

## VARIATION OF HAEMOGLOBIN IN *OTOLITHUS RUBER* (CUV. & VAL.)

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Haemoglobin phenotypes of rosy jewfish, *Otolithus ruber* were studied by cellulose acetate electrophoresis. Haemoglobin have been named Hb-A, Hb-B, Hb-C and Hb-D according to their increasing mobility. In general most of the samples gave three bands, however variations in number and size of the band, and colour intensity, were observed. In the light of these results on haemoglobin phenotypes, the intraspecific variations in the species have been discussed.

The chemical composition of haemoglobins has been established as polymorphic in several mammals. This polymorphism can easily be demonstrated by electrophoresis which was first used by Pauling *et al* (1949), for the study of abnormal human haemoglobins. In fishes, genetically controlled polymorphism was first reported in cod, *Gadus morhua* and whiting, *G. merlangus* by Sick (1961, 1965). It has also been shown that in the haemoglobin of fish there may exist an intraspecific variation during ontogeny, such as in salmon, *Salmo salar* (Kock *et al.*, 1964; Vanstone *et al.*, 1964) and herring, *Clupea berengus* (Wilkins, *et al.*, 1966). Haemoglobin polymorphism was also demonstrated in a catfish *Tachysurus caclatus* (Dwivedi and Bhosle, 1975).

The rosy jewfish, *Otolithus ruber*, is abundant in coastal waters of Goa and constitutes a commercial fishery. No information is available on the haematology and populations of this species and therefore, an investigation on the

haemoglobin phenotypes was undertaken using electrophoresis.

Fifty specimens of *Otolithus ruber* of both sexes and of different length groups were collected from the Velsao Bay, Goa, during January to February, 1975. Caudal-peduncles severed and blood was collected in tubes previously rinsed with ammonium oxalate. The erythrocytes were washed four times with cold 0.9% NaCl and lysed by adding two volumes of chilled distilled water. Lysate was stored overnight at 4°C, again centrifuged and the clear supernatant haemoglobin solution used for electrophoresis.

The electrophoresis was carried out using cellulose acetate strips (Shandon). The electrophoretic technique described by DuToit *et al* (1973), was used with some modifications. The main points of DuToit *et al*'s modified technique were the cellulose acetate strips of the size 25 X 160 mm, tris buffer of pH 6.1, and a constant voltage of 150 for one hour.

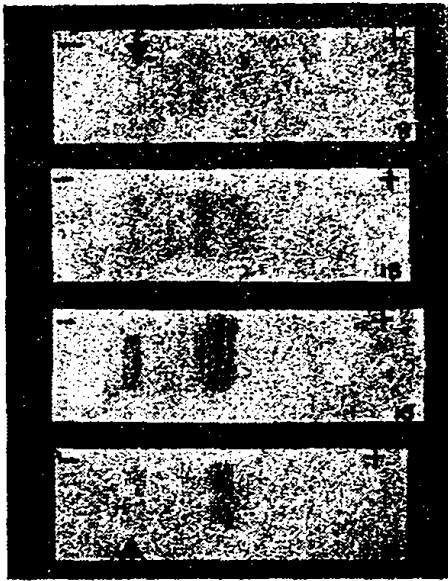


Fig. 1 (A) Haemoglobins of *O. ruber* separated on cellulose acetate strips in tris buffer of pH 6.1.

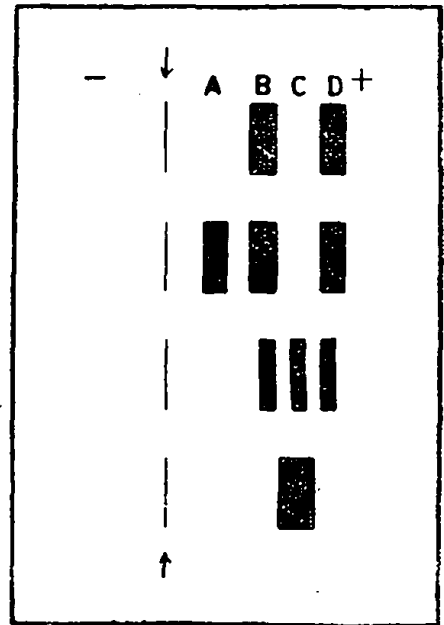


Fig. 1 (B) Digrammatic representation of Fig 1 (A) showing the haemoglobins of *O. ruber*. Arrow indicate the point of application. Alphabets indicates the name of haemoglobin.

The haemoglobins were stained with Ponsceau S. An outline of the haemoglobins of *O. ruber* is shown in Fig. 1

(A+B). Fig. 2 shows the densitometric scan and Table I represents the results of electrophoretic runs of haemoglobins

TABLE I—*Electrophoretic Data of the Haemoglobins of O. ruber*

Electro-phoresis	No. of individuals	No. of Haemoglobin band	Type of Haemoglobin	Distance from the point of application (cm)	Relative mobility (%)
Cellulose acetate	5	Two	Hb-B	1.5	65.21
			Hb-D	2.3	100
"	36	Three	Hb-A	0.7	31.81
			Hb-B	1.5	68.18
			Hb-D	2.2	100
			Hb-C	1.9	86.36
"	7	Three	Hb-B	1.6	72.72
			Hb-D	2.2	100
			Hb-C	2.0	100
"	2	One	Hb-C	2.0	100

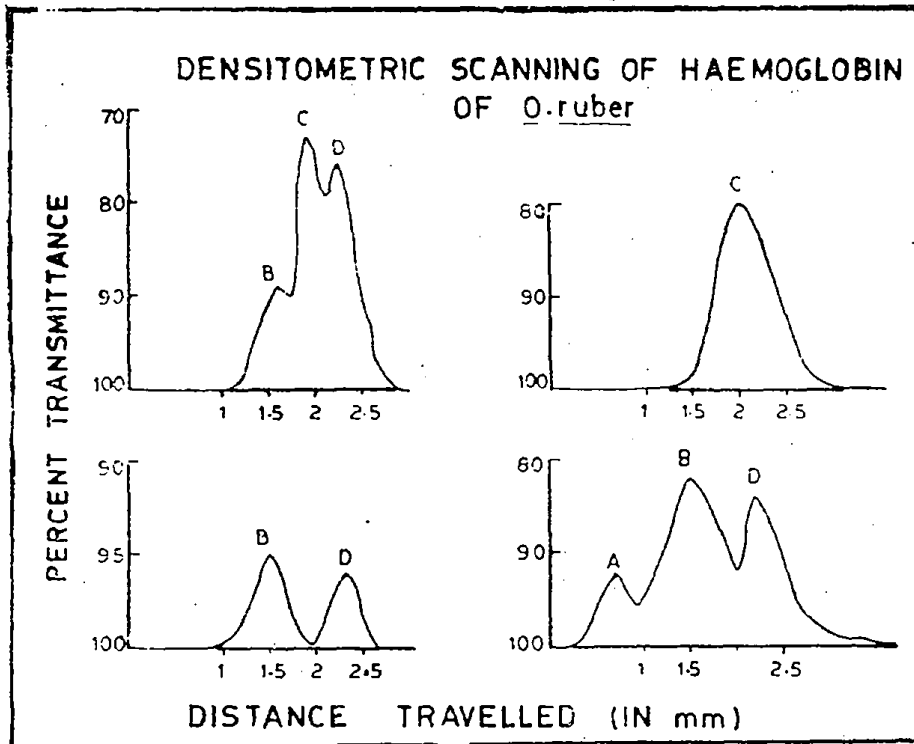


Fig. 2. Densitometric scanning of the haemoglobins of *O. ruber*. Alphabets indicates the name of haemoglobin.

of *O. ruber*. All the haemoglobins of *O. ruber* moved towards the anode in tris buffer of pH, 6.1. The haemoglobin components have been named as Hb-A, B, C and D, according to their increasing mobility pattern towards the anode. Hb-A was the slowest anodic component and did not show any variation in strength and colour intensity. It was present in most of the samples, except fourteen samples, where it was not observed. Hb-B was more rapid in its mobility than Hb-A. Hb-B showed variation in strength and colour intensity in a few samples. Hb-B gave a thin band in seven specimens as compared to the Hb- found in other specimens. Hb-C was present in nine specimens. In two specimens, it was broad and strong

whereas in others it was represented by a comparatively thin band. Hb-D gave maximum mobility but again showed considerable variation. In most of the specimens, Hb-D was a strong and broad band, but in seven specimens it gave a strong but relatively thin band, while in the other samples it was entirely absent.

The results suggest that the individual variations are demonstrated by the present technique in *O. ruber*. The intraspecific variations obtained in the present investigation do not show any relation to either sex or length, and the significance of abnormal haemoglobins of *O. ruber* is difficult to understand. In the sprat (Wilkins *et al*, 1966; Naevdal,

1968) and other clupeoid fishes (Simpson and Simson, 1966), intraspecific variations have been described but these variations are neither found to be associated with age or length nor do they have any apparent genetic basis. The number of individuals possessing the abnormal haemoglobins in the present study is too small for a satisfactory genetic analysis. However, a possible explanation for the appearance of such intraspecific variation in haemoglobin fractions of varying electrophoretic mobility is due to the substitution of the amino acid in each of the semi-molecules that constitutes the globin (Ingram, 1957). This substitution would alter the physicochemical properties of

haemoglobin completely and could explain the differences in the electrophoretic mobility. A detailed study of oxygen requirement of *O. ruber* and the biochemical properties of its haemoglobin could reveal more information about the physiological need for the multiplicity of its haemoglobins.

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