ELECTROPHORETIC STUDIES OF SERUM AND EYE LENS PROTEINS OF TWO SPECIES OF FLAT FISHES

MARIA R. MANEZES

National Institute of Oceanography, Dona Paula, Goa. 403004.

ABSTRACT

Blood sera and eye lenses of two species of flat fishes, namely Brachirus orientalis and Pseudorhombus arsius collected from June to August 1975 were analysed electrophoretically for their protein components. Total solids and total proteins were also determined. The sera and eye lens proteins of the two species showed clear differences in the number, amount and the position of the different protein components.

Introduction

Electrophoretic studies of fish serum and eye lens proteins have revealed much information concerning interspecific as well as intraspecific similarities and differences in fishes (Moore, 1945; Fugiga, 1961; Sanders, 1964; Badawi, 1971). The present investigation was undertaken to compare under uniform conditions, the electrophoretic components of sera and eye lens proteins of two species of flat fishes as regards their mobility, amount and number, so as to enable the determination of electrophoretic patterns characteristic of each The fishes investigated were species. Brachirus orientalis and Pseudorhombus arsius. Both these are widely distributed species. B. orientalis occurs along the coasts of India Sind, Andamans, China, Australia and the Persian Gulf. P. arsius is found along the coast of Africa, seas and estuaries of India and Australia

(Day, 1878; Pradhan, 1964; Norman, 1928; Misra 1943; and Munro, 1955).

MATERIAL AND METHODS

Samples of both the species (B. orientalis and P. arsius) were collected from the fish landing centres of Panjim from June to August 1975. In B. orientalis, the range in length was from 12.8 to 18.6 cm and in P. arsius from 13.6 to 22.0 cm. Blood samples were obtained from the caudal end of the fish in non-heparininized capillary tubes and were allowed to clot. The clotted blood was then centrifuged at 1,000 rpm for 5 minutes to obtain a clear sera. Samples showing evidence of hemolysis were discarded.

Eye lenses were removed from the fishes, washed in 1% saline and then ground in a test tube with 0.5 ml veronal buffer pH 8.6 and ionic strength 0.05.

Mahasagar, Vol. 8 Nos. 3 & 4, 1976

They were then centrifuged at 3,500 rpm for 15 minutes to obtain a clear solution.

total solids and total proteins, a refractometer was used.

Electrophoresis was carried out on cellogel strips using a veronal buffer pH 8.6 and ionic strength 0.05. All samples were run for 1 hour at a constant voltage of 150 volts, after which the strips were scanned using a photoelectric densitometer. For the determination of

RESULTS

Several sera and eye lens samples of B. orientalis and P. arsius were screened separately. The results abtained on the electrophoretic mobilities and the total proteins and total solids of the two species are given in Tables I and II. The

TABLE I: Electrophoretic analyses, total proteins and total solids of serum proteins.

Analyses of serum proteins	Species	Fractions (in order from slowest) to fastest)						
			1	2	3	4	5	6
Distance in mm	B. orientalis		12	. 18	24	30	39	
from the point of application	P. arsius		5 ,	12	20	26	33	41
Relative mobility	B. orientalis		31	46	61	77	100	
(as percentages)	P. arsius		12	29	49	63	80	100
		Total proteins g/100 ml 6.7			S	Total solids		
						% 10.0 8.4		
	B. orientalis							
	P. arsius	5.4						

TABLE II: Electrophoretic analyses, total proteins and total solids of eye lens proteins

Analyses of eye	Species	Fractions (in order fro					m slowest to fastest)			
lense proteins		1	2	3	4	5	6	7	8	
Distance in mm	B. orientalis	0	8	13	18	26	44	_	-	
from the point of application.	P. arsius	0	, 4	7	12	16	21	28	44	
Relative mobility	B. orientalis	0	18	29	41	59	100	_	· -	
(as percentages)	P. arsius	0	9	16	27	36	47	64	100	
		Total proteins g/100 ml				Total solids.				
	B. orientalis	0.5			2.2					
	P. arsius		2.5				1.11	4.7		

Maria R. Menezes: Electrophoretic Studies of Flat Fishes

typical electrophoretic patterns for the serum and eye lens protiens of the two species studied are given in Figs. 1 and 2. The serum pattern of B. orientalis consists of 5 fractions, of which 1, 2 and 4 are the major protein fractions. Six fractions are present in the serum of P. arsius,

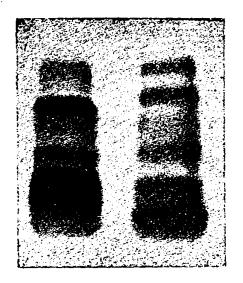


Fig. 1. Scrum protein electrophoresis patterns in B. arientalis and P. arsius.



Fig. 2. Eye lens protein electrophoresis patterns in B. orientalis and P. arsius.

fractions 1, 2, 3 and 5 are the major protein fractions. The eye lens pattern of *B. orientalis* consists of 6 fractions, of which 1 and 2 are the major proteins. Eight fractions are present in the eye lens of *P. arsius* fractions 1, 2, 3 and 6 are the major protein fractions. The differences remain constant and exhibit remarkable species specificity.

The intensity and position of the different fractions were studied by a photoelectric densitometer. The electrophoretic fractions have been assigned numbers in their order of increasing mobility (Figs. 3 & 4). The number of

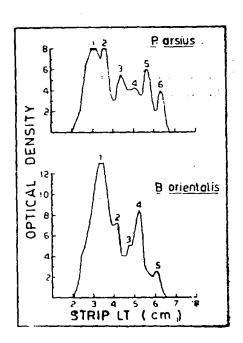


Fig. 3. B. orientalis and P. arsius, serum protein electrophoresis patterns as obtained by densitometer.

(Note: Lt = length of strip)

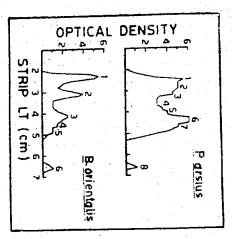


Fig. 4. B. orientalis and P. arsius, eye lens protien electrophoresis patterns as obtained by densitometer.

(Note: Lt = length of strip).

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).

CONCLUSION

The study of serum and eye lens proteins of the two species of flat fishes clearly indicate the patterns which are characteristic of the species. A comparison between them revealed a major difference in their protein fractions.

Serum proteins of B. orientalis showed 5 fractions, and of P. arsius 6 fractions. Fractions 1, 2 and 4 contain the most protein found in B. orientalis sera, and fractions 1, 2, 3 and 5 in the sera of P. arsius. The sera of the two species also differed in their final mobility. The sera of B. orientalis migrated 39 mm and P. arsius sera 41 mm. The eye lens proteins of B. orientalis showed 6 fractions, and of P. arsius 8 fractions. Fractions 1 and 2 contained most of the protein in the eye lens of B. orientalis and fractions 1, 2, 3 and 6 in the eye lens of P. arsius. The eye lens proteins of B. orientalis and of P. arsius had similar final mobilities as they migrated up to 44 mm. The number of fractions present in the serum and the eye lens was constant in each of the two species.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. S. Z. Qasim, Director of the Institute for constant encouragement and guidance while the work was in progress and also for the valuable criticism in the preparation of this paper. I am thankful to Mr. K. V. Rao and Dr. N. G. Karanth, for scrutinizing the manuscript.

REFERENCES

Badawi, H. K. 1971. Electrophoretic studies of serum proteins of four Tilapia species (Pisces). Mar. Biol., 8: 96-98.

Carpenter, B. L. 1965. Immunology and Serology. W. B. Saunders Company Philadelphia and London.

Day, F. 1878. The Fishes of India. William Dawson and Sons, London.

Fugiga, M. 1961. Use of electrophoretic serum separation in fish studies. J. Water Pollution Control Fed., 33: 250-257.

Maria R. Menezes: Electrophoretic Studies of Flat Fishes

- Mishra, K. S. 1943. A check list of the fishes of India, Burma and Ceylon. Rec. Ind. Mus., 45: 377-431.
- Moore, D. H. 1945. Species differences in serum protein patterns. J. Biol. Chem., 161: 21-31.
- Munro, I. S. R. 1955. The Marine and Fresh water Fishes of Ceylon. Department of External Affairs, Canberra.
- Norman, J. R. 1928. The flat fishes of India, with a list of specimens in the Indian Museum Part II. Rec. Ind. Mus., 30: 173-215.
- Sanders, B. G. 1964. Electrophoretic studies of serum proteins of three trout species and the resulting hybrids within the family Salmonidae. In *Taxonomic Bio-Chemistry and Serology*., pp 673-679 Charles A. Leone New York Ronald Press Company.