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PROTEIN PROFILE STUDY OF *Sarotherodon melanotheron* FROM SOUTH-WEST NIGERIAN WATER BODIES

Megbowon, I., Akinwale, M. M. A., *Ukenye, E. A., Jubrin, H., Chidume, I., Omatah, C.C. and Igwe J.C.

Department of Biotechnology,
Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria
*Corresponding Authors' email: eekwelem@yahoo.com

Abstract
Sarotherodon melanotheron is a Cichlid specie with good nutritional, economic and aquaculture potentials. The genetic difference among *S. melanitheron* populations in South-West Nigeria was investigated using 12% Sodium Dodecyl Sulphate- polyacrylamide Gel Electrophoresis (SDS-PAGE). Three populations from Pepe, Ugbonla, and Badagry were considered for the study. Thirty fish samples were analyzed; ten per location for the protein profiling. The banding pattern from sarcoplasmic protein indicated variation among the populations. Molecular weight of the protein bands varied from 10-250kDa. Ninety eight (98%) of genetic variation that was found among populations reflects a high inter-population differentiation. The protein profile produced three clusters, indicating divergence and also suggests high level of genetic variation among the studied populations of *S. melanotheron*. The existence of genetic diversity among these populations has made them suitable for use in future breeding programs for the development of improved *S. melanotheron* species.

Keywords: Genetic variation, SDS- PAGE, Protein banding, and *Sarotherodon melanotheron*

Introduction

Sarotherodon melanotheron is a Cichlid species typically found in West and Central African estuaries and lagoons (Amoussou *et al*., 2018). It has good aquaculture potentials, and therefore, represents a good candidate for aquaculture production. In view of its nutritional and economic values, understanding the protein based genetic diversity in its populations from South-West Nigerian coastal waters is elemental before designing selective breeding programs. In spite of its aquaculture potentials, our acquaintance of the genetic diversity of its natural populations is still insufficient for sustainable aquaculture practices.

Previously, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of whole proteins: muscle, sarcoplasmic, serum, liver, salivary, and a number of enzymes often have been used by some researchers as an aid in the species identification of fish (Lamy *et al*., 2008). According to Torkpo *et al*. (2006), biochemical analysis of total protein and isozyme markers reveal better diagnostic genetic variations and is usually free from genotype and environmental interactions. Thus, gel electrophoresis has become a common tool for studying variations at the genetic level. Genetic studies of the distribution of protein variations are considered essential for breeding, conservation and management of fish species (Verspoor *et al*., 2005). Hence, an effort was made in the present study to detect the protein based genetic diversity of three different *S. melanotheron* populations using electrophoresis technique-SDS-PAGE with a view to provide technical guidance for efficient management of this tilapia species genetic resources for breeding and improvement programs.

Materials and Methods

Source of Fish

Sample collection was carried out in three locations from two coastal states (Ondo and Lagos) in South-West Nigeria for *S. melanotheron* species. The geographical location in terms of longitudes and latitudes of the sampling stations are presented in Table 1. After identification, the fish were procured from the fishermen at the landing site of every station, and immediately transported to the laboratory alive and acclimatized. Ten (10) samples were randomly selected from each location for protein profiling. They were filleted and skinned with a stainless steel knife, and the muscle meat used for protein extraction.

Protein Extraction

One gram of minced fish meat was homogenized by grinding it in a mortar with 1 ml of phosphate buffer saline (PBS) containing protease inhibitor cocktail. The homogenate was centrifuged at 10,000rpm for 15min at room temperature and the supernatant (sarcoplasmic protein) was used for electrophoresis.

Qualitative Analysis of Proteins by SDS-PAGE

SDS-PAGE (12%) was carried out as outlined by Laemmli (1970), using a vertical gel electrophoresis unit to resolve the protein (SCIE-PLAS Model #TV 50, SCIE-PLAS Ltd., UK). For determination of molecular mass of each protein, a molecular weight marker kit was purchased from Fermentas, Lahore, Novagen by Merck (10–250 kDa). The gels were stained with Coomassi blue R-250, de-stained with de-staining solution (methanol, acetic acid and distilled water), and photographed with a digital camera.

Data Analysis

Protein data across the studied species were scored by their presence as '1', or absence of protein bands as '0' for each category. The binary data obtained were used to determine number of polymorphic bands, level of polymorphisms, Nei's Pairwise similarity and dissimilarity matrices. A dendrogram was constructed by using the un-weighed pair group method with arithmetic average (UPGMA) with NTSYS software.

Table 1 Geographical location of the Sampling Stations

Location	Latitude	Longitude	State
Pepe	N06^0 10$^{'}$ 01.3$^{'}$	E02^0 52.988$^{'}$	Ondo
Ugbonla	N06^0 08$^{'}$ 31.1$^{'}$	E004^0 47$^{'}$ 39.8$^{'}$	Ondo
Badagry	N04^0 25.012$^{'}$	E02^0 52.988$^{'}$	Lagos

Results and Discussion

Electrophoregram of the protein profile shown in Plate 1 a-c revealed that the three populations have different band patterns. Overall, twenty-three bands were recorded; nine bands for Badagry population, seven bands each was observed in both Pepe and Ugbonla populations. Their molecular weight varies between ten (10kDa) to two hundred and fifty kilo Dalton (250kDa). It was also observed from the profile that bands with molecular weight of 20, 25, 55 and 100kDa are common in all the studied populations. Band with molecular weight of 70kDa was not shown or produced (Table 2). Table 3 showed that the highest similarity coefficient of 0.89 was observed between Ugbonla and Badagry, followed by Pepe and Ugbonla (0.54), while Pepe and Badagry had the least (0.43). The Analysis of Molecular Variance (AMOVA) revealed that a large and significant genetic variation (98%, p<0.01) was found among populations (Table 4). The UPGMA dendrogram based on the genetic distances revealed three clusters: The three populations clustered separately (Figure 1).

The electropherogram generated by SDS-PAGE showed difference both in the banding pattern and the molecular weight of the sarcoplasmic proteins of *S. melanotheron* populations. However, the consistent presence of some similar bands (20, 25, 55 and 100kDa) in all the samples from three locations confirms that they were close in ancestral relationship. Similar observations were made by Omoniyi and Fagede (1998) in muscle protein electrophoretic studies between *O. niloticus* and *S. galileus*. About 98% of genetic variation that was found among populations reflects a high inter-population differentiation in the studied sites, and also suggests high genetic variability in the sarcoplasmic protein profiling of *S. melanotheron*. The high value in genetic variation of *S. melanotheron* from three populations also shows the very high genetic diversity among them. The SDS-PAGE pattern of the *S. melanotheron* samples from various populations shows inconsistency in many protein bands, thus showing distance ancestral relationship. Similar finding was made by Yilmaz *et al.* (2005) between two species of *Orthrias insignis euphyraticus* and *Cyprinion macrostomus*. The existence of this high genetic diversity could be attributed to genotype heterogeneity which could be used to maximize the expression of heterosis among the studied *S. melanotheron* populations.

The clustering of the samples into their different populations strongly indicates that each of the three populations is a distinct genotype, and protein electrophoresis is a useful tool for their discrimination. Dudwadkar *et al.* (2015) also reported that the diversity in protein profiles have potential for species delimitation and also serves as marker for intra- and inter- specific hybridization. It is also stated by Alege *et al.* (2014) that electrophoresis of proteins is a powerful tool for identification of genetic diversity because proteins stored in the seeds/muscles are highly independent of environmental fluctuations.

Conclusion

Protein profiling presents a unique reliable method of fish identification, and genetic differentiation of specie populations. The current study established high genetic variability in *S. melanotheron* populations which can be exploited for breeding, and conservation programmes to improve *S. melanotheron* in South-West Nigeria.

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Table 2: Summary of Muscle Proteins Electrophoregram of *S. melanotheron* Populations

Protein Marker kDa	Pepe	Ugbonla	Badagry
250	ND	ND	D
130	ND	D	D
100	D	D	D
70	ND	ND	ND
	60	ND	ND
55	D	D	D
	50	ND	ND
	45	ND	ND
35	ND	D	D
25	D	D	D
	20	20	20
15	ND	D	D
10	ND	ND	D

Legend: D= Detected, ND= Not detected

Table 3: Genetic Similarity and Difference between Populations of *S. melanotheron*

Population	Pepe	Ugbonla	Badagry
Pepe	-	0.54	0.43
Ugbonla	0.62	-	0.89
Badagry	0.85	0.11	-

Nei's genetic similarity index (above diagonal) and genetic distance index (below diagonal)

Table 4: Analysis of Molecular Variance (AMOVA)

Source	df	MS	Est. Var.	%var
Among Pops	2	25.467	2.541	98%
Within Pops	27	0.059	0.059	2%
Total	29		2.600	100%

Legend: df: degree of freedom; MS: mean square; Est. Var.: estimated variation; %Var: percentage variation

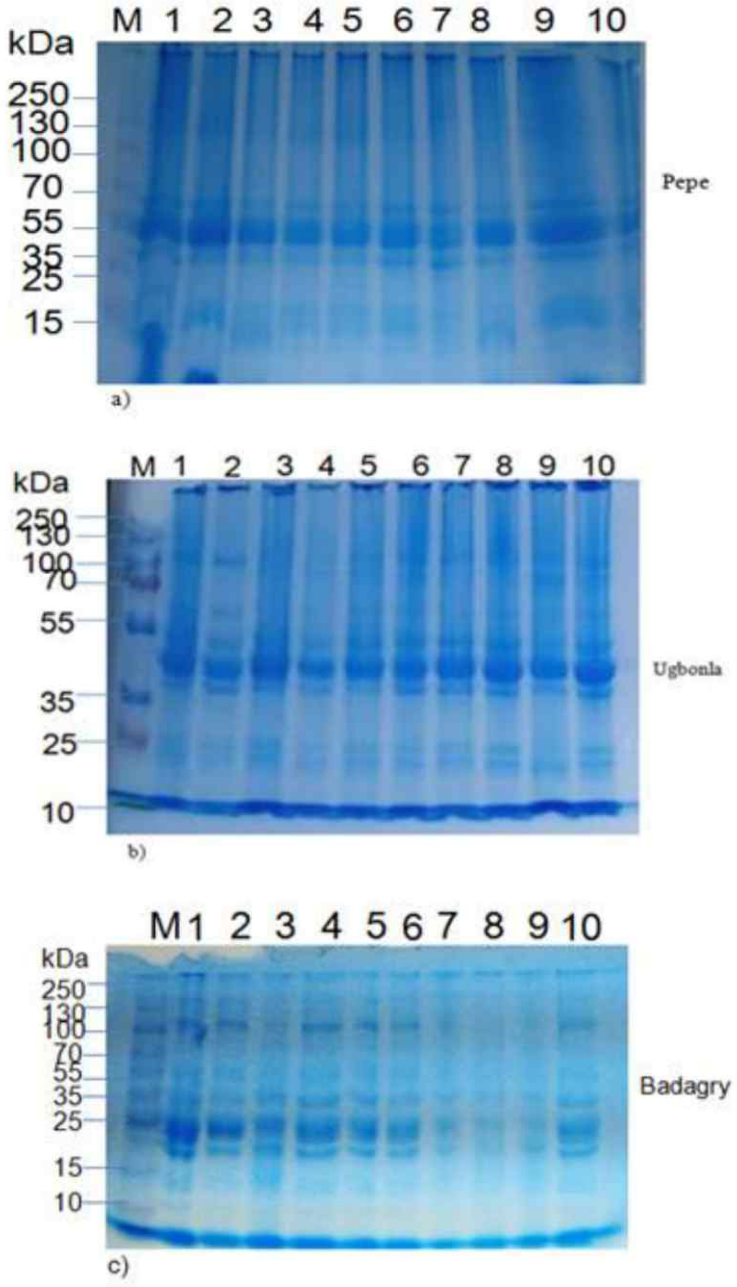

Plate 1 (a-c): Electrophoretic Protein Profile Obtained from SDS-PAGE of *S. melanotheron* Populations

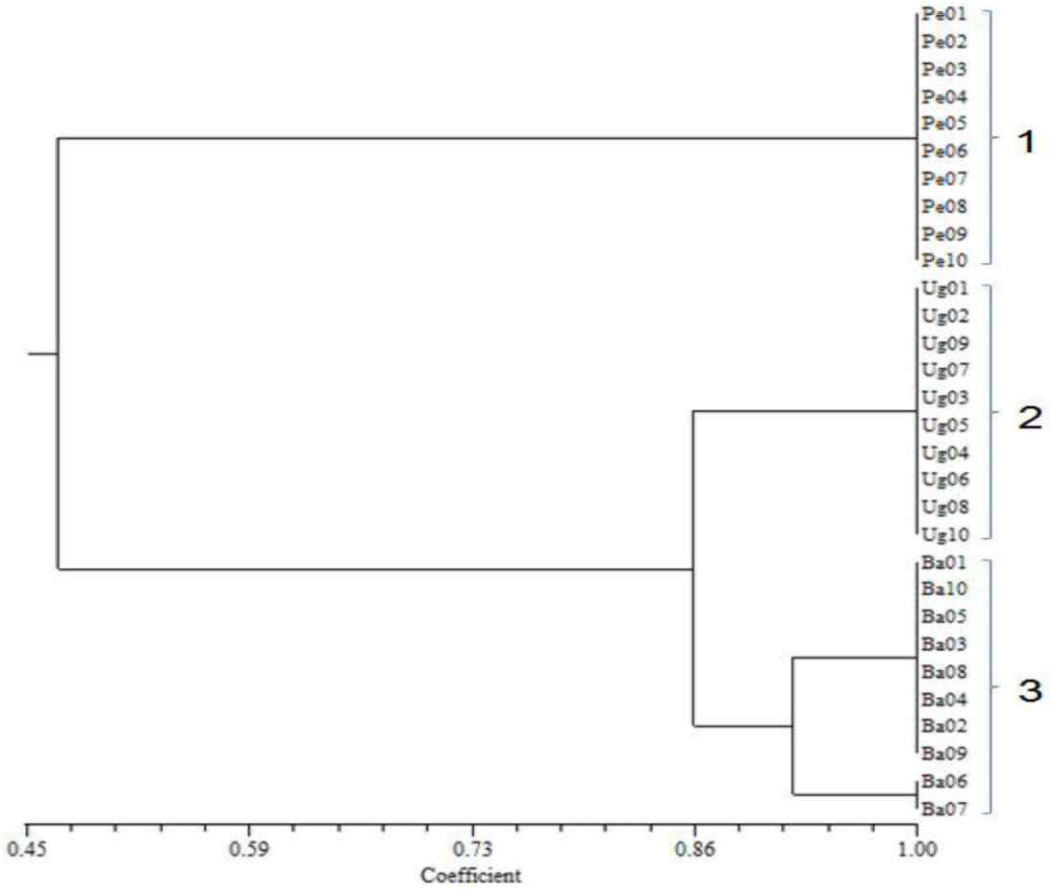

Figure 1: Dendrogram of Protein Profile for *S. melanotheron* Populations Studied

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