An array of pollutants including crude oil and its related products are known to induce stress conditions, which impair the health of fish (FEPA, 1991). Ekweozor (1989) reported that frequent spillages of crude oil and it products in creeks and rivers may have resulted in a marked reduction in the number of both freshwater and marine creatures. Earlier reports have also shown that oil pollution impact negatively on fishery resources (Afolabi *et al*., 1985). Azad (2005) observed that eggs and young stages (fingerlings) of fishes are most vulnerable to the toxic effects of crude oil and its allied products.

According to Adeola (1996), Nwabueze and Agbogidi (2010) has resulted in the reduction of rich fisheries resources of Nigerian coastal waters. Jack *et al*. (2005) reported that significant levels of total hydrocarbons were found in shellfishes in crude oil polluted stations in the Niger Delta area when compared with unpolluted sites. The eco-physiological effects of crude oil on *Machaerium lunatus* had also been reported by Bamidele and Agbogidi (2006).

Reproduction is one of the most vital functions performed by any organism. Many studies have shown that contaminant exposure causes a negative impact on some reproductive processes in fish. Some studies with petroleum and crude oil have demonstrated the potential effects of PAHs on fish reproduction. These studies have shown decreased gametogenesis, decreased gonad size and lowered egg production (Payne *et al*., 1988; Fletcher *et al*., 1998; Kiceniuk and Kahn 1981; Nwabueze and Agbogidi, 2010).

It was revealed that anthracene bio-concentrated more in the ovaries of fathead minnows than in the testes (Tilghman and Oris 1991). Reproductive output, measured as the mean number of eggs laid was also reduced in the antracene-treated fish. Hatching success was lowered in eggs treated with antracene. Although many workers have reported the effects of various PAHs on aquatic lives, there is paucity of information on the effects of PAHs especially toluene on *C. gariepinus* which is a catfish common in Nigerian waters and the most cultured fish species in Nigeria. The role of fisheries in human diet cannot be underestimated since it is a cheap source of protein in the Niger Delta area, the hub of oil industrial activities in Nigeria. The present study was undertaken to evaluate the impact of toluene at various concentrations on the reproductive, hatching success and growth performance of *C. gariepinus*.

MATERIALS AND METHODS

Experimental Animals: The broodstocks used in this study were procured from African Regional Aquaculture Centre (ARAC) Aluu, Rivers State. They were transported in the morning hours to the laboratory of Animal and Environmental Biology Department, University of Port Harcourt in airbags with pond water from the farm to avoid heat exertion. The fish were later transferred immediately into plastic aquaria after measuring their length and weight. They were acclimatized for 14 days. The holding tanks were aerated, cleaned and water was regularly renewed (Reish and Oshida, 1987). The hatched fry from the control were used for the growth performance test.

Test Material: The test material, toluene was obtained from a chemical shop in Port Harcourt, Rivers State and transported to the laboratory in corked bottles. It was stored in a cool dry place prior to use. A known volume of the toxicant (toluene) was added to a known volume of dilution water using pipette and thoroughly mixed for 30 minutes using iron stirrer to ensure sample homogeneity. After the mixing, five different concentrations of the toxicant were prepared after an initial range test to determine the initial safe concentration to be used (Vincent-Akpu, 2001). From the determined safe concentration, the following five concentrations were obtained 0.001%, 0.0015%, 0.002%, 0.0025% and 0.003% while 0.00% served as the control group. Complete randomized design was carried out (Ogbeibu, 2005). Eighteen plastic aquaria were used with 3 replicates each.

Physicochemical Parameters: The dilution water and test solution were tested for the following parameters: temperature, dissolved oxygen (DO), pH, Conductivity and TSS using standard methods (APHA, 1998). Measurements of DO was done using Winkler's method while temperature and pH were done using a pH meter and inbuilt glass thermometer respectively. The Mi 806 model combined meters was used to determine the conductivity; the meter was allowed to stabilize for 15 minutes and calibrated with KCl solution. The electrode was rinsed with distilled water and then dipped into the water sample which later stabilized and the results recorded whereas Total dissolved solids (TSS) were determined with Mi 806 model where the probe was dipped into 30ml of test water sample in a 60ml beaker; values for TSS were read from the meter.

Procedures for Measurement of Reproductive and Hatching Success: Eight (8) females and five males (5) broodstock of *C. gariepinus* were procured, taken to the laboratory and acclimatized. They were weighed and measured to determine the weight and length. The eight females were then injected in the evening with prepared hormones (ovaprim) of 0.5 ml/2kg of the fish to trigger ovulation. The injected females were stripped after 10 hours. The five males were sacrificed by a blow on the head and milt removed from the testes. Ten percent (10%) normal saline was used to dilute the extracted milt. The milt was then mixed with the stripped eggs to fertilize the eggs with the aid of feathers. The fertilized eggs were spread on Karkarbans submerged in the various concentrations of the test solution (0.001%, 0.0015%, 0.002%, 0.0025% and 0.003%) including the control. 10 ml of live eggs were exposed in each tank, dead eggs were siphoned using rubber hose and counted while the live larvae were also counted and recorded after the 96 hours.

Measurement of Growth Performance: Each Aquarium was stocked with 10 fish of known average weight for fry, fingerlings and juveniles. The fingerlings were fed with coppens feed (45% protein). A fixed feeding regime of 5% body weight per day was employed for the first 8 weeks and 4% body weight was employed for the next 8 – 12 weeks. The fish was fed twice daily, with ration divided in equal portion for 6 consecutive days and weighed in water without anaesthesia on the 7th day. The feeding regime was adjusted accordingly when necessary. The weight of the fish was measured with a top loader weighing balance (Metter PN 163) and length taken using measuring board. Tow fish were randomly selected from each test solution and weighed. The average weight was calculated and used as weight per concentration. The data was computed for weight mean gain, relative growth rate, specific growth rate and daily growth rate (Sikoki and Ekwu, 2000).

Data Analysis: Data was reported as means values. The results on growth and reproductive process were analyzed using one-way analysis of variance and their regression values obtained. All analysis was carried with the aid of Statistical Package for Social Sciences (SPSS) version 2.0.

RESULTS AND DISCUSSIONS:

Physicochemical Parameters: The study shows that the temperature was relatively the same across the different groups. The study showed increase in pH values with increased concentrations. The study also showed that DO was not affected negatively by the toxicant as the values were relatively stable across the group with the control even having the least DO value of 6.8±0.13. There was a great variation in the conductivity with the least value recorded in the control with 8.0±0.00 uS/cm and the highest value recorded in the 0.001% concentration of 18.66±0.00 uS/cm. The difference in mean conductivity values were statistical significant (P < 0.0%) (Table 1)

Table 1: Physicochemical Parameters of the different concentrations

Parameters	**Concentrations (%)**					
	0.00	0.001	0.0015	0.002	0.0025	0.003
Temperature (^{o}C)	26±0.00	26±0.00	26±0.00	26±0.00	27±0.00	28±0.00
pH	6.9±0.00	7.8±0.91	6.9±0.00	7.6±0.90	8.1±0.66	8.2±5.00
DO (Mg/l)	6.8±0.13	7.2±0.76	7.2±0.72	7.2±0.56	7.3±0.34	7.3±0.33
Conductivity (uS/cm)	8.0±0.00	18.66±0.00*	17.66±4.50*	16.33±5.33*	16.33±6.80*	16.00±7.81*

* The mean difference is significant at 0.05 levels.

The study shows that most of the physicochemical parameters such temperature, Dissolved oxygen, pH and conductivity of the test water (Table 1) were favorable to support growth and survival of *C. gariepinus*. This result is however in agreement with the reports of Vincent-Akpu, 2006, Bolarin and Halton, 1979 who reported that the physicochemical parameters of their test water supported the growth and survival of test animal (Tilapia). The study showed increase in pH values with increased concentrations. The study also showed that DO was not affected negatively by the toxicant as the values were relatively stable across the group with the control having DO value of 6.8±0.13. There was a great variation in the conductivity with the least value recorded in the control with 8.0±0.00 uS/cm and the highest value recorded in the 0.001% concentration of 18.66±0.00 uS/cm.

Growth Performance of C. gariepinus Fingerlings Exposed to Toluene for 12 Weeks: The results of the growth performance are presented in Table 2 and Fig 1. The results revealed that the mean length and weight of the *C. gariepinus* fingerlings exposed to toluene solution were affected as their growth were retarded. The various concentrations showed various impacts on the growth of the fish.

The highest growth rate was recorded in the control medium which showed a consistent growth in weight. The growth rate ranged from 3.8g to 4.5g of weight under 12 weeks.

Table 2: Growth Performance of *C. gariepinus* Fingerlings Exposed to Toluene for 12 Weeks

Growth Parameters	Concentrations (%)					
	0.00	0.001	0.0015	0.002	0.0025	0.003
Initial Mean Weight	3.6	3.7	3.6	3.8	3.7	3.8
Final Mean Weight	5.8*	3.9	3.8	3.8	3.7	3.7
Mean Weight Gain	2.2*	0.2	0.2	0.0	0.0	-0.1
Specific Growth Rate	6.86*	4.56	4.48	4.47	4.36	4.35

* The mean difference is significant at 0.05 levels

Growth is usually considered as the increase in size, weight or length of a tissue, an organ or the entire mass of an organism. The mean final weight and mean weight gained reveals that there was a steady and gradual increase from the highest concentration to the control within the study time. Weight gained is considered as an important parameter for measuring responses to experimental feed and a very reliable indicator of growth (Ogbe *et al*., 2005; Sikoki and Ekwu, 2000).

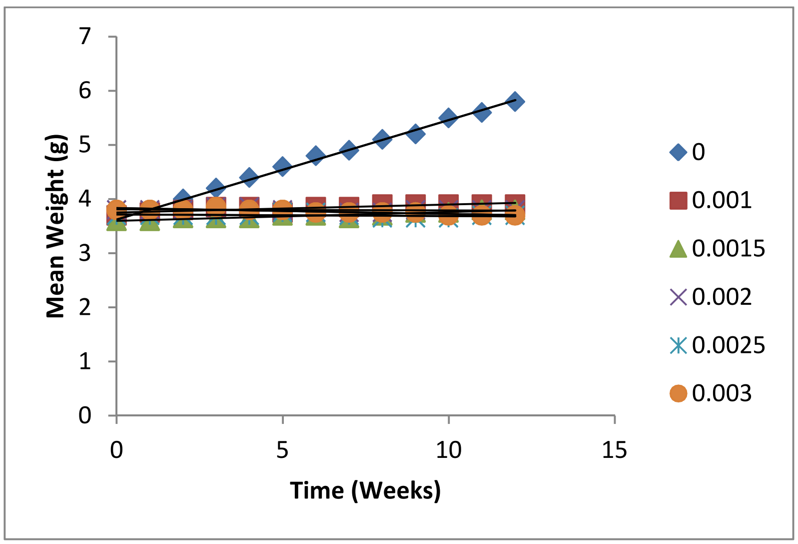

Fig 1: Plot of mean weight of fish against duration of exposure to different concentrations of toluene (time)

There were significant differences (P < 0.05) for the final weights of most of the concentrations showing that the various concentration of the toxicant had negative effects on the growth of the fish revealing that their growth was affected by exposure to toluene. Moreover, dose dependent effects of toxicant on growth have been documented (Patcher *et al*., 1981). The authors concluded that the decrease in growth of fish is mostly due to loss of appetite and not by metabolic changes. Their reports however, oppose the reports of Adiukwu (2003), Sikoki and Ekwu (2000) who opined that decrease in growth is due to metabolic changes induced by increase in plasma glucose.

Effects of Toluene on the Reproductive and Hatching Success of C. gariepinus: The results of the reproductive success showed a decrease in number of live eggs with increased concentration. The live eggs ranged from 50 to 361 in different concentrations with highest in the control (386 eggs) after the exposure. The 0.001% concentration had the second highest survived eggs. Whereas, 0.003% had the least survived eggs (50) which shows that toluene had effects on the reproductive success of *C. gariepinus* after 96 hours exposure.

The live eggs were identified by observation and appearance of larvae in the medium, while the dead eggs were found floating on the surface water as white substances and were counted (Table 3).

Table 3: Effects of Toluene on the Reproductive and Hatching
Success of *C. gariepinus*

Concentration (%)	Live Eggs	Percentage (%)
0.00	386	38.6
0.001	361	36.1
0.0015	327	32.7
0.002	280	28.0
0.0025	274	27.4
0.003	50	5.0

The results also showed a similar trend for the dead eggs which were sieved, counted and recorded. The dead eggs ranged from 152 to 242 in the various concentrations. In other words, increased concentrations resulted in increase in number of dead eggs with exception in 0.001% concentration which had an average of 205 dead eggs. The summary of these results is presented in Table 4 below.

Table 4: Dead eggs across the different concentrations

Concentration (%)	Dead Eggs	Percentage (%)
0.00	20	2.0
0.001	205	20.5
0.0015	152	15.2
0.002	180	18.0
0.0025	199	19.9
0.003	242	24.2

Effects of Toluene on Reproductive and Hatching Success of C. gariepinus: The reduction in hatching success and increased number of dead eggs of *C. gariepinus* observed in this study is in conformity with other reports. For example, Hawkes and Stehr (1982) in investigating the effects of crude oil on fish eggs, reported that the susceptibility of these early developmental stages such as eggs, larvae and juvenile fish to oil in surface water was high. Also, that hatching success was 40% lower in treated tanks than in the control and cellular formations were from 27 day –old embryos. Longwell (1978) also reported that 20% of cod eggs and 46% of Pollock eggs were dead or moribund after the wreck of the merchant off the East Coast of USA in 1976. Within the oil slick, Pollock embryos were grossly malformed in 18% of eggs. At the edge of the slick, 9% were grossly abnormal, but at greater distances, no abnormalities were found and no Cod – eggs were deformed. The dead eggs or moribund displayed a combination of cytological abnormality of the embryo cells or of the nuclear configurations, coupled with division arrest.

Tilseth *et al*., (1981) also reported that oil concentrations of 50ml/L in the immediate vicinity of the Agro merchant that there was a reduction in growth and change in buoyancy of Cod – eggs and larvae after 14 days exposure while at 250ml/L, malformations of head and jaws were recorded..

More reports supporting the results of this study showed that the water soluble fraction reduce the hatching success of fertilized Capeton eggs at concentrations of 25ml/L. Also, that 50 – 90% of Pilchard eggs died and juvenile's fish scale in plankton samples collected in the vicinity of the wreck after the Torryey Canyon incident (Johannessen, 1977; Smith, 1970). Most of these abnormalities could be attributed to alteration in the embryos cells or nuclear configurations occasioned by the toxicant effects. Other researchers have also reported that pacific herring eggs exposed for 16 days to weathered Alaska North slope crude oil in initial aqueous concentration of 0.7ppb polycyclic aromatic hydrocarbons (PAHs) caused malformations genetic damage, mortality and decreased size and inhibited swimming whereas 0.4ppb concentration caused sublethal responses such as yolk sac oedema and immortality consistent with premature hatching (Carls *et al*., 1999).

Exposure of herring eggs to petroleum hydrocarbon frequently results in small abnormal larvae with poor survival potential (Pearson *et al*., 1992). Low hatchability and high eggs mortality observed in this study is similar to the reports of several workers on the influence of petroleum hydrocarbon on the reproductive potential of fishes. Many studies have shown that contaminant exposure causes a negative impact on some reproductive success in fish ranging from decreased gametogenesis, decreased gonad size, lowered egg production and hatchability (Hedke and Puglisi 1980; Fletcher *et al*., 1982; Kiceniuk and Kahn, 1981).

*Conclusion***:** It can be concluded that toluene has an adverse effects on the growth performance and reproductive/hatchability success of *C. gariepinus*. Since toluene have these effects, it should be used with restraint and discharges containing toluene and other petroleum hydrocarbon components should be properly treated before releasing it into the environment.

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