DIFFERENTIAL SENSITIVITY OF SAGGITAL OTOLITH GROWTH AND SOMATIC GROWTH IN *Oreochromis niloticus* EXPOSED TO TEXTILE INDUSTRY EFFLUENT

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ABSTRACT-A 30-day sublethal bioassay was carried out to investigate the relative sensitivity of saggital otolith and somatic growth indices in juvenile *Oreochromis niloticus* to textile factory effluents. Somatic indices (body weight, standard length, and condition index) were measured and saggital otoliths were extracted for morphometric (length, breadth and weight) examinations. Data were subjected to one-way ANOVA which showed a significant decrease ($p<0.05$) between the weight ($7.00x10^{-3}$±$4.05x10^{-3}$g, $6.44x10^{-3}$±$9.3x10^{-3}$ g) of the right saggita of the control fish and that of the exposed fish respectively. There were no significant differences in somatic indices and left saggita measurements for all experimental groups. Allometry as indicated by correlation analysis showed a stronger ($p< 0.05$) coupling of the right saggital growth with increase in standard length unlike the left saggita. The observed differences in otolith weight have probable implications on the choice of saggital otolith that may be suitable for daily growth-ring analysis. Also implied is the fact that otolith weight show earlier sensitivity to environmental stressors than somatic indices. Otolith morphometry holds the potential for an objective and more sensitive physiological indicator of stress in *O. niloticus* than somatic indices. . [Life Science Journal. 2010; 7(2): 35 – 41] (ISSN: 1097 – 8135).

Key words: toxicity of heavy metals

I. INTRODUCTION

The sustained intensity of industrial activity has inevitably increased the levels of heavy metals in nearby land and natural waters (Tarras-Wahlberg *et al*., 2001; Jordao *et al*., 2002; Vijayakumari, 2003) and textile industries have been implicated for such practices (Akif et al.,2002; Vijayakumari, 2003; Ramadevi et al, 2006).

The toxicity of heavy metals present in process water from textile industries and other industries in its category to wild life has been proven. High mortality rate during early stages as a result of such is considered one of the major factors causing stock fluctuations. The resultant disequilibria in the ecosystem may further lead to increased environmental vulnerability hence paving way for a decline in organism population. (Laws, 1981).

Measures of growth and condition of young fishes have been used to assess the effects of environmental alterations on individuals (e.g. Karakiri *et al*, 1989, Suthers *et al*, 1992). The search for a relatively sensitive index for evaluating organism or ecosystem health have been investigated including the use of otoliths (Gibson, 1994; Able *et al*, 1999; Adams, 2002)

The potential use of otoliths as an individual record of size and growth has been recognized by some workers (Campana and Nelson, 1985; Boehlert, 1985; Reznichet et al, 1989; Pawson, 1990; Fletcher, 1995; Hare and Cowen, 1995; Agostinho, 1999; Cardinale *et a*l, 2000; Pilling *et al*, 2003) and underlying this potential is the positive relationship between otolith growth and somatic growth (Campana, 1990; Francis, 1990; Waessle *et al*., 2002). The use of otoliths in toxicology experiments has also been reported by (Zhou *et al*., 2005). Otoliths are valid structures for growth evaluation of many freshwater fish species and often are preferred over spines and scales (Hining *et al.* 2000). Fagade (1979) described in detail the structure of the otolith of *Tilapia guineensis* (Dumeril) and their use in age determination and growth evaluation.

The study investigates the relative sensitivity of morphometric measurements of saggital otoliths and somatic indices to exposures of textile factory effluents.

II. METHODOLOGY

Effluent collection and Toxicity Testing

The effluent samples for this study was collected from a textile company (Sunflag PLC) at Eric Moore, Lagos, Nigeria. Collections were made, once every week, between the months of January and March 2007 at the point of effluent discharge. These effluents were then mixed and kept in the refrigerator prior to usage.

250, 6 week old juveniles of O. *niloticus* were procured from a private fish farm in Ibadan, Nigeria. They were kept in aerated tanks and fed with Coppens® feed meal (40% crude protein) at 3% body weight for two weeks to acclimatize to laboratory conditions.

Five fishes were randomly distributed into experimental tanks and a 96hr static bioassay was conducted to determine the median lethal concentration (LC_{50}) with concentrations ranging from 0% to 40% in 2 replicates. The 96hr LC_{50} values were computed by arithmetic graphic method (Reish and Oshida, 1986).

Fractions (1/2, 12.95%; ¼, 6.47% and 1/8th, 3.24%) of the mean LC_{50} values (25.89%) were used for a 30day exposure period. The tests were conducted in triplicates and the test media were renewed every 72hrs.

Growth Studies and Otolith Extraction

After 30days exposure period, wet weights and standard length of fishes in all experimental groups were recorded.

The otoliths of 3 fishes per replicate were removed by a deep cut into the skull above the eyes and extracted otoliths were d in 70% alcohol (Brothers, 1987). Freshly extracted otoliths (right and left) were immersed in 5% hypochlorite or bleaching solution to facilitate removal of adhered tissues.

Table 1
Mean Wet Weights, Standard Lengths and Condition Indices of *O. niloticus* after 30 days exposure period (n= no of specimens=10)

Concentration (%)	Weight (g)	Standard length (mm)	Condition index (k)
0.00	12.38 ± 3.29^{a}	7.58± 0.71^{a}	2.80 ± 0.39^{a}
3.24	13.33 ± 3.14^{a}	7.85 ± 0.69^{a}	2.72 ± 0.21^{a}
6.47	12.61 ± 2.46^{a}	7.64 ± 0.53^{a}	2.80 ± 0.17^{a}
12.95	13.15 ± 2.93^{a}	7.80± 0.71^{a}	2.69 ± 0.30^{a}

Means with same superscript along the same column are not significantly different at p< 0.05
± indicates the standard deviation.

The otoliths were weighed using an analytical weighing balance 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 (length and breadth) were carried out using Adobe Photoshop® 7.

Table 2
Mean Values for left and right otolith morphometry of *O.niloticus*

CONC. (%)	LOW (g)	ROW (g)	LOL (mm)	ROL (mm)	LOB (mm)	ROB (mm
0.00	$8.33x10^{-3}±1.86x10^{-3a}$	$7.00x10^{-3}±4.05x10^{-3a}$	3.98±0.35^{a}	3.16±1.60^{a}	2.71±0.24^{a}	2.22±1.11^{a}
3.24	$9.01x10^{-2}±2.62x10^{-3a}$	$7.01x10^{-3}±2.47x10^{-3a}$	3.54±1.36^{a}	3.02±1.76^{a}	2.44±0.92^{a}	2.18±1.24^{a}
6.47	$9.33x10^{-3}±3.57x10^{-3a}$	$6.88x10^{-3}±3.0x10^{-3ab}$	3.95±0.38^{a}	3.46±1.37^{a}	2.69±0.25^{a}	2.39±0.93^{a}
12.95	$9.88x10^{-3}±1.36x10^{-3a}$	$6.44x10^{-3}±9.3x10^{-4b}$	3.98±0.24^{a}	3.46±1.37^{a}	2.70±0.09^{a}	2.72±0.18^{a}

Means with same superscript along the same column are not significantly different at p< 0.05
± indicates the standard deviation.

ROW=Right Otolith Weight
ROB = Right Otolith Breadth
LOW=Left Otolith Weight
LOL= Left Otolith Length
ROL=Right Otolith Length
LOB= Left Otolith Breadth

Physico-chemical Parameters

Surface water temperature was measured with Mercury-in glass thermometer (^{0}C) while pH was measured with pH meter. Dissolved oxygen (DO) was measured by Winkler's Titrimetric method. Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS) and Total Solids (TS) were measured as described by APHA (1992). Effluent and exposure samples were analyzed with an Absorption Atomic Spectrometer (AAS) for Iron, Zinc, Copper, Manganese, Lead, Cadmium, Nickel, Arsenic, Chromium and Cyanide.(APHA, 1992).

Data Analysis

Data were analyzed by one-way ANOVA and differences between means were considered significant at $p<0.05$ (Zar,1996). Data on otolith morphometry were further subjected to correlation analysis using Spearman's correlation coefficient. Associations or correlations between parameters were considered significant at $p<0.05$. Allometry or growth coupling between parameters was determined by a positive correlation at $p<0.05$.

RESULTS

General Observations

The fishes were observed to be stunned for about 2-3 minutes. Hyperactivity (characterized by erratic swimming and short darting movements) was generally observed across all exposure concentrations (except in control experiments) and this increased with increasing concentration... Hyperventilation as evidenced by rapid opening and closure of the operculum were also observed to increase with increase in toxicant concentration.

The gills of fish used in otolith extraction were observed to have a brighter red colouration than those of control fish. The intensity of colour increased with increasing concentration.

Effect on Growth

The mean wet weights of control fishes and those exposed to varying effluent concentrations showed no statistically significant differences after 30 days exposure. The standard length and Fulton's condition index showed no differences between the measurements obtained in all experimental groups (Table 1).

Effect on Otolith Morphometry

Morphometric measurements (otolith length and breadth) of the left and right sagittae showed no significant difference in the mean values obtained across all exposure concentrations and control. The right otolith weight however had a significantly lower weight in the highest concentration than the control exposure (Table 2).

Allometric growth

Morphometric relationships between otolith indices (weight, length and breath) and somatic indices(wet weights, standard length and condition index) for all experimental groups are summarized in Table 3. Significant associations (r= 0.376, 0.397, 0.442, $p<0.05$) were observed between all the growth dimensions (length, width and weight) of the right saggitae and standard length. Only the weight of the left saggitae had a significant relationship (r=0.477, $p<0.05$) with the standard length.

Table 3

Correlation Coefficient (r) Values between Otolith Indices and Somatic Indices.

Parameters	Otolith morphometry						Somatic indices		
	ROW	LOW	ROL	ROB	LOL	LOB	SL	CF	BW
ROW	1.00	0.951*	0.513	0.518	0.695	0.517	0.442*	-0.253	0.260
LOW	0.951*	1.00	0.522*	0.615*	0.617*	0.593*	0.477*	-2.14	0.345*
ROL	0.513*	0.522*	1.00	0.561*	0.611*	0.522*	0.376*	-0.120	0.399*
ROB	0.518*	0.615*	0.561**	1.00	0.431*	0.767*	0.397*	0.110	0.477*
LOL	0.695*	0.617*	0.611*	0.431*	1.00	0.565*	0.129	-0.178	0.074
LOB	0.517*	0.593*	0.522*	0.767*	0.565*	1.00	0.270	-0.236	0.345*
SL	0.442*	0.477*	0.376*	0.397*	0.129	0.270	1.00	-0.245	0.516*
CF	-0.253	-0.214	-0.120	0.110	-0.178	-0.236	-0.245	1.00	0.140
BW	0.260	0.345*	0.399*	0.477*	0.074	0.345*	0.516*	0.140	1.00

* Correlation significant at the 0.05 level (2-tailed)

ROW=Right Otolith Weight
LOW=Left Otolith Weight
ROL=Right Otolith Length
ROB = Right Otolith Breadth
LOL= Left Otolith Length
LOB= Left Otolith Breadth
SL = Standard Length
CF = Condition Factor
BW = Body Weight

Physicochemical Properties

The physico-chemical characteristics of effluents and exposure concentration are summarized in Table 4.Temperature values ranged from 26.02-29.00^{o}C; slightly alkaline pH values of 7.03-9.60 were recorded; dissolved oxygen content of effluent was very low (2.89mgl^{-1}) compared with all experimental groups. Other parameters like BOD, COD, TSS, TDS and TS had higher values between control and all exposure concentrations (except for control exposures). Values for BOD and COD exceeded FMENV limits in exposure concentrations (except for control exposures). The highest values for BOD, COD, TSS, TDS and TS were recorded in effluent samples and these exceeded FMENV limits.

Heavy Metal Concentrations

The mean levels of heavy metals in exposure concentrations and effluent samples are presented in Table 5. Concentrations of As, Cd, Cu, Pb, Ni, Fe, Mn and Zn were low and within acceptable limits and although Mn showed the highest availability in effluent samples, it did not exceed acceptable limits. Cyanide and Chromium levels however had values that exceeded FMENV acceptable limits.

III. DISCUSSION

Exposure of fish to pollutants especially during early development may have a wide range of specific and non specific effects (Klumpp and Von Westerhagen, 1995), which may ultimately interfere with important developmental processes (Von Westerhagen *et al*, 1988). Loss of equilibrium and hyperventilation observed in fish could be attributed to a respiratory distress due to the presence of cyanide. Solomonson (1981) reported that cyanide is a well known inhibitor of the respiratory enzyme (cytochrome oxidase) reducing tissue respiration. Other workers have also observed that metal pollution induce changes in fish ventilation and heart physiology (Hughes and Tort, 1985; Anune *et al*, 1991; Adakole and Balogun, 2005). ???High levels of cyanide may result in asphyxia for fish in natural environments where no artificial aeration exists. Respiratory distress may impair feeding ability and there has been considerable discussion on the role food in determining maximum growth rate in juvenile fish (Miller *et al*, 1991; Van der Veer and Witte, 1993; Gibson, 1994) .Underlying this factor is the concept that the quality and quantity of food are the driving forces governing size of fish as an allometric scaling factor (Brett and Groves, 1979; Reichert *et al*, 2000). The decrease in otolith weight with increasing toxicant exposure may be due to stressor induced reduction in feeding ability which may ultimately affect growth as observed by the significantly lower values between control fish and highest exposure concentration in the right sagittal otolith. Such observations were however not apparent in wet weights and standard length of fish under the same conditions. Massou et al (2004) reasoned that a variety of factors (growth rate, response lags, ontogenic transitions) may be responsible for varied relationships between otolith growth and somatic growth. Otolith weight represents an integration of the complete growth history of a fish (Burke *et al*, 1993). This is because the 3-dimensional growth of otoliths is a function of time and probably genetic predisposition whose expression is anchored on environmental events.

Labrapoulou and Papaconstantinou (2000) and Pino *et al* (2004) reported a potential advantage in the use of otolith weight as a rapid and economic method for assessment of age in both tropical and temperate fishes (Worthigton *et al*, 1995; Cardinale *et al*, 2000; Araya *et al*, 2001; Pilling *et al*, 2003)

Table 4
Summary of the Physico-chemical parameters of Effluent
Experimental groups compared with FMENV acceptable limits

Parameter	Control (mean values)	Exposure Concentrations (mean values)	Textile factory Effluent (mean values)	FMNEV Acceptable Limits
Temperature	**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**
Dissolved Oxygen	**6.72±0.25**	**5.17±2.95**	**2.89±1.50**	**4.00**
Biochemical oxygen Demand	**8.50±1.05**	**82.76±15.51**	**98.60±10.95**	**20.00**
Chemical Oxygen Demand	**10.02±0.65**	**124.99±14.38**	**308.78±35.40**	**80.00**
Total Suspended Solids	**3.02±0.25**	**19.05±3.20**	**56.63±11.87**	**30.00**
Total Dissolved Solids	**27.25±1.90**	**263.26±21.66**	**2655.30±38.96**	**500.00-1500.00**
Total Solids	**29.26±2.95**	**278.31±23.55**	**2712.90±48.76**	**2000.00**

± represents standard deviation of the values stated

As observed from the correlation results (Table 3), the length and breadth of the right saggital otolith showed a significant growth relationship ($p<0.05$) with the length-wise growth of the fish unlike the left saggital otolith. The relatively stronger relationship of the right saggital otolith suggests a selective growth coupling. This phenomenon where somatic growth synchronizes with internal structures on a particular body axis is referred to as axial symmetry. Such events of selective coupling or differential synchrony strong enough to be detected are believed to occur in species with a strong axial symmetry (Nolf, 1985; Bori, 1986).

Table 5
Summary of Levels of Heavy metals in Effluent
Experimental groups compared with FMENV Acceptable limits

Heavy metal	Control (mean values)	Exposure concentrations (mean values)	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**

--- = values indeterminable

The comparisons for differential sensitivity across otolith and somatic indices (Tables 1& 2) showed no significant differences across all exposure concentrations except for the weight of the right sagitta otolith. The sensitivity of otolith weight to toxicity regimes may be due to the fact that otolith weight unlike its length and breadth increases along a radial axis, hence allowing for detection of somatic growth in any plane. This may also explain why the weight of the left and right saggital otolith showed a better relationship ($P<0.05$) with the standard length than any other otolith measurement. Since the relationships between calcareous structures and body size in fishes are tangibly linked to changes in environmental conditions (Berghahn, 2000; Massou *et al*, 2004; Kruitwagen *et al*, 2006), otolith growth may thus be able to provide sensitive information on habitat quality.

The presence of non-biodegradable substances may be an explanation for the high COD levels observed. Suspected textile processing stages that may contribute to such physicochemical effects may include stages like sizing and desizing, scouring, dyeing and printing. The various dyes, mordant, surfactants and soap pastes used in these stages form settleable suspended solids. The biologically resistant nature of such substances and their toxic implications has been reported by Sawyer and McCarty (1978). The high TSS detected could be attributed to the vivid colors from the various dye stuffs used in textile production and this may constitute major sources of heavy metals especially chromium.

Increased concentrations of heavy metals in river sediments could increase concentration of suspended solids (Kambole, 2003) which holds dire consequences for primary production in the recipient water body. Also increased suspension in the water column could result in poor visibility for organisms with high dependence on sight, hence increasing vulnerability to predation. Sessile, interstitial or surface benthic organisms are also at risk because of the increased resident time in contaminated sediments (Chukwu and Nwankwo, 2003).

The high values of heavy metals in effluent samples (cyanide, chromium and manganese) may be due to the type of dye used in the processing of the textiles. Most synthetic dyes have been implicated as sources of heavy metals observed in textile factory effluent and studies have shown that metal exposures may lead to retarded growth in fishes (Weis and Weis, 1976; Viyakumari,2003; Ramadevi *et al*, 2006). The heavy metal profile of effluent are consistent with results published by Osibanjo (1991) in a survey of heavy metal content from Nigerian textile industries where Manganese (Mn) was the highest available metal, followed by Zinc (Zn) and Iron (Fe). Chromium and Cyanide levels exceeded FMENV limits for effluents as stated in interim Effluent Guidelines for all categories of industries in Nigeria (FEPA, 1991). Chromium has been reported to potentially damage and/or accumulate in various fish tissues thereby increasing their susceptibility to infection. (DWAF, 1996b). This may lower uptake of food and food conversion in fish leading to growth reduction. The high level of Chromium reported in effluents from Sunflag industry is consistent with values reported by Yusuff and Sonibare (2005) for effluents of Kaduna textile industry. DWAF (1996b) reported that cyanide is widespread in surface and groundwater's and originates from industrial effluents from chemical industries amongst others. Although conditions which enhance cyanide toxicity were not recorded (low pH and dissolved oxygen) in exposure concentrations due to artificial aeration, clinical symptoms of cyanide poisoning (ranging from loss of equilibrium to stupor) were observed. Patho-anatomical pointers to cyanide toxicity i.e. cherry red color of gills were also observed (Leduc, 1981; DWAF, 1996a).

IV. CONCLUSION

The ability of a water-body to support aquatic life, as well as its suitability for other uses depends on the presence or absence of contaminants in the water body. Varying levels and interactions of contaminants hold in store a range of effects for the resident organisms', especially sensitive stages like juveniles. Endeavours at measuring environmental stress from a pool of bio-indicators in fish, carries with each attempt a fundamental search for sensitivity and affordability in bioindicators.

Furthermore the differential growth relationship between morphometry of the right and left saggital otolith with fish length may have implications on the choice of otoliths to be used for microstructural studies (growth rings).

We conclude that growth studies using otoliths in juvenile sized fish may be more valid if otolith in the detected growth axis i.e. right saggital otolith (in this case) is used for such evaluations. Also since otolith weight seems to be a better detector of growth changes in juvenile *O. niloticus*, a closer look should be made on its relative importance as a sensitive parameter in pollution studies.

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