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Toxicity and Behavioural Changes in *Heterobranchus bidorsalis* Fingerlings Treated with a Micronurient Fertilizer, Agrolyser

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Abstract: *Heterobranchus bidorsalis* (mean total length, 2.94±0.34 cm SD; mean weight, 6.75±0.9 cm SD) was exposed in a daily renewal bioassay to different concentrations of agrolyser (0.00, 1400, 1600, 1800, 2000 and 2200mg/l), a micronutrient fertilizer for 96hrs to assess its toxicity. The opercular (obf), tail beat (obf) frequencies and mortality were monitored during the exposure period. The pattern of response of obf at the various concentrations of agrolyser varied very widely at the exposure duration. The tbf of the fish at the various exposure concentrations generally increased with time peaking at the 48th hour after which it began to decline. Mortality of exposed fish in all the exposure concentrations increased with duration of exposure. Exposed fish showed altered obf with exposure concentration ($p<0.05$) and time ($p>0.05$). Cumulative mortality and tbf differed with exposure time and concentrations of agrolyser ($p<0.050$). Median lethal time for the lowest concentration, 1400mg was 263.83hrs (181.41-667.75, C.L.) which was 2.5times that for the highest concentration. The 96hr lethal concentrations (LC_{50}) at 72 and 96hrs were 2975.48mg (2594.50-3995.10 C.L.) and 2340.91mg/l (2175.63-2654.56 C.L.), respectively. Although the fish appeared to be tolerant to acute levels of agrolyser, alteration in the behaviour may affect a number of activities involved with the survival of the fish in the wild and culture environment.

Key words: Opercular beat frequency, tail beat frequency, agrolyser and hybrid catfish

INTRODUCTION

Environmental factors of both natural and anthropogenic origins have been known to induce alteration of different magnitudes in the physiological and biochemical status of animals (Shilov, 1981; Vosyliene and Kazlauskiene 1999). Therefore, biomarker parameters assessment is a means of environmental monitoring, with the advantage of providing quantitative response as valuable information on ecological relevance on the acute/chronic adverse effects caused by water pollution (de la Torre *et al.*, 2005). However, these alternations are considered as an adaptive mechanism which allows the fish to cope with real or perceived stressors so that the normal homeostatic state could be maintained (Barton, 2002). Fishes exposed to toxicants undergo stress, which is a state of re-established homeostasis, a complex suite of mal-adaptive responses (Chrousos, 1998). Under stress, physiological/biochemical responses may be compromised, becoming detrimental to the fish's health and well being at which point the fish is termed distressed (Barton and Iwama, 1991). Fishes in a contaminated environment show some altered behavioural patterns which may include avoidance, locomotor activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition (Morgan *et al.*, 1991; Gormley *et al.*, 2003).

Agrochemical fertilizers and their effluents have been shown to have devastating effects on aquatic biota (Bobmanuel *et al.,* 2006; Ekweozor, *et al.*, 2001; Chukwu and Okpe, 2006; Yadav, *et al.*, 2007; de Solla and Martins, 2007; Boone, *et al.*, 2007). For example, fertilizers effluents reportedly caused altered behaviours such as opercular and tail beat frequencies and mortalities in *Clarias gariepinus, Heterobranchus bidorsalis* and their hybrid (Bobmanuel *et al.,* 2006; Ekweozor, *et al.*, 2001). Also Yadav, *et al.* (2007) observed that *Channa striatus* exposed to industrial wastewaters showed hyperactivity characterized by linear movement, jumping, opercular movement, distance movements and somersaulting depending on the concentrations. Inorganic and nitrogen-phosphate-potassium fertilizers (NPK) was toxic to *Tilapia guineensis* (Chukwu and Okpe, 2006), eggs of snapping turtle, *Chyledra serpentina* (Griffis-Kyle *et al.*, 2007) and ammonium nitrate impacted negatively on the survival, growth and develoment of embryo and larvae of amphibians- wood frog, *Rana sylvatica;* tiger salamander, *Amystoma tigrinum* (Griffis-Kyle and Ritchie, 2007).

Some of the studies indicated that the negative effects of the fertilizers on the aquatic biota may be influenced by physico-chemical parameters of the media (Chukwu and Okpe, 2006; Edwards, *et al.*, 2006). Agrolyser, a micronutrient fertilizer is manufactured by Cybernetics

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Nigeria Ltd., Lagos. Report from the manufacturer indicated that the agrochemical has been cleared, approved and recommended by regulatory agencies in the country. Unpublished reports indicated that agrolyser (a micro-nutrient) fertilizer applied at the recommended rate of 6mg/l has been used by fish and crop farmers to enhance yields from farms in field trials in different parts of the country. However, there are no published works on the toxicity of the agro-chemical to *Heterobrnachus bidorsalis* and the behavioural changes associated with exposure to acute concentration of agrolyser.

This study therefore investigated the lethal effect of agrolyser (a micronutrient inorganic fertilizer) on *Heterobranchus bidorsalis* a culturable catfish in the Niger Delta region of Nigeria with particular reference to the behavioural changes and associated toxicity.

MATERIALS AND METHODS

Two hundred fingerlings of *Heterobranchus bidorsalis* (total length 7.0± 1.0cm SD; means weight 3.0±0.7g SD) and were obtained from the African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt. They were transported in a plastic aquarium to the Fisheries Laboratory, Rivers State University of Science and Technology, Port Harcourt. The fishes was acclimated to laboratory conditions in groups of fifty in 20*l* borehole water in rectangular glass aquaria measuring 30x 25 x 25cm^3 for 7 days. The tanks were aerated throughout the acclimation period. The water was renewed daily and the fish fed with a 35% crude protein diet and 1% biomass half at 900 hours and 1600 hours respectively. Cans of agrolyser were obtained from the Rivers State Agricultural Development Programme (ADP), Port Harcourt.

Standard methods (APHA, 1998 with few modifications were employed in carrying out the experiment. A range finding test was performed by exposing five fish to five different concentrations of solutions of the agrolyser (800, 1000, 1200 and 1400mg/l). After the range finding test, the ten fish was exposed to 1400, 1600, `800, 2000 and 2200mg/l and a control. The fish was starved 24hr before and during the trails. The solutions were stirred for homogenous mixing before each aquarium was randomly stocked in triplicates with ten fish. The test solutions and control were renewed daily. Opercular beat frequency, obf; tail beat frequency,tbf and cumulative mortality were recorded 12 hourly and dead fish removed with a aquarium net. A fish was considered dead when they failed to respond to simple prodding with a glass rod.

Probit analysis (Finney, 1971) was used to calculate the median lethal concentration and time with their upper and lower confident limits. Data (obf, tbf and mortality) were subjected to analysis of variance and difference between means was separated by Duncan's multiple range test (DMRT). Other abnormal behaviours were noted and the extent of mucus production on the skin and gills of exposed fish was assessed by feeling with the fingers.

RESULTS

H. bidorsalis fingerlings exposed to various concentrations of agrolyser showed an initial rapid movements such as opercular and tail movements, accompanied by incessant gulping for air, loss of balance,and restlessness. The increase in these activities declined after six hours of contact with the toxicant. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. However, fish in the control maintained normal behaviour within the 96 hours of the experiment. The obf in fish exposed to the agrochemical was least variable at the 24th and 48thhrs at what points also they peaked (Fig. 1). Beyond this point they became more variable declining with exposure time. Tbf was depressed in all fish exposed to the chemical below the control values at all the exposure time except at the 24th hour (Fig. 2). It was depressed and less variable at the 24th and 72nd hrs, but was more variable at the 12th, 48th and 96th hrs with a peak at the 48thhr (Fig. 2). It was generally less variable and higher than the obf at these tim intervals. Mortality at the expoure concentratons increased with duration becoming more variable with time (Fig. 3). None of the exposure concentrations of the chemical caused a 100% mortality of the exposed fish.

The agrochemical produced marked changes ($p<0.05$) in the values of obf, tbf and mortality of *H. bidorsalis* at the various exposure duration and concentrations of the chemical (Table 1). The mean lethal time (MLT_{50}) of *H.*

Table 1: Changes in the opercular beat frequency (obf/min), tail beat frequency (tbf/min) and cumulative mortality (%) of *Heterobranchus bidorsalis* treated with agrolyser.

Parameter	Concentration (mg/l)					
	0.00	1400	1600	1800	2000	2200
OBF	117.93 ±14.36^a	91.33 ±28.39^b	86.93 ±25.85^{bc}	94.33 ±32.37^b	82.57 ±39.36^c	86.00±38.87^{bc}
TBF	60.20 ±38.56^a	18.87 ±8.83^b	19.73 ±8.83^b	20.73 ±10.99^b	17.71 ±10.98^b	20.08 ±16.25^b
Motality		14.00 ±10.56^d	19.33 ±15.80^{cd}	24.00 ±20.98^{bc}	30.71 ±24.33^b	42.31 ±30.59^a

Parameter	Duration of exposure (hrs)				
	12	24	48	72	96
OBF	81.00±19.88^b	123.94±10.85^a	120.78±12.42^a	74.06±20.02^c	62.40±34.00^c
TBF	24.67±9.55^b	11.39±3.57^c	41.83±20.99^a	28.76±35.71^b	25.47±22.24^b
Motality	5.00±6.18^c	8.33±9.24^c	23.89±20.90^b	32.78±25.16^a	38.67±22.85^a

Means in the same column with similar superscript are not significantly di fferent at $p<0.05$

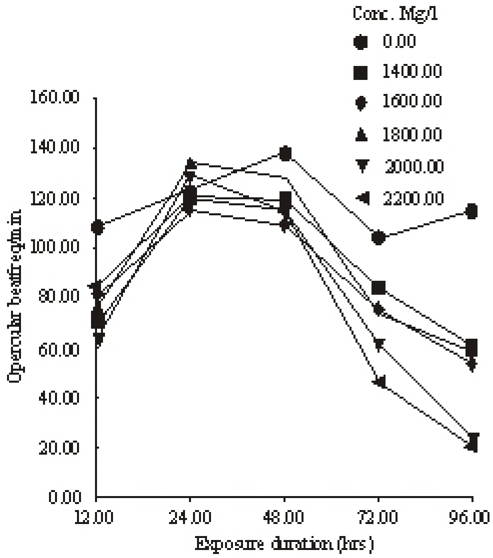

Fig.1: Opercular beat frequency (obf) of *H. bidorsalis* catfish exposed to acute levels of agrolyser

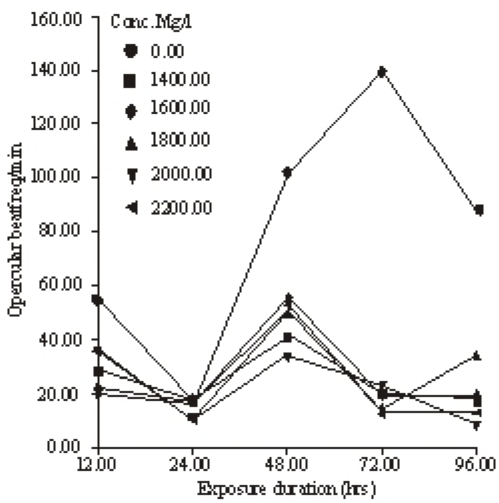

Fig.2: Tail beat frequency (tbf) of *H. bidorsalis* catfish exposed to acute levels of agrolyser

bidorsalis exposed to 1400mg/l agrolyser (139.08hrs) was about four times that of 2200mg/l.; whereas the MLT_{95} for the same 2200mg/l was 2.5times that for 1400mg/l of the agrochemical (Table 2). The respective 96hr LC_{50} and LC_{95} were 1688 .58 (1582.69-177.50) and 2340. (2175.63 -2654.56) mg/l (Table 3).

DISCUSSION

Changes in behavioural patterns exhibited by fish were possibly to counteract aquatic hypoxia condition (Kind *et al.*, 2002) possibly caused by the agrochemical. When there is impossibility of escape from hypoxic stress, physiological alternations may be evoked to compensate for low oxygen supply (Graham, 1997; Val *et al.*, 1998). Although the chemical was believed to enhance production of dissolved oxygen, this may not have been

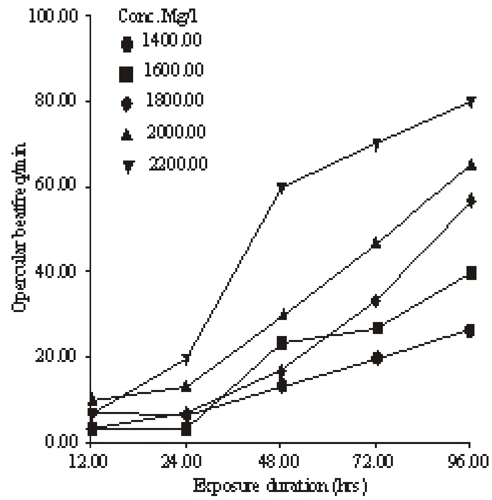

Fig.3: Cumulative mortality of *H. bidorsalis* catfish exposed to acute levels of agrolyser

Table 2: Median lethal time to death of *Heterobranchus bidorsalis* exposed to acute concentrations of agrolyser (probit) with the associated 95% confidence (lower and upper) limit

Conc. of agrolyser (ppm)	MLT_{50}	MLT_{95}
1400	139.08 (104.24-297.62)	263.83 (181.41-667.75)
1600	105.41 (87.43-147.97)	195.66 (136.38-236.48)
1800	90.46 (60.37-83.13)	167.60 (117.21-182.04)
2200	50.91 (43.11-58.57)	104.72 (91.85-125.37)

possible due to the renewal nature of the setup which did not allow phytoplankton production that would have alleviated the problem. The stressful behaviours of exposed fish such as erratic swimming, increased obf and tbf, regular visit to the surface to gulp in air, loss of balance, restlessness and finally death of fish in this study agrees with the findings of Omitoyin *et al.* (2006) in *Clarias garicpinus* to paraquat, when they exposed *Cyprinion watsoni* to copper and zinc (Shah 2002) and *Tilapia guineensis* treated with acute concentrations of chloropyrifos Chindah *et al.*, 2004) and NPK (Chukwu and Okpe, 2006)..

Hyperactivity is considered as a primary and principal sign of nervous system failure due to pesticide poisoning which effects physiological and biochemical activities (Matsumura, 1975). The disruption of the functioning of nervous system of fish might be the cause of slow and lethargic swimming, erratic swimming and loss of equilibrium (Pal and Konar, 1989). The sudden change in behaviour may be due to shock, the rise and subsequent decrease in obf and tbf may be due to fatigue resulting from suppressed metabolic rate which finally result in low oxygen demand (Jensen *et al.*, 1993). According to Kind *et al.* (2002) air breathing (regular surfacing) provides access to a high and stable supply of oxygen to the gills which acts in tandem with increased haemoglobin-oxygen affinity to preserve oxygen uptake. Increase in oxygen consumption may be associated with

Table 3: Mean lethal concentration of agrolyser on *Heterobranchus bidorsalis* (probit) with associated 95% confidence (lower and upper limit).

Duration of exposure (hrs)	Lethal concentration (LC)		
	LC_5	LC_{50}	LC_{95}
24	1681.91	4497.71	7313.50
48	1516.65(5613.53-1992.43)	3046.19 (2450.03-25177.59)	4575.74 (3251.91-55824.45)
72	985.13 (232.04-1272.48)	1980.31 (1851.68-2195.38)	2975.48 (2594.50-3995.10)
96	1036.25 (654.64-1233.54)	1688.58 (1582.69-1776.50)	2340.91 (2175.63-2654.56)

additional energy requirement for detoxification or may be due to extra activity required for avoidance reaction to the toxicant and attempt to escape from the toxic environment (Lloyd, 1992).

The accumulation of mucus in the gills surface of the exposed fish may have contributed immensely to the death of the fish in this study. Lebedeva *et al.* (1998) reported that external mucus reflects metabolic processes that take place in the fish organs, which may serve also as a criterion of the physiological status of the fish leading to the establishment of specific effects that different factors such as toxicant and the environment produce on it. The accumulation of mucus on the gills reduces respiratory activity which prevents the gill surface from carrying out active gaseous exchange and thereby causing death of the fish (Omitoyin *et al.*, 1999). Besides, the mortality in the exposed fish may have resulted from the distortion of gill architecture by the agrochemical (Obomanu *et al.*, 2007). Accumulation of mucus on the gills and distortion of gill architecture a common effect of toxicants on the gills may impair gill functions resulting in an internal toxic environment from the accumulation of nitrogenous wastes in the body leading to death. Heath (1989) corroborates this observation by stating that death of fish under toxicant action is usually due to the failure of the gills.

The level of the agrochemical used in the test was high and the $96LC_{50}$ value (4497.71mg/l) of the chemical is much lower than the recommended application rate of 600mg/hectare. If this level of agrolyser is maintained in the field, the possibility of toxic effects may be very rare.

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