

Research Article

Evaluation of the Effect of Water Soluble Fraction (Wsf) of Bonny Light Crude Oil and Sublethal Concentrations of *Lepidagathis alopecuroides* (Vahl) on Reproduction in *Clarias gariepinus* (Burchell 1822)

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Abstract: The aim of this study was to evaluate the effect of crude oil Water Soluble Fraction (wsf) and the aqueous extract of *Lepidagathis alopecuroides* on fertility and hatchability of *Clarias gariepinus*. Artificially fertilized eggs of *Clarias gariepinus* were exposed to five sublethal concentrations of *L. alopecuroides* and five concentrations of the water soluble fractions of crude oil. The results showed that there was concentration dependent reduction in hatchability of eggs exposed to *L. alopecuroides*. A highly significant ($p < 0.01$) but negative linear correlation ($r = -0.9327$) was observed between percent hatchability and the concentration of crude oil water soluble fraction. Both relationships were linear with regression equations $y = -73.68x + 65.92$ vs. $y = -0.085x + 75.03$ and coefficient of determination (R^2) = 0.870 vs. 0.915, respectively. This result confirms that both *L. alopecuroides* and crude oil water soluble fraction are capable of inhibiting hatchability of *Clarias gariepinus* and reducing the reproductive capacity of this species in the wild.

Keywords: *Clarias gariepinus*, crude oil, hatchability, plant extract, sperm viability

INTRODUCTION

The Water Soluble Fraction (WSF) of crude oil is that small fraction of oil containing components fully or sparingly soluble in water (Nwaduwe *et al.*, 2006). Organisms exposed to WSF of crude oil take up the dissolved hydrocarbon and react to their effects. The severity of the effects depends on the duration of exposure, the concentration of the components and mode of exposure. Toxicity of crude oil is dependent on the presence of fractions of toxic components like xylene, naphthalene, benzene and toluene (Noyo *et al.*, 2008).

The water-soluble fraction of crude oil has been found to reduce the growth rate and biomass turnover of some aquatic macrophytes. Kauss and Hutchinson (1975) found that aquatic macrophytes population was reduced in the presence of petroleum water soluble fraction containing harmful metallic ions like Vanadium, Nickel, Iron and Copper.

In the Niger Delta region of Southern Nigeria crude oil exploration and drilling for oil is the main stay of Nigeria economy. Oil spills in the creeks and bodies of water is a common event, Baird (2010). It is also a common event that terrestrial and marine ecosystems are destroyed during such spillage. Frequently, a lot of fish ponds are damaged due to oil slicks, fish eggs and

hatchlings suffer high rate of mortality, compared to the adult fish, which may swim out of harms way before it gets hurt (Akpofure *et al.*, 2000). Determination of median lethal concentration (LC_{50}) is one of the basic tests which provide a better understanding regarding the sensitivity of animals to crude oils. The LC_{50} value of Water Soluble Fraction (WSF) of different crude oils has been reported for three species of marine shrimp and fish (Anderson *et al.*, 1974), plants (Winter *et al.*, 1976).

The parameters which affect the LC_{50} values include species, age and habitat, duration of exposure, bioassay system (Anderson *et al.*, 1974), type of crude oil used in the test and the analytical methods applied in the bio- assay (Rand and Petrocelli, 1985).

There is, therefore, need to investigate the effect of sublethal concentrations of the crude oil water soluble fraction and *Lepidagathis alopecuroides* used as a piscicide on fertility and hatchability in the fresh water catfish *Clarias gariepinus*.

MATERIALS AND METHODS

Preparation of water soluble fraction: The crude oil used for this research work, was the Bonny Light supplied by the Nigeria Pipeline Product Marketing

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Company Limited, Subsidiary of the Nigerian National Petroleum Cooperation-Port Harcourt Refinery Branch. The crude oil was bottled and stored at room temperature (25°C) and tightly corked to avoid any possible loss of volatile compounds.

The Water Soluble Fraction (wsf) was prepared by adding 1 part of Bonnylight crude oil to 9 parts of tap water (Anderson *et al.*, 1974) in separate 2000 mL volumetric flasks and stirred for 24 h. The flasks were capped with cotton wool and an aluminum foil, to avoid evaporation of volatile oil components.

After 24 h of stirring, the mixture was then poured into a 500 mL glass stopper separating funnels in the upper layer, whilst the soluble fraction and tap water appear underneath. The aqueous phase (WSF) was drawn off through the arms near the bottom of the funnel and collected into a storage bottle. This was diluted to the following concentrations 20, 40, 60, 80,100% wsf and a control of a 100% deionized water

Preparation of plant extract: *Lepidagathis alopecuroides* was collected from Ogba town, in Ogba-Ebema-Ndoni local government area in Rivers State. The plant material was air dried for two weeks. The dried leaves were ground into fine powder with Moulinex Blender and stored in a dry air tight container and the stock solution of 4000 parts per million (4000 ppm) prepared according to Orlu and Gabriel (2011a)

Four concentrations of the extract (0.25, 0.50, 0.75, 1.00 ppm) and a control (0.00 ppm) were prepared in triplicate in 10,000 mL aquaria.

Experimental procedure: Artificial fertilization of *Clarias gariepinus* eggs was carried out according to the method recommended by FAO (2006).

The experimental fishes were acclimated in the laboratory for fourteen days, in a 40 L circular aquaria covered with a net to avoid the fish from escaping. Feed was withheld from the female broods for 48 h prior to stripping to avoid contamination of the eggs. They were administered Ovaprim™ produced by Syndel Laboratories, in Canada at 1 mL/kg (FAO, 2006). Ovaprim™ is a potent ovulating/spermiating hormone which promotes and synchronizes ovulation in many species of fish. The hormone was drawn with a 2 mL syringe and administered intramuscularly at 45° in the dorsal muscle. Ten hours after injection the males were dissected to ensure their testes were easily accessible to avoid delay in administering the milt on the eggs. Any delay will lead to the closure of the egg micropile making fertilization of the eggs impossible. The females were stripped of the eggs 10 h after administration of Ovaprim™. About 2000 eggs were introduced into a dry plastic container for fertilization (FAO, 2006) and the volume of the milt was adjusted for optimal fertilization. The fertilized eggs were spread on a mosquito net, enmeshed in the aquaria and bathed

in 200 mL of the test solution at different concentrations in triplicates. Six thousand eggs were tested per concentration; on the whole 30,000 eggs were used per test and a total of 60,000 eggs for both tests. Hatching took about 24-32 h at water temperature of 25°C. Thereafter, data was collected in triplicate on fertilized and hatched eggs, fertilized but not hatched and unfertilized eggs.

Statistical analysis: All data obtained were subjected to ANOVA and the means and standard deviation calculated using XLSTAT statistical software. Data were further subjected to Pearsons linear correlation and regression analysis to determine the influence of the crude oil water soluble fraction and the plant extract on fertility and hatchability.

RESULTS

The effect sublethal concentrations of *Lepidagathis alopecuroides* and water soluble fraction (wsf) of Bonny light crude oil on hatchability was evaluated. Percentage hatchability of the eggs of *Clarias gariepinus* was significant and dose-dependently ($p < 0.05$) reduced by treatment with aqueous sublethal concentrations of *L. alopecuroides* and wsf of crude oil. Hatchability inhibition by crude oil was linearly

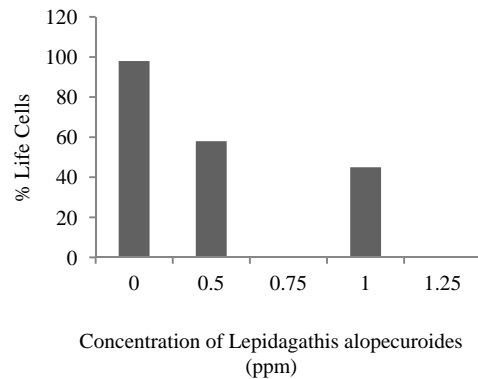


Fig. 1A: The decrease in the number of live cells from over 95% at control to about 40% at 1ppm

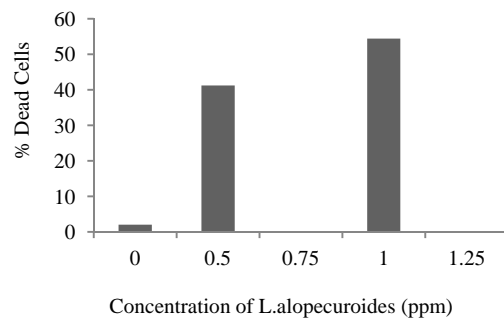


Fig. 1B: The % dead cells increased from about 5% at the control to nearly 60% at 1ppm of *Lepidagathis alopecuroides*

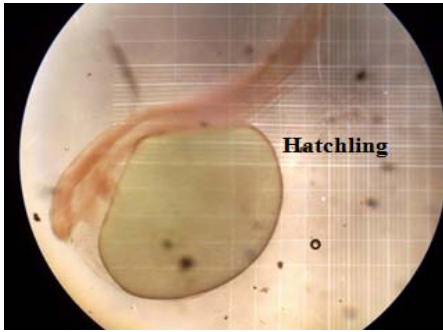


Fig. 2A: A hatchling of *Clarias gariepinus*

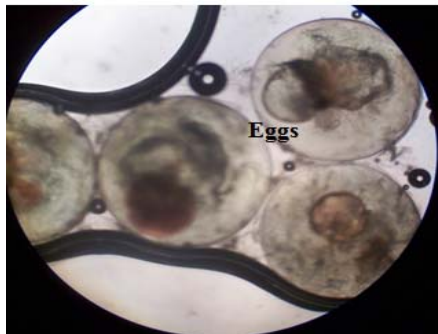


Fig. 2B: Unfertilized eggs of *Clarias gariepinus*

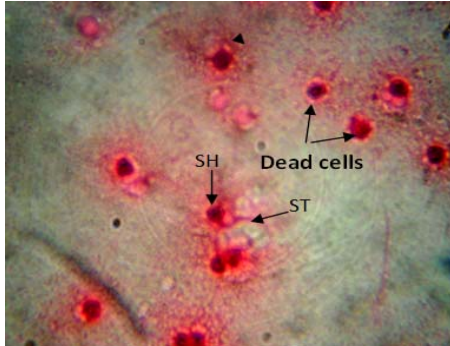


Fig. 2C: Nigrosin-eosin vital staining showing dead sperm cells at 1ppm of *Lepidagathis alopecuroides*

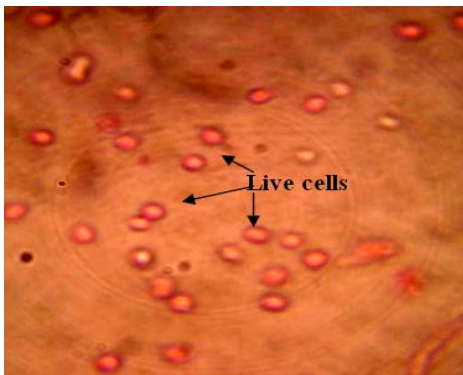


Fig. 2D: Nigrosin-eosin vital staining showing live sperm cells of control fish

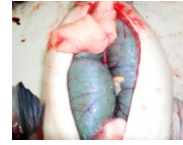


Fig. 3A: Egg sac *in situ* of *Clarias gariepinus*



Fig. 3B: The egg sac dissected out



Fig. 3C: *Clarias gariepinus*



Fig. 3D: A single egg of *Clarias gariepinus* 23 μ m (Diameter of egg measured using Ocular Micrometer)

correlated with concentration $r = -0.93$, $R^2 = 0.87$, $Y = -73.68X + 65.92$ and $r = -0.96$, $R^2 = 0.92$, $Y = -0.085X + 75.03$ for *Lepidagathis alopecuroides*.

Hatchability dropped from 70% to 10% at 1.00 ppm of *L. alopecuroides* while it declined with Crude oil water soluble fraction from 72% at control to 40 at 100%.

The effect of sublethal concentration of *Lepidagathis alopecuroides* on sperm morphology was studied in Nigrosin-eosin vital stain film and the results are hereby presented (Fig. 1A and B). The ratio of live/dead sperm cells was estimated in the control and on the treated group. The number of dead cells tended to increase with increase in concentration of *Lepidagathis alopecuroides* aqueous extracts. At 0.00 ppm which served as the control, no cells could be identified as dead in Nigrosin-eosin vital stain (Fig. 1A) at 1.00 ppm almost all the sperm cells had taken up the stain and would be classified as dead (Fig. 1B). While the number of live cells decreased with increase in the concentration of the extract.

No adverse effect was observed on the morphology of the fingerlings (Fig. 2A) and the eggs for fertilization were considered normal with a diameter of 23 μ m (Fig. 2B).

The sperm morphology observed from Nigrosin vital stain shows that high concentration of

L. alopecuroides was spermicidal and all the sperm cells appear to be dead as they have taken up the stain are considered dead Fig. 2C. Sperm cells from control (0.00 ppm) are all viable and have not taken up the stain (Fig. 2D).

Egg sac in situ and egg sac dissected out of *Clarias gariepinus* female brood stock are presented in Fig. 3A and B, respectively. A sample of the fish is shown in Fig. 3C and one egg measured with ocular Micrometer shown in Fig. 3D.

DISCUSSION

Lepidagathis alopecuroides aqueous extract applied at sublethal concentrations to the medium for hatchability led to reduction in the percentage of eggs hatched. At 0.00 ppm, the control, 75% of the eggs were hatched. There after there was concentration dependent reduction in hatchability in the groups treated. A correlation between the concentration and percentage hatchability was highly negatively significant ($r = -0.9565$, $p < 0.01$). The regression analysis carried out had the equation $Y = -0.085x + 75.03$ ($R^2 = 0.915$). The high coefficient of determination ($R^2 = 0.915$) is indicative of the fact that over 90% of the variation in hatchability between the control and the treated eggs can be attributed to the presence of the toxicant (*Lepidagathis alopecuroides*). It has been established that this plant extract is cytotoxic and spermicidal (Orlu and Ogbalu, 2011) and contains active components including saponins, alkaloids and rotenones which inhibit spermatogenesis in *Clarias gariepinus* (Orlu and Gabriel, 2011a). Saponins and rotenones are capable of causing cellular mitotic inhibition leading to arrested cellular differentiation as well as hatchability. Also reported in literature there is the possibility of hepatotoxicity of this plant extract not only in *Clarias gariepinus* (Orlu and Gabriel, 2011b) but also in rats (Orlu and Ogbalu, 2012b). Partial arrest of spermatogenesis in rats has also been reported (Orlu and Ogbalu, 2012a). This finding is in support of the report of Fasakin and Aberejo (2002) who ascertained that pulverised powder of *Piper guineense* was effective and significantly inhibited egg hatchability and adult emergence of *D. maculatus* in smoked catfish. Also in agreement with this result is the work of Elmer-Rico and Micor (2007) on brine shrimp hatchability and lethality test assay used to determine the biological activity of the *Barringtonia asiatica* (botong) seed's aqueous extract. They also observed that at lower concentrations (1-50 ppm), there was a dose dependent relationship wherein the percentage hatchability decreased as the concentration of the extract increased. The low hatching rate observed after the 12 h treatment was reported to be probably due to an alteration in the development of *Artemia* (brine shrimp) embryos.

Similar to *L. alopecuroides*, it is established also that, the water soluble fraction of crude oil contains active components including; xylene, naphthalene,

benzene and toluene that can inhibit mitotic division in fertilized eggs of *Clarias gariepinus* resulting in non hatchability due to abnormal development of the embryo. Mitotic inhibition of the fertilized eggs and abnormal differentiation and maturation could also result from the toxic effect of the harmful metallic ions like Vanadium, Nickel, Iron and Copper present in WSF of crude oil. The result of this investigation is supported by the reports of Nwaduikwe *et al.* (2006) on *Heterobranchus longifilis* and Onuoha and Nwaduikwe (1990) on *Clarias gariepinus*.

CONCLUSION

It can be concluded that sublethal concentrations of *Lepidagathis alopecuroides* and water soluble fraction of crude oil can inhibit fertility and hatchability and perhaps become toxic to the embryo in *Clarias gariepinus*, as well as, other non target marine organisms. Cleaning of accidental oil spills should be treated with the urgency it deserves to save the marine organisms from reduction in population.

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