

IDENTIFICATION OF FISH POPULATIONS WITH PARTICULAR REFERENCE TO THE PELAGIC FISH STOCKS OF THE INDIAN OCEAN REGION

by S. N. DWIVEDI

National Institute of Oceanography, New Delhi

The most essential step in any fishery management is the identification of discrete fish populations. This is particularly important for the development of Indian Ocean pelagic fisheries. The simple signal character analysis of meristic or metric characters which are usually employed do not give complete solutions to the problem, in such instances more sophisticated analysis for studying many characters together using the method of group constellations has been discussed. The development of blood groups in humans and its recent application to fish groups has been reviewed. The more recent analysis of haemoglobin components using electrophoretic techniques on paper, in agar and starch gel are discussed. The paper describes a new modification of starch gel electrophoresis method currently in use, and the application of this procedure for the study of clupeoid, scombroid and other pelagic fish populations of the Indian Ocean.

FISH POPULATION AND THEIR IDENTIFICATIONS

The progress in the identification and recognition of fish populations in the last two decades had been a very important development in fisheries management. The fish populations are known under different synonyms like, races, subspecies, subgroups. These may be defined as more or less discrete populations having separate spawning areas and can be recognised by average differences in certain characters usually morphometric. However, during the last decade serological and electrophoretic techniques have come into extensive use.

The species was once considered to be the lowest taxonomic division, it is no more so, though it is the most important category. The older concept of species from the time of Ray and Linnaneus was static and morphological. They believed that the species in nature differ by clear cut characters called 'Species character'. Now we know that some species are wide spread and consist of many local populations, and the concept of reproductive isolation was found to be the valid criterion. Species therefore may be defined as "Species are groups of actually or potentially inter-breeding natural populations which are reproductively isolated from other such groups" (Mayr 1940).

It is therefore evident that a species is heterogeneous; in other words it is a heterogeneous assemblage of populations and so are the subspecies also. The population genetists have proved that in sexually reproducing species no two natural populations are genetically identical and they usually differ taxonomically. Therefore, sub-

species are geographically defined aggregates of local populations which differ taxonomically from other such subdivisions or aggregates. Subspecies are geographically localised and consequently it is also geographically a race. These two terms are commonly used as synonyms. Now it is also accepted that the subspecies is the lowest taxonomic level which may be named. But it is not the lowest division of the species, because it is composed of numerous local populations. These local populations amongst them differ slightly in general frequencies and mean values of various quantitative characters. It may be mentioned here that the fish taxonomists recognize races within the subspecies generally without naming them.

Suitable taxonomic characters are used for identification. A taxonomic character is an attribute of an organism or a group of organisms by which it differs from an organism belonging to a different taxonomic category or resembles an organism belonging to the same category (Mayr *et al.* 1953). The most practical diagnostic characters are easily visible structures which have little variability. The determination and evaluation of a proper taxonomic character is a basic and important task in population identification. A character may be important in one case and may be entirely useless in other cases for example, mean number of vertebrae. The number of vertebrae have been extensively used for identification of herring (Runnstrom 1941) sardine (Furnestin 1945) haddock (Clark and Vladkov 1960) population but the number of vertebrae is not useful in case of tunas. Further it is highly commendable to recognize populations by one kind of character and verify them by different type of characters. Fish population which are differentiated by morphometric characters, their separation may be confirmed by blood typing, electrophoretic mobility of protein components of haemoglobin or by other suitable biochemical techniques. In the beginning (See Heinke 1898; Fage 1911) each morphometric character was analysed separately and then compared between populations. Analysis of herring populations by Runnstrom (1941) is also an example of this type. In many cases an overlap occurred in the frequency of meristic characters and many samples had to be examined. A graphic method for simultaneously comparing many samples was suggested by Hubbs and Hubbs (1953), which was a modification of Hubbs and Perlmutter (1942). Rothschild (1963) suggested an alternative method utilising Tuckey's multiple comparison procedure. A simple and effective method of coefficient of difference (C.D.) for estimating overlap between populations was introduced by Mayr Linsley and Usinger (1953). The method has been elaborated by (Gery 1962). He gave tables for a wider range of values of C.D. to evaluate non-overlap percentages at different conventional levels. This method was used to demonstrate non-overlap between races of *Lepidorhombus boschii* (Risso and Dwivedi 1964).

Since beginning meristic characters have also been used. They were compared by establishing index value of body parts. The index values are percentage of concerned body part in relation to the total length of the specimen. This in many cases, particularly when overlap occurred did not always provide a satisfactory solution. Later regression analysis and formula of allometric growth has also been used. Reeves (1940) described a method for comparing relative growth rates when absolute sizes of the specimen to be compared are different. However, the taxonomists using quantitative methods soon realised that it was not always possible to

recognize differences based on the analysis of one character only. Therefore many characters were studied and each had to be compared separately. Later methods were developed to combine many characters and examine them simultaneously. A more efficient method of Karl Pearson's Coefficient of Racial Correlation for combining many characters was used by anthropologists. It estimates the distance between two populations. This method had certain disadvantages and an improved method for calculating D^2 , a concept of distance between two populations and group constillation was introduced. The method has been explained by Rao (1952). Since then it has been used in fish taxonomy by Pillay *et al.* (1952) and Royce (1964). A detailed review of the various statistical methods used in the investigation of fish populations is given by Royce (See Marr 1955). In spite of these highly sophisticated biometrical techniques the use of these methods present difficulties regarding obtaining large samples from the same homogeneous populations and the use of these improved techniques require laborious computations. Further the morphological characters are plastic and are susceptible to be modified by the environment. Therefore ichthyologists have attempted to use those characters which are independent of the milieu, are genetic and do not change during the different phases of the life of the fish. Such methods have been borrowed from other disciplines and we find serology, immunology and analysis of protein components using electrophoretic and immunoelectrophoretic techniques have been used.

Serology dates back to the work of a Belgian Scientist Jules Bordet (1895). He demonstrated that red blood corpuscles of an animal when brought in contact with the plasma of another animal of a different species, clotting takes place. The phenomenon was called agglutination. Later a Polish doctor Landsteiner in 1900 discovered that in man agglutination of red blood corpuscles occur by mixing the blood of different individuals. Thus he made the fundamental discovery of isoagglutination. He showed that in human plasma two types of agglutinogenes occur A and B and according to their presence or absence the individual were assigned to blood groups A, B, AB, and O. These groups are hereditary and in their transmission to the next generations abide by the Mendel's law. Further the blood groups are fixed at birth and remain constant throughout the life of the individual. Studies after this fundamental discovery of Landsteiner showed that the distribution of human blood groups change from one human race to another and within the same race they change from one region to another. This discovery then became the most important point which led to the study of blood groups in different animals. Therefore the systematic serology or study of blood groups is based on the assumption that animals have evolved biochemically and morphologically. The biochemical characteristics are genetically determined and are not influenced by the environment. In a given population the frequency of occurrence of a particular gene is fixed and the quantitative differences in the occurrence of these genes can be used to identify, characterise and differentiate fish populations. This kind of work has been actively pursued in USA, Japan, Canada, Holland, France and Ireland.

In U.S.A., Sindermann and Mairs (1959) described a C blood-group system of herring. Cells from the individual fish were tested against antisera prepared by injecting herring erythrocytes into rabbits. Against this antisera some

herrings erythrocytes clumped and were called C positive. Other did not react or reacted very weakly, these are called C negative. With this reagent blood samples of more than 2000 herrings were tested along the coast of USA for frequency of antigen C and then prepoence of two groups eastern and western were shown.

In the Pacific Ocean on California coast, Vrooman (1964) demonstrated three genetically distinct subpopulations of the Pacific sardine (*Sardinops caerulea*). A northern population with 13.6% C positive factor on the coast of California, the southern population on the coast of Baja California Mexico had 6% C positive cells and third population in Gulf of California had 16.8% of C positive cells. There are many more examples available in literature.

It is interesting to mention that extracts from seeds of certain plants are used as anticoagulants. These are called Lectins. Seeds of certain plants like castor bean and Indian Licoric, agglutinate human and also other red blood cells. Some of them show specificity. They react strongly against some species and for other species slightly or not at all. This is an extensive phenomenon and now we know plant agglutinents almost completely specific for blood group antigens A ($A_1 + A_2$), A, H, and N. Strong agglutinogen are found in certain Lima beans, *Vicia cracca*. Sprague (1961) used them in the studies of oceanic skipjack. Sindermann (1962) used Lima bean (*Phaseolus limensis*), lentil *Lens culinaris* and garbanzo bean (*Cicer arietinum*) in clupeoid fishes.

The extract of the seeds can be prepared by mixing 1 gm of powered seed and 10 ml of 1.4 saline. This is kept at 37°C for three hours and then kept overnight at 4°C. The mixture is then filtered and centrifuged and the supernatant is separated. It is now ready for use. It can also be frozen and preserved for use. For tube agglutination tests 0.2 ml of extract and 0.05 ml cell suspension are sufficient and give good results.

By the use of extract of one variety of lima beans, Sindermann (1962) demonstrated evidence of population heterogeneity for four spawning populations of alewives (*Alosa pseudoharengus*) on the Atlantic coast of America off Rhode Islands, Massachusetts, Maine and Nova Scotia. The use of lectins shows great possibilities because they are specific and pick up individual and population differences. They are easy to obtain and their extracts can be prepared by simple method. The other method used is electrophoretic analysis of serum and haemoglobin. The haemoglobin consists of different kinds of protein molecules which differ in their weight and size and carry a different electric charge. These in an electric field move at different velocities. This principle is taken advantages of in electrophoretic analysis. Various media, like paper, agar, polyacrylamide and starch gel are used. These methods are now commonly used in human pathology and in other group of animals. During the last decade they have been used in fishes also. Bhuler and Shanks (1958) analysed haemoglobin of fourteen species of fresh and salt water fishes by paper electrophoretic methods and demonstrated the presence of one, two or three components of haemoglobin.

Chandrasekhar (1959) studied the haemoglobin pattern of ten species of fish by using agar electrophoretic technique and found, that the species *Labeo rhoita*, *Cirrhina mirgala*, *Ohiocephalus striatus*, *Heteropenestis fossillis* showed only

one component of haemoglobin *Labeo calbasu*, *L. bata*, *Calta* and *Tilapia mossambica* had two components, whereas *Ophiocephalus striatus* showed three components.

Sindermann and Hony (1963) conducted electrophoretic analysis in agar gel for five species of clupeoid fish from the Western North Atlantic and found all of them separated into two components and their mobilities were similar except for one species of American shad. In spite of the extensive sampling they did not find individual variation in the number or mobilities of haemoglobin fractions.

A significant progress has occurred in this field since Smithies (1955) suggested zone electrophoresis in starch gel media, which has higher resolving power.

Odenice and Shinnars (1962) used this method to study cod muscle tissue. Sick (1963) used starch gel electrophoresis to study the possible genetic differences as denoted by haemoglobin patterns of American, European and Japanese eels (*Anguilla*). Each of the group was found to be monomorphic. The American and European eels showed two components with similar mobilities and could not be distinguished from each other, whereas the Japanese eel showed a different pattern which had three components.

The author in a set of 41 experiments used starch gel medium to examine electrophoretic components of haemoglobin and their comparative mobilities, to study differences between the species and individual variation within the species. The method used is essentially same as described by Smithies (1955). In the absence of the sophisticated apparatus manufactured by Buchler and Co. two simple glass plates with four supporting 6 mm thick strips of plexiglass were used. The uniform size of the gel; $230 \times 130 \times 6$ mm was assured by making suitable markings on the glass plates, Hydrolysed starch manufactured by Connaught Medical Research Laboratories was used. 12.5% Starch block concentration gave good resolution and the block could be handled with ease during the process of cutting and staining. The gel was prepared in borate buffer of $pH 8.65 \pm 0.30$. The haemoglobin samples were inserted on filter paper strips 6 mm wide of suitable lengths. When two superimposed strips were used to put the sample, the resulting bands were more dark.

A current of 250 volts and 50 amperes was applied at the room temperature and it was not possible to obtain more than three components. If higher voltage was applied the gel was heated and experiment could not be run for more than three hours. In a cold room at lower temperature, $2^{\circ}C$, it was possible to apply higher voltage without producing any adverse effects due to heat. This enabled application of a current of 250 volts 15 amperes for 24 hours. The haemoglobin of ocean white fish (*Caulolatilus princeps*) separated into eight components (Fig. 1). The duration of the experiment of 24 hours is very long. Therefore higher voltage was tried, a current of 500 volts at 30 amperes showed very efficient resolution without any endosmosis and the period of experiment was reduced to 3 hours 30 minutes. The same method was tried for other species sardine (*S. caerulea*) and higher resolution was obtained.

This method of starch gel electrophoresis at low temperatures is an efficient method, it enables high resolution in very short time and many samples can be examined simultaneously. It can be used for studying variations and differences between populations and is an easy tool which can be applied to the pelagic fish stocks of the Indian Ocean.



FIG. 1. Showing the Starch-gel Electrophoretic pattern of the haemoglobin of ocean white fish (*Caulolatilus princeps*)

ACKNOWLEDGEMENTS

I am grateful to Dr N. K. Panikkar, Director, National Institute of Oceanography, New Delhi, for valuable advice in the preparation of this paper and to Dr. M. B. Schaifer and Dr J. L. Kask, for kindly providing facilities for work at the Inter-American Tropical Tuna Commission, La Jolla, California.

REFERENCES

- Bhuler, D.R., and Shanks, W.E. (1959). *Science*, **129**, 899.
- Chandrasekhar, N. (1959). Multiple haemoglobins in fish. *Nature, Lond.*, **184**, 1652-1653.
- Clark, J.R., and Vladykov, V.D. (1960). Definition of Haddock stocks of the North West Atlantic. *Fishery Bull. Fish Wildl. Surv., U.S.*, **60**, 283-296.
- Dwivedi, S.N. (1964). Ecologie, morphologie et biologie comparées des deux espèces du genre *Lepidorhombus*, *L. megastoma* (Lionvan) et *L. boscii* (Risso). Etude de bur races et populations. *Revue Trav. Off. (scient. tech.) Pech. marit.*, **28**, 321-400.
- Fage, L. (1911). Recherches sur la biologie de l'anchois *Engraulis encrassicholus* LINNE, races, age et migrations. *Annls Inst. Oceanogr., Monaco*, **2**, 1-40.
- Furnestin, J. (1945). Contribution a l'étude de biologie de *Sardina pilchardus*, WALB. *Revue Trav. Off. (scient. Tech.) Pech. marit.*, **13**, 221-386.
- Gery, J. (1962). Le probleme de la sous-espece et sa definition statistique (a propo du coefficient de Mayr Linsley Usinger). *Vie Milieu*, **13**, 521-541.
- Heincke, C. (1898). Naturgeschichte des Herring. I. Die lokal formen und die wanderungen des Herring in den Euroasschen Muren. *Abh. dt. Seefischer.*, **2**, 128.
- Hubbs, C.L., and Hubbs, C. (1953). An improved graphical analysis and comparison of series of samples. *Stst. Zool.*, **2**, 49-57.
- Hubbs, C.L., and Perlmutter, A. (1942). Biometric comparison of several samples with particular reference to racial investigations. *Am. Nat.*, **76**, 582-592.
- Marr, J.C. (1955). The use of morphometric data in systematic racial and relative growth studies in Fishes. *Copeia*, No. 1, 23-31.
- Mayr, E. (1940). Speciation phenomenon in birds. *Am. Nat.*, **74**, 249-278.
- Mayr, Linsley, E.G., and Usinger, R.L. (1953). *Methods and Principles of Systematic Zoology*. McGraw-Hill Book Co., Inc, New York, 328 p.

- Odense, P. H., and Shinnars, C. W. (1962). Direct vertical starch gel electrophoresis of cod muscle tissue. *Can. J. Biochem. Physiol.*, **40**.
- Pillay, T. V. R., Pillay, S. R., Ghosh, K. K. (1963). A comparative study of the populations of Hilsa *Hilsa ilisha* (Ham) in Indian waters. *Proc. I. P. F. C.*, 10th session 10-25 Oct. 1962, Sec. II: 37-62.
- Poulik, M. D., and Smithies, O. (1958). *Biochem. J.*, **68**, 636.
- Rao, C. R. (1962). *Advanced Statistical Methods in Biometric Research*. London Weley Publications, 390 p.
- Reeves, F. C. R. (1940). Relative growth in the snout of ant eaters. A study in the application of quantitative methods in systematics. *Proc. zool. Soc. Lond.*, Ser A, **110**, 47-80.
- Royce, W. F. (1964). A morphometric study of yellowtail tuna *Thunnus albacare* (Bonnaterre). *Fishery Bull. Fish Wildl. Surv. U. S.*, **63**, 395-443.
- Rothschild, B. J. (1963). Graphic comparisons of meristic data. *Copeia*, No. 4, 601-603.
- Runnstrom, S. (1941). Racial analysis of herring in Norwegian waters. *Rapp. Norwegian Fish. Mar. Invest.*, **6**, 1-110.
- Sick, K. (1962). Haemoglobin pattern and chromosome number of American, European, and Japanese eels (*Anguilla*). *Nature, Lond.*, **193**, 1001-1002.
- Sindermann, C. J., and Honey, K.A. (1963). Electrophoretic analysis of the haemoglobin of Atlantic clupeoid fishes. *Copeia*, No. 3, 534-537.
- Smithies, O. (1955). *Biochem. J.*, **61**, 629.
- Sindermann, C. J. (1962). Use of plant hernaagglutinins in serological studies of clupeoid fishes. *Fishery Bull. Fish Wildl. Surv. U. S.*, **63** (1).
- Sindermann, C. J., and Maris, D. F. (1959). A major blood group system in Atlantic sea herring. *Copeia*, No. 3, 228-232.
- Sprague, L. M. (1961). Erythrocytes of the oceanic skipjack *Kastuwonus pelamis* recognised by phytoagglutinins. *Genetics*, **46**, 901.
- Vrooman, A. M. (1964). Serologically differentiated sub-populations of the Pacific sardine, *Sardinops caerulea*. *J. Fish. Res. Bd Can.*, **21**, 691-701.