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EVALUATION OF WILD AND CULTURED AFRICAN MUD CATFISH (*Clarias gariepinus*; Burchell, 1822), FROM WATER BODIES AROUND SOKOTO METROPOLIS, USING SDS-PAGE

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ABSTRACT

This research was carried out in Usmanu Danfrodiyo University, Sokoto, to establish genotypic variations between *C. gariepinus* from two ecotypes (cultured and wild), using SDS-PAGE. Ninety (90) live samples of *C. gariepinus* were collected from three fish habitats, 30 samples from each ecotype were determined. The positions of the proteins were determined using ColorPlus Prestained Protein Marker, Broad Range (7-175 kDa). Bands were presented in increasing order of alphabets, following increasing order of molecular weight of the bands. The results revealed genotypic variations between the ecotypes. Conclusively, there was significant difference (P<0.05) between the genotypes of the wild and cultured *C. gariepinus* populations studied. The wild *C. gariepinus* population group from river Rima can be proteotypically (genetically) differentiated by the absence of allele B, and G while the cultured *C. gariepinus* from Magatakada farms can be distinguished by absence of allele E across the populations' banding profiles. This finding presents baseline genetic information for further research and proper management of *C. gariepinus* from these water bodies.

Keywords: Genotype; variation; ecotype; band; allele; genetic.

INTRODUCTION

Clarias gariepinus is one of the commercially important fish species that occur naturally in the Nigerian freshwater bodies. Scientifically, sound management of any fish resources relies on basic knowledge of the biology of the species, including information on population structure; such information influences the development of strategies for its management and conservation.

The species in the wild interbreed and crossbreed randomly, due to uncontrolled mating, and this, may be responsible for evolutionary changes due to natural selection. The common hatchery practices in Nigeria and the world at large involve indiscriminate mating of different fish breeds to produce hybrids which can lead to loss of the pure lines within the aquaculture realms and alter their gene frequencies; what could be seen as a pure line of fish breed is probably a hybrid of multigenetic series of crosses from different species and genera. Consequently, there is mix up in the identity of *C. gariepinus* from indigenous to the most

widely cultured Dutch improved *Clarias*. The problems stated above undoubtedly can lead to loss of the real identity of the pure lines within the aquaculture system and this cause introgression, which shifts the gene pool towards a negative genetic drift.

One of the two basic approaches for identification used in fish genetics and taxonomy is the molecular method. Molecular systematics, however, grew out of population genetics (Hillis, 1987), because of the need for detailed knowledge of the cytogenetic make-up and molecular assessment of organisms to establish genetic evolutionary relationships between species, genera, families, orders and so on (Farooq *et al*., 2011). Studies of genetic variants of proteins have been conducted in the past and successfully applied to diverse fishery problems and hybrid verification (Aspinwall and Tsuyuki, 1963; Theophilus, 1988). Although good numbers of studies have been conducted on the fundamental analysis of *C. gariepinus* serum proteins, to identify them and also to note genetic polymorphism of some protein fractions (Theophilus *et al*., 1988), little information is available on the genus *Clarias* of the river Rima. Therefore, this research focused on the study of the prototypic genotypes of wild and cultured *C. gariepinus* from river Rima using serum proteins for Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) to evaluate the genotypes of the wild *C. gariepinus* from river Rima and its conspecifics from the cultured habitats and also to provide a baseline genetic information of the fish population groups based on their prototypic genotypes.

MATERIALS AND METHODS

Study Area

This research was conducted in Usmanu Danfodiyo University, Sokoto, located on latitude 13^0 07'38.9''N and longitude 5^0 12'19.0E within Sokoto ecological segment. Sokoto is located in the savannah agro-ecological zone (Latitude 13^0 00'27.0''N and Longitude 5^0 15'05.6''E), which is about 350m above the sea level. The climate is semi arid (SERC 2012). The area received an average annual temperature of 30.26^0C with average rainfall of 260 mm and an average annual relative humidity of 48.54% in the year 2012 (SERC, 2012).

Fish Sampling

Ninety (90) live samples of 100g to 600g of *C. gariepinus* were collected from the river Rima Kwalkwalawa area and from Magatakada and Premier Fish Farms, all within the Sokoto metropolis. The wild samples were randomly selected from the caught population made by the fisher-men at the bank of the river.

Blood Sample Collection

Blood samples (2ml) were drawn from the caudal vein, beneath the vertebral column using syringe with hypodermal needle, the blood samples were collected into a plain vacuum tube (Majolagbe *et al.,* 2012). The blood sample collection was carried out in the Laboratory of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto. These samples were taken to the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto, where the serum electrophoresis was

carried out using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Serum Protein Extraction

The blood samples were homogenized with extraction buffer (800 µl of 0.1 M tris-HCl at pH 7.6), vortex for 1 min. and centrifuged at 3000 rpm for 12 min. at the temperature of 4^0C. The supernatant was collected into Eppendorf tubes and kept in a deep freezer till use. Electrophoresis preparation, condition staining and destaining were all carried out following Lemmni Method (1970). The protein extract for all samples were applied to 12.5% Polyacrylamide Gel.

Gel Casting and Electrophoresis

The gel was polymerized in horizontal gel caster; a thin layer of isopropanol was added to smoothen the gels surface and poured into the caster before the comb was placed inside to make wells for the samples. Samples were loaded into individual well and run for 2 hours at 150V and 0.5 mini-ampere with electrophoresis power source machine. The gel was removed from the cells and the images were scanned and stored in a computer system for further analysis.

Band Scoring

Each gel was scored visually by placing it in a light box which allows electrophoretic protein bands to be seen clearly. Gels were scored visually: presence (1) or absence (0) of protein bands classification was used. Reference Marker (RM) was measured out. The positions of the proteins as enumerated by Gatehouse (1979) and Machuka (2001) were determined using ColorPlus Prestained Protein Marker, Broad Range (7-175 kDa). Bands were presented in increasing order of alphabet following increasing order of molecular weight of the bands (Sneath and Sokal, 1973).

Table 1: Optimised composition of 12.5 % gel used for the SDS-PAGE

Reagent	Volume
40% Acrylamide	3.125 (ml)
2% Bisacrylamide	1.70 (ml)
Tris buffer (3.0M Tris-HCl, pH 8.8)	1.25 (ml)
ddH_2O	4.75 (ml)
10 % SDS (Sodium Dodecyl Sulphate)	100 (µl)
10 % APS (Ammonium persulphate)	50 v
TEMED (Tetramethylenediamine)	8 (µl)

RESULTS AND DISCUSSION

The produced protein bands from SDS-PAGE electrophoresis profiling of the populations is presented in Plate 1. The profile revealed wide range of molecular weight allele and polymorphism of allele across genotypes in the population groups. The allele had molecular weight range of <7 to >175kDa and these were obtained from a total of 9 bands (alphabets A-I). Most of the bands were within 17 and 175kDa except band H and I with lower molecular weight (7 and <7 kDa respectively). Absence of band A (175 kDa) was unique to Magatakada Farm population group (CSMF), lane 1 fish sample, while absence of band B (80 kDa) was unique to sample on lane 1, Premier Farm population group (CSPF) on lane 6 and all across the lanes of WSRR, lane 7, 8 and 9. C (58 kDa) was present across lane 1 to 9, the three fish population groups studied. Absence of band D (46 kDa) was unique to CSMF on lane 2 only. Absence of band E (30 kDa) was unique to individuals across the CSMF fish sample group, lane (1 to 3) and wild population group, from river Rima (WSRR) fish samples on lane 8 and 9. Absence of band F (23 kDa) was observed in CSMF lane 1 and 2 and WSRR on lane 9. Band G (17 kDa) was absent in CSMF, lane 2 and all across the three fish samples of WSRR population group on lane 7, 8 and 9. Band H (7 kDa) was absent across all the three fish population groups from lane 1 to 9. Band I (<7 kDa) was absent only in CSMF fish sample on lane 2.

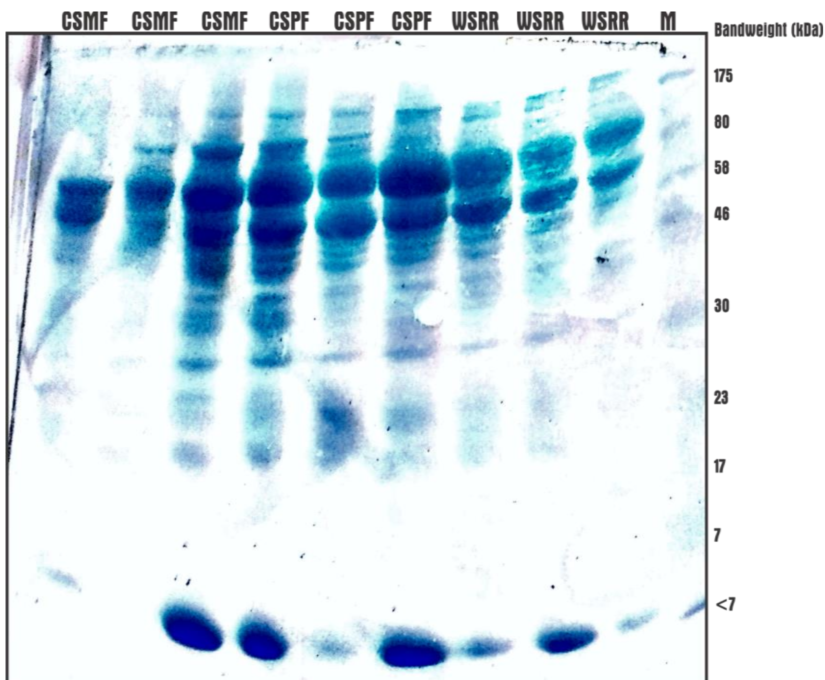

Plate 1: Scanned phenogram of the SDS-PAGE

Solomon *et al.* (2015) described genetic variations caused by inbreeding, crossbreeding and other practices leading to dilution of gene pool as the major cause of

variations in cultured and wild species of *C.gariepinus.* In another report, Gottileb (1971) stated that gel electrophoresis has the advantage that it can directly equate variations in protein banding patterns to genes encoding these proteins. The protein banding patterns of the *C.gariepinus* population groups studied were discovered to differ based on the ecotypes. This signifies a notable alteration in the gene pool of the fish population groups from the wild to the culture media.

Table 2: SDS-PAGE Scored Bands Profile

Band Class	M (kDa)	CSMF	CSMF	CSMF	CSPF	CSPF	CSPF	WSRR	WSRR	WSRR
A	175	0	1	1	1	1	1	1	1	1
B	80	0	1	1	1	1	0	0	0	0
C	58	1	1	1	1	1	1	1	1	1
D	46	1	0	1	1	1	1	1	1	1
E	30	0	0	0	1	1	1	1	0	0
F	23	0	0	1	1	1	1	1	1	0
G	17	1	0	1	1	1	1	0	0	0
H	7	0	0	0	0	0	0	0	0	0
I	<7	1	0	1	1	1	1	1	1	1

Keys: 1= Band present; 0 = Band absent
CSMF= cultured population group from Magatakada Farm; CSPF=cultured population group from Premier Farm; WSRR= wild population group from river Rima; M= molecular marker.

Bands A and H aligned with the Marker at 7kDa and 175kDa molecular weight respectively. A and H could be linked biochemically and genetically with α-amylase inhibitor and a polymorphic form of prostatic growth factor respectively. Gatehouse *et al.* (1979) and Machuka (2001) determined position of proteins using low and high molecular weight markers in Kilodalton such as: phosphorylase B, 94; bovine serum albumin, 67; ovaalbumin, 43; carbonic anhydrase, 30;trypsin inhibitor, 20.1 and α–amylase inhibitor, 14.4 while Chevalter *et al.* (1993) linked 175kDa molecular weight with a major prostatic growth factor (PGF-1) this could be responsible for the noted difference in the growth pattern of the *C. gariepinus* population groups studied from the different sampling locations. However, the position of allele H was observed to be closed in between 7 and 17kDa which corresponds to the molecular weight of α –amylase inhibitor, corresponding to MBP-β-galactosidase. This inhibitor has possibility of being useful in treating obesity and diabetes mellitus resulting from defects in insulin secretion (Ali *et al*., 2006). Proteinase inhibitors are potential model systems that are used to study basic evolutionary processes and changes, example of which is functional diversification (Christeller, 2005). Meanwhile Oyebola *et al.* (2013) reported existence of functional disparity in pectoral spine sub-species of *C. gariepinus*; the sub-species genotypes were 100% canonically discriminated and were biochemically separated by the presence or absence of alpha-amylase inhibitor. According to the authors, specimens that possessed smooth anterior portion of pectoral spines were those that inherited the band that corresponded to the biochemical compound. Therefore, insignificance of many

morphometric phenotypes from among the *C.gariepinus* population groups studied in this research can be linked to the absence of allele H across the three *C.gariepinus* population groups studied both between ecotypes and population groups. The growth superiority recorded by the Premier Farm population group as observed in the current study could be linked with the mixture of genetic materials that originated from the cross combinations of wild sourced parents and the pure hatchery sourced parents for series of breeding exercise that generated the present fish filial population group sourced from this very sampling location. The wild population were observed to lack allele B which corresponds to 80kDa somewhere between 67kDa and 94kDa, corresponding to MBP-truncated-β-galactosidase, presence of this allele helps the maintenance of blood vessels, tissue growth and healing factors. This probably explains why the wild population group has the least mean body weight (BW) and total length (TL) among the three *C.gariepinus* population groups. While other variations observed could result from the differences in the banding pattern of the different fish population groups studied.

CONCLUSION

The genotypes of the cultured *C. gariepinus* population groups from the two evaluated fish farms and the wild *C. gariepinus* from river Rima showed different protein banding profiles and the studied *C. gariepinus* population groups were characterized by heterogeneity of genotypes. The wild *C. gariepinus* population group from river Rima can be proteotypically (genetically) differentiated by the absence of allele B, and G while the cultured *C. gariepinus* from Magatakada farms can be distinguished by absence of allele E across the populations' banding profiles. The *C. gariepinus* population group from Premier farm had all the bands possessed by the two other fish population groups, except for lane 6 without allele B. the missing allele from the latter *C. gariepinus* population group confirms its ontogenic relationship with the wild samples from river Rima.

Further, research is hereby recommended for in depth knowledge of the underlying genetic differences and to establish a possible way to harness the vast genetic resources endowed upon the wild for improvement of the cultured stock.

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