Comparative Electrophoretic Studies of Muscles Proteins for Two Species *Tilapia zillii* and *Orochromis aureus* in Fresh Water Fishes of Iraq

Fawziah Sh. Habeeb and Amal A. Mahdi

Department of Aquaculture and Marine Fisheries; Marine Science Center, University of Basrah, Iraq

Abstract. In this study sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was applied to the lateral muscles proteins of two species from fresh water fishes *T. zillii* and O. *aureus* (Cichlidae) (fish taken from Basrah marshes (AI Suawib marsh) and (AI-Dabab marsh).The eectrophoretic showed that there were deferences in the number of proteins band of two species 4 protein bands of *T. zillii* and 3 for *O. aureus*. The molecular weight of protein bands ranged from 123.296 mg to 18.356 mg for *T. zilii* and from 73.7 91 mg to 16.84 mg for *O. aureus*. The electrophoretic analysis of lateral muscle proteins revealed that SDS-PAGE can be considered agood taxanomic criterion to differentiate among fish species .

Key word: SDS- PAGE**,** electrophoresis**,** *T. zillii***,** *O. aureus*

Introduction

It has been found that cichlids are the most species-rich group of fishes, which include 1625 species, they are great scientific and economic interest for their importance either in fisheries , aquaculture and they are an unusual group of fish that have undergone a rapid speciation event (11, 13, 17, 42). The cichlids divided into three major groups: Pelmatochromine cichlids, Haplochromine cichlids and Tilapiine cichlids, the last group comprise over 70 species, which are all commonly referred to as the "tilapias" (6, 24, 41 , 40) .

The tilapiines have been divided into three major genera primarily on the basis of breeding habits or reproductive behavior: *Oreochromis*, the maternal mouthbrooders, *Sarotherodon*, the biparental and paternal mouthbrooders , and *Tilapia*, the substrate spawners (32, 41, 42, 43) tilapias are endemic to Africa, but interest in their aquacultural potential led to nearly worldwide distribution (32), it have been introduced into nearly

every tropical and subtropical country in the world (33), and presently these fishes found in freshwater and estuary in Africa, middle east, Indian coast , south and middle America and Philippine (16).

Several species of tilapias are herbivorous, readily reproduce in small ponds (32), grows well on artificial feeds, resistant to diseases (36), and it have ability to adapttemperature, alkalinity, and salinity (4, 28).

Oreochromis aureus (Steindachner, 1864), commonly called the Blue Tilapia which is a native of africa and middle east (19). Another species of this genus (*O. niloticus*) was Introduced to the Tigris River basin in Iraq but didn´t apparently survive winterkill (7), while *T. zilli* Introduced for fish farming from Egyp also didn´t apparently survive (7, 8).

A specimen was caught in the Khabour river, presumably a fish farm escapee and redbelly tilapias (*T. zillii*) are now established in the Syrian Euphrates (7). Ellewi (12) recorded *T. zillii* at Al-Musayyib on the Euphrates river in Iraq, and Mutlak & Al-Faisal (27) founded two species (*T. zillii* and *O. aureus*) from south of the main outfall drain in Basrah city.

Several tilapiine species share similar morphological features and can be easily hybridized (16, 17), and considerable inter population variation has been detected in many species (3, 40). which make it difficult to differentiate tilapia based on morphological characteristics only (22, 23).

In the past, the identification of ish species was carried out mainley by examining the external morphological characteriestics. In the present day, electrophoreses of sarcoplasimic proteins, serum proteins, liver proteins, muscles proteins and a number of enzymes often have been used by som reserchers as an aid in the species identification of fish (15, 25, 31, 47). eiectrophoresis has been as atool for examining biochemical variation in afish populations (8, 45), Which have proved to be useful in species identification (31, 2). It can give an independent estimate of the level of variation within a population without an extensive survey of morphological and other quantitative traits (2, 26, 38) because the electrophoresis is a simple, rapid and highly sensitive tool to analyze protein, It is used to isolate individual components of protein or nucleic acids (8, 45). In

polyacrylamide gel electrophoresis (PAGE), proteins migrate in response to an electrical field through pores in a polyacrylamide gel matrix, pore size decreases with increasing acrylamide concentration of pore size and protein charge, size shape determines the migration rate of the protein (21).

Several authors were performed electrophorus studies for tilapiine fish among them : Brummett *et al.* (5) which compared three populations, each of *O. aureus*, *O. mossambicus* and *O. urolepis honorum* and two each of red tilapia derived from hybridization of *O. urolepis honorum* females and *O. mossambicus* males, for electrophoretic mobility of their enzymes and constructed dichotomous keys based on relative electrophoretic mobility of isozymes for the identification of the species. Zaki *et al.* (48) used iso electric focusing (IEF) technique to identification of hybrid and the pure parental stain of *Oreochromis* species. Nagl *et al.* (28) were studied classification and phylogentic relationships of 42 tilapiine species inferred from mitochondrial DNA sequences. El-Alfy *et al.* (9) were studied the tissue specific lactate dehydrogenase (LDH) isozyme pattern of tilapine fishes, *O. niloticus*, *O. areus* and *T. zillii* using horizontal starch gel electrophoresis. El-Alfy *et al.*(10) used random amplified polymorphic DNA (RAPD) techniaue to assessy the genetic variation among three tilapiine species. Bakhoum *et al.* (2) were studied genetic evidence for natural hybridization between Nile tilapia, and esterase isozymes using electrophoretic analysis and hybrid index. Espinosa- Lemus *et al.* (13) performed study for the morphometric and genetic characterization of tilapia stocks for effective fisheries management in two Mexican reservoirs. Saad *et al.* (34) were monitored the genetic polymorphism in some tilapia species (*T. zillii*, *Sarotherodon galilaeus* and *O. niloticus*) by fin tissue isozyme distributions. Shair *et al.* (37) were studied genetic variation investigation of tilapia grown under Saudi Arabian controlled environment. Toniato *et al.* (40) were discriminated among tilapia species of the genera, *Oreochromis*, *Tilapia* and *Sartherodon* by using Polymerase Chain Reaction – Restricted Fragment Length Polymorphism of 5S Ribosomel DNA (PCR-RFLP of 5S rDNA).

In Iraq, there were a few studies for this species of fishes: Ellewi (12) which recorded *T. zillii* for the first time in Iraq, and Mutlak and Al-Faisal (27) which found two species (*T. zillii* and *O. aureus*) in Basrah city. While Qadoory (33) was studied the reproductive

cycle of *T. zillii* in the Al-Swaib and Al-Ghatira marshes, south of Iraq. The aim of this paper was to describe the taxonomic study of two species of *T. zilli* and *O. aureus* from Basrah province since *Tilapia* has not been previously examined by electrophoresis analysis.

Materials and Methods

Extracts for proteins has been carried out using sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) according to the procedure described by Wong *et al* (46).

1-Exract ion of fish proteins

Extract ion of muscles has been carried out according to Normen (30), Fish were cleaned with tap water the skin and fine were removed manually. The muscle samples were taken from the area between the dorsal fine and lateral line. The samples were freez-dried/24h- and grounded with electrical mortur (1gm-dry wt), 5ml. buffer solution of Tris-HCl (pH 7.5) was added to it and kept for 1h. The mixture was centrifuged (600 rpm / 15min) at (4°C).

Producing two layers, the soluble fraction consists principally of sarcoplasmic proteins was stored at 0°C until analysis.

2- Sample preparation:

A sample 100 ml volume of protein solution mixed with 100 ml buffer solution of Tris – HCl (pH 6.7- 6.9) and 80 ml of 10% of distal water D.W.

The samples were located in the water bath for 2-3 min and added to it mixture of (20-30) ml to bromophenol blue solution with 30 mg of glucose substance.

3-Electophresis Process :

Slide glasses overlaid with polyacrylamide gel were placed in vertical starch gel electrophoresis apparatus (Maxi Vertical Electrophoresis) which provided to British Company (Cleaver Ltd Scientific) and the program (UVI band software program) which provided from company (44). The electrode buffer was added to the positive and negative

poles contains, switch on electrical cycle with 90 V until bromophenol blue stain reached the all slides and after the hole gel was stained, using a modification of the method (UVI band advanced software), then switch off the electrical cycle and picked up the gel.

The Results

Figure 1 (A1) Shows the results of electrophoresis analysis to lateral muscles protein for *T. Zillii* (which taken from Al-Suwaib marshe, north of Basrah city). (B1) Shows the results (from Al –Dabab marsh, south of Basrah) there were 4 protein bands which available in there densities, volumes and molecular weights. (A2) Shows the results for *O. aureus* (which taken from AI –Suwaib marsh) (B2) shows the results (from AI –Dabab marsh) south. There were 3 protein bands which available in there densities, volumes and molecular weights which ranged

Between 18.356 mg to 123.296 mg for *T. zilli* and between 16,89 mg to 73.791 mg for *O . aureus* (B1) shows the result for *O.aureus* (which taken from AI –Suwaib marsh) and (B2) shows the results (from AI –Dabab marsh south of Basrah city) .

Our results appeared that there were difference in a number, thickens and molecular weights of protein bands between one species which found in different area and between two species.

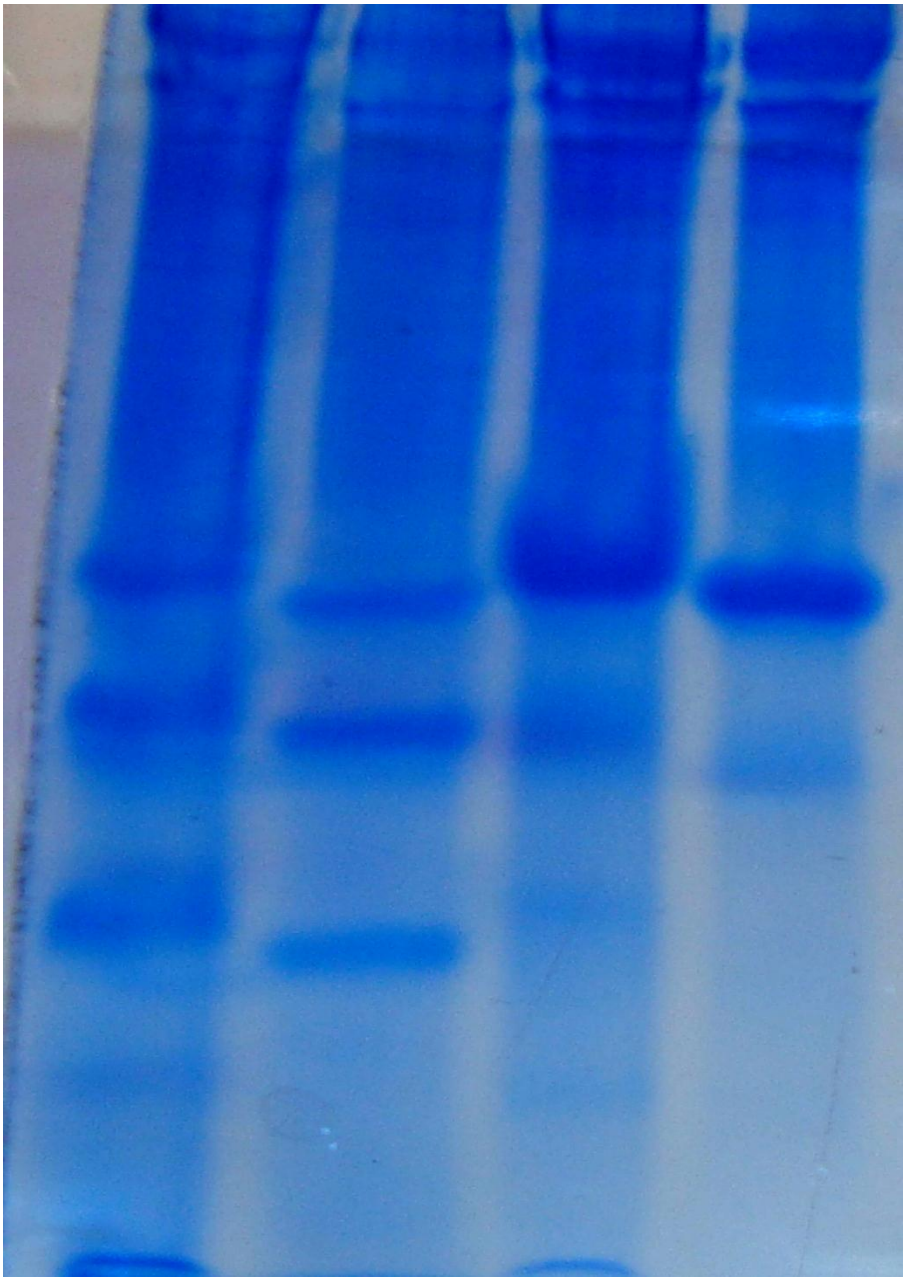

Fig (1): The lateral muscles proteins bands of *Tillapia zillii* and *Orochromis aureus* (1st lane belonged to *T. zillii* from (Al-Suwaib marsh) 2 nd lane belonged to *O .aureus* from (Al –Suwaib marsh) (3rd lane to *T. zillii* from AI –Dabab marsh) (4th lane to *O. aureus* from Al - Dabab marsh) .

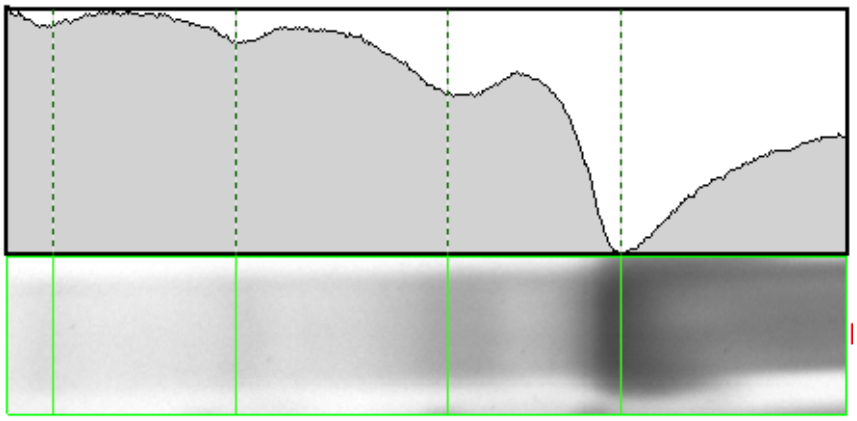

Fig. (2): Shows the number of the bands for *T. zillii* (Al –Suwaib marsh north of basrah)

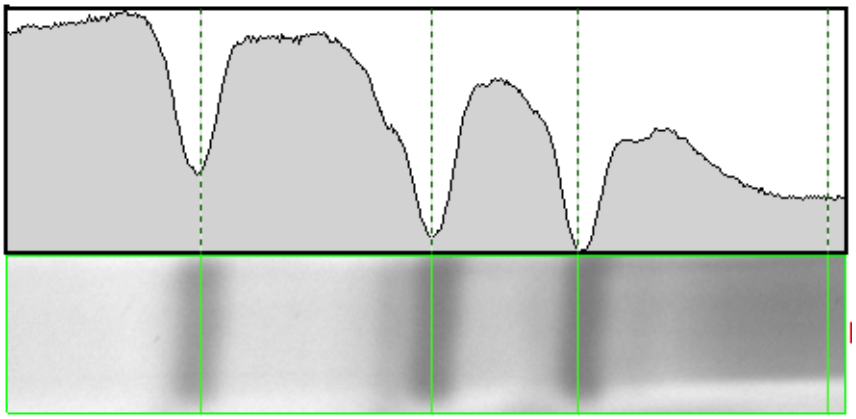

Fig (3): Shows the number of the bands for *T. zillii* (Al –Dabab marsh south of basrah city).

Table (1): Shows the molecular weight of the bands for *T. zillii*.

Bands	AL-Suwaib marsh (MWmg)	AL-Dabab marsh (MWmg)
Band1	123.296	120. 432
Band2	73.791	79.359
Band3	34.562	32.896
Band4	18.356	18.637

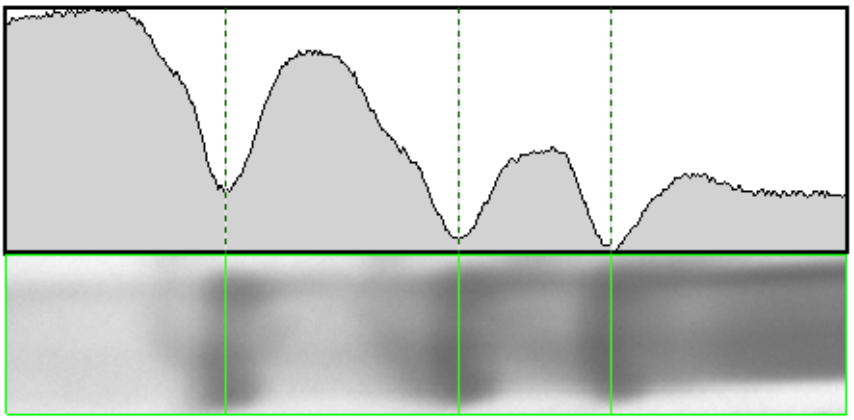

Fig. (4): Shows the number of bands for *O. aurues* from (Al- Suwaib marsh.

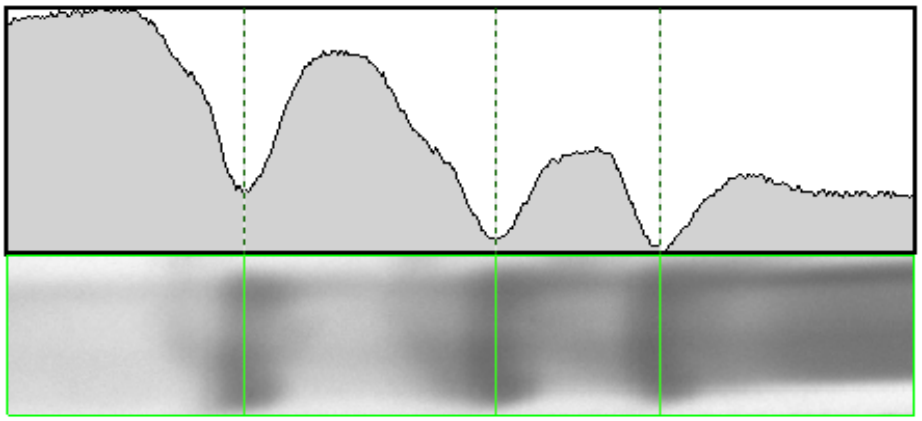

Fig. (5): Shows the number of the bands for *O. auraes* which taken from Al-Dabab marsh.

Table (2): Shows the number of protein bands for *O. auras*.

N	**AL-Suwaib marsh (MWmg)**	**AL-Dabab marsh (MWmg)**
Band1	101	98.342
Band2	76.821	89.235
Band3	16.791	16.842

Discussion

In general taxonomic studies are based on morphometric measurements and anatomical characteristics .Electrophoresis of muscle proteins, serum proteins have been widely used in the classification of fish. These kinds of studies brought about a new look to taxonomical evaluation .Discrimination of related taxa can be easily made according to their electrophoretic results of serum proteins (39 , 47). Unfortunately, there has been no taxonomical study with these species by muscles protein electrophoresis in Iraq. Our result shows that there were difference in a number , thickness and molecular weights of

protein bands between one species which found in different area and between two species .

The differences in thickness of protein bands for *T .zillii* and *O.aureus* in to lnvstigated areas may be relation to different in food items which were found in lnvestigated areas this results is similar with Niolson (29) which refers that the differentiation in food items is the pinoeir *et al* (31) refers to the different in number of isolated protein bands belonged to the food and feeding habits of the species and its environment niche . But in anether study for (35) by using SDS –PAGE the result showed that the total numbers of protein bands to liver of *T .zillii* were found 10 , 8 , 7 bands which taken from three ecologically different localities (unpolluted, agricultural-pollted and industrial –polluted) which different in afood and anather environment niche . molecular weight was ranged from 12.76 to 114.47 KDa . ln a previous study by using SDS-PAGE , the protein bands to liver of *T. zillii* and *O.aureus* were separated and analyzed by using SDS –PAGE, The protein band numbers of these fishes 7 and 8 ,respectively . ln the other research the electrophorsis analysis of liver proteins of *T .zilli*i and *O. aureus* were separated b*y* using SDS –PAGE showed that there were differences between the two species in both the number of bands and the molecular weight of the proteins bands . ln the study mentioned protein band numbers of *T. zillii* have shown similarity to protein band numbers of *O .niloticus* mentioned above .However the protein bands of *T . zillii* and *O .aureus* studied were found to be similarity .A study conducted to investigate the effects of different proteins levels in various fish species which was well correlated with their taxonomic level (1) . in conclusion these fishes are easily distinguished by SDS –PAGE taxonomically .

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