

SERUM PATTERNS OF FLAT FISHES

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ABSTRACT

Blood serum of a flat fish *Psettodes erumei* was analysed by cellogel electrophoresis. The fishes chosen for this study were of different sizes and sex. The patterns were found to be remarkably similar in numbers and mobilities of fractions, irrespective of the size and sex. However, on comparing the serum pattern of this fish with the serum pattern of the two other flat fishes *Brachirus orientalis* and *Pseudorhombus arsius*, a clear difference was observed between them.

In recent years, biochemical techniques have been applied as a tool for the study of taxonomy of fishes. One of the major biochemical techniques used in the study of fish population has been electrophoresis. The number of fractions, the quantity of protein in each fraction in electrophoresis and their physiochemical properties have been utilized as taxonomic characteristics. Certain electrophoretic properties of blood proteins serve to differentiate specific taxonomic groups and to illustrate similarities of proteins within families or even in higher taxonomic units (Dessauer and Fox, 1956; Sibley, 1960).

Electrophoretic studies of serum proteins, muscle proteins, plasma proteins and hemoglobins, especially of higher vertebrates have disclosed species specific patterns (Deutsch and Mcshan, 1949; Badawi, 1971; Moore, 1945) and in some cases, variant patterns with frequencies characteristic of particular geographic areas or races (Ridgway, Klontz and Matsumoto, 1962; Dessauer and Fox, 1956).

In the present study, electrophoretic analysis has been made of serum proteins of a flat fish *Psettodes erumei*, which is widely distributed in estuaries and coastal regions. This study was undertaken to determine whether individual variations in serum patterns exist in the species examined and whether such physiological variables as age, sex or condition of maturity exert any influence on electrophoretic characteristics.

Specimens of *P. erumei* were collected from fish landing centres of Panjim (Goa) from January to May 1977. The range in total length was from 17.2-26.5 cm, the counts for the dorsal fin rays were from 47-54, anal fin rays 35-39, pectoral fin rays from 13-15 and vertebrae 23-25.

Blood samples were obtained from the caudal end of the fish in non-heparinized capillary tubes and were allowed to clot. The clotted blood was then centrifuged at 1000 rpm for 5 minutes to obtain a clear serum. Electrophoresis was carried out on cellogel strips (2.5 × 14 cm) using a veronal buffer of pH 8.6

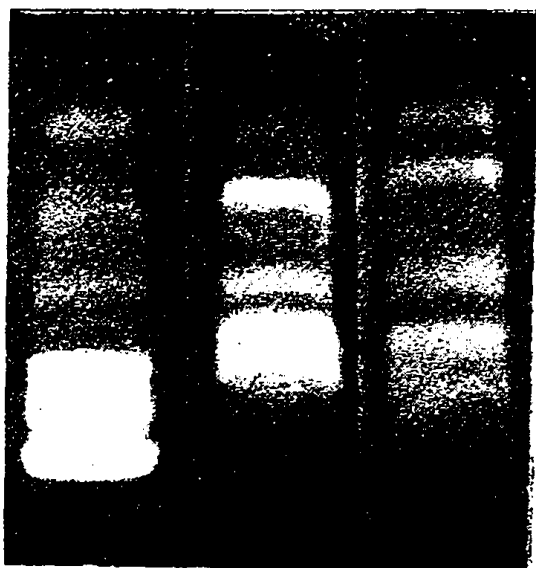


Fig. 1. Serum protein electrophoresis patterns in *P. erumei* (A), *B. orientalis* (B) and *P. arsius* (C).

and 0.05 M. Sera samples of 0.005 ml were used. All runs were for 1 hour at a constant voltage of 150 volts. The electrophoretic strips were stained with 0.2% ponceau S in 3% trichloroacetic acid and finally washed in several changes of 5% glacial acetic acid until the places on the strip containing no proteins were completely dye-free. The strips were scanned with a photoelectric densitometer. For the determination of total proteins and total solids, a refractometer was used.

About 60 specimens of different sizes and sex were screened separately. The electrophoretic pattern for all the specimens studied were found to be almost similar. The serum pattern in all the cases consists of 7 fractions of which the first 3 fractions of slower mobility are the major protein fractions (Fig. 1A). The results obtained on the electrophoretic mobilities and the total proteins and total solids are given in Table I. Because pooled samples of serum were used in the analyses of total proteins, the extent of individual variation was obscured. The intensity and position of the different fractions were studied by a photoelectric densitometer (Fig. 2). The electrophoretic fractions have been assigned numbers in the order of increasing mobility. The number of crests was found to correspond to the number of distinct proteins and the areas under the crests were proportional to their concentrations as noted earlier by Carpenter (1965). With the exception of some individual variation

Table I. Values of electrophoretic mobilities, total proteins* and total solids[†] of *P. erumei*, *B. orientalis* and *P. arsius* serum.

Analyses of serum proteins	Species	Fractions (in order from slowest to fastest)						
		1	2	3	4	5	6	7
Distance in mm from the point of application	<i>P. erumei</i>	0	6.7	11.8	20.86	29.3	31.3	42.4
	<i>B. orientalis</i>	12	18	24	30	39	—	—
	<i>P. arsius</i>	5	12	20	26	33	41	—
Relative mobility (%)	<i>P. erumei</i>	0	15.8	27.84	49.22	69.14	73.9	100
	<i>B. orientalis</i>	31	46	61	77	100	—	—
	<i>P. arsius</i>	12	29	49	63	80	100	—
Percentage of protein in each fraction	<i>P. erumei</i>	41.15	19.75	24.69	8.23	2.47	1.65	2.06
	<i>B. orientalis</i>	50.4	12.8	4.0	24.8	8.0	—	—
	<i>P. arsius</i>	32.48	21.37	10.26	3.42	17.09	15.38	—

*Total proteins (g/100 ml)

†Total solids %

<i>P. erumei</i>	8.5	12.3
<i>B. orientalis</i>	6.7	10.0
<i>P. arsius</i>	5.4	8.4

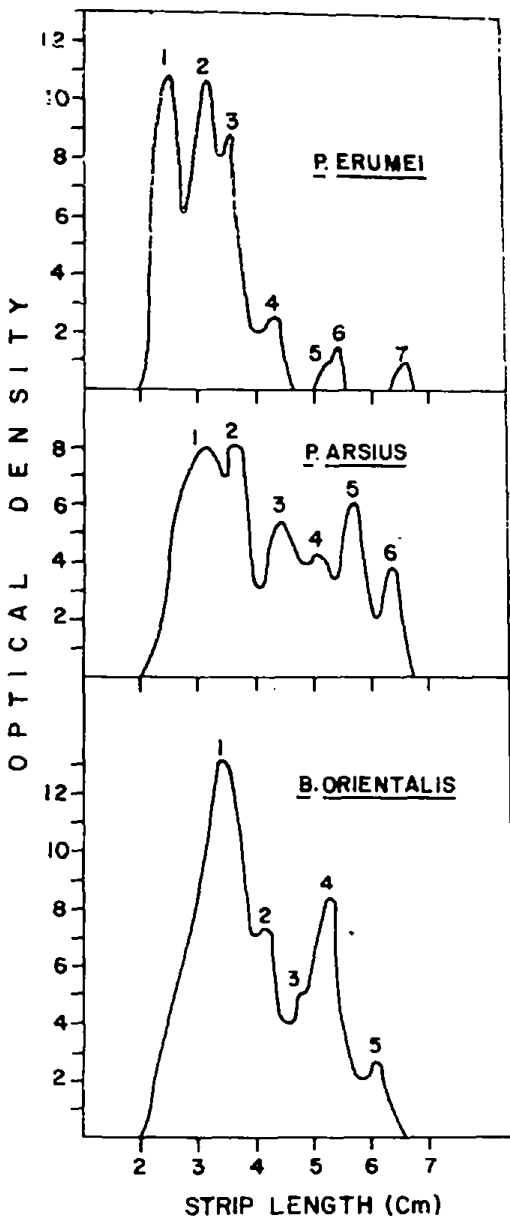


Fig. 2. *B. orientalis*, *P. arsius*, and *P. erumei* serum protein electrophoretic patterns as obtained by densitometer.

in the staining intensities of the minor components, the patterns exhibit remarkable species-specificity and no significant differences were noted.

On comparing the serum pattern of this fish with that of the other two flat fishes, *Brachirus orientalis* and *Pseudorhombus arsius* (Menezes, 1975), a clear difference was observed in number, intensity and mobility of fractions. The serum pattern of *B. orientalis* showed 5 fractions, *P. arsius* 6 fractions and *P. erumei* 7 fractions. The fraction present at the point of application in *P. erumei* serum was absent in the serum pattern of the other two species (Fig. 1).

From the data presented here, it is evident that the electrophoretic patterns of the 3 species of flat fishes are distinct. Each species can be identified by its serum electrophoretic pattern. The differences in electrophoretic patterns among the species can be classified into three categories: (a) the number of protein fractions present; (b) the migratory distances of these fractions and (c) the staining intensity of each of these fractions.

It has been suggested that the observed species differences in serum protein electrophoretic patterns may provide a molecular basis for determining phylogenetic relationships (Bertini and Cei, 1962; Cei and Bertini, 1961). These studies have also indicated that with uniform analytical methods, comparative studies of electrophoretic patterns of the serum proteins offer a promising approach for expanding our

present knowledge of the comparative biochemistry and physiology of the blood.

It would be interesting with the background of the present data, to compare other morphologically similar but geographically isolated species of flat fishes.

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