

attributed them to the reproductive cycle. In the present study, no clear cut seasonal trend was evident in biochemical constituents. Reproductive stages occur throughout the year in the brown and red algae studied and there were no specific fruiting periods¹³. Thus, one can expect conspicuous seasonal changes in the biochemical composition of those plants where there is a definite seasonal rhythm in the growth and reproductive cycles.

The authors wish to acknowledge Drs S.Z. Qasim and A.G. Untawale of NIO, Dr M.S. Murthy of Saurashtra University, Rajkot and Shri M. Umamaheswara Rao, Andhra University, Waltair, for critically evaluating this paper and for useful suggestions.

References

- Himmelman J H & Carefoot J H, *J exp mar biol ecol*, **18** (1975) 139.
- Untawale A G & Dhargalkar V K, *Report on the seaweed resources of the Goa coast* (National Institute of Oceanography, Goa) 1975, 1-135 pp.
- El Wakeel S K & Riley J P, *J Cons perm int Explor Mer*, **22** (1957) 180.
- Folch J M Lees & Stanley G H S, *J biol Chem*, **226** (1956) 497.
- Dubois M, Gillas K A, Hamilton J K, Rebus R A & Smith F, *Anal Chem*, **28** (1956) 350.
- Carefoot J H, *J mar biol Ass, UK*, **47** (1967) 565.
- Dhargalkar V K, *Intern Seaweed Symp on Marine algae of the Indian Ocean region, Bhavanagar*, 9-12 January, 1979 (Abstract).
- Mann K H, *Mar Biol*, **12** (1972) 1.
- Carefoot J H, *Mar Biol*, **18** (1973) 228.
- Sumitra-Vijayaraghavan, Wafar M V M, Jacob P G, Balasubramaniam T, Rajagopal M D & Krishna Kumari L, *Mahasagar—Bull natn Inst Oceanogr*, **8** (1975) 9.
- Rajagopal M D, Sumitra-Vijayaraghavan & Wafar M V M, *Mahasagar—Bull natn Inst Oceanogr*, **8** (1975) 187.
- Paine R J & Vadas R L, *Mar Biol*, **4** (1969) 79.
- Umamaheswara Rao M, Personal communication.

Immunochemical Analyses of Soluble Lens Proteins in Some Marine Fishes

MARIA R MENEZES

National Institute of Oceanography, Dona Paula, Goa 403 004

Received 18 July 1979

Soluble eye lens proteins of 10 fishes, belonging to the families Clupeidae, Hemirhamphidae, Lactariidae, Scombridae, Stromatidae, Psettodidae, Bothidae and Soleidae were studied by immunoelectrophoresis using the lens antiserum of *Sardinella longiceps*. Each species had a specific antigenic complex. The degree of antigenic correspondence among the species varied in accordance with their taxonomic relationship.

Immunogenic studies on marine fishes have been made¹⁻⁴ though most of these are concerned with the identification of sub-populations using red cell

techniques. Immune response in teleost fishes has received attention because of the interest in the immunogenetics of these animals and also for a better understanding of their evolutionary development⁵. In this communication immunochemical correspondence of the soluble lens proteins of some of the common fishes of Goa is presented.

Sardinella longiceps, *S. fimbriata* (Clupeidae); *Hemirhamphus georgii* (Hemirhamphidae); *Lactarias lactarias* (Lactariidae); *Rastrelliger kanagurta* (Scombridae); *Parastromateus niger*, *Pampus argenteus*, (Stromatidae); *Psettodes erumei* (Psettodidae); *Pseudorhombus arsius* (Bothidae) and *Brachirus orientalis* (Soleidae), collected from the fish-landing centres of Panaji were used.

Antiserum for the study was produced by the following method⁶, in a rabbit using *S. longiceps* lens protein. Rabbit (2 to 3 kg) was bled from the marginal ear vein and the blood was set aside for the preparation of the pre-immune control serum. The animal was then injected subcutaneously with 0.5 ml of *S. longiceps* lens extract (30-50 mg in 1 ml saline) mixed with 0.5 ml of Freund's complete adjuvant. On the 15th day, 1 ml of the lens extract without the adjuvant was injected intravenously into the marginal ear vein. The same procedure was repeated on the 22nd day. One week after the 3rd injection, the rabbit was bled from the marginal ear vein and treated for antibody activity. The blood was collected in a precooled centrifuge tube and allowed to clot. The clotted blood was then centrifuged at 3,500 rpm for 10 min to obtain a clear antiserum.

Immuno-electrophoresis was carried on agar plates using 6 × 11 cm glass plates and 1.5% Difco's Bacto-agar in veronal buffer (pH 8.6) of ionic strength 0.05. The method of Afonso⁷ was suitably modified for the present work.

Two holes (slots) were bored on the agar gel plate and 3 µl of the antigen (lens protein) were deposited in each well. Soon after, the plate was subjected to electrophoresis using a constant voltage of 150 v for 45 min. Then the plates were kept in a humid chamber for preliminary diffusion for 3 hr at a constant temperature (30°C). About 20 µl of the antiserum was then placed in-between the 2 antigen wells whose long axis parallels the axis of electrophoretic migration. The plates were again left to diffuse for about 24 hr in a humid chamber at 30°C. The plates were then washed in 1% sodium chloride and developed in 0.2% aqueous acetic acid. Contact prints of the plates were taken.

Results of the reaction of various test antigens with the antisera are given in Fig. 1. Clear patterns of precipitate were obtained with each of the antigen used. The preimmune serum did not show any reaction with the test antigens.

The proteins of the lens are chosen for investigation because they have the advantage of an extremely wide range of serological cross reaction⁸. An antiserum prepared against lenses of any of the vertebrate species gives precipitin reactions with lenses of all other vertebrates⁹. This is in striking contrast to the much more intensively studied serum proteins, which have a considerably more limited range of cross reaction¹⁰.

Antiserum of *S. longiceps* lens showed 4 components in the homologous reaction with *S. longiceps* lens. With *S. fimbriata* lens also it showed 4 components but there was some slight variation in the design of the precipitate lines formed and in their mobilities. Reaction with *H. georgii* lens showed 5 components while with *L. lactarius* 3, *R. kanagurta* 2, *Parastromateus niger* and *Pampus argenteus* 3,

Psettodes erumei and *Pseudorhombus arsius* 1, and *B. orientalis* 2 components. Since closely related species display similar reactions and share some precipitate bands in reactions of identity, it is possible to get a good idea of their relationships.

S. longiceps and *S. fimbriata* lens showed very similar reciprocal reactions. *H. georgii* lens shared some precipitate bands with *Sardinella* sp., but the reciprocal reactions were somewhat weaker. *L. lactarius* and *R. kanagurta* lens appear to stand somewhat apart from the *Sardinella* lens complex. The reactions of *Parastromateus niger* and *Pampus argenteus* lens, however, showed similarity to each other. The 3 flat fishes *Psettodes erumei*, *Pseudorhombus arsius* and *B. orientalis* lens showed very little taxonomic affinity for the antisera prepared against *S. longiceps* lens. Thus,

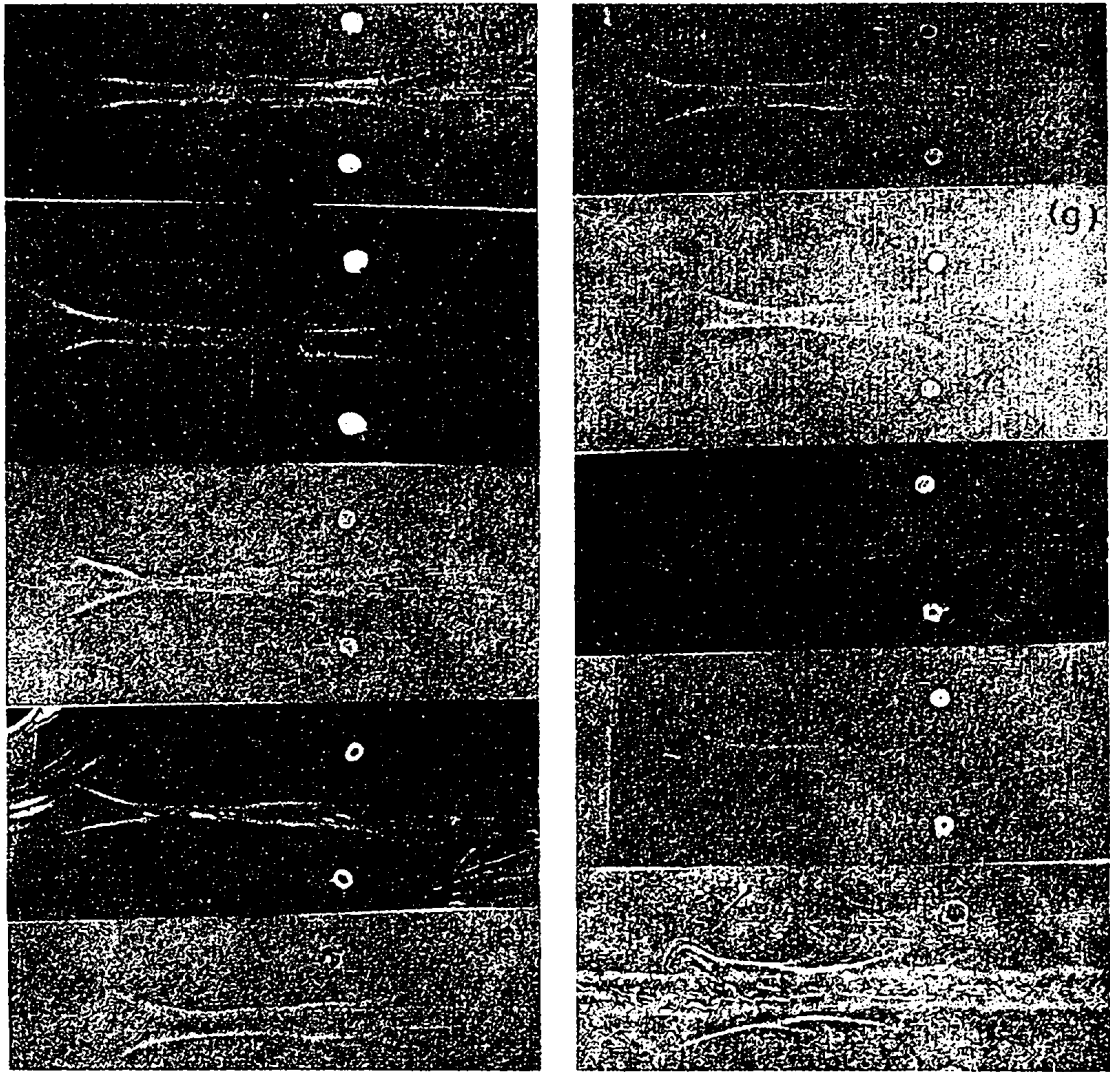


Fig. 1— Reaction of anti *S. longiceps* lens serum with lens protein from various fishes [a, *Sardinella longiceps*; b, *S. fimbriata*; c, *Hemirhamphus georgii*; d, *Lactarius lactarius*; e, *Rastrelliger kanagurta*; f, *Parastromateus niger*; g, *Pampus argenteus*; h, *Psettodes erumei*; i, *Pseudorhombus arsius*; j, *Brachirus orientalis*]

the overall picture on the immunoelectrophoretic patterns of different species suggests that in the evolutionary sequence the flat fish species should be placed farther away from the other fishes.

Squid lens antisera, prepared in the rabbit was found to have 4 to 5 components when tested with squid lens in immunodiffusion assays¹¹. No cross reactions, however, were noted with a number of vertebrate lenses. Similarly, antisera against vertebrate lens failed to show any cross reaction with the squid lens⁹. This shows that the invertebrate lens does not share any immunochemical similarity with the vertebrate lenses and that the cephalopod eye evolved along a distinct path from that of the vertebrates. The phylogenetic relations among the vertebrate lenses by the reactions obtained with antihuman lens serum and other vertebrate lenses showed a decrease in the number of shared components when they get farther and farther removed phylogenetically^{8,12,13}. In this study also, the immunoelectrophoretic patterns (Fig. 1) clearly show a decrease in the number of shared components with an increase in the phylogenetic distance. It has been found that fishes which are more closely related on a morphological basis tend to have more common antigens.

The author wishes to express her gratefulness to Dr S.Z. Qasim, Director, for continued interest and guidance throughout the work and also for the valuable suggestions in the preparation of this paper. Thanks are also due to Dr T.S.S. Rao, for critically going through the manuscript.

References

- 1 Cushing J, *Science*, **115** (1952) 404.
- 2 Ridgway G, Cushing J & Durall G, *U S Fish Wildl Serv Spec Sci Rept Fish*, **257** (1958) 1.
- 3 Suzuki A, Shimizu Y & Morio T, *Nankai Regional Fish Res Lab (Japan) Rept*, **8** (1958) 104.
- 4 Sprague L M & Vrooman A M, *Ann N Y Acad Sci*, **97** (1962) 131.
- 5 Ridgway G J, Hodgins H O & Klontz G W, *Phylogeny of immunity* (University of Florida Press) 1966, 199.
- 6 Menezes M R, *Morphometry and serology of some fishes from the Goa region*, M.Sc. thesis, University of Bombay, 1979, 144.
- 7 Afonso E, *Clinica Chim Acta*, **10** (1964) 114.
- 8 Hektoen L, *J infect Dis*, **31** (1922) 72.
- 9 Manski W, Halbert S P & Auerbach T P, in *Taxonomic biochemistry and serology*, edited by C A Leone (The Ronald Press, New York) 1964, 545.
- 10 Nuttall G H F, *Blood immunity and blood relationship*, (Cambridge University Press, London) 1904.
- 11 Manski W, Halbert S P & Auerbach T P, *Archs Biochem Biophys*, **92** (1961) 512.
- 12 Manski W, Auerbach T P & Halbert S P, *Fedn Proc*, **19** (1960) 334.
- 13 Manski W, Auerbach T P & Halbert S P, *Fedn Proc*, **20** (1961) 41.

Biochemical Composition of the Bivalve Molluscs, *Villorita cyprinoides* var. *cochinensis* (Hanley) & *Meretrix casta* (Chemnitz)

P T LAKSHMANAN* & P N KRISHNAN NAMBISAN†
Department of Marine Sciences, University of Cochin,
Cochin 682 016

Received 7 April 1978; revised received 9 July 1979

Biochemical components in *M. casta* and *V. cyprinoides*, collected from Cochin Backwaters, varied with season and species. An inverse relationship between protein and carbohydrate was observed in both the species, the sum of carbohydrate and protein being a constant. Increase in lipid level was accompanied by an increase in total phosphorus of the 2 clams. Calorific values and lipid content were higher in *V. cyprinoides*. The carbohydrate minima in the 2 species during January-February were attributed either to spawning or low concentrations of plankters.

Meretrix casta and *Villorita cyprinoides* var. *cochinensis* comprise one of the major components of shell fisheries in the southwest coast of India. Though some information is available on the chemical¹⁻⁸ and biochemical⁹ composition of molluscs, detailed and systematic information on the biochemical composition of these species is lacking. Such information would be desirable to fix the best season for harvesting from the point of view of high nutritive value. Hence an attempt has been made to investigate the biochemical composition of these 2 bivalves. Interrelationships between the different biochemical components have also been worked out.

Monthly collections were made from September 1976 to August 1977. *M. casta* was obtained from a place about 2 km southeast of Cochin barmouth (sal; $32 \pm 0.5\%$ in summer and $5 \pm 1\%$ in rainy season) and *V. cyprinoides* from a place about 6 km northeast of the barmouth (sal., 20% in summer and 1% in rainy season). No data could be collected for March 1977.

The animals were brought to the laboratory and kept in sea water of habitat salinity for a day so as to remove the pseudofaecal materials. Whole soft portions from 15-20 animals of almost the same size were then removed and the wet weight of the combined material was determined. It was homogenised and dried to constant weight at 60°C , the dried material was finely powdered and an aliquot was ashed at $500-550^\circ\text{C}$ in a muffle furnace. The residual weight was taken as the ash content. Various biochemical constituents were determined by standard methods: carbohydrate and lipid¹⁰, nitrogen¹¹ and total phosphorus¹² were estimated. The amount of protein

*Present address: Central Institute of Fisheries Technology, Cochin 682 029

†For correspondence