
EFFECT OF TEXTILE FACTORY EFFLUENT ON OTOLITH AND SOMATIC GROWTH PARAMETERS IN *Clarias gariepinus*

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Abstract

Are otolith parameters more sensitive than somatic indices in detecting stress conditions in fish? This question was investigated using juvenile-sized *Clarias gariepinus* in a 30-day exposure bioassay to a textile factory effluent. A series of static bioassays were initially conducted with concentrations of 0.00–40.00% and the 96hr LC₅₀ value derived by probit analysis was 20.06%. Nominal concentrations of the LC₅₀ values (6.69%, 3.34% and 2.23%) and control (0.00%) were used in a static/renewal bioassay. The differential growth in saggittal otolith and somatic parameters across exposure concentrations were examined by taking somatic and otolith morphometric measurements. Effluent samples and exposure concentrations were analyzed for some physico-chemical parameters while effluent samples were also analyzed for cyanide and heavy metals: Pb, Cu, Mn, Zn, Fe, Ni, Cd, As and Cr. The weight of the right otolith of lower exposure concentrations (0.00, 2.23, 3.34%) was significantly heavier ($p < 0.05$) than those of the highest exposure concentration (6.69%). No other significant differences were observed across somatic and otolith parameters examined. Effluent sample was characterised by low DO, high TS, TDS and TSS while exposure concentrations and effluent sample had alkaline pH and BOD and COD values that exceeded the Federal Ministry of Environment (FMNEV) limits for effluents discharged into surface waters. The values of Cr and cyanide also exceeded FMNEV limits. These constituents may elicit adverse environmental and public health effects if continued and unregulated discharge of effluents is not restricted. The relative sensitivity of otolith parameters over somatic parameters, and the relative sensitivity between otolith parameters were demonstrated. The suitability of otolith weight as a probable early-detector of stress in natural environments is suggested.

Keywords: otolith, somatic indices, textile factory effluent, *Clarias gariepinus*.

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Introduction

The roles of anthropogenic activities in the deterioration of water quality of natural water systems have been widely studied with respect to sustainable fish production and management (Heath and Claassen, 1999; Eastwood and Couture, 2002; Ashraf, 2005). Activities such as urbanization and industrial processes (Van Vuren *et al*, 1999) produce effluents which elicit toxic responses in fish (Wedemeyer *et al*, 1990) when introduced into aquatic habitats. Studies have shown that organism responses under toxic conditions can be utilized as sentinels of stress to forestall potential harm to populations or ecosystems (Ham *et al*, 1997; Adams *et al*, 1999; Adams, 2000). According to Heath (1987) and

Forrester *et al* (2003), pollution-related effects had been reported to include altered physiological functions and growth reduction/deformities.

Measures of growth and condition of young fishes have been used to assess the effects of environmental perturbations on individual fish species (Gibson, 1994; Adams, 2002; Gilliers *et al*, 2006) and some of such measures have entailed the use of otoliths. The extensive use of otoliths in detecting changes in the growth rate of various fish species has been appreciated (Hining *et al*, 2000; Reimchen and Nosil, 2001; Strelchek *et al*, 2003) while its growth-response to physiological and environmental events had been affirmed (Campana and Neilson, 1982; Volk *et al*, 1999).



Clarias gariepinus is a tropical species of commercial importance because it constitutes a major source of fish protein in Nigeria (Fagade, 1998). Some authors had reported the otolith morphology of other local fish species like *Tilapia guineensis* (Fagade 1980a), *Chrysichthys nigrodigitatus* (Fagade, 1980b) and *Brycinus nurse* (Saliu and Fagade, 2004) but no clear relationship has been made between otolith growth and toxic conditions in the environment. This study investigated the relative ability of otolith and somatic parameters to produce responses usable as early detectors of stress in *C. gariepinus*.

Materials and methods

Collection of effluents

Whole unfiltered effluent samples were collected from the point of discharge of a textile company at Eric Moore, Surulere Lagos State, Nigeria. Collections were made once every week between the months of January and March 2007. These were kept at 4°C and mixed to ensure evenness of stock solution prior to usage for bioassay procedure.

Procurement of test organisms

Early juveniles (6 weeks old; 18.51±2.34g) of *C. gariepinus* were procured from a commercial fish farm in Ibadan, transported to the laboratory in oxygenated waterproof bags and kept in holding tanks (250 L capacity) filled with dechlorinated tap water for a period of two weeks to allow them acclimatize to laboratory conditions (pH 7.03±0.05; DO 6.72±0.25mg l⁻¹; temperature 26.02±1.95). Fish were fed daily with Coppens® feed meal (40% crude protein) at 3% body weight and uneaten food was siphoned out to prevent accumulation of metabolites.

Bioassay procedure

Range finding test

A range finding test was conducted with 10 fishes per exposure concentration (77.74l) in two replicates with concentrations of 0.00%-30.00% in a static system for 48 hours. Standard procedures for bioassay as described by APHA/AWWA/WPCF (1995) and Reish and Oshida (1986) (12g of fish to 5L of water) were used.

96hr static bioassay

Test concentrations were made by serial dilutions with dechlorinated tap water using similar procedure for conducting the 48hr range finding test. Exposure concentrations of 0.00%, 10.00%, 15.00%, 20.00%, 25.00%, 30.00%, 35.00% and 40.00% were prepared in two replicates and ten juveniles were introduced into each concentration. The experimental set-up was

observed for 96hr and dead fish (fish with no opercula movement) were promptly removed. The 96 hr LC₅₀ values were computed by probit analysis (Reish and Oshida, 1986).

Sublethal exposure (Growth studies)

Nominal fractions of the LC₅₀ value were used in a static/renewal bioassay for a period of 30 days. These gave concentrations of 6.69%, 3.34% and 2.23% respectively. Exposure concentrations and control (0.00%) were prepared by serial dilution in 2 replicates (94.92L) and 10 fishes (20.15±1.24g) were introduced into each concentration. The exposure concentrations were renewed every 72hr for the duration of the experiment. After the exposure period, wet weights and standard lengths of fishes were recorded. Three fishes were selected from each exposure concentration, sacrificed by medullar transection (Lucky, 1977) and their otoliths were removed by a deep cut into the skull above the eyes. The right and left saggital otolith of each fish were extracted and fixed in 70% alcohol (Brothers, 1987). The saggital otoliths were weighed using the Ohaun compact analytical weighing balance (Mettler Instruments) before measurement by image analysis. Image analysis techniques were used with modifications as described by Lombarte (1990) where otolith image was acquired with a high resolution scanner and measurements were carried out using Adobe Photoshop® 7. Condition index of fishes across exposure concentrations was calculated using Fulton's condition index:

W/L^3 , where W=wet weight and L= standard length.

Physico-chemical parameters

Physico-chemical parameters like temperature (temp.), pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) were measured for exposure concentrations and raw effluent using standard methods (APHA/AWWA/WPCF, 1995). Heavy metal content of effluent sample was analysed using a HACH DR890 Atomic Absorption Spectrometer (AAS). Metals analysed included Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Lead (Pb), Cadmium (Cd), Nickel (Ni), Cobalt (Cu), Arsenic (As) and Chromium (Cr). Values obtained were compared with acceptable limits of effluents discharged into surface waters in Nigeria (FEPA, 1991).

Statistical analysis

Mortality of fish during acute exposure period (quantal response) was analysed using the probit method (Finney, 1971). Data on otolith morphometry and somatic parameters were analysed using one-way ANOVA (differences between means were considered significant

($p < 0.05$) and Spearman's correlation coefficient. Correlations between parameters were considered significant at $p < 0.05$.

Results

Temporary loss of equilibrium was observed in fish at all exposure concentrations. Hyperactivity was noticeable across all exposure concentrations alongside spiral patterns of movement. Hyperventilation as indicated by the rapid opening and closure of the operculum was also observed. These behavioural observations were absent in untreated control exposures. Dead fish had their opercula distended. The 96hr LC_{50} values were 20.10% and 20.01% for replicates 1 and 2 respectively with a mean value of 20.06% (Table 1). 100% mortality was recorded in the 30.00% to 40.00% exposures, 80% mortality was recorded in the 25.00% exposure, and 60% mortality was recorded in the 20.00% exposure while no mortalities were recorded in the 15.00%, 10.00% and 0.00% (untreated control) exposures.

Table 1: Acute toxicity of textile factory effluent on *C. gariepinus* after 96hr.

Mortality Interval	R1; $y = -2.640x + 105.0$	R2; $y = -2.563 + 100.1$
LC_{10}	35.99%	35.15%
LC_{50}	20.10%	20.01%
LC_{90}	5.68%	5.90%

Mean measurements of somatic parameters subjected to one-way ANOVA, showed no significant ($p > 0.05$) increases across exposure concentrations (Table 2). Similar results were observed when otolith parameters were subjected to ANOVA except for the weight of the right otolith which showed significant differences ($p < 0.05$) between the highest exposure concentration (6.69%) and other lower exposure concentrations (0.00%, 2.23%, and 3.34%) (Table 3).

Morphometric relationships between standard length, body weight, otolith length, otolith breadth and otolith weight for all experimental fish are summarised in Table 4. Significant ($p < 0.05$) positive correlations were observed between the left and right otolith weight and somatic parameters (body weight, total length and standard length).

Table 2: Mean wet weights, standard lengths and condition indices of *C. gariepinus* juveniles after 30 days exposure period ($n = 10$).

Conc. (%)	Wet Weight (g)	Standard length (cm)	Condition index (k)
0.00	23.47 ± 6.67 ^a	13.83 ± 1.30 ^a	0.93 ± 0.21 ^a
2.23	28.10 ± 4.49 ^a	14.48 ± 1.26 ^a	0.92 ± 0.10 ^a
3.34	24.18 ± 1.71 ^a	13.91 ± 0.61 ^a	1.07 ± 0.05 ^a
6.69	24.62 ± 6.09 ^a	13.37 ± 1.45 ^a	1.01 ± 0.08 ^a

Means with same superscript along the same column are not significantly different ($p > 0.05$).

Table 3: Mean values for the left and right otolith morphometry of *C. gariepinus*.

Conc.	Left otolith weight (kg)	Right otolith weight (g)	Left otolith length (mm)	Right otolith length (mm)	Left otolith breadth (mm)	Right otolith breadth (mm)
0.00	4.00 × 10 ⁻² ± 1.86 × 10 ^{-3a}	4.67 × 10 ⁻³ ± 8.20 × 10 ^{-3ab}	2.81 ± 0.17 ^a	2.93 ± 0.15 ^a	1.90 ± 0.06 ^a	1.98 ± 0.13 ^a
2.23	4.50 × 10 ⁻² ± 1.10 × 10 ^{-3a}	4.83 × 10 ⁻³ ± 1.30 × 10 ^{-3ab}	2.84 ± 0.21 ^a	2.80 ± 0.17 ^a	2.04 ± 0.17 ^a	2.03 ± 0.25 ^a
3.34	4.30 × 10 ⁻³ ± 8.20 × 10 ^{-3a}	5.00 × 10 ⁻³ ± 1.10 × 10 ^{-3b}	2.77 ± 0.17 ^a	2.79 ± 0.22 ^a	2.15 ± 0.21 ^a	2.10 ± 0.18 ^a
6.69	3.83 × 10 ⁻³ ± 7.50 × 10 ^{-3a}	4.17 × 10 ⁻³ ± 7.50 × 10 ^{-3c}	2.71 ± 0.19 ^a	2.68 ± 0.23 ^a	1.87 ± 0.11 ^a	2.02 ± 0.08 ^a

Means with same superscript along the same columns are not significantly different ($p > 0.05$).

Table 4: Correlation (r) values for otolith and somatic parameters of *C. gariepinus*.

	Total length	Standard length	Left otolith weight	Right otolith weight	Condition index	Left otolith length	Left otolith breadth	Right otolith length	Right otolith breadth
Body weight	0.883*	0.898*	0.573*	0.531*	-0.133	0.512*	0.457*	0.335*	0.284*
Total length		0.952*	0.467*	0.420*	0.414*	0.513*	0.498*	0.366*	0.350*
Standard length			0.483*	0.433*	-0.494*	0.499*	0.478*	0.352*	0.359*
Left otolith weight				0.972*	0.074	0.282*	0.257*	0.123	0.104
Right otolith weight					0.119	0.227*	0.202	0.074	0.061
Condition index						-0.125	-0.184	-0.085	-0.209
Left otolith length							0.841*	0.601*	0.549*
Left otolith breadth								0.517*	0.556*
Right otolith length									0.749*

Note: *indicates significant correlation between parameters ($p < 0.05$).

The values for physicochemical properties are presented in Table 5. Temperature values were within normal ambient temperature for tropical waters. Slightly alkaline pH values of 7.64 ± 0.60 were recorded in all exposure concentrations with effluent sample having a higher alkalinity value of 9.60. Dissolved oxygen content of the dye factory effluent was very low (2.89 mg l^{-1}) but increased in all exposure concentrations (5.17 ± 0.40

mg l^{-1}). Other parameters like BOD and COD had values higher than the Federal Ministry of Environment (FMENV) acceptable limits in exposure concentrations and effluent sample. The values recorded for TSS, TDS and TS were low in exposure concentrations but markedly high in effluent sample and exceeded the values stipulated by FMENV for effluents discharged into surface waters.

Table 5: Physico-chemical characteristics of textile factory effluent and exposure groups.

Parameters	Control	Exposures	Textile Factory effluent	FMENV (acceptable limits)
Temperature (°C)	26.02 ± 1.95	26.94 ± 3.40	29.00 ± 1.95	29.00
pH	7.03 ± 0.05	8.24 ± 0.53	9.60 ± 0.85	6.00-9.00
Biochemical Oxygen Demand (BOD)	8.50 ± 1.05	82.76 ± 15.51	98.60 ± 10.95	20.00
Dissolved Oxygen (DO)	6.72 ± 0.25	5.17 ± 2.95	2.89 ± 1.50	4.00
Chemical Oxygen Demand (COD)	10.02 ± 0.65	124.99 ± 14.38	308.78 ± 35.40	80.00
Total suspended solids (TSS)	3.02 ± 0.25	19.05 ± 3.20	56.63 ± 11.87	30
Total dissolved solids (TDS)	27.25 ± 1.90	263.26 ± 21.66	2655.30 ± 38.96	500.00-1500
Total solids (TS)	29.26 ± 2.95	278.31 ± 23.55	2712.90 ± 48.76	2000.00

Note: $X \pm SD$ represents means + standard deviation of the values recorded. FMNEV= Federal Ministry of Environment.

Table 6 shows the levels of cyanide and heavy metals in exposure concentrations and effluent sample. Heavy metals were not detected in exposure concentrations and untreated control, but textile factory effluent sample contained cyanide and chromium in concentrations that exceeded acceptable limits for effluents discharged into surface waters (FMNEV). Metals like Ar, Cd, Pb, Ni, Fe, Mn, and Zn were detected at low concentrations in effluent sample and their values were within acceptable limits stipulated by the FMNEV for heavy metals content in textile industry effluents discharged into surface waters.

Table 6: Levels of cyanide and heavy metals of textile factory effluent compared with FMENV acceptable limits.

Heavy metal (mg l ⁻¹)	Textile factory effluent (mean values)	FMENV (acceptable limits)
Cyanide	0.223	0.1
Chromium	0.693	0.1
Arsenic	0.015	0.1
Cadmium	0.035	<1.0
Copper	0.310	<1.0
Lead	0.085	<1.0
Nickel	0.355	<1.0
Iron	0.700	20.0
Manganese	2.123	5.0
Zinc	0.870	<1.0

FMNEV= Federal Ministry of Environment.

Discussion

Pollution by metals is a major source of toxic pollutants in the world (Lloyd, 1992) and its presence in Nigerian freshwaters has been reported (Calamari and Naeve, 1993; Oguzie, 2000; Obasahon and Oronsaye 2000; Omoregie *et al*, 2002). This in turn should attract important concerns as regards its potential effect on fish in receiving waters. Temporary loss of equilibrium, hyperventilation and hyperactivity reported in exposure concentrations (with the exception of control exposures) shortly after juvenile *C. gariepinus* fish were introduced into exposure concentrations is an indication of the toxic effect of the textile factory effluent eliciting behavioural changes in exposed fish. This observation is consistent with the report of Little *et al* (1993) that behavioural responses of fish are often the first responses affected by toxic stress and rapidly developing fish (fingerlings and juveniles) are typically more sensitive to toxicity effects than adult fish. Behavioural changes commonly occur 75% earlier than the onset of significant mortality and hypo or hyper-activity as well as diurnal rhythms can disrupt feeding patterns and increase vulnerability

to predation in natural waters (Little *et al*, 1993). Increased vulnerability to predation for fish in polluted waters may result in higher mortalities and portend negative implications for fish abundance. Srivastava *et al* (2007) observed hyperexcitation, convulsions and rapid opercula movement when *Labeo rohita* and *Channa punctatus* were exposed to paper mill effluent. Pathan *et al* (2009) also reported that the freshwater fish, *Rasbora daniconius* showed hyperexcitation, erratic swimming, convulsions, jerky movement and rapid opercula movement exposed to paper mill effluent.

While some authors had reported a similar range of concentration of 20.06% recorded in this study for fish exposed to textile effluents; others had however documented varying range of concentrations for other industrial effluents discharged into surface waters. For example, Muley *et al* (2007) reported a 96hr LC₅₀ concentration of 18-22% for fingerlings of the freshwater fish *Labeo rohita* exposed to textile mill effluent. Vanerkar *et al* (2004) however, reported higher 96 hr LC₅₀ values of 35-41% for physico-chemically treated effluent of a herbal pharmaceutical wastewater on the fish *Lebistes reticulata*. The higher LC₅₀ value may be due to the process of treatment that the effluent had gone through but it is important to note that this effluent was still toxic to fish. Pathan *et al* (2009) recorded a lower 96hr LC₅₀ value of 16.5% for *R. daniconius* (weight, 4.0-5.5g). The lower LC₅₀ values recorded by these authors when compared with the value recorded in this study (20.06%) may be due to the differences in weight of the fishes used (test organism were 20.51g). Murthy (1986) noted that the magnitude of toxic effects is dependent on length and weight, corporal surface/body weight and advancement in physiology which may confer more resistance to toxicity in different fish species.

The ability to detect changes in growth rate of a fish using otolith-fish length relationship has been reported by Strelchek *et al* (2003), and evident in the correlation table is a strong relationship between saggital otolith parameters and standard length. Such significant positive correlations between the left and right saggital otolith validates the use of otoliths in growth studies of fish (Bradford and Geen, 1987). The concurrent correlation of the left and right saggital otolith parameters with the standard length of the fish suggests the absence of selective symmetry (or axial symmetry) in *C. gariepinus*. The observed sensitivity of otolith weight to the exposure regimes unlike other otolith parameters and all somatic parameters may be attributable to the 3-dimensional sensitivity of otolith weight to growth of a fish i.e. growth changes in any plane of a fish have a corresponding effect on otolith weight. Reasons why only the right saggital otolith showed a significant sensitivity ($p < 0.05$) are unknown.

The alkaline pH, high levels of BOD and COD in

effluent sample and exposure concentrations (except control exposures) is due to the general nature of the dyes present as the major component of textile factory effluents. Wynne *et al* (2001) similarly observed that textile effluents are highly coloured, saline (alkaline) and contain non-biodegradable compounds. Such high alkalinity is a reflection of the ability of wastewaters to neutralize acids and is undesirable for bacteria (most of whom require slightly acidic conditions) responsible for breakdown of compounds to non harmful substances in natural waters. Yusuff and Shonibare (2004) recorded high levels of pH, BOD, COD, TSS in wastewater from textile industries in Kaduna, Nigeria. They attributed high levels of BOD to the pollution strength of the effluent and were of the opinion that high COD implied toxic conditions and the presence of biologically resistant organic substances. The low level of dissolved oxygen (DO) in effluent sample may be attributed to the high level of organic loads and corresponds with the high total solids content of the effluent. Enormous amount of organic loads will require high levels of oxygen for chemical oxidation and decomposition. The discharge of such effluents into aquatic systems may have grave implications for the survival of aquatic organisms that require a range of 5.4- 8.5mg^l⁻¹ to sustain aquatic life (Fakayode, 2005). Increased DO level of 5.17mg^l⁻¹ across exposure concentrations was due to artificial aeration. Total solids, TDS and TSS values in effluent sample was higher than the FMNEV acceptable limit and such high levels could be attributed to the intense colour of various dyestuffs used in textile mills. AEPA (1998) reported that textile effluents are high in suspended solids and implications of increased suspended particles could range from reduced primary production to poor visibility of sight-dependent organisms, hence increasing their vulnerability to predation. The high TSS observed can be further attributed to the dye content which in turn contains heavy metals that bind to river particles thereby having the potential to cause increased concentration of suspended solids (Kambole, 2003).

The high levels of cyanide and chromium in the textile factory effluent is in line with the observations of Benavides (1992) and Yusuff and Sonibare (2004) that the dyeing process usually contributes some heavy metals like chromium, lead, zinc and copper to wastewaters and are traceable to the type of dye used in the textile processing activities. Cyanide is a notable metabolic disruptor and inhibitor of respiration in fish (Solomonson, 1981) and causes increased toxicity under low oxygen conditions. This may explain why increased hyperventilation was observed shortly before death, with dead fish having their opercular distended. Hexavalent chromium compounds have the capacity to enhance UV-induced skin cancer in humans (Costa and Klein, 2006) when incorporated into drinking water. This observation

should be of primary health concern and may explain why FEPA (1991) recommended that effluents discharged by textile industries in Nigeria should be properly monitored and managed for compliance to acceptable stipulated limits for constituents of compounds in wastewater in view of the fact that they contain high levels of pollution parameters like suspended solids, BOD, COD, nitrogen, phosphate, toxic chemicals, pH, heavy metals like chromium, sulphides, oils and grease.

The hope of sustainable fisheries lies in the ability to manage existing resources by taking into cognizance the incidences of pollution occurring in local aquatic systems. Studies have shown that fish populations living in metal-polluted environment undergo changes that can ultimately affect the various levels of biological organization and *vice-versa* (Sherwood *et al*, 2000; Adams, 2002; Gomez *et al*, 2008). Regardless of lethal effects, loss of adaptive capacity of fish species may ultimately predispose fish to mortality and should be of concern. This on a short term can result in population decline of local fauna in affected habitats. Thus, the need for early detectors of stress in the environment in form of integrated responses such as observed in otolith growth is not only pertinent but expedient considering the relative affordability of extracting otolith data. The relative suitability of otolith weight over somatic indices as an early detector of stress is suggested. Further research on its suitability as an early sentinel in other tropical fish species is recommended.

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