

**IDENTIFICATION OF THE PROTEIN PATTERN IN THE RESULTING HYBRID  
AND THE PURE PARENTAL STRAIN OF *OREOCHROMIS SPECIES* USING IEF.**

Zaki, M. I.  
Professor of Aquaculture  
National Institute of Oceanography and Fisheries, Alexandria.  
Tel. (03) 422-1959 / Fax. (03) 545-7611

El-Gharabawy, M.  
Assistant Professor of Aquaculture  
National Institute of Oceanography and Fisheries, Alexandria.  
Tel. (03) 422-8723 / Fax. (03) 545-7611

Ghabrial, S. G.  
Researcher  
National Institute of Oceanography and Fisheries, Alexandria.  
Tel. (03) 854-347 / Fax. (03) 545-7611

**Abstract**

The IEF technique is used as a tool for identification of *Oreochromis spp.* And its hybrid by the comparative analysis of their protein pattern and separating macro molecules differing in iso-electric points (PI). The similarities of differences of protein pattern between the pure parental species and their hybrid were investigated. This is done by using the genetic markers to distinguish the hybrid from its parents in order to control and identify the brood stock.

**Introduction:**

The precise identification of fish species is of a prime importance to clarify the taxonomic position of such species in Egypt as a pre-step for rearing, artificial spawning and hybridization which is required for fish farm.

Electrophoresis is the main method for the analysis of the biochemical systematics in various taxa. In most cases species were examined for sufficient number of proteins by means of high resolution gradient gel electrophoresis or polyacrylamide gel isoelectric focusing, species-specific protein patterns were found (El-Gharabawy & Zaki, 1990 and El-Gharabawy *et al.*, 1995).

There are relatively few biochemical studies on fish soluble muscle tissue proteins and knowledge in this regard is still fragmentary (O'Maoileidigh *et al.*, 1988 and Basaglia, *et al.*, 1991).

Identification of *Oreochromis spp.* which are used in the aquaculture techniques according to their morphological differences is not completely satisfactory. The present study aims to test the possibility

of using isoelectric focusing technique as a tool for identifying the species under consideration and its hybrid by the comparative analysis of their soluble protein of muscle and gill.

### Materials and Methods:

A sample of muscle was taken from both *Oreochromis niloticus* and *Oreochromis aureus* and their hybrid after 12 and 30 days. Also, the same weight was obtained from the gill of both species and their hybrid (0.5 gm), each was homogenized with 5 ml Tris-HCl buffer (pH 8.00). The homogenates were centrifuged at 6000 rpm for 10 minutes. The clear supernatant was pipetted and kept frozen. Silver staining technique method of Heukeshoven and Dernick (1985) was applied.

Technical procedure for sample application and isoelectric points (PI's) of protein were performed as described by Pharmacia LKBC (1987).

### Results and discussion:

According to the pH gradient from 3 to 9, twenty seven protein fractions were separated in *Oreochromis niloticus* muscle and exhibited a distinct electrophoretic pattern which could be clearly identified at PI's 4.3 and 8.5 . Twenty eight protein fractions were separated in *Oreochromis aureus* muscle and may be identified by its bands at PI's 9.25 from a total of twenty eight protein fractions. As shown in Fig. (1), each of the examined tissue exhibited a distinct electrophoretic pattern and could be clearly differentiated.

Twenty seven protein bands were isolated from the gill of *O. niloticus* which could be identified by the specific bands at PI's of 3.83, 5.30, 6.43, 7.75, 9.10 and 9.20 . Also *O. aureus* had twenty seven gill protein fractions with a specific bands at PI's of 5.25, 6.90, 7.53 and 8.25 . However, twenty nine protein fractions were separated from the gill of the hybrid.

From Fig. (1a & b) and Fig. (2), it appears that there are definite variations between electrophoretic patterns of both muscles and gills of the different species of *Oreochromis* and their hybrid.

Twenty protein fractions were separated from *O. niloticus* fry and exhibited a distinct electrophoretic pattern and identified at PI's of 5.85 and 8.30 . Hybrid after 12 days had twenty protein fractions and exhibited a distinct band at PI's 5.60, 6.50 and 6.60 . However, the hybrid of age 30 days had twenty two protein fractions and exhibited a distinct band at PI's 3.73, 4.20, 4.78, 5.15 and 6.60.

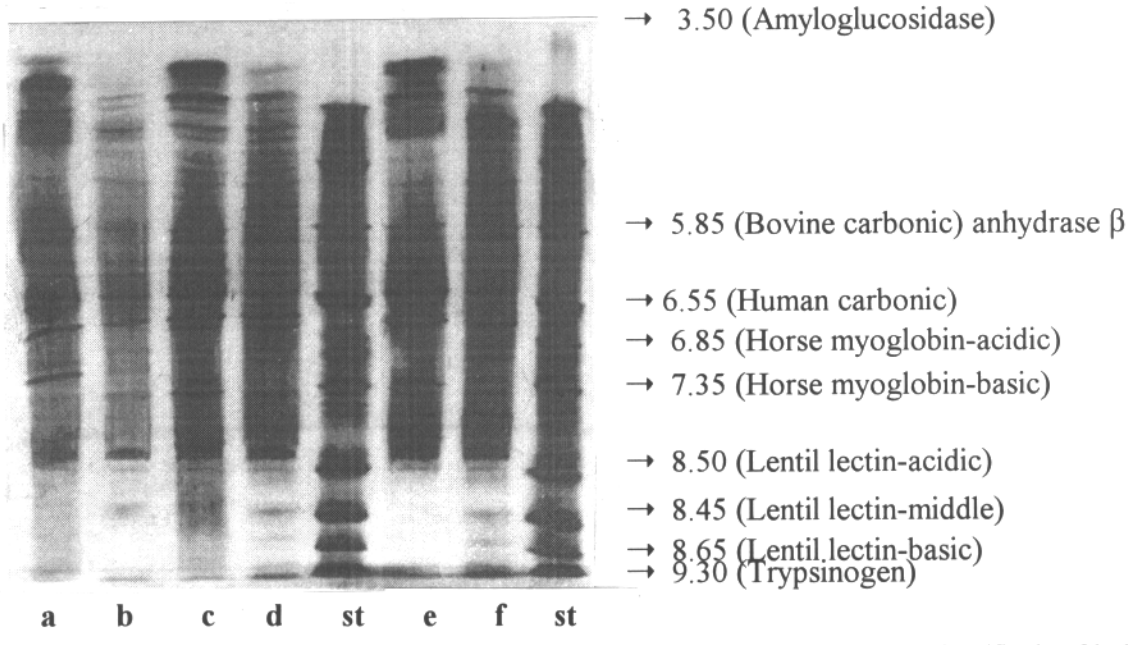
*O. niloticus* and *O. aureus* were found to be the most suitable species for aquaculture in Egypt. Crossing male *O. aureus* with female *O. niloticus* Results in a hybrid of at least 80% males which are characterized by having higher growth rate than their parents. Wohlfarth *et al.*, 1983 showed that the hybrid of *O. niloticus* and *O. aureus*. posses the best combination of production traits among a number of inter specific hybrid under condition of Israeli aquaculture.

The use of biochemical methods such as isozyme and protein electrophoresis techniques as measures for species identification have been widely applied in fish (El-Deeb, 1983; Basaglia 1992 and El-Garabawy *et al.*, 1995).

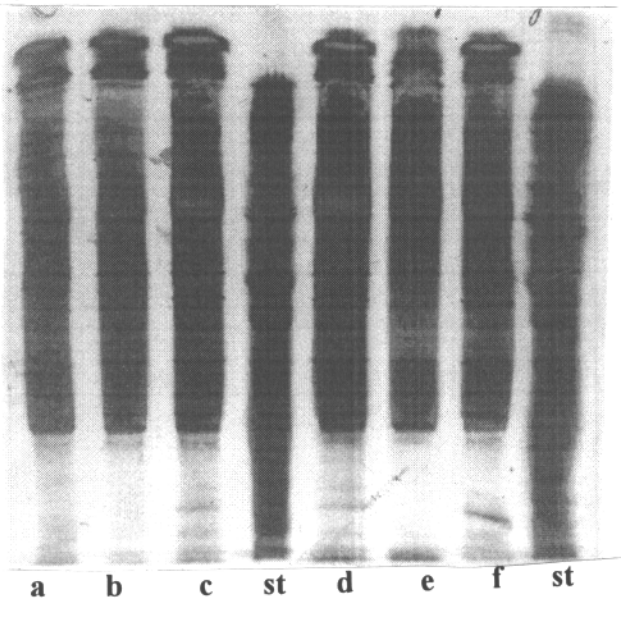
Nieder and Bussse, (1992) studied the blood sera of 9 species of Blenniidae. They found that band patterns in electeopherograms are constant in each species and differ characteristically between species.

The number of protein bands for the fry of *O. niloticus*, hybrid of age 12 days and the hybrid of age 30 days, could be identified at certain PI values regardless of the total number of bands.

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**Figure 1(a).** IEF of phast gel (IEF 3-9) of protein in *Oreochromis species*. a, c and e (flesh of hybrid, *O. niloticus* and *O. aureus*). b, d and f (gills of hybrid, *O. niloticus* and *O. aureus*) respectively, St (standard protein from 3-10).

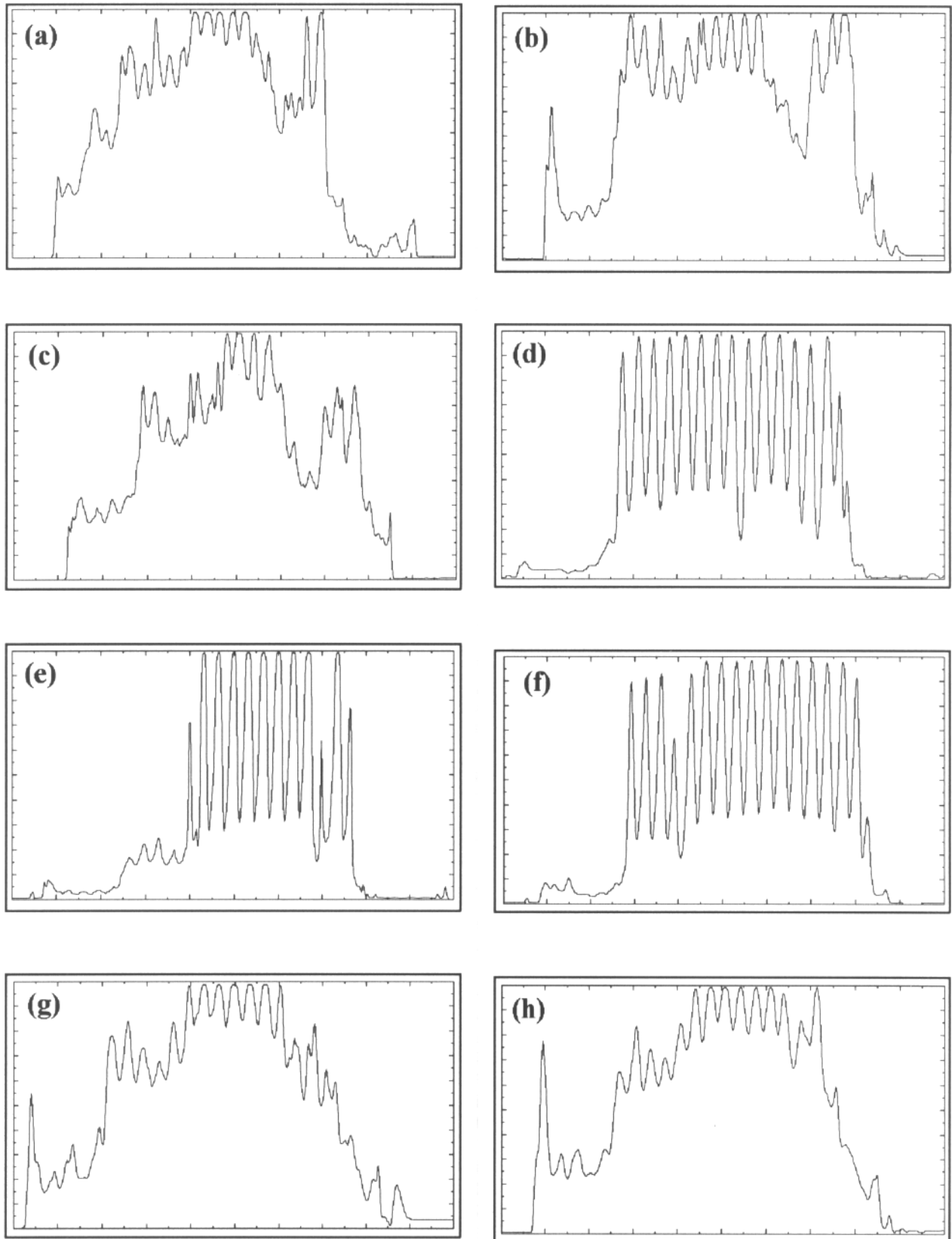


**Figure 1(b).** IEF of phast gel (IEF 3-9) of protein pattern in muscle of *Oreochromis species*. a (*O. niloticus* adult), b (*O. aureus* adult), c & d (*O. niloticus* fry), e & f (hybrid after 12 & 30 days) and St (standard protein).

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**Fig. (2):** The electrophoretograms and densitograms of flesh and gills of *Oreochromis* species: a, b, c, d, e and f (muscles of *O. niloticus*, *O. aureus*, hybrid after 3 months, *O. niloticus* Fry, hybrid after 12 days and hybrid after 30 days), g, h and i (gills of *O. niloticus*, *O. aureus* and hybrid).



We would like to notify that the resulting hybrid has specific bands, which are biased to the female gamete, a similar result obtained by Ghabrial, (1990) on the developmental criteria of embryological stages of the same species.

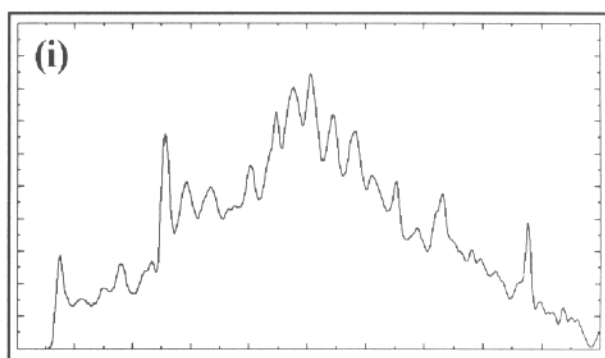


Fig.(2) continued.

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