# Electrophoretic Studies on Eye Lens and Muscle Proteins of some Flat Fishes off Bombay Coast

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Electrophoretic profiles of eye lens protein and muscle proteins of *Cynoglossus macrolepidotus, Pseudorhombus elevatus, Zebrias quagga* and *Asopia cornuta* show species specific band pattern. A total of 8, 9, 10 and 9 bands in eye lens proteins and 7, 5, 9 and 5 bands in muscle proteins were observed in the above mentioned fishes respectively. The use of electrophoretic profile of eye lens proteins and muscle proteins in fish taxonomy is discussed.

Fish taxonomy is primarily based on their morphological characters. In recent years, however, electrophoretic technique has been employed as a tool for identification of fish species. Many authors had used this technique for identification of different fish species (Tsuyuki *et al.*, 1965; Cowie, 1968; Devadasan & Nair, 1971; Taniguchi, 1969; Hasnain & Siddique, 1974; Dwivedi & Iftekhar, 1976; Dhulkhed & Rao, 1976; Rao, 1985; Kochar, 1988).

The soluble proteins of the eye lens have great value in taxonomic studies. For comparative studies these proteins may provide more data than the serum proteins, because the latter are produced by a variety of cells, where as the eye lens proteins are synthesized by only one cell type present in the eye as a single layer (Rourke, 1959).

According to Tsuyuki & Roberts (1966) muscle myogens are practically unaltered by factors other than genetic. It is species specific and extremely invariable within a given species. Taniguchi (1969) observed that electrophoretic pattern of lizard fish muscle protein was very stable for at least 45 days when the muscle was kept at -20°C. Therefore it is evident that the electrophoretic characters of the muscle proteins, such as number of components and percentage and mobility of each component are more reliable than those of serum proteins.

The application of studies on protein patterns is receiving increasing attention. Genetic information coded into DNA molecules are translated through a series of reactions into proteins. The analysis of proteins, therefore are considered to be the simplest indirect approach to genetic linked species identification (Marmur *et al.*, 1963). Morphometric characters such as branchiostegal rays, fin rays, scales though useful for quick examination of the fish species, show sufficient inter-species overlap and also need large number of samples. In contrast, muscle myogen pattern show species specificity and reproducibility which enables identification on the basis of single analysis. The myogen pattern is virtually independent of sex differences. In the present investigation muscle and eye lens proteins are studied.

## Materials and Methods

Specimens of *C. macrolepidotus, P. elevatus, Z. quagga* and *A. cornuta* were collected from the landing center at Versova, Bombay in fresh condition. They were packed in ice and brought to the laboratory.

Each fish was cleaned well and eye lenses were removed carefully from cortex and homogenised in 0.5 ml of 0.9% saline (pH 7) in a mortar in chilled condition (0-2°C). The homogenates were centrifuged at 5000 rpm for 30 min and the supernatents were used for electrophoresis.

Dorsolateral white muscles were taken for electrophoresis. They were homogenised with 1 ml of 0.9% saline. The homogenates were centrifuged at 5000 rpm for 30 min and supernatents were used for the experiment.

Method of Davis & Lindsay (1967) was followed for polyacrylamide gel electrophoresis. Acrylamide of 7.5% was used for the analysis of muscle and eye lens proteins. Tris-glycine at pH 8.3 was used for upper and lower chambers of electrophoretic tank. 50 µl of eye lens and muscle extracts were mixed with 40% sucrose solution (1:1) and were loaded on top of gel. Tracing dye bromophenol blue was also added to the solution. Upper and lower receivers were connected to a constant electric supply. Entire apparatus was kept in refrigerator during electrophoresis.

Electrophoresis was carried out for two and half hour with a current of 5 mA/gel tube and total voltage of 250 mV for 8 tubes. After completion of the experiment the gels were romoved and were kept over- night for staining in kenacid blue in labelled test tubes and were destained in 7.5% acetic acid. The gels were washed several times till they became perfectly transparent leaving only the protein fractions stained. Gels were read quantitatively with the help of densitometer connected to a scanner.

**Results and Discussion**

A total of 8, 9, 10 and 9 bands were observed in eye lens proteins of *C. macrolepidotus, P. elevatus, Z. quagga* and *A. cornuta* respectively. A total of 7, 5, 9 and 5 bands were observed in muscle proteins of above mentioned fishes respectively. The densitogram of the electrophoretic pattern of the four species of fish under study are shown in Figs. 1 to 6.

A total of 8 bands were observed in eye lens proteins of *C. macrolepidotus*. Rao (1985) also observed 8 bands in eye lens proteins of *C. macrolepidotus*. According to Rao (1985) intensity of individual protein band and percentage protein varied depending on size(age) and sex. Kasinathan *et al.* (1972) reported 9 protein bands in *C. bilineatus* (Lac) and these bands showed quantitative differences in total contents of different bands. Similar results are obtained in the present work. The amount of protein in band no.8 was highest i.e. 21.60%. Muscle

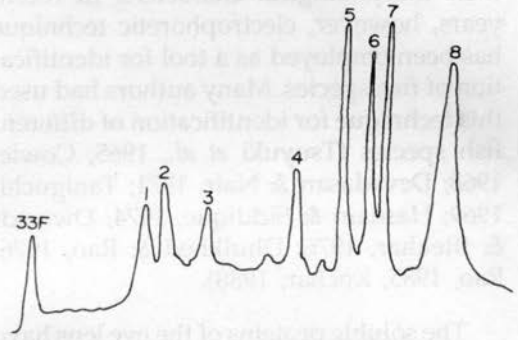


Fig. 1. Densitogram of eye lens proteins of *C. macrolepidotus*

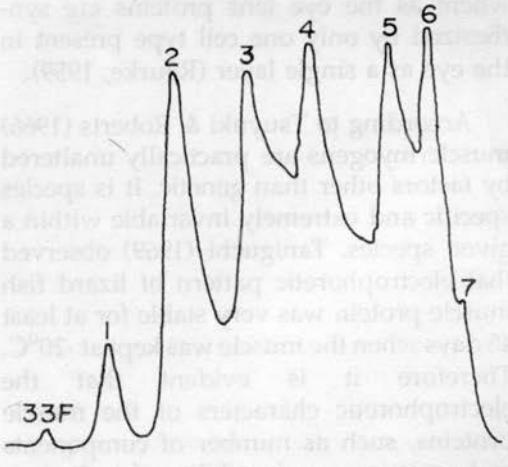


Fig. 2. Densitogram of muscle proteins of *C. macrolepidotus*

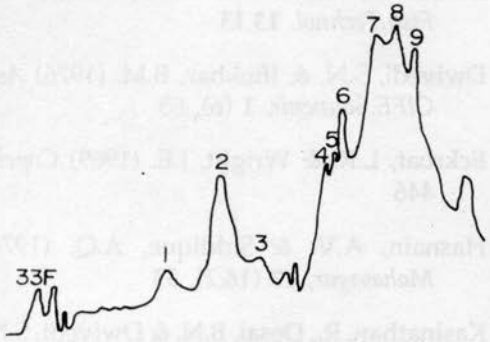


Fig. 3. Densitogram of eye lens proteins of *P. elevatus*

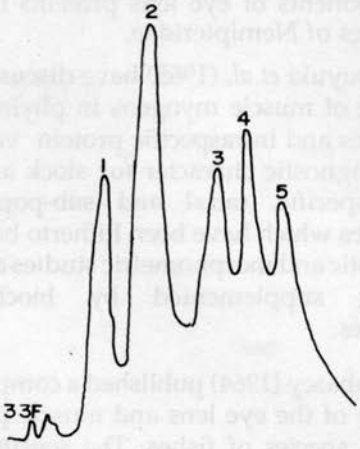


Fig. 4. Densitogram of muscle proteins of *P. elevatus*

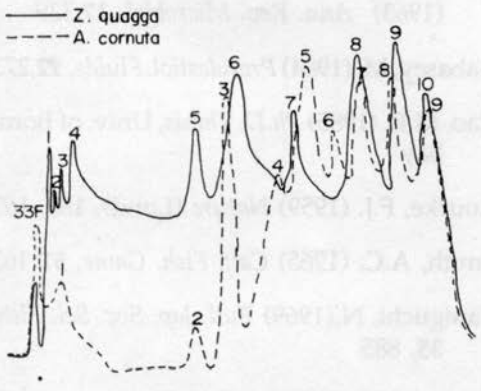


Fig. 5. Densitogram of eye lens proteins of *Z quagga*. and *A. cornuta*

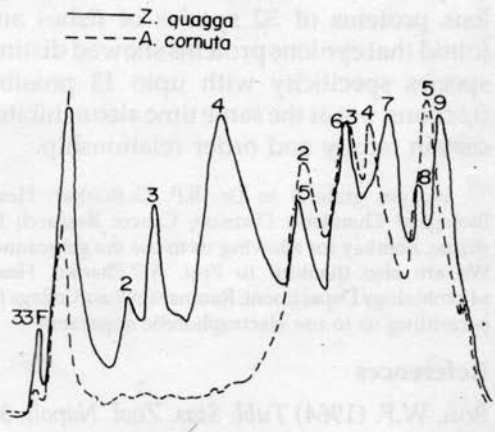


Fig. 6. Densitogram of muscle proteins of *Z. quagga* and *A. cornuta*

protein in *C. macrolepidotus* showed presence of total of 7 bands and percentage of protein in band no. 2 was highest i.e. 34.59%. From the densitograms it is clearly observed that each species has its own band pattern. For example eye lens proteins of *Z. quagga* and *A. cornuta* when compared, shows that band no.4 and 6 of *A. cornuta* are missing in eye lens protein of *Z. quagga* where as muscle proteins of *A. cornuta* lack band no. 2, 3 and 4 which are prominent in band pattern of *Z. quagga*.

When the above mentioned four species are compared in band pattern, it is observed that all of them show presence of minimum of 8 bands in eye lens proteins with species specific band pattern. If the percentages of proteins in bands are compared they show variation. Chakraborty (1980) also observed such definite species specific pattern in the

components of eye lens proteins in three species of Nemipteridae.

Tsuyuki *et al.* (1965) have discussed the value of muscle myogens in phylogenetic studies and intraspecific protein variation as diagnostic character for stock analysis. Interspecific, racial and sub-population studies which have been hitherto based on meristic and morphometric studies are now being supplemented by biochemical studies.

Rabasey (1964) published a comparative study of the eye lens and muscle proteins of 35 species of fishes. The sensitivity of his technique enabled him to stress the large number of protein fractions upto 11. In present work *Z. quagga* showed 10 fractions of eye lens proteins. Bon (1964) studied eye lens proteins of 32 species of fishes and found that eye lens proteins showed distinct species specificity with upto 13 possible fractions, but at the same time also exhibited certain family and order relationship.

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