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Evaluation of Response Patterns in Somatic and Otolith Features of Laboratory-Reared and Wild *Clarias gariepinus* Exposed to Industrial Effluent

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Abstract: This study was aimed at comparing the responses of somatic and otolith features in *Clarias gariepinus* under chronic exposure conditions to industrial effluents in the laboratory for 60 days and in the wild for 6 months. Fish were collected upstream and downstream bi-monthly from a river receiving composite mixtures of industrial effluent while laboratory-reared *C. gariepinus* were exposed to the same effluent mixtures in 60 days static renewal/bioassay using concentrations of 6.11, 3.05 and 2.23%, respectively and control series. A total of 21 variables representing saggital otolith and somatic data from both wild and laboratory fish were subjected to factor analysis. For laboratory reared fish, PC 1 indexed as 'otolith factor', PC 2 indexed as 'condition factor' and PC 3 indexed as 'paired fin factor' accounted for 26.15, 19.01 and 12.55% of the total variance, respectively. For wild fish, otolith factor (PC 1) and condition factor (PC 2) accounted for 38.24 and 22.69% of the variance respectively. The first 3 components and the first 2 components for laboratory and wild fish accounted for more than 50% of total variance in data. Reliability index (Cronbach's alpha (α>0.70) showed that the 'otolith factor' had strong internal consistency and is reliable as a primary and viable index of stress for both laboratory and wild fish. The complementary role of condition factor in stress detection was also highlighted. The emergence of paired features (otolith, pectoral and pelvic fins) as sensitive parameters in toxicity responses may be an indication of the onset of asymmetry in these structures.

Keywords: *Clarias gariepinus*, condition factor, industrial effluents, otolith factor, paired fin factor, reliability index

INTRODUCTION

Fish play an important role in the monitoring of aquatic pollution because they respond with great sensitivity to changes in their environment (Van der Oost *et al*., 2003). A number of researchers have demonstrated the potential value of various combinations of fish parameters to provide evidence of toxicity or stress responses ranging from condition factor (Dutil and Lambert, 2000), fin-erosion (Sinderman, 1980; Reash and Berra, 1989), retarded fin regeneration (Verma, 2005) and otolith parameters (Adeogun and Chukwuka, 2010, 2011) among others.

The use of bioassays to establish or predict the eco-toxic nature or potentials of a wide range of anthropogenic substances including industrial waste water has been reported (Thomas *et al*., 1986; Rojickova-Padrtova *et al*., 1998; Clement *et al*., 1996; Pandard *et al*., 2006) with its main advantage hinged on its ability to integrate the effects of all contaminants including additive, synergistic and antagonistic effects (Thomas *et al*., 1986; Pandard *et al*., 2006). On the other hand the incidence of phenotypic divergence i.e., morphological, physiological, ecological and behavioral changes in early life stages of fish under artificial environmental conditions or circumstances compared to their counterparts in the wild has been documented by a number of authors (Taylor, 1986; Swain *et al*., 1991a, b; Fleming *et al*., 1994; Thorpe, 2004; Von Cramon-Taubadel *et al*., 2005; Jonsson and Jonsson, 2006; Uglem *et al*., 2011).

In view of the foregoing, the incidence of phenotypic divergence in fish under chronic toxic conditions in controlled environments (laboratory conditions) may likely result in divergence of useable biomarkers of toxicity indicative of situations due to contamination by toxicants in the wild. This study thus seeks to elucidate and compare patterns of response to toxicity in laboratory exposed fish and fish exposed in the wild to composite mixtures of industrial effluents and explore the existence of overlapping variables applicable to both laboratory and wild fish.

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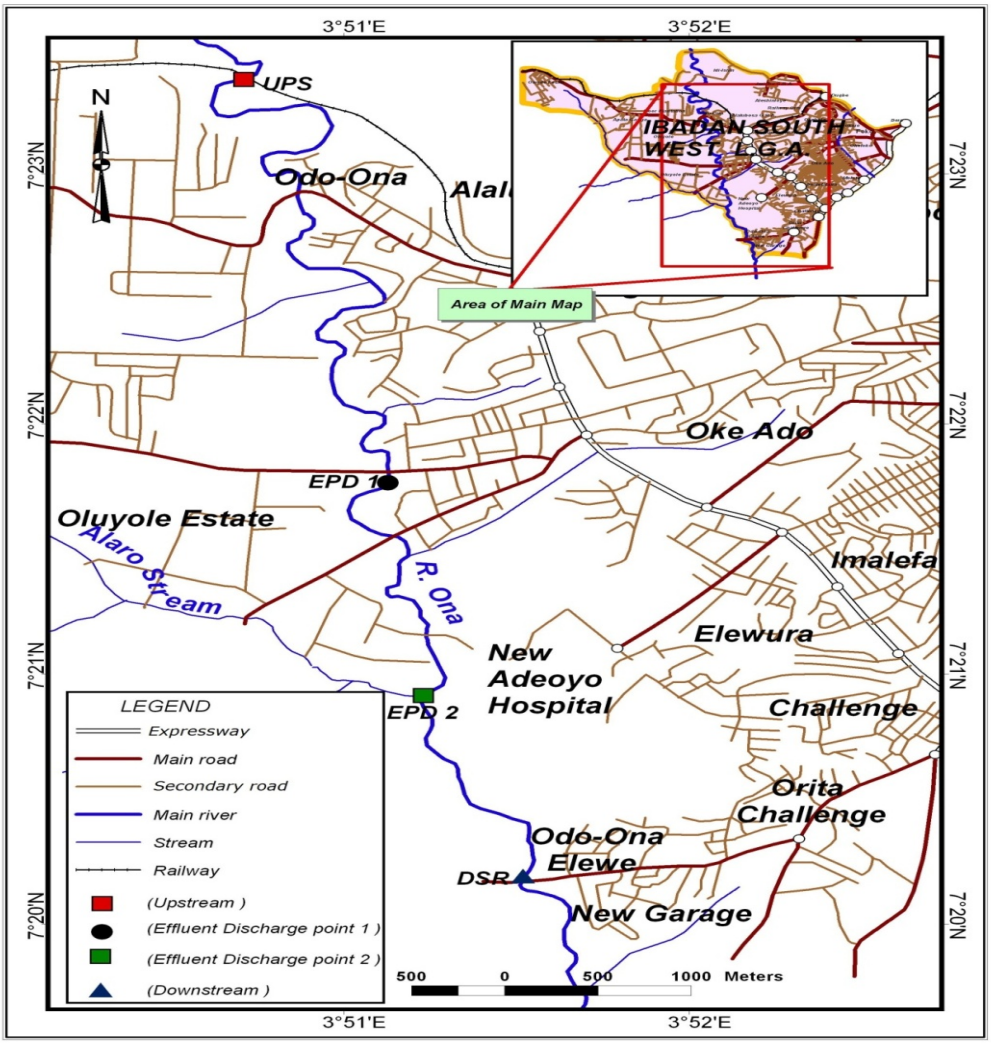

Fig. 1: Map of Ona river showing sampling points

MATERIALS AND METHODS

Study area: Ona river (7^0 19'-7^0 42'N; 3^0 48'-3^0 55'E) is part of a dense network of inland watercourses that flows southwards adjoining other rivers emptying into the Lagos lagoon. It flows in a North south direction towards Ogun river and companies located along this river include a beverage industry, agricultural production and processing industry and food industry. Channelled effluents from these industries are connected by a network of canals and empty into Ona river at designated points (Fig. 1). Fish samples were collected upstream and downstream of effluent discharge points.

Collection of wild *C. gariepinus*: Live samples of juvenile and adult *C. gariepinus* were collected Upstream (UPS) and Downstream (DSR) of effluent discharge points with the help of a local fisherman using stationary surface gill nets. Fish were anesthetized on ice and transported to the Fisheries and Hydrobiology Research laboratory, Department of Zoology, University of Ibadan, Ibadan, Nigeria for further analysis.

Collection of laboratory reared *C. gariepinus* and acclimatization: Juvenile *C. gariepinus* (body weight; 9.97±4.13 g) raised in outdoor tanks at the Department of Zoology, University of Ibadan were acclimatized to laboratory conditions for 2 weeks under adequate aeration in a large glass aquaria (80×150×250 cm) during which water was changed every 3 days. Fish were fed with 50% crude protein diet at a rate of 4% of body weight per day. Uneaten feed were siphoned out

after feeding to prevent accumulation of metabolites and all test organisms were exposed to a 12 h photoperiod at 29-32°C. Stocking density was ensured at 12 g of fish to 5 L of water as specified by Reish and Oshida (1986).

Chronic exposure: Laboratory-reared fish were exposed to the same composite effluents (1:1v/v) discharged into Ona river that wild fish are constantly exposed to. Static/renewal toxicity test was conducted for 60 days from March to April 2010 using nominal fractions of a predetermined 96 h LC_{50} value which gave concentrations of 6.11, 3.05 and 2.23%, respectively and 20 juveniles were introduced into each exposure concentration. Experimental concentrations included an untreated control (0.00%) with no effluent added in 3 replicates. The same biomass to volume ratio (12 g of fish to 5 L of water) used during acclimatization period was maintained for the duration of the exposure period. Exposure concentrations were renewed every 3 days with fresh effluent mixture to maintain the requisite concentrations and fish were fed at 4% of body weight.

Morphometric measurement and meristic counts: For measurements and counts, 10 specimens of wild *C. gariepinus* were collected bi-monthly from Ona river upstream and downstream of effluent discharge points for 6 months (February to July 2010). Randomly selected samples of laboratory-reared fish (3 fish per exposure) from all exposure concentrations including the control were collected at an interval of 7 days to evaluate the effect of toxicant on growth parameters of fish. Body Weight (BW) of fish were measured with an Ohaun compact digital weighing balance (Mettler Instruments) and Total Length (TL), Body Depth (BD), Left Pelvic Fin length (LPvF), Right Pelvic Fin length (RPvF), Left Pectoral Fin length (LPF), Right Pectoral Fin length (RPF) were measured with an Absolute Digital caliper (Tresna Instruments Inc.) to the nearest 0.1 mm according to the methods described by Barel *et al*. (1977). Meristic counts including the Number of Left Pelvic Fin rays (NoLPvF), Number of Right Pelvic Fin rays (NoRPvF), Number of Left Pectoral Fin rays (NoLPecF) and Number of Right Pectoral Fin rays (NoRPecF) were recorded in fish. The abbreviations specified for each variable were used throughout the text.

Otolith extraction and analysis: The right and left sagittal otolith of each fish were extracted after medullar transection of fish skull (Lucky, 1977) and fixed in 70% alcohol (Brothers, 1987). Digitized otoliths (Lombarte, 1990) were measured using Adobe Photoshop ® CS4 and weighed to the nearest 0.000 g with an Ohaun digital weighing balance, PA213 Model (Mettler Instruments).

Data analysis: All data were size-adjusted to normalize the data (Reist, 1986). Data were thereafter subjected to one way ANOVA or Paired samples t-test where applicable to test for significant difference in means of parameters between exposure concentrations or across pollution gradients respectively. Differences in means were considered significant when $p<0.05$. Factor analysis using Principal Component Analysis (PCA) as method of extraction was applied to elucidate latent response patterns of paired morphometric, merisitc and otolith variables. Test for internal consistency within extracted variables and removal of weak variables was carried out using Cronbach's reliability test. In computing Cronbach's alpha coefficient of reliability, an alpha of at least 0.7 was considered to indicate a reliable set of items (De Vaus, 2002). Multivariate analysis was carried out with SPSS 18.0 (SPSS Inc. USA).

RESULTS

Otolith and somatic variables in laboratory-reared *C. gariepinus*: The test for difference in means (ANOVA) between control and exposure set-ups for all parameters showed that 3 un-paired variables (TL, SL and BW) and 5 paired variables (LOL, ROL, LOB, ROB and LPF) out of the 21 tested variables emerged as sensitive factors to the exposure regimes. The paired variables showed the highest spread among the sensitive variables with otolith variables showing the earliest sensitivities (Fig. 2).

From the factor analysis, a total of 6 components/ factors were extracted by the PCA and 80.23% of the total variance in the data was accounted for by these factors. Principal Components (PC) 1, 2 and 3 were selected for consideration because they accounted for the larger part of the total variations in the data i.e., 57.71% out of 80.23% (Table 1). The first PC represented the changes in otolith morphometric measurements i.e., ROL, LOL, ROB, LOB and was indexed as 'otolith factor'. Otolith factor accounted for 26.15% of the total variance and indicated a strong correlation ($p<0.05$) or allometry (growth relationship) between the left and the right saggital otolith. The ROL and LOL showed the highest loadings within this factor suggesting their critical role in the variations accounted for by the 'otolith factor'. Principal Component 2 (PC2) comprised parameters fundamental to the computation of condition factor i.e., TL, SL, BW and other complementary variables i.e., ED, LOW and ROW thus

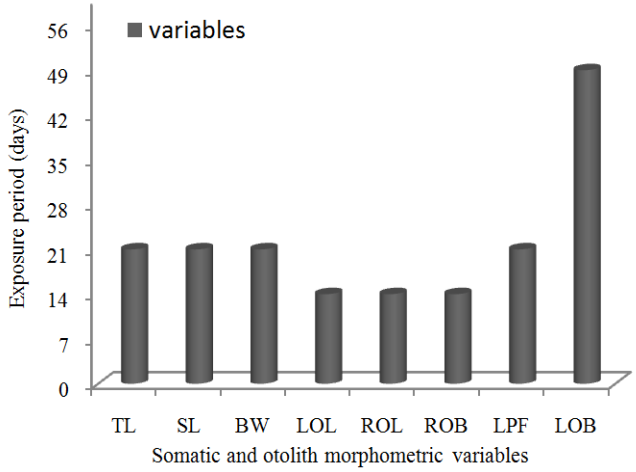

Fig. 2: Time dependent sensitivity of otolith and somatic morphometric variables in laboratory-reared *C. gariepinus* exposed to industrial effluent (extracted from mean differences of all parameters using ANOVA)

Table 1: Varimax rotated factor loadings for otolith and somatic variables of laboratory-reared *C. gariepinus*.

	Component					
Variables	1	2	3	4	5	6
ROL	0.870[a]					
LOL	0.809[a]					
ROB	0.777[a]					
LOB	0.695[a]					
LOW		0.819[b]				
ROW		0.781[b]				
BW		0.763[b]				
TL		0.759[b]				
SL		0.718[b]				
ED		0.628[b]				
LPvF			0.885[c]			
RPvF			0.883[c]			
LPF			0.868[c]			
RPF			0.859[c]			
NoRPecF				0.979[d]		
NoLPecF				0.978[d]		
NoRPvF					0.841[e]	
NoLPvF					0.828[e]	
BD						0.780[f]
Variance (%)	26.149	19.012	12.549	8.753	7.899	5.871
Cummulative (%)	26.149	45.161	57.710	66.463	74.362	80.233

Extraction method: Principal component analysis; Rotation method: Varimax with Kaiser normalization; Same superscript letters: Clusters of parameters with high significant loadings for each factor

it was indexed as 'Condition factor'. Condition factor accounted for 19.01% of the total variance and the ROW and LOW also showed the highest loadings compared to other parameters constituted within this factor suggesting that the weight of the right and left saggital otolith had a critical role in the variations accounted for by this factor. Principal Component (PC3) represented the changes in size of paired-fins, was indexed as the 'paired fins' factor and accounted for 12.55% of the total variance. The constituents of this factor suggest the strong allometric relationship between the pectoral and pelvic fins.

The parameters contained in each of the PCs were selected with the consideration of using these few parameters as dependable scales to discriminate between exposed fish and unexposed fish. These groups of parameters were subjected to reliability test to check internal consistency and eliminate weak parameters. After the reliability test using Cronbach's alpha (Table 2), the parameters in PC1 and PC3 remained

Table 2: Reliability index of extracted components for laboratory-reared *C. gariepinus*.

Parameters	Component 1 (otolith factor)	Component2 (condition factor)	Component 3 (paired-fin factor)
ROL	0.745		
LOL	0.712		
ROB	0.653		
LOB	0.617		
TL		0.965	
SL		0.951	
BW		0.895	
ED		0.676	
LOW		0.465	
LPF			0.787
RPF			0.794
LPvF			0.789
RPvF			0.789
Cronbachs's Alpha	0.838	0.701	0.907

Component values: Corrected item-total correlation

Table 3: Somatic and otolith parameters of wild *C. gariepinus* upstream and downstream of effluent discharge

	t-test for equality of means		
Parameter	t	df	Sig. (2-tailed)
TL	1.032	13	0.321
SL	0.908	13	0.380
HL	0.293	13	0.774
ED	-1.041	13	0.317
BD	0.665	13	0.518
BW	-1.177	13	0.260
LOL	-0.201	13	0.844
ROL	-0.144	13	0.888
LOB	-0.132	13	0.897
ROB	-0.224	13	0.826
LOW	1.783	13	0.098
ROW	-3.905	13	0.002*
LPL	-1.652	13	0.122
RPL	-1.758	13	0.102
LPvL	1.296	13	0.217
RPvL	0.749	13	0.467
NoLPecF	-0.394	13	0.700
NoRPecF	-0.743	13	0.471
NoLPvF	2.393	13	0.032*
NoRPvF	2.393	13	0.032*

*: Occurrence of significant differences ($p<0.05$) between compared groups

unchanged while PC2 was trimmed-down to TL, SL, BW, ED and LOW. All PCs showed strong reliability indexes based on the corrected item-total correlation and Cronbach's alpha values ($\alpha>0.70$). Otolith factor (PC 1) had a reliability index of 0.838, PC 2 (condition factor) had an index of 0.701 and PC 3 (paired fins factor) had a reliability index of 0.907.

Otolith and somatic variables in wild *C. gariepinus*: The paired t-test for difference in mean values of measured parameters in fish sampled upstream and downstream of industrial effluent discharge points indicated that only 3 parameters i.e., ROW, NoLPvF and NoRPvF showed significant ($p<0.05$) sensitivity to the pollution gradient existing in the stretch of the river studied (Table 3).

Factor analysis of data from wild fish showed that a total of 5 components/factors were extracted which accounted for 93.12% of the total variance in the data. Principal Components (PC) 1 and 2 accounted for a higher proportion i.e., 59.93% of the total variance in the data and were thus selected for interpretation of response patterns in wild fish (Table 4). The first PC comprised parameters (LOL, LOB, ROL, ROB and ED) fundamental to fish balancing and coordination, thus PC 1 was indexed as 'Otolith factor'. This factor highlighted the allometric relationship first between the left and right saggita and second between otolith factors and Eye Diameter (ED) of the fish. Otolith factor accounted for 38.24% of the total variance in the data.

Principal Component 2 (PC2) was indexed as the 'Condition factor' based on the presence of parameters (TL, SL, BD, BW, NoLPvF and NoRPvF) that define an elaborate picture of fish condition. This factor highlighted the strong positive relationship between changes in number of pelvic fin rays and general fish condition and accounted for 21.69% of the total variance.

The changes in pectoral fin length was represented by PC 3 and indexed as 'Pectoral fin factor'. It comprised only the length of the Left and Right Pectoral Fin i.e., LPF and RPF and accounted for 14.26% of the total variance.

The parameters contained in each of the PCs were selected with the consideration of using these few parameters to discriminate between fish upstream and downstream of discharge points. These groups of parameters were subjected to reliability test to check for internal consistency and eliminate weak parameters. After the reliability test using Cronbach's alpha (Table 5), the parameters in PC1 remained unchanged while BW was eliminated from PC2 to strengthen the Cronbach's alpha. All PC's showed strong reliability indexes based on the corrected item correlation and Cronbach's alpha values. Items constituting PC3 were dropped from the reliability analysis because of the few number of items and to align with to the assumption that scales borne out of a greater number of items are more reliable (De Vaus, 2002).

From the reliability test (Table 5) ROL and LOL showed relatively higher item-total correlation of 0.907 indicating that these variables contributed greatly to the stability of the 'otolith factor'. The same applied to the role of TL and SL to the stability of the 'condition factor' with a total index of 0.717.

Table 4: Varimax rotated factor loadings for otolith and somatic variables of wild *C. gariepinus*

	Component				
Variables	1	2	3	4	5
TL		0.777[b]			
SL		0.735[b]			
BD		0.712[b]			
BW		0.759[b]			
NoLPvF		0.946[b]			
NoRPvF		0.946[b]			
ED	0.749[a]				
LOL	0.989[a]				
ROL	0.971[a]				
LOB	0.946[a]				
ROB	0.961[a]				
LPL			0.978[c]		
RPL			0.979[c]		
LPvL					0.969[e]
RPvL					0.976[e]
NoLPecF				0.954[d]	
NoRPecF				0.891[d]	
Variance (%)	38.238	21.691	14.264	10.566	8.387
Cummulative (%)	38.238	59.929	74.174	84.740	93.126

Extraction method: Principal component analysis; Rotation method: Varimax with Kaiser normalization; Same superscript letters: Clusters of parameters with high significant loadings for each factor

Table 5: Reliability index of extracted components for wild *C. gariepinus*

Parameters	Component 1 (otolith factor)	Component 2 (condition index)
LOL	0.980	
ROL	0.966	
LOB	0.924	
ROB	0.951	
ED	0.721	
TL		0.992
SL		0.977
NoLPvF		0.734
No.RPvF		0.734
BD		0.435
Cronbachs's Alpha	0.907	0.717

Component values: Corrected item-total correlation

DISCUSSION

A wide range of morphological, biochemical or physiological metrics' have been proposed as condition indices or measures of fitness (Stevenson and Woods, 2006). From the laboratory study, the 'otolith factor' appeared to be the most sensitive group of variables to toxicity regimes followed by the 'condition factor'. This agrees with previous studies (Adeogun and Chukwuka, 2010, 2011) which reported the relative sensitivity of otolith parameters over somatic parameters in *Oreochromis niloticus* and *C. gariepinus* exposed to textile factory effluent. Otolith growth as a physiological index have also been reported to respond differently to environmental factors than somatic growth (Lombarte and Lleonart, 1993) and the chemical processes involved in otolith growth are more directly affected by the physico-chemical properties of surrounding waters than the metabolic processes involved in body growth (Casselman, 1990). The emergence of the pelvic and pectoral fins as sensitive parameters accounting for about 12% of the total variance is also worthy of note. The response of paired morphological characters to unfavorable environmental conditions has been documented (Almeida *et al*., 2008). According to Almeida *et al*. (2008) goldfish under stressful environmental conditions showed a significant reduction in locomotive parameters (length of pectoral fin) and sensory parameters (first barbels). He further stated that the incidence and degree of stress responses of some morphological characters may be related to its functional importance to the fish. All extracted factors i.e., otolith factor, condition factor and paired fin factor were subjected to reliability tests to validate the variables making up each factor. The stability of the 'otolith factor' was demonstrated with a Cronbach's alpha = 0.838 (Table 2). For the 'condition factor' all variables were retained except the ROW, thus having a reliability index = 0.701. The stronger allometric relationship of the LOW with the elements of condition index compared to the ROW was also reported by Adeogun and Chukwuka (2010, 2011) and this suggests the potential of otolith weight as a direct index of fish condition. Although the deposition of otolith material as an extra-cellular process has been reported to be under different physiological control than somatic cellular growth (Simkiss, 1974) there may be some form of biochemical factor responsible for the synchrony of these 2 variables.

The results of the one-way analysis of variance depicted in Fig. 1 also provides confirmatory evidence of the relative sensitivity of otolith variables over somatic variables and the relative sensitivity of paired variables to toxic conditions over non-paired variables. The paired-samples t-test for difference in mean variables between fish collected upstream and fish collected downstream of effluent discharge (Table 3) showed that only paired variables i.e., ROW and NoLPvF and NoRPvF showed significant response ($p<0.05$) to the pollution gradient created by the discharge of industrial effluent into the river.

From the factor analysis of somatic and otolith variables of wild fish exposed to industrial effluent (Table 4), it was evident that the otolith variables accounted for the highest variance 38.24% while the condition index accounted for 21.69% of the total variance. The high loadings of the LOL and ROL confirm observations from the laboratory exposures that these variables are critical to the variances accounted for by the 'otolith factor'. The increased variances accounted for by the 'otolith factor' for wild fish (38.24%) compared to 'otolith factor' in laboratory fish (26.15%) may be attributable to the availability of all ecological cues that maximize the responses of these features, for example, the increased demand on otoliths for navigation and coordination in the wild. Patterson (2004) documented the ability of natural physical, chemical or biological stimuli to trigger adaptive mechanisms in various life history stages of a fish. Other reports have shown that the limited availability of ecological requirements characterized by artificial environmental conditions can affect the morphology, physiology and behavior of fish (Balon, 2004; Kihslinger and Nevitt, 2006; Barber, 2007).

The allometric relationship of the pelvic fin rays (NoRPF and NoLPF) with major variables representing the 'condition factor' and its validation by the Cronbach's reliability index ($\alpha = 0.717$) suggests that the number of pelvic fin rays has considerable potential as a fitness index. This supports early reports which have documented the use of pelvic fin rays as an equally accurate alternative to otolith micrometry for fish growth analysis (Gust, 2001; Zymonas and McMahon, 2009). Earlier reports have also documented variations of meristic characters in response to environmental factors and have been noted for many species (Hubbs, 1922; Taning, 1952; Weisel, 1955; Lindsey, 1958, 1962; Fowler, 1970).

The appearance of otolith factor as an overlapping variable useable as a measure of stress for both laboratory and wild *C. gariepinus* is worthy of note. The higher performance of the otolith parameter in wild fish exposures (38.24% variance) compared to the laboratory exposure (26.15% variance) may be attributable to the ecological circumstance of the fish i.e., artificial or wild (Balon, 2004). Its prospects as a measurable index of stress in wild fish are more tangible especially in its ability to incorporate developmental stress during the life history of the fish. It has also been documented that water current as an ecological factor increases the neural sensitivity of fish to changes in water quality (Chagnaud *et al*., 2006).

Furthermore the emergence of paired features (i.e., otolith features, pectoral and pelvic fins) as sensitive parameters widely represented in the top three extracted components in both laboratory and wild fish exposure to industrial effluent strongly suggests the importance of investigating fluctuating asymmetry in these paired structures and its implication on fish survival.

Reports have shown that the incidence of developmental instability or fluctuating asymmetry in bilateral structures will affect the ability of the individual to maintain homeostasis during development when faced with stress, either genetic or environmental (Møller and Swaddle, 1997; Dongen, 2006). Asymmetry in functionally important traits for fish such as fins probably makes locomotion less efficient, as has been observed for some domesticated animals (Møller and Swaddle, 1997; Knierim *et al*., 2007).

CONCLUSION

The difference in response patterns to industrial effluent exposures in laboratory-reared and wild fish was demonstrated with 'otolith factor' emerging as the principal variable in toxicity response assessment of *Clarias gariepinus*. Although otolith parameters emerged as the overlapping variable useable for toxic response assessment in both laboratory and wild fish, a higher variance was accounted for in wild fish than for laboratory fish, an occurrence attributable to the ecological circumstances under which both exposures occurred.

The sensitivity and importance of paired features (otolith, pectoral and pelvic fins) to toxicity regime was also demonstrated in this study. Investigations into the incidence of fluctuating asymmetry in paired structures due to environmental stress and its usability as a confirmatory variable in toxicity studies is recommended.

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