E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(1): 41-43

www.fisheriesjournal.com
Received: 21-11-2019
Accepted: 25-12-2019

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Evaluation of the hatching and growth performance of *Clarias gariepinus* exposed to benzene (Burchell, 1822)

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Abstract
The study was conducted to determine the effects of benzene on the hatching and growth of *Clarias gariepinus*. The experiment was carried out for a period of 5 weeks. The brood stock (two males and two female of *Clarias gariepinus*) used for hatching the eggs were maintained for 3 weeks on a diet of 40% crude protein (3% body weight). The research was carried out in triplicate of five different concentrations of Benzene in addition to the control in eighteen plastic bowls with ne following concentrations of Benzene: 0ml/l (control), 0.03ml/l, 0.06ml/l, 0.09ml/l, 0.12ml/1 and 0.15m1/l respectively. The results showed that 0. 15m1/l concentration of benzene caused 100% mortality to the exposed eggs after the 5th day while 0.12ml/l caused 100% mortality after the seventh day. The research revealed that the toxicant; benzene, can affect both the hatching and growth of *C. gariepinus*. However, at a concentration of 0.12ml/l of C_6H_6, there was no observable increase in length after week 1 and 2. This was also observed at a concentration of 0.15ml/l of C_6H_6 for week 1 and 2. The highest mean length observed was 1.5 cm at a concentration of 0ml/l of C_6H_6. The study therefore opined that benzene should be used with restraint and prevented from entering into the environment in order to reduce hatching success and retarded growth performance leading to reduction or shortfall in annual fish yield in the wild.

Keywords: benzene, *Clarias gariepinus*, broodstock, concentration, hatching

Introduction
The aquatic environment is a reservoir of toxic chemical which find their way into lake, rivers, and sea, through industrial, domestic and agricultural activities. The declining market value and poor circulation of nation's wealth had led to youth restiveness and vandalism of petroleum pipeline resulting in oil spillage causing much harm to aquatic organisms leading to a decline in fish species and reduce yields from aquaculture products [11]. The effects of oil spillage in aquatic environment may involve subtle changes that over a long period could damage the composition of aquatic ecosystem or damage their ability to survive [9]. Petroleum spillage in aquatic environment prevents oxygen penetration which may lead to death of fishes and other aquatic organisms, and putrefaction of underlying vegetation, and release of hydrogen sulphide [1].

There are large volume of derived petroleum products which includes gasoline, toluene, benzene, naphthalene, engine oil, phenol and other industrial effluents which are indirectly or directly discharged into the aquatic environment thereby causing imbalance in the ecosystem. These imbalances on aquatic lives are caused by either the physical nature of the spilled petroleum product (physical contamination and smothering) or by its chemical components (toxic effects and accumulations leading to tainting). The effect of benzene which is one of the active chemical components in petroleum (crude oil) on the hatching and growth performance is our major concern in this study.

Life continuity of African catfish depends on the laying of eggs and successful hatching and growth of the fish. *C. gariepinus* is air-breathing catfish found throughout African and the Middle East. They are freshwater dweller and live in lakes, river, streams as well as human-made habitats. *C. gariepinus* are large, eel-like fish usually of dark grey or black colour on the back fading to a white belly. This study therefore seeks to review the effect of benzene toxicant on the hatching and growth of *Clarias gariepinus*.

Materials and Method

Sample collection and acclimatization

Adult catfishes *Clarias gariepinus* were collected from African Regional Aquaculture Centre (ARAC) Aluu, Rivers State and then transported in the early morning hours with airbags with pond water to University of Port Harcourt Choba Fish Farm, of the Fisheries Department, Faculty of Agriculture. The broodstock which comprised of two male and two females (within 1500g — 1800g) were fed with a commercial pellet diet (3% of body weight per day) and kept in plastic holding tank containing tap water (temperature 28°C, pH 7,dissoived oxygen 7.25mg/l, alkalinity 22.0mg/i). After 3 weeks of acclimatization, fishes were used for the experiment.

Identification of suitable organism

In the experiment, two males and two female fishes were selected based on the external morphological features which the matured male fish was identified by a slightly pointed genital papilla and females by a swollen abdomen and reddish swollen vent. In addition, the maturity of the ripe female was confirmed by a slight pressing of the ventral side of the fish for oozing of eggs.

Hormone administration and collection of gametes

The females were artificially induced by intra-muscular injection with 0.2m1 of ovatide hormone (product) per kilogram body weight. After 14 hours of hormone administration, eggs were stripped into circular plastic bowl and the testes were removed from the male fish and sperm was pressed into a dry sterile petri-dish. Stripped eggs were then fertilized with the normal saline diluted sperm suspension. After 2mins of gentle stirring, the fertilized eggs were weighed and 1 gram of egg which was equivalent to average of 280 eggs were immediately introduced into the different concentrations of benzene treated water for the eggs bioassay test.

Experimental setup

Percentage Concentration of Benzene in the test water

Table: Percentage Concentration of Benzene in the test water

% of C_6H_6 used	Conc. in/l of water	Conc. in ml/17ltrs of water
0.1	0.03	0.5
0.2	0.06	1.0
0.3	0.09	1.5
0.4	0.12	2.0
0.5	0.15	2.5

The stock solution was prepared by addition of the following concentration of Benzene per liter of water. Six different concentrations used include 0ml/l, 0.03ml/l, 0.06ml/l, 0.09ml/l, 0.12ml/l and 0.15ml/l for hatching success and growth performance to be ascertained. To obtain benzene toxicity, the average of 280 fertilized eggs were randomly selected and exposed to the respective concentrations, each experimental treatment and control was executed in three replicates. The experiment was conducted using 17liters of water in each experimental plastic bowls and mortality counts recorded for benzene exposed group as well as the control.

Results

The study reveals a relative stable physico-chemical water quality of test water as presented in Table 1. Temperature was at optimum with a value of 28^0C, dissolved oxygen was 7.25mg/l, pH was 7 (neutral), conductivity was 110mg/l and alkalinity was 22.0mg/l. These values were within World Health Organization (WHO), limits.

Table 1: Physico-chemical parameters of test water

Initial Water Quality Parameters	Results/Values
Temperature	28^0C
Dissolved oxygen	7.25mg/l
pH	7
Salinity	0
Conductivity	110mg/l
Alkalinity	22.0mg/l

Mortality count of eggs and fry at different benzene concentrations, at different time interval were considered, Table 2. The egg stage lasted between 0-48hrs which indicated that the eggs were not uniformly hatched. One gram of eggs (280 eggs) were distributed into the 18 different experimental bowl (control and benzene exposed bowl), only unfertilized eggs which was white in colour were recorded as mortality count and delay in the hatching process was also observed.

Table 2: Mortality count of the eggs and fry at Different Concentrations, at different time interval.

Time of Monitoring	Benzene Concentration and Mortality Count					
	0mg/l	0.03mg/l	0.06mg/l	0.09ml/l	0.12ml/l	0.15ml/l
24 hrs (Day 1)	56	61	50	42	31	25
48 hrs (Day 2)	2	107	132	176	235	246
72 hrs (Day 3)	1	3	3	3	5	6
92 hrs (Day 4)	0	3	3	4	3	3
120 hrs (Day 5)	1	1	2	1	3	0
144 hrs (Day 6)	1	0	1	2	2	0
168 hrs (Day 7)	1	1	1	2	1	0
192 hrs (Day 8)	0	2	2	3	0	0
336 hrs (Day 14)	0	0	5	7	0	0

The result on Table 3 showed that hatching was delayed in the experimental bowls with benzene exposure. At the end of the 14 days of the experiment the control recorded the highest hatching success with the least survival in 0. 09m1/l concentration. Similarly, at a concentration of 0.15ml/l, there was no hatching between 96hours and 336 hours exposure.

Table 3: Hatching Success of eggs.

Time of Monitoring	Hatching Success at different Concentration					
	0mg/l	0.03mg/l	0.06mg/l	0.09ml/l	0.12ml/l	0.15ml/l
24 hrs (Day 1)	224	-	-	-	-	-
48 hrs (Day 2)	222	112	98	62	14	9
72 hrs (Day 3)	221	109	95	59	9	3
92 hrs (Day 4)	221	106	92	55	6	0
120 hrs (Day 5)	220	105	90	54	3	0
144 hrs (Day 6)	219	105	89	52	1	0
168 hrs (Day 7)	218	104	88	50	0	0
336 hrs (Day 14)	218	102	86	47	0	0

Growth is considered as increase in length from Table 4, the length increased from the highest concentration of exposed tanks to the control within the study time. At a concentration of 0.12ml/l of C_6H_6, there was no observable increase in length after week 1 and 2. This was also observed at a concentration of 0.15ml/l of C_6H_6 for week 1 and 2. The highest mean length observed was 1.5 cm at a concentration of 0ml/l of C_6H_6.

Table 4: Growth performance of *C. gariepinus* fingerlings exposed to benzene for 2 Weeks using Total Length (cm) as the measurement parameter.

Week	Length of Fish Sample at different Concentration					
	0ml/l of C_6H_6	0.03ml/l of C_6H_6	0.06ml/l of C_6H_6	0.09ml/l of C_6H_6	0.12ml/l of C_6H_6	0.15ml/l of C_6H_6
Week 1	1.2cm	1.0cm	0.9cm	0.8cm	-	-
Week II	2.7cm	2.1cm	2.8cm	1.8cm	-	-
Mean Length Gained	1.5cm	1.1cm	1.1cm	1.0cm	-	-

Discussion

The study demonstrates that organisms in the early stages of embryonic development are usually more sensitive to toxicological effect. Thus examining organism at these stages can help evaluate the sub lethal effect of toxicants distinguishing the nature of the toxicological effects. The acute toxicity of benzene on the hatching success of African catfish fertilized eggs presented above indicate the increasing mortality rate with increasing concentrations from 0.03 to 0.1 5m1/1 and the hatching success of eggs increased with decrease in concentration of toxicant (Benzene). More reports supporting the result of this experiment showed that the water soluble fraction reduced the hatching success of fertilized capeton eggs at concentrations of 25m1/l, also50-90% and pilchard eggs died and juveniles' fish scale in plankton samples collected in the vicinity of the wreck after the Torryey canyon incident [5, 10]. Exposure of herring eggs to petroleum hydrocarbon frequently results in small abnormal larvae with poor survival potential [7]. Contaminant exposure causes negative impact on some reproductive success in fish rearing ranging from decrease gonad size, lower eggs production and hatchability [4, 3]. The increasing number of Mortality of eggs of *Clarias gariepinus* observed in this study is in Conformity with other reports which says that increasing toxicity of Chemical (Crude oil products) adversely affect the early stages of fish. Growth is considered as increase in length. The length increases from the highest concentration of exposed tanks to the control within the study time. Length gained is considered as important parameter measuring responses to feed intake and reliable indicator of growth [6, 8]. The result of the growth performance is however in agreement with the report of Daniel and Edafe [2], who reported that the mean length of *C. gariepinus* fingerlings exposed to toxicant (toluene solution) were affected as their growth were retarded with increase in the concentration of toxicant.

Conclusion

The negative effect of benzene on the eggs of *Clarias gariepinus* increases with increasing concentration and exposure time, structural abnormalities were also slightly observed (such as head shape, hemorrhages were found in the group exposed to benzene). Various concentrations of toxicant had a negative effect on the growth of fish. Consumption of the benzene accumulated aquatic organisms from polluted aquatic habitat may pose harmful effects on human health. This implies that there is need to ensure proper treatment and disposal of benzene contaminated water and industrial effluents into our water bodies.

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