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Sublethal Effects of Ammoniacal Fertilizer Effluents on three Commercial Fish Species from Niger Delta Area, Nigeria

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ABSTRACT: Sublethal effects of various concentrations of fertilizer effluents on the tail beat frequency per minute (TBF min^{-1}) and opercular beat frequency per minute. (OBF min^{-1}) of *Orechromis niloticus, Clarias gariepinus* and hybrid (*Heterobranchus bidorsalis* (female) **x** C. *gariepinus* (male)) were examined in a static 96hr. bioassay under laboratory conditions. Effluent concentration, ammmonia (ionised and un-ionised), urea and pH significantly affected TBF and OBF of the three species. Ammonia particularly, the un-ionised form exacted the most significant effect on TBF and OBF. OBF appears be a more sensitive indicator of stress than TBF and may therefore be more suitable for detecting sublethal physiological effects of pollutants. This study further revealed that the fertilizer effluents have variable compositions whose values may far exceed the allowable limits and hence may pose great danger to the aquatic environment.

Aquatic pollution is significant to fisheries and aquaculture industries. Studies have been conducted on the toxic effects of effluents (waste waters) on various commercial fish species (Wai-Ogosu 1987, Ojuola and Onuoha, 1987). Some of such studies were done with increasing interest in finding physiologic correlate of activity that can be monitored under field conditions (Thorpe, 1987). Investigations into the relationship of such parameters as heart rate, tail beat frequency and respiration rate with activity have been attempted (Ross *et al*. 1981). Changes in opercular rhythm have been documented as sensitive indicator of physiological stress in fish subjected to sublethal levels of pollutants (Davis, 1973). However, none of the above dealt with the toxicity of ammoniacal fertilizer effluents on tilapia and *Clarias* species which are common estuarine and pond – reared fish in Africa.

This study investigates the sublethal effects of wastewater from a fertilizer company in the Niger Delta area of Nigeria on the tail beat frequency (TBF) and opercular beat frequency (OBF) of fingerlings of three commercial fish species – *Oreochromis niloticus*, *Clarias gariepinus* and hybrid (*Heterobranchus bidorsalis* (female) **x** *C. gariepinus* (male)). The information obtained from this study would be helpful in predicting the effect of effluent in the field.

MATERIALS AND METHODS

Fingerlings of *Oreochromis niloticus, C.gariepinus* and hybrid (mean total length 3.0 – 7.9cm; mean weight, 0.6 – 2.3g) were acclimated to laboratory conditions in glass tanks for a period of two weeks. The fish were fed compound diet (30% protein) at 3% body weight *ad libitum.* The tanks were cleaned daily and water exchange was done every 48 hours. Mortality during acclimation period was less than one percent. The industrial ammoniacal effluent was obtained in 50*l* plastic containers each from the National Fertilizer Company of Nigeria, Onne near Port Harcourt on three occasions. These represent different ranges of the discharge at the outfall. The physico-chemical characteristics of the wastewater were determined according to APHA (1980) and shown in Table 1. Twelve 50*l* glass aquaria were used for the exposure of the three species, respectively. Test solutions were prepared by serial dilution from each effluent sample. Serial dilutions were prepared on a volume to volume (v/v) ratio so that the percentage (%) concentration in each test solution is obtained by using the formula below (FAO, 1984):

$$\text{Volume percent} = \frac{\text{Volume of effluent}}{V_E + V_{DW}} \times 100$$

Where, V_E = Volume of effluent, V_{DW} = Volume of dilution water.

The determined volume of effluent was added to the desired quantity of dilution water and stirred vigorously to disperse the effluent. Screening test was done in 1*l* conical flask with single fish. Concentrations of the effluent that caused death within 30 minutes were omitted from the definitive test (Gurure, 1987). Each aquarium held 20*l* of test medium. Test organisms were randomedly introduced into each aquarium with each holding 20 fingerlings within an hour after preparation of test solution. The fish were not fed 24 hours before and during the biossay. The test media were analyzed for physico-chemical parameters (APHA, 1980, Tables 2) at the Environmental Laboratory of the Nigerian National Petroleum Corporation, Port Harcourt, Nigeria.

Each fish species was exposed to four levels of the effluent with a control. The test media were analyzed for their characteristics (Tables 2). Every treatment was replicated thrice. The mean TBFm^{-1} and OBF min^{-1} were record at 6, 24, 48, 76 and 96 hours for all species and treatments, respectively. The

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relationship between TBF min^{-1} and $OBFmin^{-1}$ and the various physico-chemical parameters of effluent were determine with the linear regression model of Statistical Analysis System, SAS, (1985). Mean and standard error (S.E.) were calculated for the TBF and OBF of the various species exposed to the different effluent concentrations. Root mean square error (R.M.S.E) and coefficient of determination (R^2) were used to establish the fitness of the model chosen.

RESULTS AND DISCUSSION

Table 1 shows that the values of the physico-chemical parameters of all treated effluents fall within the acceptable limits, except the values for ammonia in the first and second samples, dissolved oxygen, urea, and phosphate in all the samples (FEPA, 1991). The physico-chemical characteristics of various concentrations of effluents employed in the bioassay are shown in Table 2. The values of pH, temperature and conductivity are within the FEPA (1991) guidelines/standards for those parameters. But the free ammonia, urea, phosphate were 118.2,4.1 and 30 times, respectively higher than the limits; and the DO values 0.35 times below the 5mg/l limit set by FEPA (1991). There was a progressive increase in ammonia, percent un-ionized ammonia (UIA), pH, phosphate, urea and conductivity values with increase in effluent concentration in the bioassay for *O. niloticus* and hybrid. But this was not true for ammonia and un-ionized ammonia for *C. gariepinus* bioassay. The above may imply an increased toxicity with the raised values of the physicochemical parameters (Table 2). The mean OBF and TBF and their standard errors for *O. niloticus, C. gariepinus* and hybrid exposed to various percent concentrations of effluent are shown in fig. 1. Table 3 shows the relationships between OBF and TBF of the various species and the physico-chemical parameters of the effluence

Powell and Fielder (1982) stated that toxicity of pollutants to fish usually increased with temperature which may be due to an increased uptake of toxin to an added environmental stress e.g. reduced oxygen solubility (Ananthakrishnan and Kutty, 1974). The recorded values of water temperature were within tolerable range for these tropical species and may not have affected the toxicity of the effluent and hence the OBF and TBF of the exposed fishes.

It has been observed by Thomas and Rice (1975) that increased opercula rate may be caused by decreased efficiency in oxygen uptake, transport or increase metabolic rate. The dissolved oxygen levels in the bioassay solutions were generally low (1.54 – 24 ppm) and may be accounted for by respiration of fish species, fast decomposition of organic matters due to static condition and reduction of other gases e.g. CO_2 among others. The low dissolved oxygen value only had significant influence on $TBFmin^{-1}$ of *C. gariepinus* contrary to the raised ventilation volume in sockeye salmon exposed to bleached kraft pulp mill effluent (Davis, 1973). The observed response may be due to the ability of the fishes to tolerate very low oxygen levels (Kutty, 1984).

Although phosphate levels did not significantly affect OBF and TBF in all fish species, yet high levels in aquatic environment may indicate pollution (Sehgal, 1980). Such levels are known to have adverse effects on the embryonic development and survival of common carp (Toor *et al*. 1983). Hence we may safely suspect that aquatic organisms exposed to the effluent may suffer similar fate as reported for carp. The high level of urea in the stock and test solutions may be due its usage in the production of fertilizer (Tables 2). The urea molecule is dipole in aqueous solution, and is less toxic than ammonia (Forster and Goldstein, 1970). Very high conductivity values reflect the presence of a large number of dissolved ions such are characteristic of industrial wastewaters (Chukwu, 1993). But all the values recorded in this study were far below that reported by other authors, which may be why conductivity caused significantly raised TBF in the hybrid only.

Ammonia had significant effect on the TBF and OBF of *O. niloticus* and OBF of the hybrid respectively, with no ammonia effect on *C. gariepinus* (Tables 3) due to the absence of ammonia in the stock solution (Table.1).

Table 1:Physico-chemical composition of different ranges of source NAFCON effluent used for the bioassay.

Date of Collection	NAFCON effluent 4 / 6 / 93	NAFCON effluent 24 / 6 / 93	NAFCON effluent 23 / 5 / 93	FEPA limit (FEPA 1991)
Fish species	*O. niloticus*	Hybrid	*C. gariepinus*	
S/No. Parameters				
1. Temp.,at Collection (^{0}C)	31	30	30	35
2. Temp., when analysed(^{0}C)	27	26	27	
3. pH	7.68	8.4	6.5	6 - 9
4. Appearance	Slightly turbid	Colloidal	Slightly turbid	Clear
5. Odour	Free	Mildly Pungent	Nil	Free

6. Oil (ppm)	Nil	Nil	Nil	-
7. NH_3 (ppm)	5.90	9.12	0.00	0.1
8. Conductivity*	202.8	253.5	74.99	40
9. BOD at 20 ^{0}C (ppm)	1.36	4.25	1.11	50
10. DO (ppm)	3.50	3.1	2.0	5
11. TDS (ppm)	92.18	115.3	340.9	2000 max.
12. Urea (ppm)	158.93	192.1	137	100
13. Phosphate (ppm)	125.03	351.3	389.7	5
14. Zinc (ppm)	0.6	0.5	0.9	
15. Iron (ppm)	0.4	0.4	0.8	

*(Ohms/cm)

Table 2: Characteristics of bioassay medium for (a) *O.niloticus*, ***(b)** C. gariepinus and* ***(c)** Hybrid*

(a) *O.niloticus*	Concentration (%)				
Parameter	0	20	30	40	50
Temperature(^{0}C)	25	26	26	26	26
pH	6.60	7.72	7.92	8.07	8.12
Ammonia*	0.00	4.52	7.39	8.38	10.71
Conductivity (ohms/cm)	30	86	109	125	140
Urea	18.17	217.98	136.24	381	395
Phosphate*	3.05	97.55	68.59	116.0	134.0
Dissolved Oxygen(DO)*	1.74	1.96	1.54	1.98	1.68
UIA^+ (%)					
(b) *C. gariepinus*	0	40	60	80	100
Temperature (^{0}C)	24	25	25	27	26
pH	6.60	7.00	7.05	70.4	7.05
Ammonia	0.00	0.00	0.00	0.00	0.00
Conductivity (ohms/cm)	28	42	250	231	425
Urea	9.42	115.0	113.0	123.0	141.0
Phosphate	11.74	91.34	120.0	994.3	215.0
DO	2.04	1.26	1.46	1.42	1.34
UIA^+ (%)	0.30	0.60	0.64	0.63	0.64
(c) *Hybrid*	0	40	50	60	70
Temperature (^{0}C)	25	25	26	25	27
pH	6.63	7.50	7.62	7.83	8.20
Ammonia	0.00	2.73	3.48	4.97	11.82
Conductivity (ohm/cm)	30	95.2	108.2	134.2	408.3
Urea	22.7	86.28	199.81	322.0	408.3
Phosphate	3.56	42.2	66.30	111.8	150.7
DO	2.03	1.75	1.97	1.99	1.86
UIA^+ (%)	0.25	1.79	2.33	3.70	8.33

*ppm + UIA – Un-ionized ammonia

Table 3: Relationship between TBF/OBFmin^{-1} of the three speciesand (a) Concentration (%) of effluent, (b) Urea, (d) Ammonia, (d) Uni-ionised ammonia, UIA and (e) pH

Fish species	Relationship	Regression equation	R.M.S.E.	R^2
(a) Concentration (%) of effluent				
O. *niloticus*	TBF	y = 59.82+ 0.54x	10.72	0.71^*
	OBF	y = 0.008+ 0.81x	7.73	0.91^{**}
C. gariepinus	TBF	y = 47.71+ 0.12x	0.57	0.91^{**}
Hybrid	TBF	y = 99.63+ 0.45x	11. 32	0.44^{ns}
	OBF	y = 71.57 – 0.72x	4.97	0.91^{**}
(b) Urea				
O.niloticus	TBF	y = 74.49+ 0.01x	14.87	0.45^{ns}
	OBF	y = 70.38+ 0.01x	20.48	0.40^{ns}
C. gariepinus	TBF	y = 6.63 + 0.04x	12.73	0.28^{ns}
	OBF	y = 148.14 – 01x	0.57	0.91^{**}
Hybrid	TBF	y = 110.43+ 0.001x	14.87	0.03^{ns}

	OBF	y = 61.40 – 0.01x	7.76	0.78**
(c) Ammonia, NH_3				
O.niloticus	TBF	y = 62.09+ 3.48x	11.27	0.68*
	OBF	y = 48.19+ 5.41x	5.78	0.95**
C. gariepinus	TBF	y = 54.18+ 0.0x	13.00	0.00
	OBF	y = 137.2+ 0.0x	1.66	0.00
Hybrid	TBF	y = 107.39+ 1.06x	14.12	1.13ns
	OBF	y = 66.05 – 3.15x	4.24	0.94***
(c) Uni-ionised ammonia, UIA				
O.niloticus	TBF	y = 60.81+ 5.41x	10.23	0.74*
	OBF	y = 48.97+ 7.75x	9.29	0.88**
C. gariepinus	TBF	y = 57.87 – 6.56x	14.98	0.006ns
	OBF	y = 143.03 – 10.37x	0.75	0.85**
Hybrid	TBF	y = 107.8+ 1.36x	14.33	0.10ns
	OBF	y = 66.32 – 4.51x	4.49	0.93***
(d) pH				
O.niloticus	TBF	y = 100.45+ 23.95x	10.00	0.75*
	OBF	y = 175.6+ 33.48x	10.32	0.85**
C. gariepinus	TBF	y = 93.00 – 5.58x	14.93	0.01ns
	OBF	y = 200.22 – 9.05x	0.75	0.85**
Hybrid	TBF	y = 13.41+ 16.64x	10.19	0.55ns
	OBF	y = 230.0 – 23.6x	5.03	0.91**

ns = non-significant, *, ** and *** = significant at 0.10, 0.05 and 0.01 level of probability, respectively.

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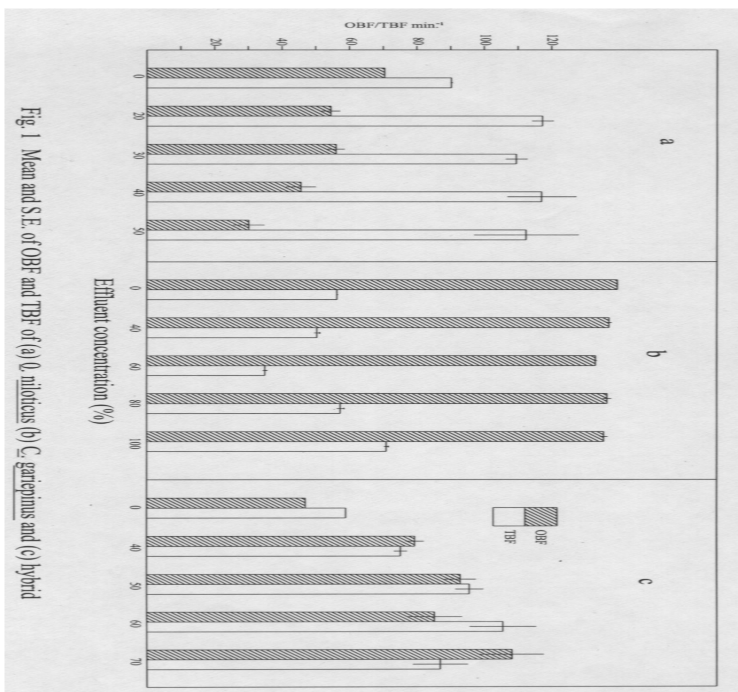

Fig. 1 Mean and S.E. of OBF and TBF of (a) O. niloticus (b) C. gariepinus and (c) hybrid

The response pattern of the three species to un-ionized ammonia and pH are similar, and the most sensitive parameter was OBF. Nonetheless the degree of responses (TBF and OBF) differ as indicated by the slope function values in the regression equation of un-ionised ammonia and pH against TBF and OBF. (Table 3) Ammonia toxicity has been shown to be dependent on its concentration in the un-ionised form; and pH and temperature are major determinates of the proportion of ammonia present in the un-ionized form (EIFAC, 1973). Kuma and Krishnamoorthi (1983), implicated ammonia as a major pollutant in fertilizer wastewater as noted in this study. Un-ionised ammonia was thought to be lipophilic which easily diffuses across respiratory membranes (Kormakik and Cameron, 1981), accounting for their more toxic effects. Also it was shown to produce impairment of cerebral energy in fish (Walker and Schenker, 1970).

Many of the characteristics associated with acute ammonia toxicity were noted in the *O. niloticus* and hybrid

Figure 1 shows that the fish species had variable OBF and TBF responses to the various grades of the effluent. The responses and their levels have some relationship to the toxicity to the stock solutions from which the test solutions are prepared. The OBF response of *C gariepinus* in the control is abnormally high when compared to the control of other species. Moreover, the responses of *O niloticus* and hybrid are more variable than that of

C gariepinus are shown in the S.E. values. It was observed that the TBF and OBF in *O. niloticus* and hybrid rose proportionally with increment in effluent concentration, but declined with time as reported in *O. niloticus* exposed to acetellic (Omorogie and Ufodile, 1991).) The fish increased its activity to cope with rise in the level of stress (concentration), but the hyperactivity waned with the depletion of fish energy (Davis, 1973). However, the OBF responses of *C gariepinus* and hybrid declined with increase in the concentration of the test solutions. (Table 3).

Generally, the results of this study showed that OBF is a more sensitive physiological tool than TBF for monitoring environmental stress in fish as a result of exposure to NAFCON effluent as noted in fish exposed to sublethal levels of pollutants (Drummond *et al*, 1973). Besides, these effluents have variable compositions whose values may sometimes far exceed the allowable limits thereby posing great danger to the aquatic biota. There is therefore the need for the authorities concerned to ensure that treated effluent discharges comply with acceptable standard to save our environment from destruction.

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