

EFFECT OF CHRONIC EXPOSURE TO WATER SOLUBLE FRACTIONS OF FORCADOS CRUDE OIL ON THE GROWTH AND DEVELOPMENT OF AFRICAN CATFISH (*Clarias gariepinus*) LARVAE

O. O. FAFIOYE

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ABSTRACT

Clarias gariepinus eggs were artificially fertilized and incubated in water (oxygen: 4.3 ± 1.2 mg/l; Temperature: $26.5 \pm 1.0^\circ\text{C}$; alkalinity 27.5 ± 0.7 mg/l; pH: 6.7 ± 0.3) containing sublethal concentrations (1.0 and 1.5 mg/l) of water soluble fractions of Forcados crude oil and control. Larvae were reared for ten days, while yolk sac metamorphosis and growth rate were compared and the mortality rate evaluated daily. Egg hatching ranged between 30-36 hours in control and 30-45 hours in treated concentrations indicating the toxicant as a possible egg hatching inhibitor. Delayed hatching and a reduction in the percentage of hatched eggs in the exposed eggs compared to the unexposed eggs clearly demonstrated the negative impact of the Forcados crude oil.

KEYWORDS: Metamorphosis, larvae, growth, hatching.

INTRODUCTION

Oil is a complex mixture of substances and its composition varies from place to place depending on age and condition of formation (Smith, 1972). Oil spillage and pollution are possible consequences of the oil exploration with accompanying negative effects on fishery resources of Niger – Delta, Nigeria.

Fish reproductive, developmental and behavioural processes are very sensitive to exposure to hydrocarbons, while eggs and young stages are more vulnerable than adults (Sabo and Stegemann, 1977). Crude and fuel oils have been found to be toxic to fin fish eggs and larvae at concentrations 0.5 – 10ppm (Craddock, 1977; Brungs *et al.*, 1978). However, information on the controlled rearing and toxicity testing of catfish larvae is scanty and restricted to relatively few papers (Mitchell and Bennett, 1972; Hogendoorn, 1980; Hecht, 1981; Ufodike and Omoregie, 1991).

Clarias gariepinus is economically important in Nigeria and its growing for food and culture has led to increased interest in its exploitation. To supply fish for artificial spawning and rearing of the larvae of this fish, both natural and laboratory conditions are practiced on large scale (Bakiker, 1984).

However, high mortality occurs in both artificial and natural spawning of catfish due to the mining of Forcados crude oil (Ezenwaji, 1992). It is therefore necessary to investigate the impacts of water soluble fractions of Forcados crude oil on the reproduction and larval growth of *C. gariepinus*.

MATERIALS AND METHODS

Nine hundred fertilized eggs (mean diameter range = 0.92 – 0.98mm) attached to shredded synthetic bags were obtained from an artificially spawned single female *C. gariepinus*. The eggs were laid in aerated dechlorinated tap water at 26.0°C .

Two sublethal concentrations of 1.0mg/l and 1.5mg/l of Forcados crude oil, determined from a range finding test conducted for lethal toxicity of the same crude oil on catfish (Fafioye, 2002), and a control were used in triplicates. In a static renewal exposure test, 100 fertilized eggs were exposed to each 50-litre glass experimental chambers half filled with aerated dechlorinated tap water and oil. Experimental tanks were cleaned and the concentrations renewed every day. The

test media physico-chemical parameters measured include temperature ($26.5^\circ\text{C} \pm 1.0^\circ\text{C}$), dissolved oxygen (4.3 ± 1.2 mg/l), pH (6.7 ± 0.3) and alkalinity (27.5 ± 0.7 mg/l) for both incubation and subsequent experimental work.

During incubation, continuous aeration was applied to minimise stress and favour hatching. Hatching time of the eggs was monitored in each chamber on an hourly basis and the number of hatched eggs recorded. The behaviour of the larvae with regards to swimming and feeding, with the time of mouth opening, and mortality rate, were observed and recorded. Swim-up larvae (fry) were fed with ground dried chicken offal twice daily.

The relationship between larval growth and yolk sac depletion during the first ten days was studied by taking measurements of yolk sac and total length of ten larvae picked at random in each chamber (Henderson *et al.*, 1983). Larval total length was measured from the tip of the upper jaw to the distal end of the caudal fin, while yolk sac was measured along the longitudinal axis only (Henderson *et al.*, 1983). Daily mortality rate was recorded and dead fish were promptly removed.

Data on egg hatchability were analysed by standard deviation, while two-way analysis of variance (ANOVA) was used to examine the relationship between yolk sac depletion and larval growth and the oil concentration. Probabilities of *F* ratios <0.05 were considered significant as in the SPSS for Windows 2000 package.

RESULT

Hatching of eggs occurred between 30 and 36 hours in the control and between 30 and 48 in the treated chambers after fertilization (Table 1). Non-hatched eggs floated up after 36 hours of exposure, while the treated eggs were more buoyant than the untreated / control eggs. More eggs (38-69) significantly ($P < 0.05$) hatched in the control than in any of the treated (14-55) chambers to show that the concentrations affected the rate of hatching of *C. gariepinus* egg. The higher the concentration, the fewer the number of hatched eggs and the larger the duration of hatching. Values recorded for hatched eggs significantly reduced in the various concentrations and control and exposure period. Newly hatched larvae in control and experimental tanks were inactive and either remained suspended in the media or attached to the side or bottom of the chambers with their ventral side displayed towards the surface of water throughout the first day.

On the second day, the larvae in control tank occasionally went into jerking motion, darting rapidly upwards to the water surface and then sinking passively back to resting position. This behaviour occurred late on the third day with low intensity in the larvae exposed to toxicity test. The third and fourth days witnessed an increase of this activity, followed by mouth opening and fast metamorphosis of the yolk sac. Swim-up

larvae (fry) began feeding after the fourth day (control) and fifth day (experiment) when their yolk sacs have completely metamorphosed. The rate of feeding amongst the hatchlings was insignificant ($P>0.05$). Fry in the control consumed more food than those in the treated tanks and the consumption was significantly different ($P<0.05$).

Table 1: Mean hatchability of *Clarias gariepinus* eggs in sublethal concentrations (1.0 and 1.5mg/l) of water soluble fractions (WSF) of Forcados crude oil.

Concentration (Mg/l)	No of eggs/tank	No of eggs that hatched/Hours				
		12-h	24-h	30-h	36-h	48-h
0.0 (Control)	100	-	-	38	69	69
1.0	100	-	-	21	51	55
1.5	100	-	-	14	38	47

Key:- - Means no hatching.

Figure 1 shows the relationship between the larval growth and yolk sac depletion in both treated and control experiments during the first ten days of larval life. Rapid yolk sac metamorphosis occurred in the first three days of larval life and this reflected in the initial steep growth (First growth phase). No significant ($P>0.05$) growth was noticed in the treatments after the yolk sacs absorption between 4th and 5th day

(Control), 4th to 6th day (1.0 mg/l) and 4th to 7th day (1.5mg/l). However, differences in values between the treatments were significant ($P<0.05$). The second growth phase witnessed a gradual increase in growth, which began on the 6th, 7th and 8th day for control, 1.0 mg/l and 1.5 mg/l respectively, and became prominent on the following subsequent days.

Total length and Yolk sac depletion (mm)

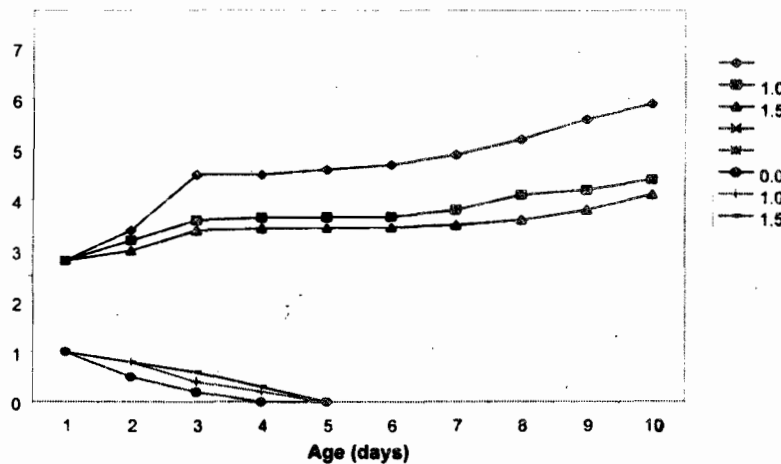


Fig 1. Mean relationship between yolk sac depletion and larvae growth of *Clarias gariepinus* exposed to sublethal concentrations of water soluble fractions (WSF) of Forcados crude oil.

Figure 2 shows the daily cumulative mortality curve of *C. gariepinus* larvae exposed to treatments and control for a ten-day period after hatching. Critical period of high mortality was observed between the period of mouth opening (3rd and 4th day) and the second growth phase (8th day). Peak

mortality occurred on the 4th day and was highest (38%) in 1.5 gm/l and lowest (30%) in control experiment. There was a significant difference ($P<0.05$) between the mortalities of the control and the treated experiments and between the treatments.

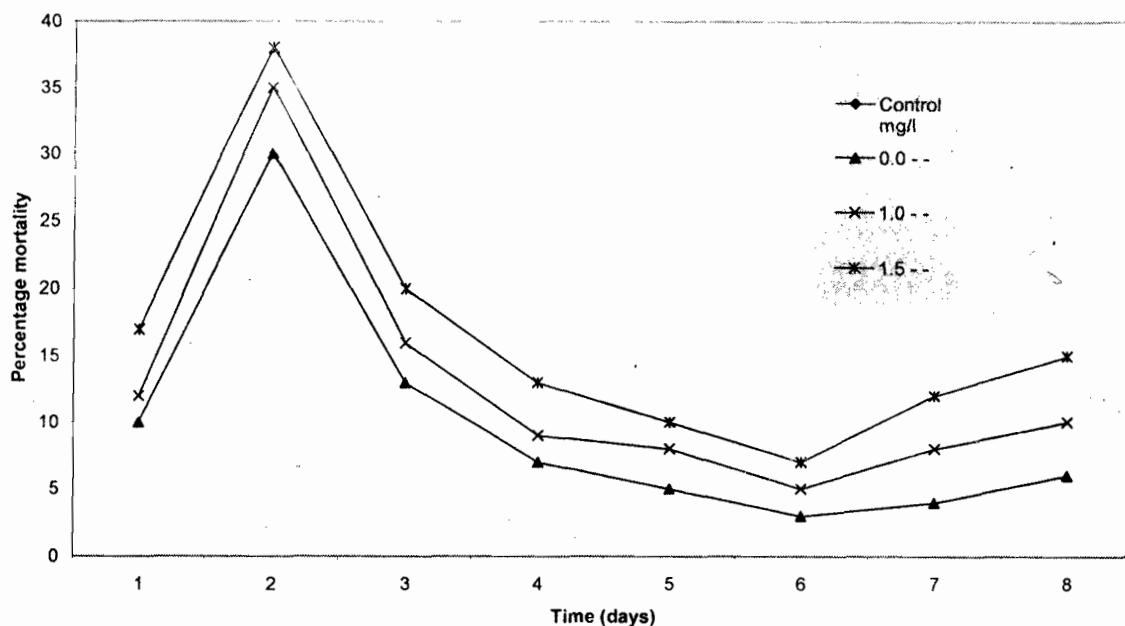


Fig 2. The daily cumulative mortality curve of *Clarias gariepinus* larvae exposed to sublethal concentrations of water soluble fractions (WSF) of Forcados crude oil for 10 days period after hatching.

DISCUSSION

Hatching hours documented were normal for the control experiment (Aguigwo, 1988), while the delayed hatching recorded for the treatment showed the negative impact of this toxicant. A delayed hatching was recorded in flagfish, *Jordanella floridae* eggs treated with RJ-5 jet fuel (Jenkins *et al.*, 1977) and rainbow trout, *Salmo gairdneri* embryos (Millemann *et al.*, 1984) exposed to coal - derived synthetic fuels. Low survival during incubation experiment may be due to unnatural confinement of the eggs and changes in their density. These observations agree with earlier reports of Hogendoorn (1980) and Abohweyere (1990). The floating nature of the undeveloped or non-hatched eggs in both treated and control tanks is normal (Millemann *et al.*, 1984). This may reflect egg buoyancy since a change in the relative buoyancy of eggs often occurs during development (Coombs and Hiby, 1979 and Millemann *et al.*, 1984). However, buoyancy of the treated eggs was higher than the untreated eggs and this may be partly due to the viscosity of the toxicant and its adsorption on the eggs and/or impact of aromatic hydrocarbon of the oil on the physiology of the eggs (Green and Trett, 1989). These findings may have strongly contributed to a high variability in the success of incubating *C. gariepinus* eggs. Similarly, the delayed hatching and reduction in the number of hatched eggs in the treated experiments compared with the control may be due to the effects of water soluble fractions of Forcados crude oil which result was significantly different ($P < 0.05$). This finding agrees with Henderson *et al.*, (1983) documentation on rainbow trout, *Salmo gairdneri* subjected to hydrazine.

The various behavioural patterns such as jerking motion, darting rapidly upwards the surface of water and then sinking passively back to resting position recorded for the larvae are similar to those earlier reported by Westlake *et al.*, (1983), Devlin *et al.* (1985) and Petersen *et al.* (1986) in rainbow trout (*S. gairdneri*), fathead minnow (*Pimephales promelas*) and sunfish (*Lepomis humilis*), respectively. Impact of the toxicant seemed unnoticed in the larval growth within the first three days. This may be due to closed-mouth nature of the larvae, which disallowed swallowing of the toxicant.

However, from the 4th day onward, there was reduction in growth of the treated larvae compared with the control. This may be due to fry drinking/swallowing of the toxicant leading to physiological impairment. Similar results were obtained when coho salmon fry were subjected to toluene and naphthalene (Moles *et al.*, 1981). However, Mitchell and Benneth (1972) found no mortality in different concentrations of water soluble fractions of crude oil as a result of loss of volatile material due to their non-renewal experimental method. Therefore the daily renewal of the concentration in this experiment ensured a steady intensity of the toxicant for a relevant result. The significant difference ($P < 0.05$) between growth in the control and treated and between the treatments also attests to the relevance of this work.

The three days yolk metamorphosis was reflected in the initial steep growth and which suggests that larvae survived on the endogenous food reserve during this period. The second growth evolved from the larval acceptability and intensification on exogenous food. This shows that chicken offals food met all requirement of artificial feed (Little *et al.*, 1994). The low mortality increase phase reflects this submission, which is similar to the earlier reports on cutthroat trout (Woodward *et al.*, 1981 and 1985).

The disparity in the growth increase between the treated larvae and control, showed that this toxicant causes growth retardation which is economically insignificant and therefore, detrimental to fisheries boosting worldwide. The retarded growth rate period may indicate partial acceptability of the exogenous food, which might be a contributing factor to high mortality, and shrinkage of unfed larvae between the days. Previous studies showed that over 90% larval mortality occurred during the first ten days after egg hatching (Kuo *et al.*, 1973 and Devlin *et al.*, 1985). However this submission is confirmed only during the first three to four days in control and experimental larvae respectively when mouths were not opened. The reason for this may be due to contamination of the reserved food (yolk) by the toxicant.

It may be deduced that long - term exposure to the water soluble fractions of Forcados crude oil as 1.0mg/l concentration is likely to cause physiological impairment of *C.*

gariiepinus seedlings necessary for stocking fish farms or natural aquatic ecosystem and thereby rendering the world fisheries resources declining.

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