POPULATION GENETIC STUDIES ON THE OIL SARDINE *(Sardinella longiceps)*

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN MARINE SCIENCE OF THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
KOCHI - 682 022

BY

MOHANDAS N. N.

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
P.B. No. 1603, KOCHI - 682 014
INDIA

JANUARY 1997

Dedicated
to
My Grandma

DECLARATION

I hereby declare that this thesis entitled **POPULATION GENETIC STUDIES ON THE OIL SARDINE** (Sardinella longiceps) has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

MOHANDAS N.N.

KOCHI-682014
JANUARY, 1997

CERTIFICATE

This is to certify that the thesis entitled **POPULATION GENETIC STUDIES ON THE OIL SARDINE** (Sardinella longiceps) is a bonafide record of the work carried out by Mr. **MOHANDAS N.N.** under my guidance and supervision and that no part thereof has been presented for the award of any other degree.

Dr. M.K.GEORGE, M.Sc., Ph.D.
Senior Scientist
Central Marine Fisheries
Research Institute
KOCHI - 14.

KOCHI-682014
DECEMBER 1996

ACKNOWLEDGEMENTS

I acknowledge my deep sense of gratitude to Dr. M.K. George, Senior Scientist for his guidance and supervising the work, without which this work would not have been possible. I would like to extend my sincere thanks to Dr. P.S.B.R. James, former Director of CMFRI, for kindly permitting me to carry out the work at CMFRI and to Cochin University of Science and Technology for granting me the Ph.D. registration.

I am grateful to Dr. George John, Director D.B.T., New Delhi for his guidance and encouragement while he was my first supervisor. I also thank Dr. A.G. Ponniah, Senior scientist, N.B.F.G.R., Lucknow for his valuable help and suggestions.

I must thank Dr. A. Noble, Dr. P.P. Pillai, scientists of CMFRI, for their regular advice and help during the course of my work.

I remain deeply indebted to Drs. Peer Mohammed, K.C. George, P.C. Thomas,. I.D. Gupta, N.K. Varma, Vijaya Gopal, and Suseelan, scientists of CMFRI for their encouragement and help during the investigation period.

I place on record my gratitude to Mr. E.V. Radhakrishnan, Dr. N. Neelakanta Pillai, Dr. K.K. Sukumaran, Dr. Kuriakose, Scientists, CMFRI for helping me during specimen collection.

I am indebted to Dr. G. Mukundan, former Director, CAS in Animal genetics, Dr. Ragunanandanan, both of veterinary college,

Kerala Agricultural University, Dr. P.U. Varghese, MPEDA, Cochin, Dr. N.R. Menon, Dean Faculty of Marine Science, Cochin University of Science and Technology and late Mr. Sadananda Rao for their valuable suggestions and help extended during the study period.

I express my deep sense of gratitude to Dr. Joshi K.K., scientist CMFRI and his family members for their valuable advice and constant encouragement from the very beginning of this research work.

I would like to show my sincere gratitude to Mr. Sathyanandan, scientist CMFRI for the computer software support required for statistical analysis of morphometric data.

I would like to thank Mr. K. Diwaker, Lab-Assistant for ICAR adhoc project and now technical assistant at CMFRI Mandapam for assisting me in laboratory work. Also express my sincere thanks to Mr. P. Raghavan, Mr. Nandakumar, Mr. Kurup, Mr. John, Mr. Davis and Mr. V. Chandrasekhar for their timely help.

I wish to thank all my colleagues and friends especially Dr. K.K. Vijayan, Dr. C.P. Balasubramanyam, Mr. Reji Mathew, Mr. Rexi Rodrigus, Dr. Paramananda Das, Mr. Dinesh babu, Mr. Tomy Prince, Dr. S. Vijaya Kumar, Dr. Venkitakrishnan, Dr. Gopalakrishnan, Mr. Madhu, K., Mr. Sini Joys Mathew, Mrs. Kalpana Deepak, Miss Bindu Paul, Mr. Paulton M.P., Miss V. Sapna, Mr. V.R. Unnikrishnan, Mr. P.N. Asokhan, Mr. Sathya Reddy, Mr. Sathya Narayana Reddy and Mr. S.K. Kareem for their ready and untiring help.

Above all I am very much indebted to my employer C.P. Aquaculture (India) Pvt. Ltd. and its staff members namely Dr. Pinij K., Mr. Paibool I., Mr. Pairach, S., Mr. Aekapan R., Mr. Chuchai K., Mr. Boonyarit Y., Mr. Udomsak, A. and Mr. Ganesh A. for their encouragement and help.

I also thank Mrs. Aisha Suresh, Coastal Impex for typing the thesis.

I also thank ICAR for awarding me the research fellowship under the ICAR Ad-hoc scheme, "Population genetic studies on oil sardine, Sardinella longiceps to identify distinct genetic stocks".

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

The Indian oil sardine, Sardinella longiceps, is a major commercial fishery of India. The fishery presently exploited is composed of populations drawn mainly from Mangalore/Karwar, Calicut, Cochin and Quilon from the west coast. It is also caught from Mandapam and Madras on the east coast.

The oil sardine fishery is exploited and managed as unit stock. In other words, it is assumed that the fishery is supported by interbreeding populations. On the contrary, the preliminary morphomeristic studies of its sample populations had revealed that the fishery may be composed of two or more heterogeneous populations (Devanesan and Chidambaram, 1943; Prabhu and Dhulkhed 1972; Antony Raja, 1973). On the other hand, it is well known that a thorough knowledge on the population genetic structure of the fishery is essential for scientific exploitation and conservation of any fishery resources. Besides, a recurring problem inherent with the oil sardine fishery of India is the short and long term fluctuations experienced in its annual abundance. The probable causes of the problem remain undetected and unexplained inspite of exhaustive information available on its biology and fishery (Anon, 1979).

An important question associated with the above problems of oil sardine fishery is that whether the fishery is composed of geographically/genetically isolated heterogeneous populations. The only investigation that has attempted to study the population genetics of the species was that of Venkitakrishnan (1995). The

biochemical genetic analysis of the polymorphic enzyme in the above investigation revealed that populations of S. longiceps are heterogeneous in nature.

The objective of present investigation was to study the population genetic structure of S. longiceps by applying three different basic population genetic techniques such as cyto-genetics, non-enzymatic biochemicalgenetics (general protein) and morphomeristics/metrics. The reasoning behind choosing these three basic methods may be explained as follows. Under the concept of evolution, every species believed to be undergoing micro and macro evolutionary process, resulting in the expression of significant genetic variations at levels of species specific chromosome morphology/structure, gene controlled protein structure and polygene controlled morphomeristics and metrics (Ayala and Kiger, 1980).

Naturally, the best materials and methods to study the genetic variability in the oil sardine Sardinella longiceps are its chromosomes, proteins and morphomeristics/metrics present in sample populations of the species.

The findings of the present investigation are presented in three separate chapters Cytogenetics, Biochemical genetics (only general proteins) and Morphometrics; starting with a review of literature appropriate to each subject matter.

2. CHAPTER - I

CYTOGENETICS

2.1 REVIEW OF LITERATURE

Every species has its own cytogenetic identity described as its diploid chromosome number (2n) and species specific chromosome morphology/structure. The current status of fish cytogenetics, in terms of techniques, fish karyology and evolution of fish karyotypes was given by Rishi (1989). Fish cytogenetics related to taxonomy and evolution was reviewed by Manna (1989). A vital part of the cytogenetic studies is the standardisation of the procedures for the preparation of the karyotype of the species. A number of known techniques have been applied and found successful in many fish species. A practical problem in chromosome preparation of teleost fishes is large number of chromosome and their small size compared to that of other vertebrates (Gold, 1979).

Most of the fish cytologists follow the procedures involving preparation of mitotic chromosomes from actively dividing somatic tissues of live specimens or from embryos. The soft organs such as kidney, spleen and liver have proved to be good source of chromosomes (Davisson et al., 1972; Gold, 1974). The earlier work of Tjio and Levan (1956) revolutionised cytogenetic studies. In 1960, Wolf et al., worked on cell culture methodology reported by mammalian cytologist Nelson-Rees et al. (1967). Wolf and Quimbly (1969) developed an innovative method using cells cultured from suitable tissues of fresh water and marine fishes.

The most advanced technique for obtaining chromosomes from the fish is from cultured leucocytes. A series of papers by Labat et al., (1967); Ojima et al., (1970) in carp and gold fish; Heckman and Brubaker (1970) and Heckman et al., (1971) in gold fish and trout; Kang and Park (1975) in Anguilla anguilla; and Thorgaard (1976) in the rainbow trout have shown the advantages of the leucocytes method. Another method employed successfully is invitro cell culture. The review of this method was made by Roberts (1964); Ojima and Hitotsumachi (1967); Gold (1979); Ojima (1982); Blaxhall (1983); Hartley and Horne (1983).

In fish chromosome preparation studies, a popular procedure followed is the method of giving colchicine injections to the fishes and take squashes of the suitable tissue such as testes (Roberts, 1964; Ohno et al., 1965), kidney (Catton, 1951), corneal and conjunctive epithelium (Drewry 1964; Sick et al., 1962) gill epithelium (McPhail and Jones, 1966; Lieppman and Hubbs, 1969), embryological material like, blastula of early embryo (Swarup, 1959; Simon and Dollar 1963), sectioning of testes (Nogusa, 1960) growing various tissue invitro (Roberts, 1964; 1966; 1967.) Eventhough, several methods have been put forward to obtain chromosomes from different tissues, the direct or invivo method has been found to give good result (McPhail and Jones 1966; Stewart and Lewin, 1968; Denton and Howell, 1969; Gold, 1974; Kligerman and Bloom, 1977; Chourrout and Happe, 1986; Reddy and John, 1986; Cucchi and Baruffaldi, 1989, 1990; Gold et al., 1990).

The method described by Kligerman and Bloom (1977) for obtaining well spread metaphase from solid tissues of fishes was reported to be superior to other methodologies, because it produced high quality metaphases that can be located easily. Earlier work on chromosome preparation for karyotyping by the use of peripheral blood culture has been done by number of workers (Ojima et al., 1970; Yamamota and Ojima, 1973; Legrande, 1975). A culture technique described by Blaxhall (1983), using separated peripheral blood lymphocytes from fish, yields well spread chromosomes for karyotyping and banding techniques. The method of chromosome preparation from lymphocyte culture of 30 Atlantic salmon was studied by Hartley and Horne (1984).

A recent invivo type methodology of chromosome preparation, using phenylhydrazine and cobalt chloride were employed, (Cucchi and Baruffaldi, 1989). Fan and Fox (1990) developed a method for the preparation of fish chromosomes from abdominal cavity fluid cells.

The characteristics of fish karyotypes are often used for taxonomic differentiation of species. Generally it has been considered that karyotypes had undergone specific patterns of rearrangements within different evolutionary lineages (White, 1973). Hence, species specific karyotypic differences between species are useful in systematic studies. For example, most authors classified salmonidae species on the basis of one armed and two armed chromosomes according to the guidelines of Levan and Sandberg (1964). This species also showed intraspecies

(Roberts, 1968; Grammeltvedt, 1974; Barshene 1978) and even intra-individual (Barshene, 1981; Hartley and Horne, 1984) chromosome polymorphisms. Such a polymorphism is due to Robertsonian translocations (Hartley and Horne, 1984,b). Though, the number of chromosomes ranged from 54 to 60, the number of arms (NF) was generally 72 (Boothroyd, 1959, and Roberts, 1968; 1970).

The rainbow trout (Salmo gairdneri) is the most extensively studied fish for cytogenetics and it showed a great deal of chromosome polymorphism of the Robertsonian type at both inter and intrapopulations (Thorgaard, 1976; 1983; Hartley and Horne 1982; Ueda et al., 1983). Chromosomal number and polymorphism present in rainbow trout, Atlantic salmon and brown trout were well described by Hartley and Horne (1984). In many salmonid species, the chromosomes which will undergo polymorphism due to Robertsonian translocation have the common number of arm (NF) (Allendorf and Thorgaard, 1984; Hartley, 1987). The Q-band chromosomal polymorphism (Phillips and Zajicek, 1982) and chromomycin A_3 chromosomal polymorphism (Phillips and Ihssen, 1985) were reported in lake trout and also demonstrated that those polymorphism are heritable (Phillips and Ihssen, 1986; Phillips et al., 1989).

Gold and Avise (1977) studied the karyotype of nine genera of North American minnows (Cyprinidae). Later works (Gold and Avise, 1977; 1984; Gold and Amemiya 1986) have focussed on karyotypic differentiation among North American cyprinid fishes.

Amemiya and Gold (1988) examined variations of chromosomal NORs among North American cyprinid species. Cataudella et al., (1987) reported results of cytogenetic studies of six different stocks of the common carp, Cyprinis carpio, from natural and artificial environments in Italy. He studied karyotypes from somatic cells and cultured blood cells, using G-banding, C-banding and NOR of the Cyprinus carpio. The differences in chromosome arm number have been found between fishes of the family Salmonidae from Europe and North America.

Karyological studies on nine genera of North American minnows (Cyprinidae) by Gold and Avise (1977) revealed that all had the diploid chromosome number, 50. The halploid (n) karyotype of 24 acrocentric chromosome was found throughout several diverse orders of the sub-class Teleostei and appeared to be the predominant karyotype in recently evolved perciformes (Roberts, 1964; 1967; Denton, 1973). This led to the hypothesis that the 24 acrocentric chromosome complement may be ancestral to all modern fishes (Ohno, 1974). The chromosome numbers varied from 58 to 64 among rainbow trout sampled from 29 locations ranging from Alaska to California (Thorgaard, 1983). The salmoniform species were found to have higher chromosome number (n=36) than cypriniformi species (n=25), Simon (1963) found that the diploid chromosome number in five species of Onchorhynchus ranged from 52 to 74 and the arm number from 102 to 112. Perhaps the karyotypically more variable taxa is the genus Salmo. Its chromosome numbers (2n) ranged from 54 to 80 and arm number ranged from 72 to 102 (Svardson, 1945; Wright, 1955; Rees, 1957;

Simon and Dollor, 1963; Roberts, 1967; 1968; 1970; Nygren et al., 1968; 1972; Gold and Gall, 1975; Hartley and Horne, 1984b), whereas,the North American region had NF = 72 (Boothroyd, 1959; Roberts, 1968; 1970). Two species of the same genus can have identical chromosome number (2n) like Anguilla anguilla; 38 and Anguilla rostrata, 38 (Sola et al., 1984); Leporinus elongatus; 54, L. locustris; 54, L. striatus; 54 (Galetti et al., 1984) or very different numbers Salmo salar; 56 and Salmo trutta 80 (Phillips and Ihssen,1985).

Karyomorphology of more than 125 fishes of India has been reported (Rishi, 1989). Most of the work on the chromosome of teleost species has been reported in a series of papers by Natarajan (1969; 1970); Subrahmanyam and Ramamurthy (1971). Chatterjee and Mahjhi (1973) showed that both sexes of Mugil parsia possess 48 acrocentric diploid chromosomes and without distinguishable sex chromosomes. Rishi (1973) investigated eighteen marine teleosts belonging to fifteen diverse families by using cytological methods. Natarajan and Subrahmanyam, (1974) studied on the karyotype of 16 teleost species belonging to 15 families and 7 orders such as anguilliformes, cypriniformes, siluriformes, synbranchiformes, scorpeaniformes, perciformes and tetroadontiformes. The somatic chromosomes of both sexes and meiotic stages of the female fish Trichogaster faciatus were described by Rishi (1975).

Based on morphometric data of the metaphase chromosomes of the kidney, Khuda-Bukhsh (1975) determined the diploid number of both sexes of Puntis japonicus. Rishi (1976) described the mitotic chromosomes of both sexes of Callichrom bimaculatus. Khuda-Bukhsh and Manna (1977) carried out studies on somatic and germinal chromosomes of aquarium fish Mollinesia latipinna. Giemsa banding in fish chromosome have been done by Rishi (1979). Karyomorphological analysis of somatic chromosomes of 3 female species Mystus gulio, Eutropichithys vacha and Mastacembelus armatus were carried out by Manna and Khuda-Bukhsh (1978). Chromosomal homogenity of the cat fishes Heteropneustes fossilis and Clarias batrachus were reported by Rishi (1978). Rishi and Jaswant Singh (1982) studied karyotypic data on five estuarine fishes, Etroplus suratensis, Glossogobius giuris, Mugil peigleri, Tricanthus brevirstris and Strongglura strongglura. Das (1983) reviewed the status of cytogenetic studies in marine fishes from India. Out of 1400 species listed, the diploid number of chromosomes range from 16 to 239. The modal number (2n=48) was observed in 460 species. While diploid number of 46 was next in frequency in about 225 species. About 140 species had the diploid number of 50. The work on chromosomal evolution in Indian murrels belonging to the genus Channa (Dhar and Chatterjee, 1984) indicated the two chromosomal variety of Channa punctata with 2n number as 34 and 32.

Khuda-Bukhsh and Barath (1987) and Manna and Khuda-Bukhsh (1977) showed that Cyprinus carpio and Labeo calbasu had distinct diploid chromosome number of 100 and 50 respectively. Hybrid

individuals of Cyprinus carpio V- Labeo calbasu contained 100 chromosomes. Karyotypic analysis of two Indian air breathing fishes Channa punctatus and Heteropneustes fossilis revealed that the diploid chromosome number was 32 and 56 respectively (Zhang, 1990). Studies on two Indian marine species, Otolithus cuvieri and Nibea diacanthus revealed a diploid count of 48 acrocentric chromosome in both species (Chakraborthy and Kagwade, 1989).

Several procedures are available for chromosome preparation from live somatic cells of different tissues or from cultured cells. The range of species specific chromosome number vary extensively from 16-239. There is a remarkable variations of chromosomal number at intra and inter-species levels. Majority of the fish species have 48 (2n) chromosomes and the reason of which remains as a debatable issue. The chromosome number between populations may also vary indicating some form of genetic heterogenity within the species.

2.2. MATERIALS AND METHODS

2.2.1. MATERIALS

2.2..1.1. Source of Experimental animal

The Oil sardine, Sardinella longiceps were collected during 1989-91 from Cochin, Calicut, Mangalore (West coast) and Mandapam (East coast). Live specimens were caught by both mechanised and non-mechanised vessels from different stations. The gear types used by non-mechanised vessels were gill nets, thanguvala and shore seine; and trawl nets by mechanised vessels. Samples from Cochin were obtained by mid-water trawling of "Cadalmin" research vessel of CMFRI and that of local commercial trawlers. Specimens collected at Mandapam was captured by shore seine.

2.2.2. METHODS

Live specimens collected from Calicut, Mangalore and Mandapam were brought to the local laboratory of CMFRI where it was treated with colchicine. The treated and fixed tissues were removed and placed in cold conditions. Then these tissues were brought to the CMFRI laboratory at Cochin, where it was processed for preparing metaphase plates.

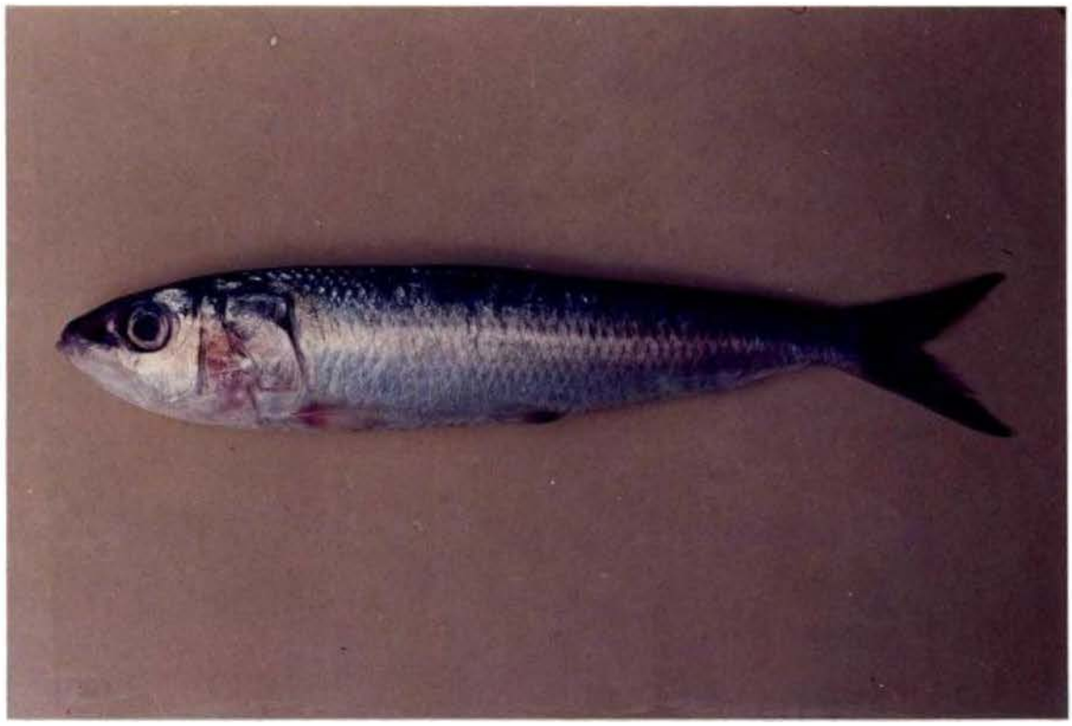
Live specimens collected from Cochin area were brought to laboratory at Cochin and kept alive until treated with Colchicine. Then the desired tissue was removed for preparing metaphase plates. All the tissues were properly labelled and stored for analysis.

PLATE 1 a. Test animal (*Sardinella longiceps*)

b. Test animal (*Sardinella longiceps*) in the laboratory condition

PLATE-1

A



B

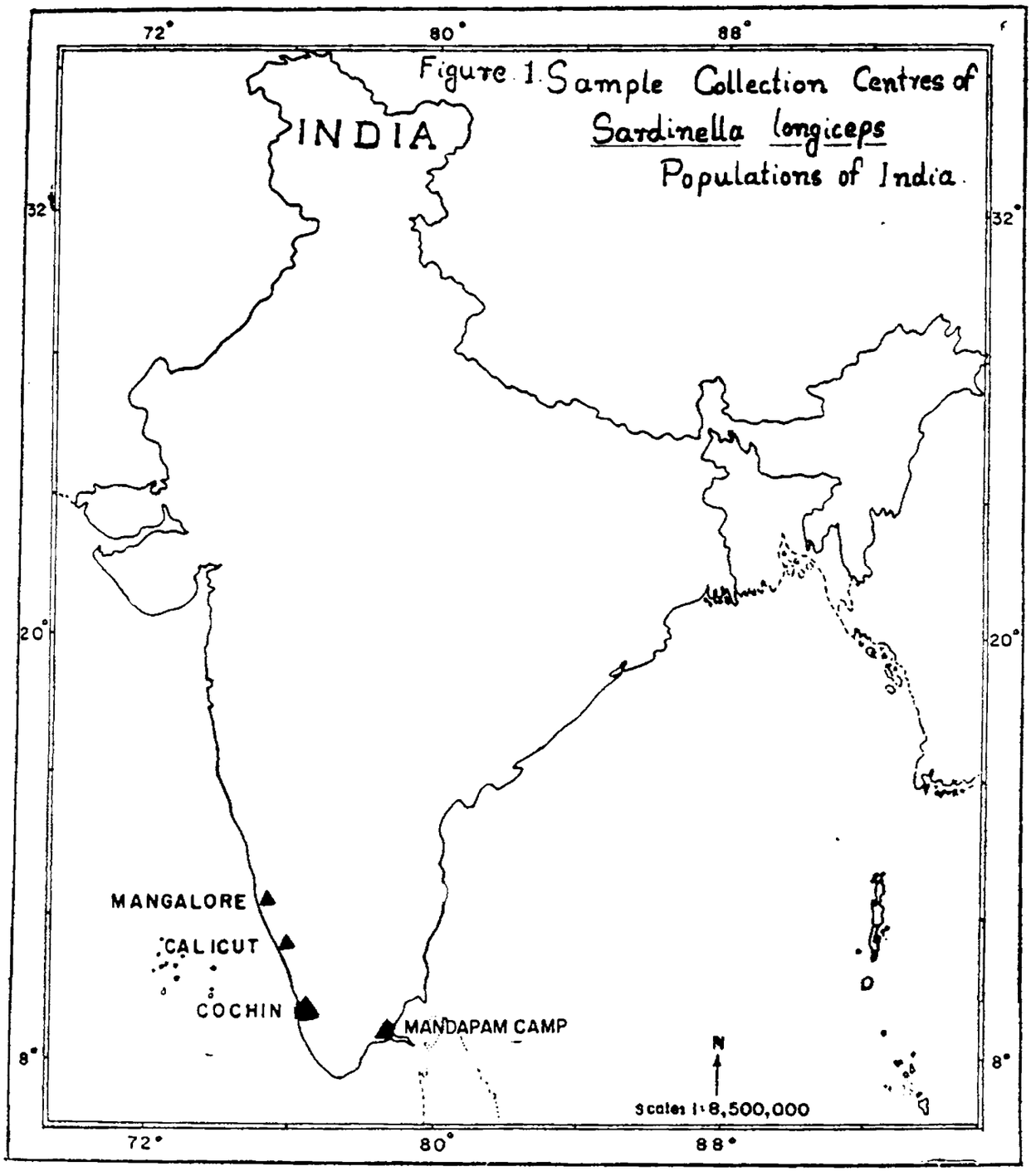


Figure 1. Sample Collection Centres of *Sardinella longiceps* Populations of India.

The process of analysis has been worked out on the basis of following aspects (a) Pretreatment. (b) Colchicine treatment and slide preparation c) Karyotype preparation.

2.2.2.1. **Pretreatment:**

The live specimens collected at Cochin, Calicut, Mangalore and Mandapam were brought to the local laboratories of CMFRI where the sample were kept alive before chemical treatment.

2.2.2.2. **Colchicine treatment and slide preparation**

For the standardisation of chromosome preparation methodology, different known methods of cytogenetic studies were applied. Modifications were also made in order to suit the test species. The following methods were tested for standardisation of methodology.

1. Denton and Howell (1969)
2. McPhail and Jones (1966)
3. Reddy and John (1986)
4. Le Grande and Fitzsimons (1976)
5. Chourrout and Happe (1986)
6. Kligerman and Bloom (1977)

1. Denton and Howell (1969):

Oil sardine of 5 cm. size was allowed to swim in a well aerated beaker containing colchicine solution (0.01%) for 3 hours. After 3 hours the animal was sacrificed and the gills and kidney tissues were dissected out. The tissues were treated with

hypotonic solution of 0.3% KCl (dissolved 300 mg. of KCl in 100 ml distilled water) for 20 minutes. The cell suspension obtained was then centrifuged at 2000 rpm for about 5 minutes. The supernatant was discarded, fresh fixative (methanol, glacial acetic acid, 3:1 ratio) was added and the material kept in refrigerator.

Before dropping the cell suspension on the slides,they were removed from the refrigerator and allowed to reach at room temperature. Suspensions were dropped from a height of 15 cm. on to the slides which is chilled in 50% alcohol and ignited. The slides were stained with Giemsa solution for 25 minutes. After staining, the slides were rinsed in distilled water and dried. The dried slides were stored in slide boxes for further microscopic examination.

2. Mc Phail and Jones (1966):

The fish was given a 0.005% colchicine 1 ml/100 gram body weight. This solution was injected into the anterior dorsal musculature and allowed to reside in well aerated tank for 2 hours. Then the gill and kidney tissues were removed from the sacrified fish. The tissues were hypotonised in 0.4% KCl at room temperature for 30 minutes and stained in 2% Giemsa stain for 20 minutes. The stained tissue were shaken lightly on a clean slide until a light slurry of cells was deposited on it. Large pieces of tissue were removed. The slurry was immediately covered with a clean cover glass and squashed manually using a rubber stopper.

3. Reddy and John (1986):

The laboratory reared fishes were injected intramuscularly with 0.005% colchicine (1ml/100 gm body weight) and kept in well aerated tank for 3 hours. The specimens were sacrificed and the gill and kidney tissues dissected out. After cleaning the tissues, it was transferred to 1% sodium citrate solution and cut into small pieces. Incubation was done at room temperature for 30 minutes and then transferred to a glass tissue homogeniser and gently agitated. After removing the large tissue particles, the cell suspension was centrifugated for 5 minutes at 2000 rpm. The supernatant liquid was decanted. About 4 ml of fixative (methanol; acetic acid, 3:1 ratio) was mixed to the material and allowed to stand for 20 minutes. The material was again centrifugated before giving the change of fixative and kept under refrigeration over night. The slides were prepared as in method 2.

4. LeGrande ad Fitzsimons (1976):

Collected oil sardines were given an intramuscular injection of 0.005% colchicine (1 ml/100 gm of body weight). After 3 hours the fishes were sacrificed then kidney and gill tissues were dissected out. The tissue was minced in 2-3 ml of 1.0% sodium citrate solution at room temperature and allowed to stand for 30 minutes. After citrate treatment, the suspension was centrifuged for 5-7 minutes at about 2000 rpm. The supernatant was decanted, and cell button fixed with absolute methanol; glacial acetic acid (3:1) solution. After three changes in fixative, they were stored in the refrigerator till the spreads were made. Before

dropping the cell suspension on the slides, they were removed from the refrigerator and allowed to reach the room temperature. Suspension were dropped from a height of 15 cms, on to the slides stored in chilled 50% alcohol and air dried. The slides were stained in a Giemsa solution for 25 minutes.

5. Chourrout and Happe (1986):

The fishes were injected 0.005% colchicine into dorsal muscle. The kidney and gill tissues were dissected out from specimens after 3 hours of colchicine injection. Each tissue was transferred to 2 ml of 0.4% KCl solution for 30 minutes at room temperature. The tissue suspension was centrifugated at 2000 rpm, for about 7 minutes. The supernatant was decanted and the fixative (methanol; acetic acid, 3:1) added to the residue, resuspended and kept for 25 minutes at about 700 rpm. The supernatant was poured off and fresh fixative added. The mixed material was stored in refrigerator. Before dropping the cell suspension on the slides, they were removed from the refrigerator and allowed to reach room temperature. Suspensions were dropped from a height of 15 cm on to the slide stored in chilled 50% alcohol and air dried. The slides were stained in a Giemsa working solution for 25 minutes.

6. Kligerman and Bloom (1977):

The fishes were allowed to reside in a well-aerated tank after an intramuscular injection of 0.001% of colchicine (1 ml/100 gm body weight of fish). After 3 hours the fishes were

sacrificed by pithing and the kidney and gill tissues were dissected out. Individual tissue were transferred to 10 times of their volume of 1% sodium citrate or 0.4% KCl, hypotonic solution for 30 minutes. The blood vessels, mucus, and other impurities were removed. The tissues fixed in methanol glacial aceticacid 3:1 solution by slowly adding the fixative drop by drop. Two changes of fixative was done. The tissues were kept in a refrigerator. After 1 hour the fixative was again changed. For preparing slides, a few pieces of tissue were removed from the fixative and touched to a piece of filter paper to remove excess fixative. The tissue was then placed in an embryo cup and 5-8 drops of 50% acetic acid was added to it. The tissue was minced gently for about 1 minute to form a cell suspension. This was dropped on to clean, grease-free slide, warmed between 40^{o}C and 50^{o}C, using a pasteur pipette. Drop the suspension from a height of about 8-15 cm and immediately after dropping, it was withdrawn back into the pipette, leaving a ring of cells approximately 1 cm dia, on the slide. Care was taken in applying the cells as too many cells per ring will impede metaphase spread resolution. Two to three rings were made on one slide. The slides were air dried and stained in 2% Giemsa stain for 25-30 minutes. The fresh slides were observed under microscope. Mounting of the slides were done in DPX.

2.2.2.3. <u>Karyotype preparation</u>:

Metaphase plates of well spread chromosomes with distinct morphology were used for karyotyping. Since the prints meant for

METHODOLOGY ADOPTED :

The fishes were allowed to reside in a well aerated tank after an intramascular injection of 0.1% colchicine(1ml/100 gram body weight) and kept alive in a well aerated tank for 2 to 2.5 hours. After this the fishes were sacrificed by pithing and the kidney and gill tissues were dissected out. Individual tissue were transferred to 10 times of their volume of 0.4% KCl, hypotonic solution for 30 to 40 minutes at low temparature. The blood vessels, mucus and other impurities were removed. The tissues then fixed in methanol glacial acetic acid 3:1 solution by slowly adding the fixative drop by drop. Two changes of fixative was done. The tissues were kept in a refrigerator. After 1 hour the fixative was again changed. For preparing slides, few pieces of tissue were removed from the fixative and touched to a piece of filter paper to remove excess fixative. The tissue was then placed in an embryo cup and 5-8 drops of 50% actic acid was added to it. The tissue was minced gently for about 1 minute to form a cell suspension. This was dropped on to clean grease-free slide, warmed between 40°C and 50°C using a pasteur pipette. Drop the suspension from a height of about 8-15 cms and immediately after dropping, it was withdrawn back into the pipette, leaving a ring of cells approximately 1 cm dia on the slide. Care was taken in applying the cells as too many cells per ring will impede metaphase spread resolution. Two to three rings were made on one slide. The slides were air dried and stained in 2% Giemsa stain for 25-30 minutes. The fresh slides were observed under microscope. Mounting of the slides were done in DPX.

karyotyping should be as large as possible without loss of definition, prints with good magnification were used for the study. The individual chromosomes were cut out from a photographic print with good contrast. The homologous pairs were arranged to a hard white paper according to the morphology and total length.

2.3. RESULTS

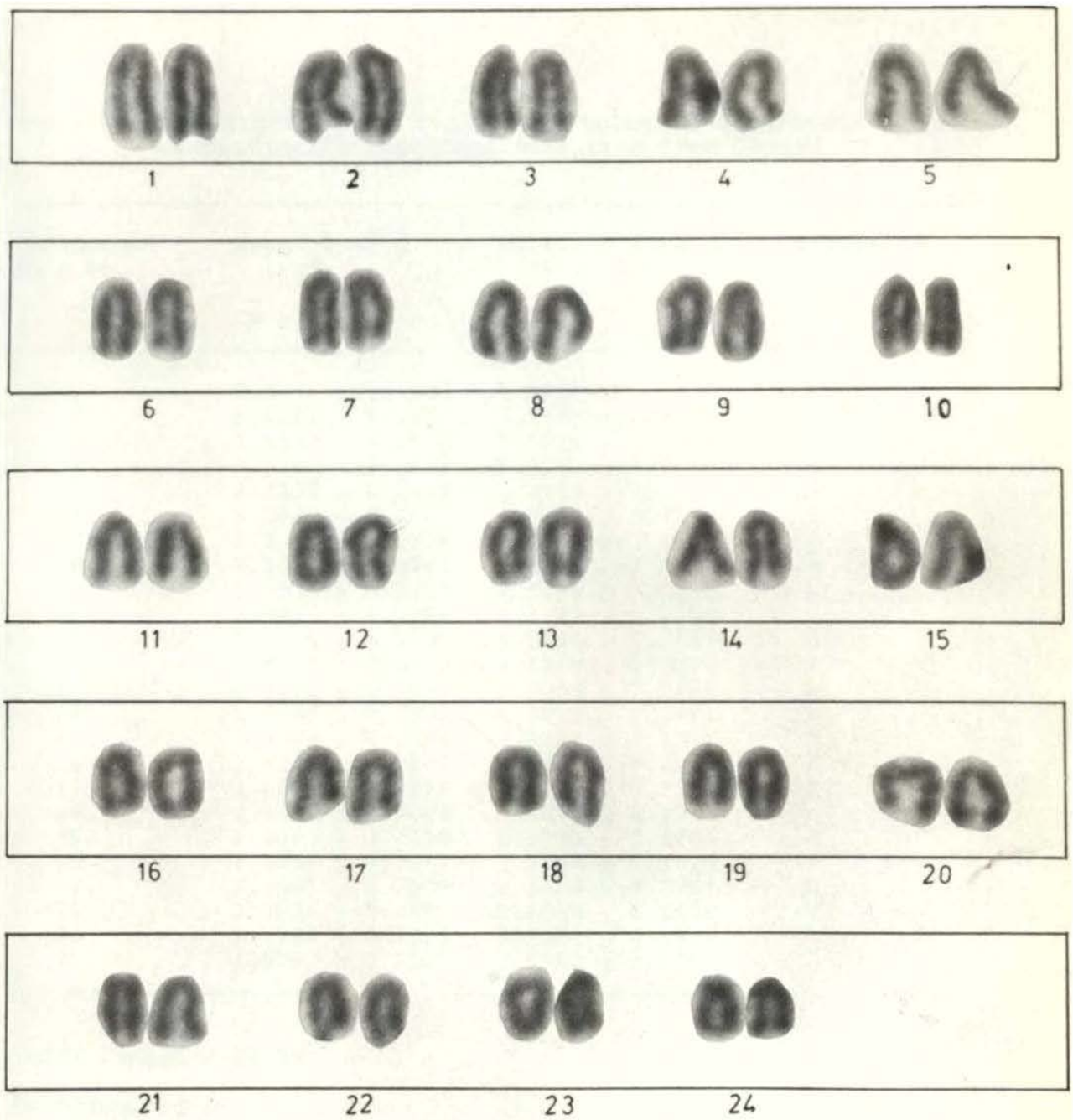
A total number of 415, oil sardine specimens collected from 4 centres like Cochin (114), Calicut (101), Mangalore (100) and Mandapam (100) were analysed by cytogenetic techniques. A total of 1660 metaphase plates were prepared. Karyotype of the species obtained from the four centres are shown in Plates No.2 (Cochin), Plate No.3 (Calicut) Plate No. 4 (Mangalore) and Plate No.5 (Mandapam). The modal diploid chromosome number (2n) of the species was 48 and it was observed in 74.79% metaphase from Cochin; 78.61% Calicut, 79.15% from Mangalore and 77.31% from Mandapam (Figure 2 & 3). All chromosomes were acrocentric in shape with (2n) number 48 and an NF value of 48.

The total length of the chromosomes of the species samples from Cochin, Calicut, Mangalore and Mandapam were 68.39 μm (Table 1); 61.41 μm (Table 2); 68.54 μm (Table 3) and 61.36 μm (Table 4) respectively. The 24th and Ist pairs of chromosome of the species were having the minimum and maximum length respectively in all the four centres (Table 1-4). The minimum - maximum length of paired chromosomes in Cochin, Calicut,

Mangalore and Mandapam varied from 1.9762 μm to 3.8171 μm (Table 1); 1.7432 μm to 3.6687 μm (Table 2), 1.9632 μm to 3.8270 μm (Table 3), 1.7398 μm to 3.6904 μm (Table 4) respectively. Again, the minimum - maximum (24th pair - Ist pair) relative length of paired chromosome for Cochin, Calicut, Mangalore and Mandapam varied from 2.8892 to 5.5806 (Table 1), 2.8385 to 5.9738 (Table 2), 2.8640 to 5.583 (Table 3), 2.8349 to 6.0133 (Table 4) respectively. A close comparison of chromosome length characteristic values between regions showed very interesting, apparently two distinct groups. The total length, minimum-maximum range of length and minimum-maximum range of relative length were closely comparable between Cochin and Mangalore (Ist distinct group) and these values between Calicut and Mandapam (2nd distinct group).

PLATE 2. Karyotype of *Sardinella longiceps* collected from Cochin

PLATE 2. KARYOTYPE OF FISH *SARDINELLA LONGICEPS*



2n : 48
NF : 48
COCHIN

10 /u

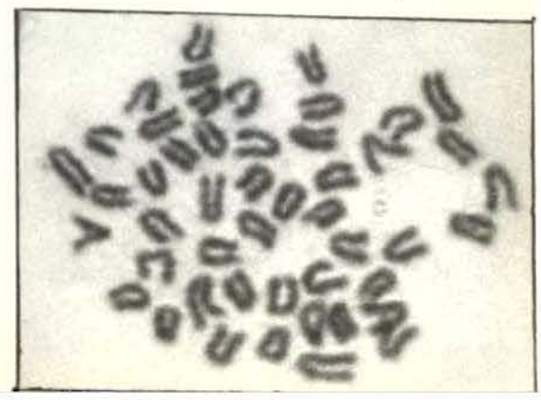


Table 1. **Total chromosome length and relative chromosome length of *Sardinella longiceps* collected from Cochin.**

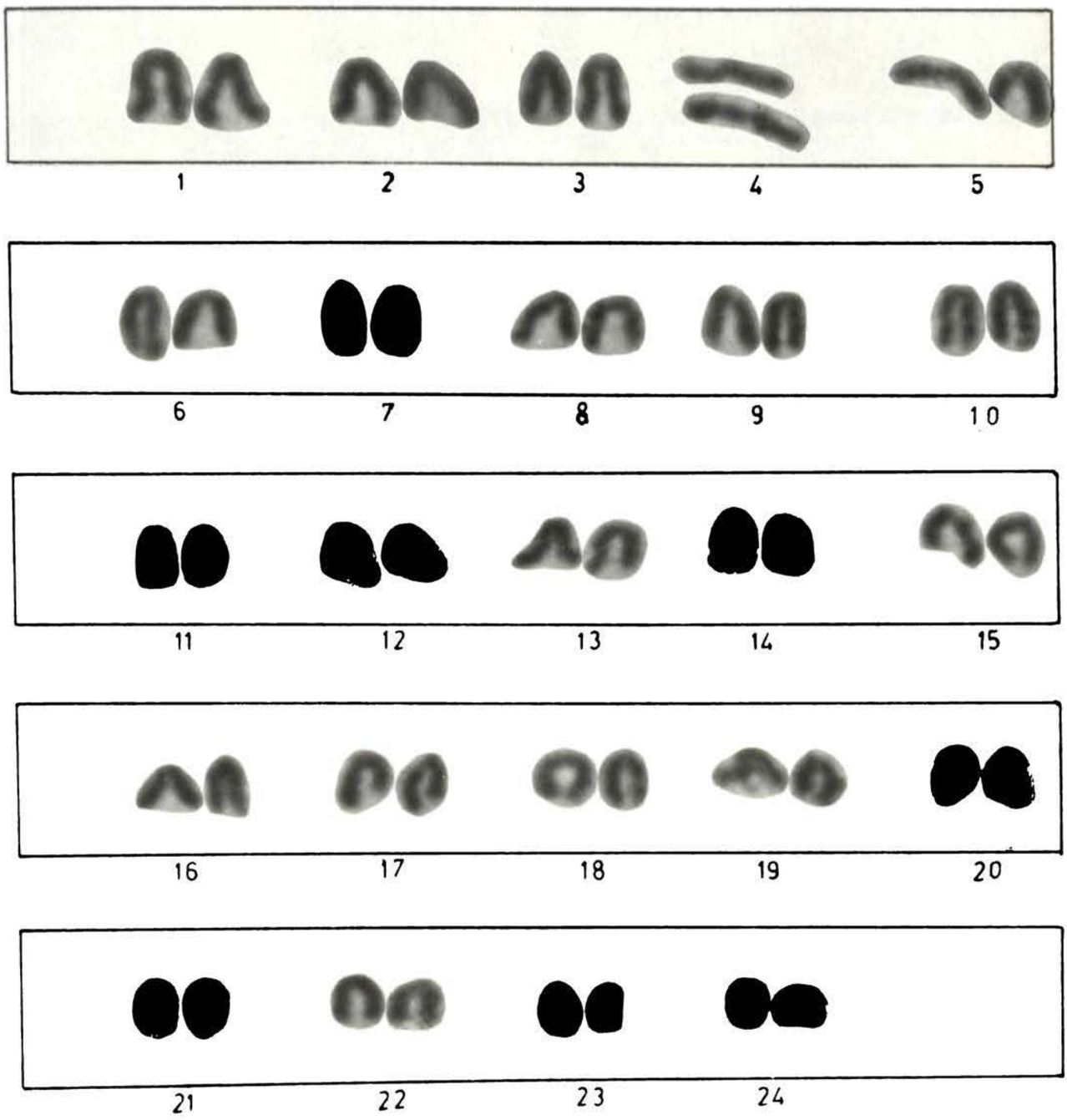
Chromosome Pair No.	Total length (um ±) ($\bar{x}$ ± S.D.)	Relative length (% ± S.D.)	Chromosome type
1.	3.8171 ± 0.1538	5.5806 ± 0.1230	A*
2.	3.6551 ± 0.1820	5.3438 ± 0.1759	A
3.	3.4904 ± 0.2535	5.1030 ± 0.2049	A
4.	3.3740 ± 0.2900	4.9328 ± 0.2589	A
5.	3.3280 ± 0.2424	4.8655 ± 0.2318	A
6.	3.2646 ± 0.2867	4.7729 ± 0.2550	A
7.	3.2136 ± 0.2774	4.6983 ± 0.2638	A
8.	3.1027 ± 0.3201	4.5362 ± 0.3087	A
9.	3.0916 ± 0.3123	4.5199 ± 0.3120	A
10.	2.9923 ± 0.2484	4.3747 ± 0.2081	A
11.	2.9298 ± 0.2961	4.2834 ± 0.2817	A
12.	2.8288 ± 0.3190	4.1357 ± 0.3085	A
13.	2.7554 ± 0.3072	4.0284 ± 0.2586	A
14.	2.7236 ± 0.2861	4.9819 ± 0.2751	A
15.	2.7004 ± 0.2584	3.9480 ± 0.2341	A
16.	2.6324 ± 0.2529	3.8486 ± 0.2067	A
17.	2.6079 ± 0.2559	3.8127 ± 0.2141	A
18.	2.4336 ± 0.2068	3.5579 ± 0.2216	A
19.	2.4303 ± 0.2410	3.5531 ± 0.2385	A
20.	2.4014 ± 0.2263	3.5108 ± 0.1816	A
21.	2.3689 ± 0.2009	3.4626 ± 0.1764	A
22.	2.1584 ± 0.1803	3.1556 ± 0.1586	A
23.	2.1226 ± 0.2085	3.1032 ± 0.1837	A
24.	1.9762 ± 0.1907	2.8892 ± 0.1561	A

Total length = 68.39

*Acrocentric

PLATE 3. Karyotype of *Sardinella longiceps* collected from Calicut

PLATE 3. KARYOTYPE OF FISH *SARDINELLA LONGICEPS*



2n : 48
NF : 48
CALICUT

10 µ

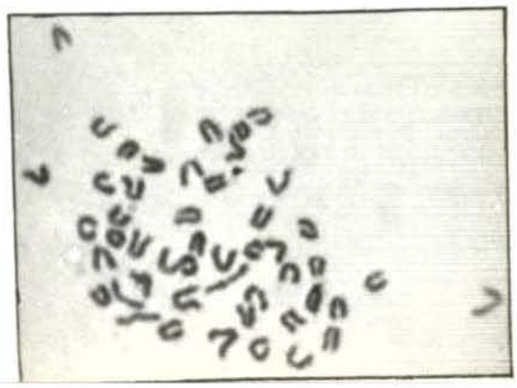


Table 2. **Total chromosome length and relative chromosome length of *Sardinella longiceps* collected from Calicut.**

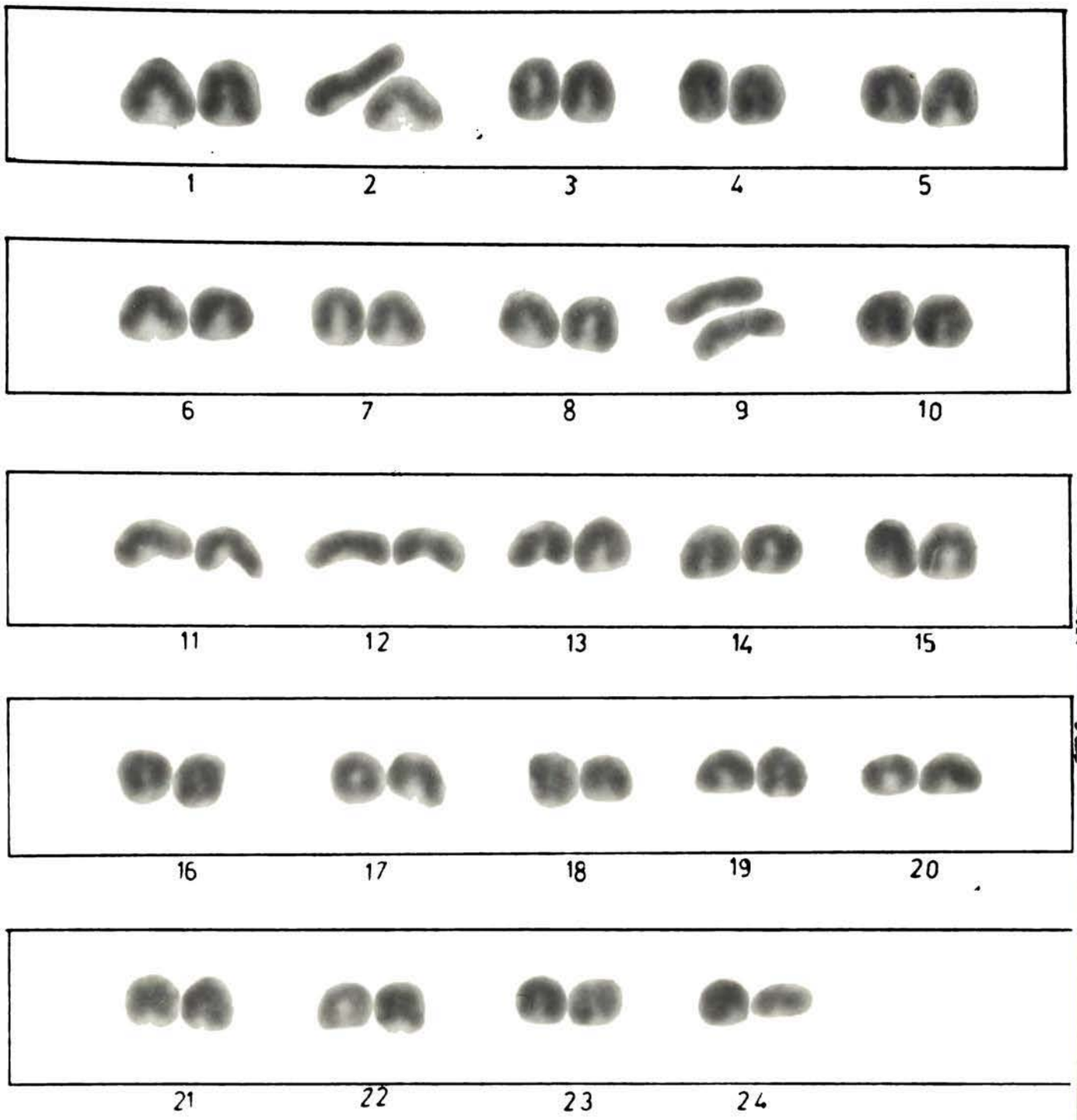
Chromosome Pair No.	Total length (um ±) ($\bar{x}$ ± S.D.)	Relative length (%) ($\bar{x}$ ± S.D.)	Chromosome type
1.	3.6687 ± 0.4221	5.9738 ± 0.4028	A*
2.	3.5334 ± 0.6214	5.7535 ± 0.5821	A
3.	3.3538 ± 0.4748	5.4611 ± 0.4480	A
4.	3.2979 ± 0.4117	5.3700 ± 0.3925	A
5.	3.1309 ± 0.4098	5.0981 ± 0.3824	A
6.	3.0554 ± 0.4022	4.9752 ± 0.3517	A
7.	2.9420 ± 0.4205	4.7905 ± 0.3402	A
8.	2.7955 ± 0.4146	4.5520 ± 0.3295	A
9.	2.7606 ± 0.4564	4.4951 ± 0.3012	A
10.	2.5926 ± 0.2658	4.2216 ± 0.2137	A
11.	2.5707 ± 0.2551	4.1859 ± 0.2096	A
12.	2.4513 ± 0.3238	3.9915 ± 0.2514	A
13.	2.3979 ± 0.3008	3.9045 ± 0.2183	A
14.	2.3429 ± 0.2869	3.8150 ± 0.2276	A
15.	2.2951 ± 0.2794	3.7371 ± 0.2010	A
16.	2.2635 ± 0.2377	3.6857 ± 0.1976	A
17.	2.2094 ± 0.2690	3.5976 ± 0.1526	A
18.	2.1344 ± 0.2744	3.4755 ± 0.1687	A
19.	2.1109 ± 0.2488	3.4377 ± 0.1520	A
20.	2.0694 ± 0.2255	3.3696 ± 0.1450	A
21.	1.9385 ± 0.2812	3.1565 ± 0.1230	A
22.	1.9128 ± 0.2963	3.1146 ± 0.1058	A
23.	1.8417 ± 0.2834	2.9989 ± 0.1824	A
24.	1.7432 ± 0.3445	2.8385 ± 0.2015	A

Total length = 61.41

* Acrocentric

PLATE 4. Karyotype of *Sardinella longiceps* collected from Mangalore.

PLATE 4. KARYPTYPE OF FISH *SARDINELLA LONGICEPS*



2n : 48
NF : 48
MANGALORE

10 µ

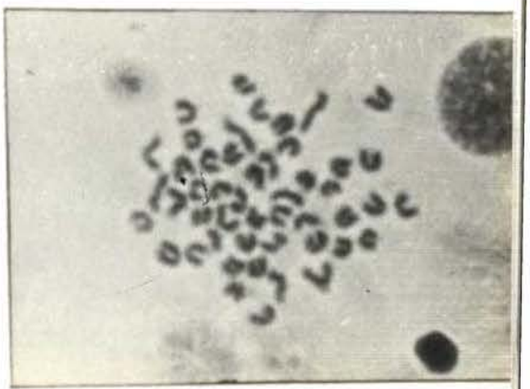


Table 3 Total chromosome lengths and relative chromosome lengths of *Sardinella longiceps* collected from Mangalore.

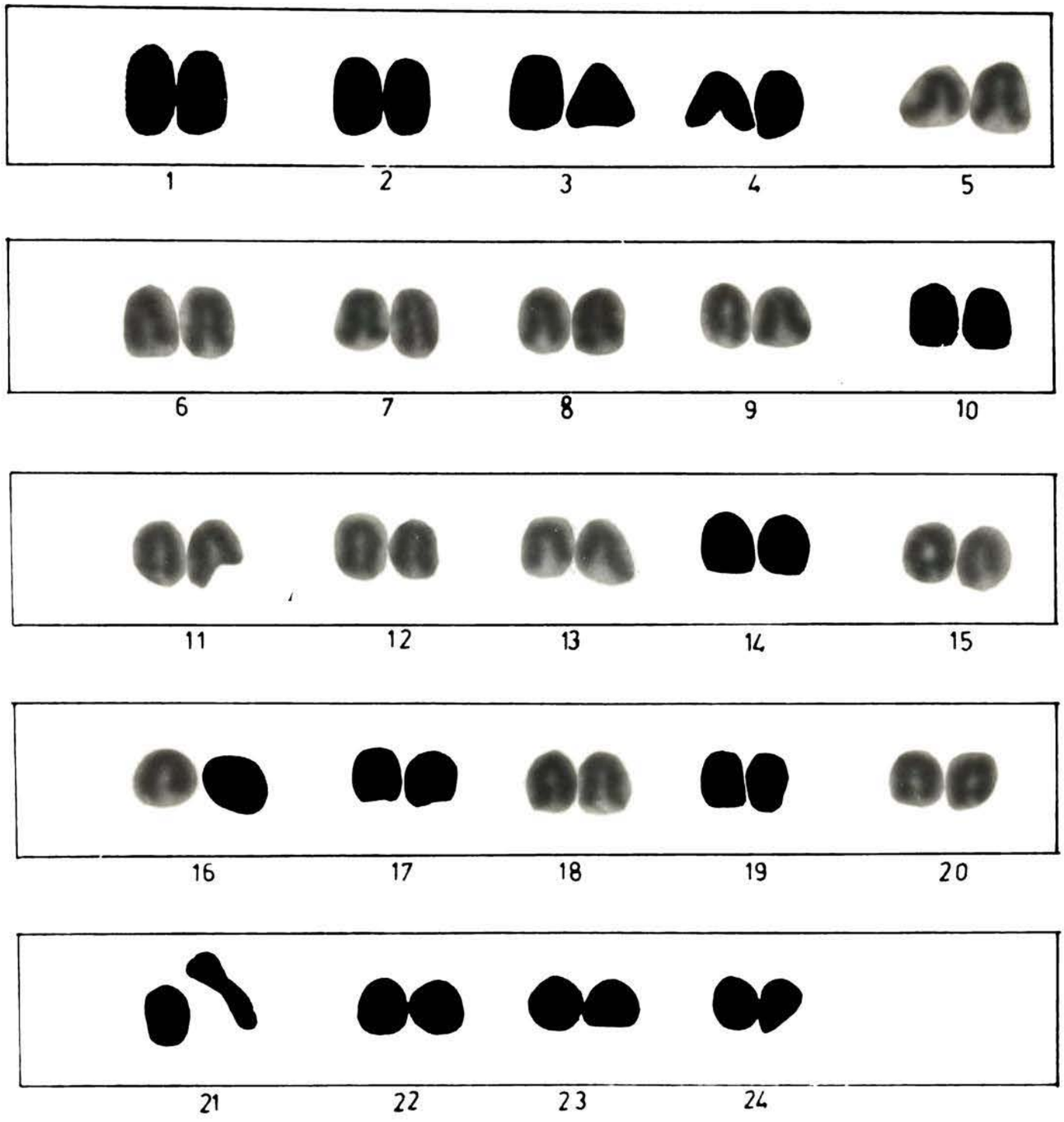
Chromosome Pair No.	Total length (um ±) ($\bar{x}$ ± S.D.)	Relative length (%) ($\bar{x}$ ± S.D.)	Chromosome type
1.	3.8270 ± 0.1539	5.5831 ± 0.1284	A*
2.	3.6534 ± 0.1838	5.3298 ± 0.1569	A
3.	3.4924 ± 0.2518	5.0950 ± 0.2304	A
4.	3.3744 ± 0.2896	4.9228 ± 0.2418	A
5.	3.3282 ± 0.2422	4.8554 ± 0.2038	A
6.	3.2628 ± 0.2887	4.7600 ± 0.2587	A
7.	3.2134 ± 0.2775	4.6879 ± 0.2436	A
8.	3.1144 ± 0.3358	4.5435 ± 0.3291	A
9.	3.0975 ± 0.3192	4.5188 ± 0.3087	A
10.	3.0678 ± 0.3966	4.4755 ± 0.3108	A
11.	2.9237 ± 0.3026	4.2653 ± 0.2581	A
12.	2.8236 ± 0.3243	4.1193 ± 0.3074	A
13.	2.7603 ± 0.3022	4.0269 ± 0.2697	A
14.	2.7311 ± 0.2788	4.9843 ± 0.2516	A
15.	2.7058 ± 0.2532	3.9474 ± 0.2234	A
16.	2.6320 ± 0.2533	3.8397 ± 0.2031	A
17.	2.5162 ± 0.2471	3.7747 ± 0.2412	A
18.	2.5162 ± 0.2770	3.6708 ± 0.2625	A
19.	2.4349 ± 0.2538	3.5522 ± 0.2487	A
20.	2.4034 ± 0.2252	3.5050 ± 0.2070	A
21.	2.3689 ± 0.2006	3.4559 ± 0.1539	A
22.	2.1434 ± 0.1744	3.1269 ± 0.1485	A
23.	2.1207 ± 0.2100	3.0938 ± 0.1279	A
24.	1.9632 ± 0.1822	2.8640 ± 0.1585	A

Total length = 68.54

* Acrocentric

PLATE 5. Karyotype of *Sardinella longiceps* collected from Mandapam.

PLATE 5. KARYOTYPE OF FISH *SARDINELLA LONGICEPS*



2n : 48
NF : 48
MANDAPAM

10 µ

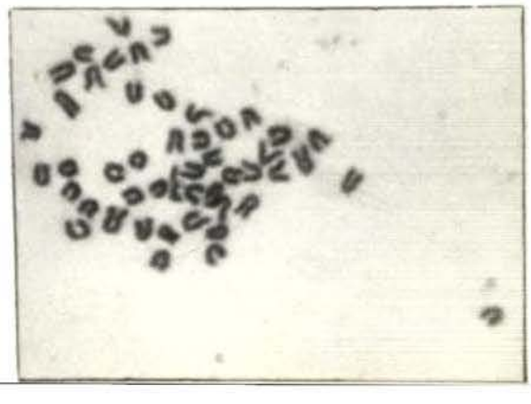


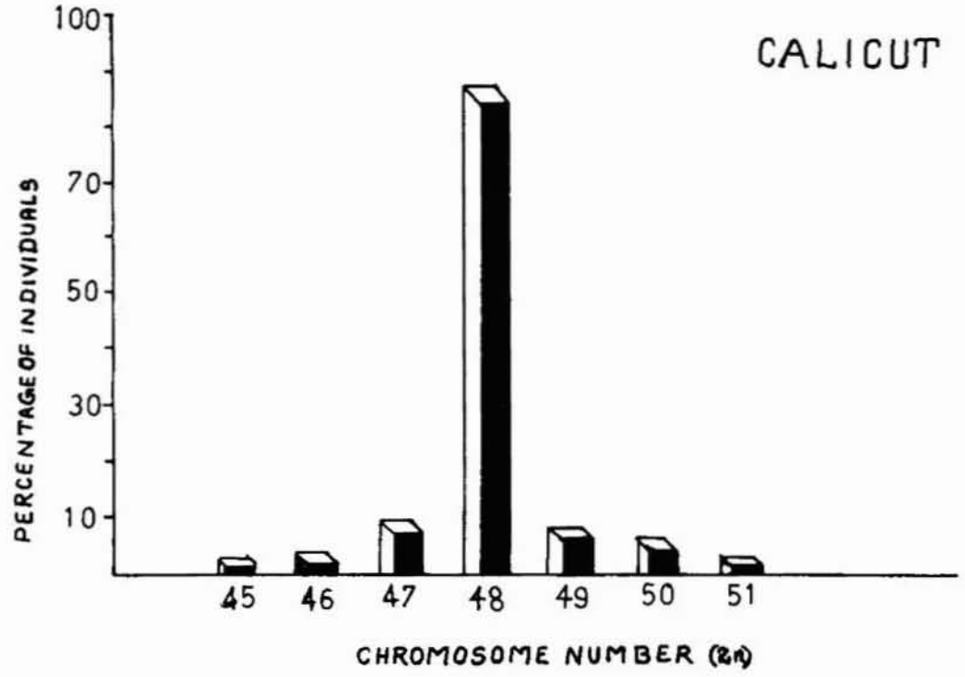
Table 4 Total chromosome lengths and relative chromosome lengths of *Sardinella longiceps* collected from Mandapam.

Chromosome Pair No.	Total length (um ±) ($\bar{x}$ ± S.D.)	Relative length (%) ($\bar{x}$ ± S.D.)	Chromosome Type
1.	3.6904 ± 0.4874	6.0133 ± 0.4782	A*
2.	3.4831 ± 0.5725	5.5755 ± 0.5263	A
3.	3.3537 ± 0.4750	5.4647 ± 0.4530	A
4.	3.2972 ± 0.4124	5.3726 ± 0.4016	A
5.	3.1296 ± 0.4111	5.0995 ± 0.4072	A
6.	3.0386 ± 0.3934	4.9512 ± 0.3859	A
7.	2.9356 ± 0.4269	4.7834 ± 0.4185	A
8.	2.8375 ± 0.4224	4.6236 ± 0.4057	A
9.	2.7640 ± 0.4530	4.5038 ± 0.4182	A
10.	2.5913 ± 0.2671	4.2224 ± 0.2537	A
11.	2.5694 ± 0.2563	4.1867 ± 0.2318	A
12.	2.4436 ± 0.3187	3.9817 ± 0.3078	A
13.	2.3965 ± 0.3023	3.9050 ± 0.2574	A
14.	2.3446 ± 0.2851	3.8204 ± 0.2160	A
15.	2.2959 ± 0.2785	3.7410 ± 0.2091	A
16.	2.2381 ± 0.2350	3.6469 ± 0.2275	A
17.	2.2105 ± 0.2678	3.6019 ± 0.2439	A
18.	2.1353 ± 0.2739	3.4793 ± 0.2586	A
19.	2.1100 ± 0.2497	3.4381 ± 0.2139	A
20.	2.0674 ± 0.2855	3.3681 ± 0.1954	A
21.	1.9426 ± 0.2756	3.1654 ± 0.2520	A
22.	1.9152 ± 0.2956	3.1207 ± 0.2491	A
23.	1.8399 ± 0.2860	2.9980 ± 0.2549	A
24.	1.7398 ± 0.3480	2.8349 ± 0.3137	A

Total length = 61.36

* Acrocentric

Figure-2. Percentage of chromosome plates showing modal value



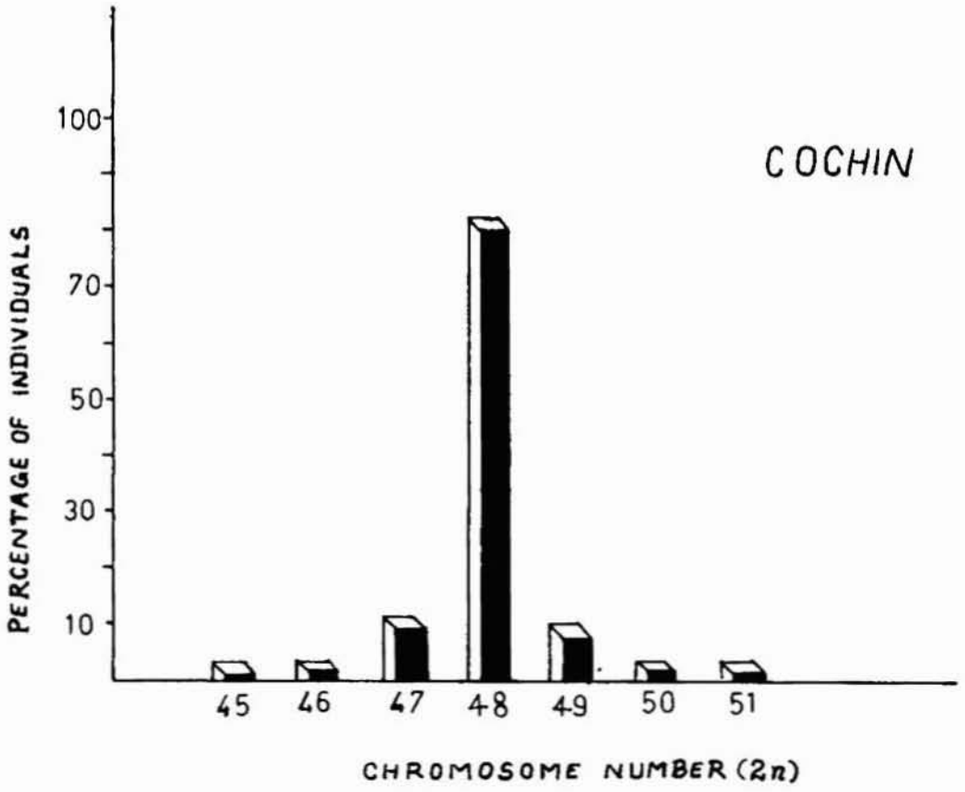
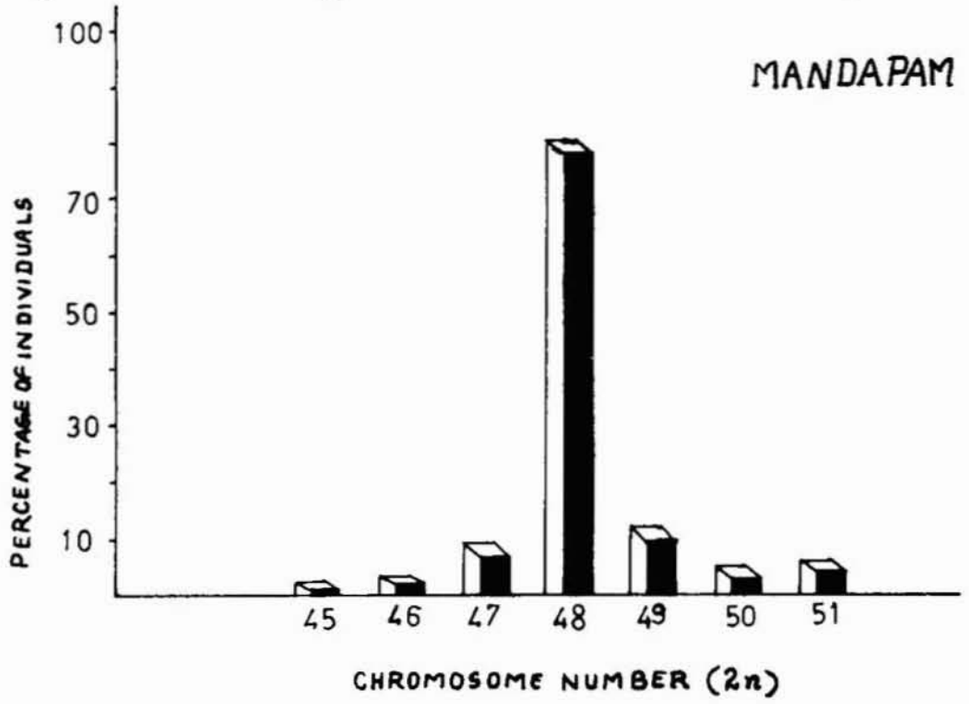


Figure-3. Percentage of chromosome plates showing modal value



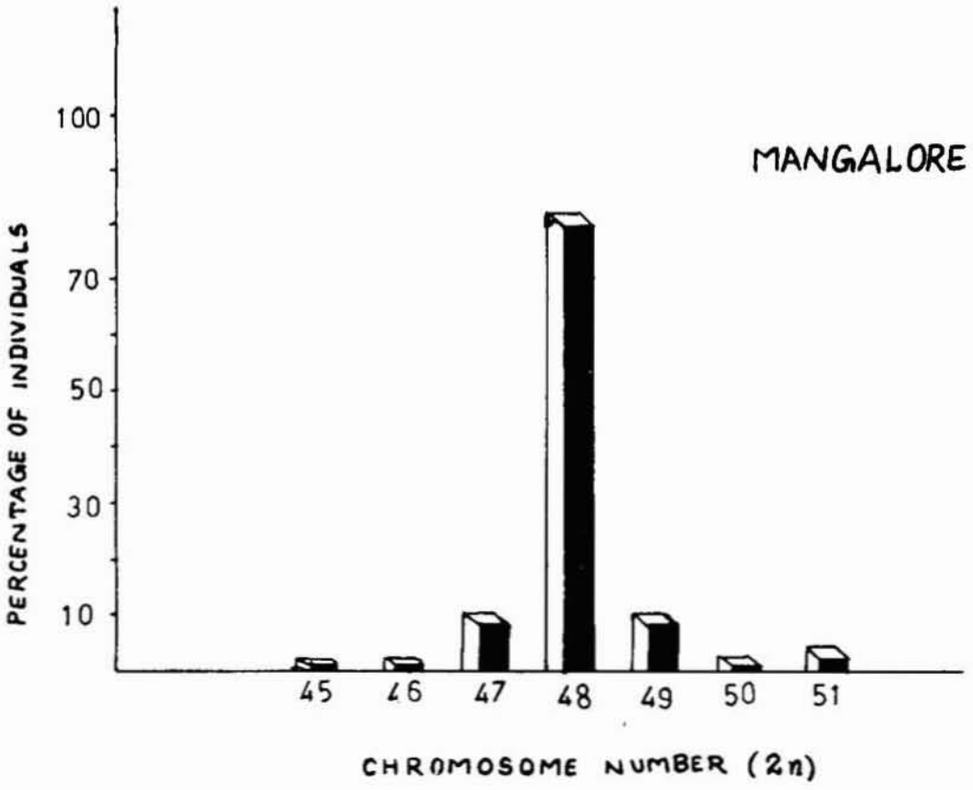


Table 5. **Chromosome numbers in Clupeidae fishes.** (Doucette and Fitzsimons, 1988)

	Species			Reference
1.	*Alosa pseudoharengus*	48	48	Mayers and Roberts, 1969
2.	*Clupea harengus*	52	52	Roberts, 1966
		54	66	Skvortsova, 1975a, 1975b
		54	68±2	Skvortsova, 1975a
3.	*Clupea harengus membras*	54	69±1	Skvortsova, 1975b
4.	*Clupea harengus pallsi*	52	58	Krysanov, 1978
		52	60	Skvortsova, 1975a, 1975b; Ohno et al., 1968; Ohno et al. 1969
		54	58	Krysanov 1978
5.	*Caspialosa kessleri*	48	48	Vasil'yev, 1980
6.	*Dorosoma cepedianum*	48	50	Fitzsimons and Doucette, 1981
7.	*D. petenense*	48	50	Fitzsimons and Doucette 1981
8.	*Gadusia chapra*	46	46	Khuda-Bukhsh, 1979.
9.	*Brevoortia patronus*	46	50	Doucette and Fitzsimons, 1988
10.	*B. smithi*	46	50	Doucette and Fitzsimons, 1988
11.	*B. tyrannus*	46	50	Doucette and Fitzsimons, 1988
12.	*Harengula clupeola*	28	52	Doucette and Fitzsimons, 1988
13.	*Sardinella melanura*	44	52	Rishi, 1973
14.	*Sardinella longiceps*	48	48	Present study

2.4. DISCUSSION

The earliest studies in fish cytogenetics began with the pioneering works of Retziat (1890) and Kastschenko (1890). However,due to lack of reproducible techniques for obtaining high quality metaphase spreads in large number of small fishes, research on this regard made little progress until recent times.

As a result of advances in technical and technological innovations of later period, many fish karyotypes have been described and the informations were used increasingly in the studies of evolution, cytotaxonomy, population genetics, mutagenesis and aquaculture (Booke 1968; Denton 1973; Manna 1983). In the initial period, invivo method of chromosome preparation was followed (Meredith 1969; Evons et al., 1972; Stock et al., 1972, and Kligerman and Bloom 1977). This method suffered from low mitotic index. Later, further improvement made in the protocol for invitro chromosome preparation helped to make rapid progress in cytogenetic investigations. (Labat et al. 1967, Heckman and Brubaker, 1970, Heckman et al., 1971). In the present investigation, the method of invivo chromosome preparation of Kligerman and Bloom (1977) was adopted with modifications according to the protocol of Reddy and John (1986). The invivo method has got several advantages and some disadvantages over the invitro method. Although both methods are employed with varying success, each suffers from certain disadvantages. The invivo preparation method leads to sacrifice the fish and the results may be affected due to varying changes

in the physiological state of the test specimens. Though, cell culture technique, invitro provides excellant mitotic index and resolution of chromosome details, the required special laboratory skills and equipments are not always available. Moreover, the high cost of cellculture, for karyotyping a large sample of adult fish would preclude its use in all but very specialised laboratories. Since large number of adult individuals, had to be examined for racial markers, in vivo method of chromosome karyotyping was followed in the present investigation. The general procedure adopted in the present study has also been extensively followed in population cytogenetic studies of fishes by others (Garcia et al., 1988, Moran et al., 1989).

However, the karyotyping of marine pelagic species like Sardinella longiceps was a challenging job as it was vulnerable to the laboratory stress conditions. In all the experiments, the fish could not be acclimatised to the laboratory conditions as done easily in estuarine and freshwater fishes. However, keeping the animals in well aerated sea water collected from the area where from the fish was caught enabled to hold them alive for a maximum of 48 hours. Due to its vigorous forward movement, the snout hits the side of the container causing severe snout injury and this ultimately leads to mortality of some specimens. Hence, an attempt was made to complete the procedure upto the tissue fixation in the fishing boat itself. Unexpectedly the results obtained by this procedure were not promising because the chromosomes appeared broken and deformed with fuzy edges due to some unknown reasons. However, quality metaphase plates were

obtained when tests were conducted in the laboratory conditions. In this respect, various methods reported by others, were tried for making readable metaphase plates (Mcphail and Jones 1966, Denton and Howell 1969; Kligerman and Bloom 1977, Reddy and John 1986). Most of these methods employed deposition of cells on slides followed by air drying or flame drying. The best results were obtained by modifying and incorporating the essential steps of colchicinisation, hypotonic treatment, fixation, cell suspension preparation, deposition of cells and air drying (Kligerman and Bloom 1977, Reddy and John 1986).

Chromosomes with optimum contractions were obtained with colchicine 0.1% solution (1 ml/100 gm body weight) exposure for 2 to 2-1/2 hours. Colchicine was used to arrest quickly proliferating cell population at the metaphase stage. Shortening of chromosomes was observed with a higher dose of colchicine or longer duration of colchicine exposure (Denton 1973). A variability of response to colchicine existed between individuals in which some fish did not respond to the treatment, the reason for which is unknown and such a phenomenon was also reported by Gjedrem et al., (1977), Hartley and Horne (1983) and Chourrout and Happe (1986). In this study exposure to hypotonic solution (0.4% KCl) for 35-40 minute at low temperature gave better results than that of the exposure compared to the 1% tri-sodium citrate hypotonic solution. As a result most of the metaphases formed were well spread and the chromosome morphology was quite undisturbed and very clear (Plate 2). Hypotonic treatment

carried out in cold condition had definite advantage since, cell swelling was controlled and the chance of cell bursting was less than that of the hypotonic treatment at room temperature. However, inspite of all these satisfactory conditions, a few individuals gave poorly spread metaphases, again the cause of which is unknown.

Following hypotonic treatment, the tissues were fixed in freshly prepared 3:1 methanol - glacial acetic acid solution. In theory, the alcohol component hardens the tissue and also causes shrinkage. The acetic acid component alternatively counteracts some of the shrinkage caused by the alcohol and is desirable because of its rapid penetrability (Humason, 1979). The 4% Giemsa staining solution with a pH of 6.8 yielded good results.

The present description of the karyotype in the Indian oil sardine S. longiceps is the first report of its kind. The observed modal diploid number of the species was 48, (NF 48) all acrocentric in shape at Cochin, Calicut, Mangalore and Mandapam (Plates 2-5). Analyses of the metaphases in S. longiceps further revealed that there is no obvious differences between males and females. Though, there are a few reports on the presence of identifiable sex chromosomes in fishes (Lieder 1963; Chen 1969; Ebling and Chen 1970; Uyeno and Miller 1971; Rishi and Gaur 1976; Thorgaard 1977), the current concept holds that sex chromosomes in fishes are in a low grade of differentiation and hence morphologically not distinguishable (Dhar and Chatterjee 1984).

Literature reveals that karyotype informations for only 16 out of approximately 340 species of clupeiformes and elopiformes are available. (Doucette and Fitzsimons, 1988). Table-5 shows the summary of previous karyomorphological studies on clupeidae. Though, vast number of fish species inhabit in a diversified environmental conditions, the degree of karyotypic diversity is surprisingly very low. A good percentage of species has 48 acrocentric chromosomes. hence, the diploid number 48 with uniarmed chromosomes, has been suggested by many workers as primitive among teleost fishes (Ohno 1970; Fitzsimons 1972; Legrende 1975) or as the most fundamental karyotype of fishes (Denton, 1973). It also appears to be the dominant karyotype in the perciformes (Chiarelli and Capanna, 1973). This has lead to the suggestion that 48 acrocentric chromosome complement may be ancestoral.

Such wild assumptions on the evolutionary status of the extant species based on their observed karyotypes have no significance to the main objectives of the present study. Hence a study of karyotype of S. longiceps and its populations was undertaken from the view point of fisheries management of its resources. To achieve the objectives, the species specific chromosome number, its morphology, and intraspecies polymorphism of chromosome number and its morphology were examined in the sample populations of the species. In this respect the effort spent in the standardisation of the methodology and its application enabled to discover specific karyotype details on S. longiceps. It is interesting to report that the species also has

48 acrocentric chromosomes. All the three populations of the species tested from the west coast and the fourth sample tested from the east coast of India had identical chromosome number and morphology (Plates 2-5, Table 1-4). This agrees with the reports that polymorphism in chromosome number and morphology is not common though not rare among fishes. (Allendorf and Thorgaard, 1984, Manna, 1989). A reason suggested for the rarity of fish chromosomal polymorphism is that chromosomal rearrangements in fishes tend to be fixed rapidly, perhaps, due to small effective population size (Wilson et al., 1985).

Inspite of the presence of the same diploid chromosome number (2n = 48) among many of the diverisified fish species, the phenomenon of intraspecies variations in the species specific chromosomal number and morphology have been reported in some species of fishes. Several studies have demonstrated the utility of such chromosome polymorphism as a markers for cytogenetic differentiation of fish stocks within the species (Roberts 1968; Gold and Gall, 1975; Hartley and Horne 1982, 1984; Thorgaard and Allen 1987; Moran et al., 1989; Garcia et al., 1988; Fan and Fox 1990).

In this respect the present discovery of polymorphism in the total mean length and relative length of 48 chromosomes among the four populations of the species S. longiceps is significant. Two distinct type of chromosome lengths within the species, were recorded (Table No.1-4). The total mean lengths of oil sardine populations from Cochin (68.39) and Mangalore (68.54) were very

closely comparable. On the other hand the values of Calicut (61.41) and Mandapam (61.3698) were another comparable type. Naturally, these region or population oriented differences were also reflected in the percentage of minimum maximum range of relative lengths of paired chromosomes of these two distinct groups. These values varied from 2.8349 - 6.0133 / 2.8385 - 5.9738 in Mandapam/Calicut groups to 2.8640 - 5.5831/2.8892 - 5.5805 in Mangalore/Cochin group. It may be reasoned that measurements of chromosome lengths may be affected by variable factors such as physiological state of the fish and experimental condition existed at the time of taking the data. A close scrutiny of these factors and the very nature of the data itself help to reject the possibility that the observed difference were due to certain artefacts.

It is true that samples from Calicut and Mangalore received their colchicine immediately after they were caught where as the samples from Cochin and Mandapam were administered the injection after these were brought and kept alive at the respective laboratories. The physiological stress occurred to the former groups must have been different from the latter. On the contrary what is interesting is, the chromosomal lengths of the samples between Calicut and Mangalore (61.41/68.54) as well as that of Cochin and Mandapam (68.39/61.36) themselves were different, whereas closely similar values were shown between Cochin/Mangalore (68.30/68.54) and between Calicut/Mandapam (61.4/61.36) though experimental conditions were different at their places as discussed above. In other words, the higher

chances of affecting the length values though existed between two heterogeneous regions, it did not occur and hence, the observed difference and lack of differences were due to natural facts and not due to artefacts.

The above facts and figures supported the present conclusion that some form of population genetic heterogenity exist within the species, S. longiceps. Though, total lengths of chromosomes are usually measured in fish cytogenetic studies, no reports of comparison of such values between populations or any report on significant differences indicating population/stock varieties were come across by the investigator. However, it is interesting to discuss a relevant report on the data presented on the total mean length of two samples of Channa punctata and their modal chromosome numbers. Based on chromosome number (2n), the species was differentiated C. punctata variety A (2n=34), and C. punctata variety B (2n=32). The data on total chromosome lengths, though they were significantly different not reported as a marker for differentiation of the varieties. The C. punctata Variety A showed a mean total length of 47.07 /um and C. punctata var. B a total length of 39.74 /um (Dhar and Chatterjee, 1984). it may be argued that the apparent higher total length of chromosomes of C. punctata Var. A may be attributed to an extra pair of chromosome (17th pair) possessed by C. punctata Var A than that of C. punctata Var. B. Even if that additional length (0.88) produced by the 17th extra pair is not considered, the value of the chromosome length of the two examples will still continue to be

significantly different. Besides, the minimum-maximum total length range and percentage relative length of chromosomes of the two sample also reflected significant difference of karyomorphology of the varieties of the species C. punctata.

Thus, the present report of significant differences of total mean lengths, and relative lengths of chromosomes of oil sardine populations is comparable to the above analysed data of C. punctata. Therefore, it is recommended that total mean lengths of chromosomes may be critically examined and used as a karyomorphological marker for differentiation of genetic stocks of marine as well as fresh water fish species.

The present observation of significant differences in the total mean lengths of chromosomes of S. longiceps tested between Cochin/Mangalore and Calicut/Mandapam were further corroborated by morphometric and protein phenotype heterogenity of oil sardine populations as observed in the other chapter of the present study and the biochemical genetic heterogenity of Cochin, Calicut, Mangalore and Mandapam oil sardine populations as reported by Venkitakrishnan (1995).

3. CHAPTER - II

BIOCHEMICAL GENETICS (GENERAL PROTEINS)

3.1. REVIEW OF LITERATURE

Many investigators have recommended and applied biochemical genetic techniques for the identification of gene controlled electrophoretic phenotypes of proteins and enzymes present in different tissues of fishes. Then the analysis of the observed genotypes and their allelic frequencies have been used as a basis for stock identification (Crow and Kimura, 1970; Utter et al., 1974; 1987) It is evident from different reports that general proteins and some enzymatic proteins were useful for stock identification programmes. Electrophoretic patterns of muscle proteins have been widely used in fish systematics and identification of its genetically different stocks (Tsuyuki et al.,1967; Eckroat and Wright, 1969, Uthe and Ryder 1970; Smith et al., 1980; Anderson et al., 1983; Menezes, 1990; Vijayakumar, 1992). Avise (1974) have reviewed systematic studies using electrophoretic data and concluded that electrophoretic techniques are extremely valuable tool for systematics.

The electrophoretic techniques and staining procedures for detection of different kinds of protein systems published by Smith (1968), Brewer (1970), Shaw and Prasad (1970) enabled different researchers to discover and publish large volumes of information on the electrophoretic characteristics of different enzyme systems and some non-enzymatic proteins. The major reason for worldwide application of biochemical genetic techniques involving gel electrophoresis is the implication of genetic stock concept in the fisheries management of commercially important

fish resources (Moller, 1968; 1970; Utter et al., 1974; Allendorf and Utter 1979).

The very basis of search for different genetic stocks is the concept of evolutionary processes occurring within the species. The degree of evolutionary diversification between identified discrete stocks may also be expressed in terms of genetic identity and genetic distance measured according to the method of Nei (1972). Its application in biochemical genetics of populations was also explained using models (Ayala and Kiger, 1980). Gordon (1947) is believed to have been the first to show that reproductively isolated populations within the same species of fish differed in gene frequency. Later improved biochemical techniques were used to examine single protein markers, such as esterase, haemoglobin and transferrin polymorphism in the Atlantic cod, Gadus morhua (Sick, 1961; Moller, 1966; Jamieson, 1967) and tuna species, (Fujino, 1967; Fujino and Kang 1968a, 1968b).

The potential of simple proteins like that of muscle as genetic markers was shown in different species of fishes. Starch gel electrophoretic patterns of muscle myogen in the marine species, Anoploma fimbria was first described by Tsuyuki et al. (1965a). Large scale population studies of the cod Gadus morhua revealed four variant patterns in its muscle extracts (Odense et al., 1966). Studies on freshwater species also showed intraspecific variation in muscle myogen patterns (Utter et al., 1966; Tsuyuki et al., 1967). Electrophoretic and immuno-

electrophoretic techiniques have demonstrated the presence of codominant allelic variations in the serum proteins and their genetic implications in the systematics and stock identification of various cichlids (Avtalin and Wajdani, 1971; McAndrew and Majumdhar, 1983).

Biochemical genetics of fishes have been reported by many workers on pup fish (Turner, 1974) Minnow (Avise et al., 1975), Mexican cichlids (Sage and Sealander, 1975); Rainbow trout (Allendorf and Utter, 1979); Bone fish (Shaklee and Tamaru, 1981); Brown trout (Ryman et al., 1979; Ferguson et al., 1981); Cuthroat trout (Busack and Gall, 1981); Sturgeon (Phelps and Allendorf, 1983); Atlantic herring (Rayman et al. 1984); Pacific herring (Grant and Utter, 1984) using electrophoretic methods. Electrophoresis was carried out using the water soluble muscle proteins of six grey mullets (Mugilidae), caught off the Mediterranean coast of Isreal (Herzberg and Pasteur, 1975). Eight wild populations of high sierra golden trout, Salmo aguabonita and one domestic stock of rainbow trout, Salmo gairdneri were examined for biochemical variation in eight proteins systems (Gall et al., 1976). Variation in eight systems was determined by at least 10 loci in both golden and rainbow trout and all the alleles identified in rainbow trout were observed as a electrophoretically identical phenotypes in golden trout. Mangaly and Jamieson (1978) investigated genetic variability in the natural populations of European hake by tracing the geographic distributions.

Since most of the biochemical processes involve catalytic participation of large number of enzyme systems, they have been investigated as potential source of genetic variation studies. Thus major portion of literature available on modern fish stock identification studies are based on enzyme allelic frequencies (Hodgins et al., 1969; Northcote et al., 1970; Williscroft and Tsuyuki, 1970; Wright and Atherton, 1970; Utter and Hodgins, 1972; Aspinwall, 1973; Utter et al., 1973; Allendorf et al., 1976; Bailey et al., 1976; Mangaly and Jamieson, 1978; Busak et al., 1980; Grant et al., 1980, Wishard et al., 1980; Winans, 1980; Ferguson and Mason, 1981; Shakle, 1983; Ryman, 1983; Withler, 1985; Koljonen, 1986; Okazaki 1986; Utter et al., 1992). Shaklee and Salini (1985) studied genetic variations in the muscle proteins of Lates calcarifer. Utter et al (1987) outlined the applications of genotypic and allelic data obtained by electrophoresis in stock identification of fishes. Smith et al., (1989) reviewed the electrophoretic data on marine fishes in relation to the stock concept. Electrophoretic technique has been applied on stock identification for the commercially important marine species (Wishard-Seeb and Gunderson, 1988; Jamieson and Birley, 1989) on estimating the stock component of ocean caught salmon (Beachmam et al., 1987; Utter et al.,1989). Shaklee et al. (1989) proposed the genetic nomenclature for protein coding loci in fish in order to promote and facilitate genetic studies in fish.

The essence of the above review is that all tissue proteins/enzymes are potential genetic markers and any one or

more markers may be selected and analysed by electrophoretic method to study the genetic variability within the species and among its populations. Considering all the inherent constraints facing the present investigation, only muscle and eye lens proteins were selected and analysed by simple discgel electrophoresis method.

3.2. MATERIALS AND METHODS

3.2.1 Field collection

The population samples of Oil Sardine, *Sardinella longiceps* were collected from four widely separated regions, Mandapam of the east coast and Cochin, Calicut and Mangalore of west coast of India (Fig. 1). These locations were selected considering distributional abundance of the species in the region (Anon, 1979).

The west coast population samples were obtained from purse-seine nets of commercial vessels during 1988-1991. The east coast sample was collected from shore seines and kept frozen until transported to Cochin. Frozen fish samples from Calicut, Mangalore, Mandapam were transported in ice box to the laboratory within 12 hrs of collection. The ice preserved fresh sample from Cochin was transported immediately to the laboratory. All the collected samples were then stored in deep freezer until used for analysis. The electrophoresis of proteins was completed within one week of collection.

3.2.2. Sample preparation

Tissue samples of eye lens and skeletal muscle of each fish were dissected out from the thawed fishes in cold condition. Only white muscle tissue was used for electrophoresis. These tissue samples packed in aluminium foil and labelled properly were kept over night at - 4^{o}C. The cut out tissues were processed within fourty eight hours. Just before every experiment, tissue samples were allowed to thaw at room temperature. Homogenisation of tissue was done in the cold double distilled water using Remi mechanical homogeniser. The homogenized tissue was centifuged using refrigerated centrifuge at 5,000 rpm for 30 minutes. The clear supernatant obtained was then used for electrophoretic analysis.

The electrophoretic method followed for separation of general proteins was according to the techniques of disc electrophoresis (Davis, 1964). The details of reagents used and procedures followed for electrophoresis are given below.

3.2.3. Reagents for stock solutions

1. Gel buffer (Tris-HCl pH 8.9)

TRIS	--- 36.6 grams
IN HCl	--- 4.8 ml
TEMED	--- 0.26 ml

2. Tank buffer (Tris Glycine - pH 8.3)

Tris Buffer (0.05 M)	--- 6 grams
Glycine (0.38 M)	--- 28.6 grams

All the buffer reagents were dissolved separately in double distilled water (DDW) and made upto 100 ml. The pH of the solution was checked with Toshiniwal battery operated pH meter. The pH was adjusted by adding either HCl or 1 N NaOH solution as required. The solution was filtered with Whatman filter paper and kept in an amber coloured bottle at $4^{o}C$ until used. The gel buffer was used without further dilution. Just before use, one part of tank buffer stock solution was diluted with nine parts and the solution was used as tank buffer.

3. 40% Acrylamide solution

   40 grams of acrylamide was dissolved in 100 ml of DDW (W/V) and filtered through Whatman filter paper. The solution was left at $4^{o}c$ in an amber coloured bottle.

4. 2.1% Bisacrylamide solution

   2.1 gms bisacraylamide was dissolved in 100 ml DDW (W/V)

5. Ammonium per sulphate solution: 280 mg of ammonium per sulphate was dissolved in 100 ml DDW (W/V)

6. 40% Sucrose solution

   40 gram of sucrose was dissolved in 100 ml of DDW and kept at $4^{o}c$ until used.

7. Marker dye (Bromophenol blue 0.1%)

   100mg of Bromophenol blue crystals dissolved in 100ml of DDW (W/V) 1 part of 0.1% bromophenol blue was mixed with same part of 4% sucrose solution. Then the whole mixture was diluted with DDW in 1:1 ratio n(V/V)

3.2.4. **Staining solution**

1. 1 gram of amido black was dissolved in 1 litre of DDW (W/V).

2. Coomassive Brilliant blue 0.2%. 2 grams of coomassive brilliant blue was dissolved in a mixture of Methanol, DDW and acetic acid prepared in 5:4:1 proportions.

3.2.5. **Fixative**

10% Trichloroacetic acid (TCA):-

200 grams of TCA was dissolved in 2 litres of DDw.

3.2.6. **Destaining solution**:

7% acetic acid solution.

3.2.7. **Apparatus used**

Cylindrical perspex tanks manufactured by M.C. Dalal and Co with facilities to run 12 tubes at a time was used. A power pack manufactured by Biochem Company (Model LK 890) was used for supplying the power.

3.2.8. **Procedure**

The stock solutions, like, gel buffer, acrylamide/ bisacrylamide and ammonium per sulphate were brought to room temperature before preparation of the gel. To obtain 7.5% gel, 3.5 ml of gel buffer, 7 ml of acrylamide, bisacrylamide, 3.5 ml of DDW and 14 ml of ammonium per sulphate were thoroughly mixed. Care was taken to avoid trapping air while preparing the gel. Gel

tubes of 100mm length and 6mm inner diameter were carefully inserted into the rubber grommet. The mixed solution was carefully added with the help of a glass syringe to a height of 80 mm. Immediately, water was layered carefully above the gel by a plastic syringe without disturbing the gel bed to a height of about 2-3mm. The function of the water drop is to overlay the gel solution so that it polymerises with a flat surface.

The gels polymerised in about 20-30 minutes at room temperature. After polymerisation the water from the gel tube top was decanted. The samples were applied by means of micropipette over the gel. Sucrose solution containing marker dye was added to the sample to separate the sample from the buffer and to ensure a uniform flow of current. The gel tubes were then inserted into the silicon grommets of the upper buffer tank reservoir in such a way that the upper end of tubes were just above the lower surface of the upper tank. The upper and lower buffer tanks were filled with the tank buffer.

The upper tank was fixed to the lower tank of the electrophoretic apparatus and the apparatus was connected to the power pack. The power pack was switched on for 30 minutes before connecting to the apparatus so as to get a uniform current flow. The current was regulated to have a uniform flow of 12 milli amperes for 10 minutes and then it was adjusted to 36 milli amperes. The power was switched off when the marker dye had migrated to about 5mm from the lower end of the gel. The run usually takes 80 to 120 minutes at $4^{O}C$. After the electrophoresis

the gel tubes were removed from the grommets and then the total length of the gel and migration distance of the marker dye were measured. The gels were then removed from the gel tubes by forcing a jet of water between the gel and the inner wall of the gel tubes using a syringe without damaging the gels.

3.2.9. Staining procedure for general proteins

1. The protein present in the gel were fixed by placing the gels in 10% trichloroacetic acid (TCA) solution for 10 minutes.
2. The gels were stained in amido black solution for 10 minutes.
3. Destaining was carried out by soaking the gels in 7% acetic acid until excess stains were thoroughly washed off from the gel.

3.2.10. Standardisation of electrophoresis

The objective of standardisation was to evolve a suitable standard electrophoretic procedure to obtain better separation and resolution of muscle and eyelens tissue proteins of *Sardinella longiceps*. Following experiments were conducted to get a better separation and resolution of the proteins.

1. **Tissue extraction**

   Tissue extract was prepared in both Tris buffer solution pH 7.5 and Double distilled water (DDW). Better results were obtained with DDW.

2. Gel compositions

Different gel compositions were tested to get different pore size of gels and to find out the one that gives better resolution and separation of maximum number of bands. Best results were obtained with 7.5% gel.

3. Buffers

A variety of buffer systems given in different literature have been tried for standardisation. Of these, discontinuous buffer system, Tris-glycine buffer pH 8.3 (Tank buffer) and Tris - HCl, pH 8.9 gel buffer gave best results.

4. Quantity of sample for optimal resolution

Experiments were carried out to find out the optimum quantity of sample which gave good resolution after homogenising 100mg of tissue in 1 ml of DDW. Different concentrations tried were 40 µl, 60 µl, 100 µl and 120 µl. From the results, it was concluded that 60 µl for eyelens and 60 µl for muscle tissue gave better separation without trailing effect. The above described standardised methods were followed for final electrophoresis of tissue proteins studied in the investigation.

3.3. RESULTS

3.3.1. Eye lens proteins

The electrophoretic patterns of eye lens proteins in Sardinella longiceps population from Cochin, Calicut, Mangalore and Mandapam were almost identical (Plate 6, Fig. 4). A total of eight bands were present in all the specimens tested from all the regions. The first band was seen at the point of application of sample. The other seven bands migrated towards the anode at different rates (Plate 6) If each band is assumed to be under the control of an independent locus, eight loci may be responsible for the observed patterns. All specimens tested showed four strongly stained thick bands followed by three, lightly stained narrow bands. Besides, the band at the cathode region was also of narrow type.

3.3.2. Muscle Proteins

The electrophoretic patterns of muscle proteins in Sardinella longiceps are shown in Plates 7,8 and Fig. 5,6. The patterns could be differentiated into three types. The first type consisted of a single, well stained band at the bottom of the gel where the sample was applied. The second type consisted of two large thick bands in all the samples except in Mandapam and Calicut where an additional single banded phenotype also existed. The third type consisted a group of lightly stained narrow bands in most of the specimens. On the basis of presence or absence of a particular band at a particular region five independent loci

PLATE 6. Disc gel electrophoretic pattern of eyelens protein in S. longiceps

Fig. 4. Zymogram patterns of eyelens tissue of S. longiceps

PLATE - 6

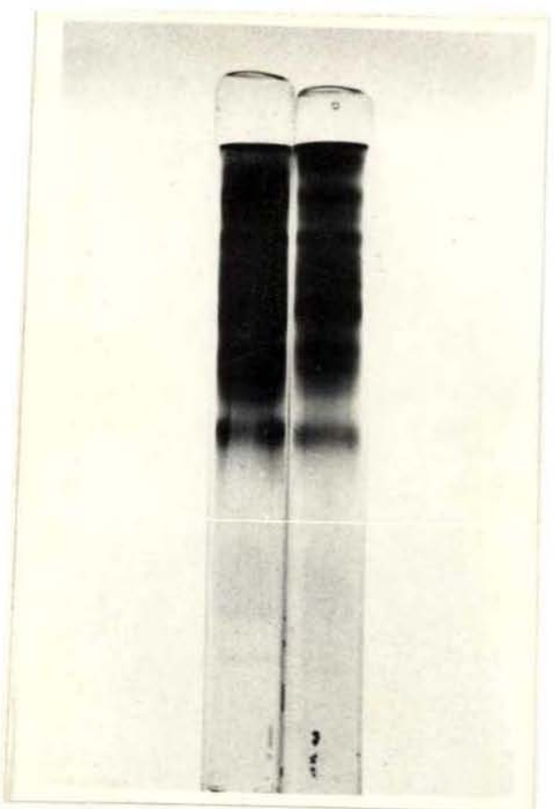
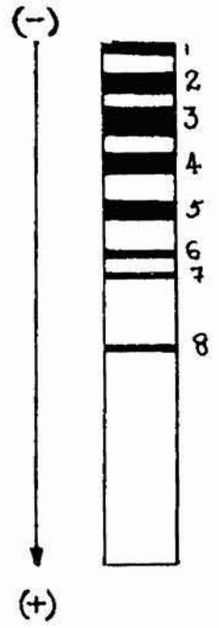


Figure-4.



could be assumed to be present as shown in the Fig. 5,6. The slowest moving protein fraction representing first locus remained at the bottom of the gel. The region recognised as the second locus showed two bands with identical migrations in all the specimens of Mangalore and Cochin population samples and in some specimens of Calicut and Mandapam sample. The second locus showed some variations in the gel position of these two bands in certain number of specimens indicating, some form of polymorphism at the second locus in Calicut and Mandapam populations (Fig.5, 6). The variation was mainly due to difference in the gel position of one of these two bands (SF). Thus according to the comparative slow and fast electrophoretic migratory nature of these two bands, the two banded patterns in Mangalore, Cochin, Calicut and Mandapam, could be designated as SF. The single banded phenotype was designated as SS or FF depending on its migration rate (Fig.5). A single narrow band (SS) appeared at the gel position 20 mm appeared to be the product of a third locus in all the four populations. A comparison of protein fractions moving faster than that of third locus indicated bands of fourth and fifth loci. The two banded and single banded phenotypes were present at fourth and fifth loci in Mangalore whereas all the specimens in Cochin and Calicut were two banded at these two loci. On the contrary, all the specimens in Mandapam were single banded at these two loci. Thus the fourth and fifth loci also appeared to be polymorphic in the species. The protein fractions at fourth and fifth loci were also designated as SF to indicate the double banded phenotypes while on comparison, the single band phenotypes

observed in Mandapam was equal to the SS at the fourth and FF at the fifth locus (Fig. 5,6).

Thus an over all comparison of all these five apparent loci in different populations of S. longiceps tested from Cochin, Calicut, Mangalore and Mandapam indicated three polymorphic loci (second, fourth and fifth) and two monomorphic loci (first and third). Assuming the observed phenotype variations were due to allelic variations at each of these three different loci, allelic frequencies and expected genotype frequencies were also estimated. The results are shown in Tables 6, 7. The frequencies of S and F alleles were equal in samples from Cochin and Mangalore as these regions had only the presumed two banded heterozygotes. The major reason for the occurrence of different allelic frequencies at second locus in the populations of Calicut and Mandapam was due to the presence of single banded SS and FF phenotypes in addition to SF type at the second locus in the respective regions (Fig. 5,6, Table 6). The presence of both two banded and single banded phenotypes at fourth and fifth loci produced its own allelic frequencies at Mangalore. The allelic frequencies at the fourth and fifth loci also were equal in Cochin and Calicut as only the assumed heterozygotes were present in these two regions (Fig. 5, Table 6). The Mandapam population on the other hand showed cent percent S and F alleles at the fourth and fifth loci respectively.

The significant differences in the phenotypic patterns and their assumed allelic frequencies at one or more of the assumed

loci in all the four populations suggest that these populations are heterogenous. However, the question is whether these phenotypic variations are genetic in nature. For this purpose the observed phenotypic frequencies were compared with that of the Hardy-Weinberg equilibrium condition to determine the nature of the observed variations. The chi square values at all the polymorphic loci in Cochin and at the second locus in Mangalore were highly significant (Table 7). The obvious reason for such significant deviation from the Hardy-Weinberg equilibrium condition was due to cent percent heterozygote genotypes and lack of homozygotes in the samples. The chi square values at the fourth and fifth loci were just significant in Mangalore. The chi square values were also highly significant at fourth and fifth loci in Calicut, the reason being lack of homozygotes at these loci. It is interesting to record apparently fixed alleles at fourth and fifth loci in Mandapam populations.

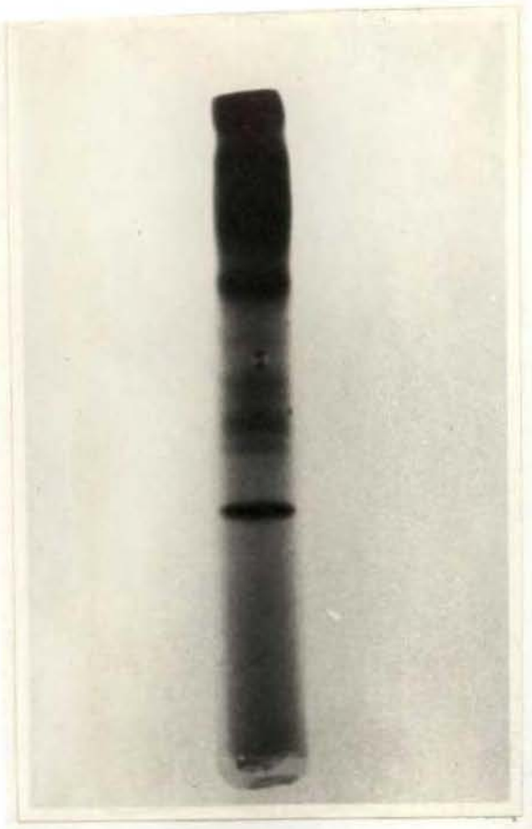
As the observed-expected frequencies of protein phenotypes were significantly different from that of Hardy-Weinberg equilibrium condition, the observed variations in the muscle protein phenotypes can not be considered as strictly genetic in nature. However, these populations are phenotypically very different among themselves. In this respect the Mandapam stock is phenotypically very distinct from the other three populations.

PLATE 7. Disc gel electrophoretic pattern of muscle protein of S. longiceps collected from Cochin (A) and Calicut (B)

Fig. 5. Zymogram patterns of muscle Protein in S. longiceps from Cochin (A) and Calicut (B)

PLATE-7

A



B

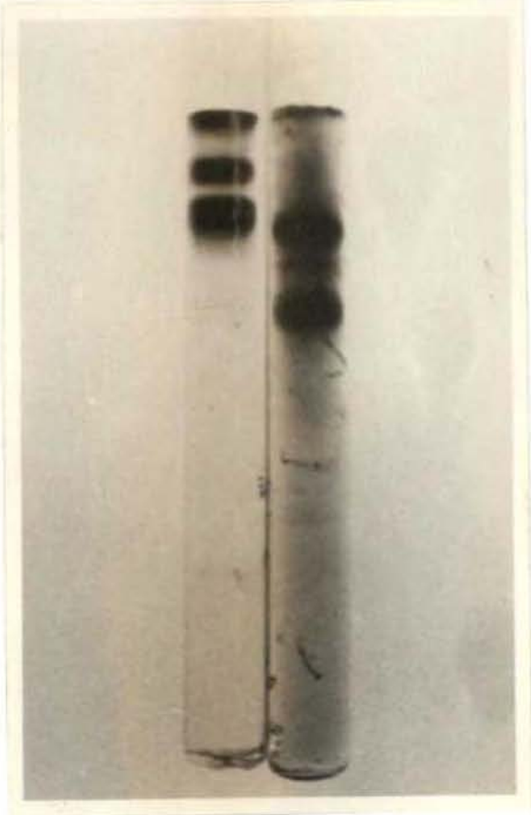


Figure-5.

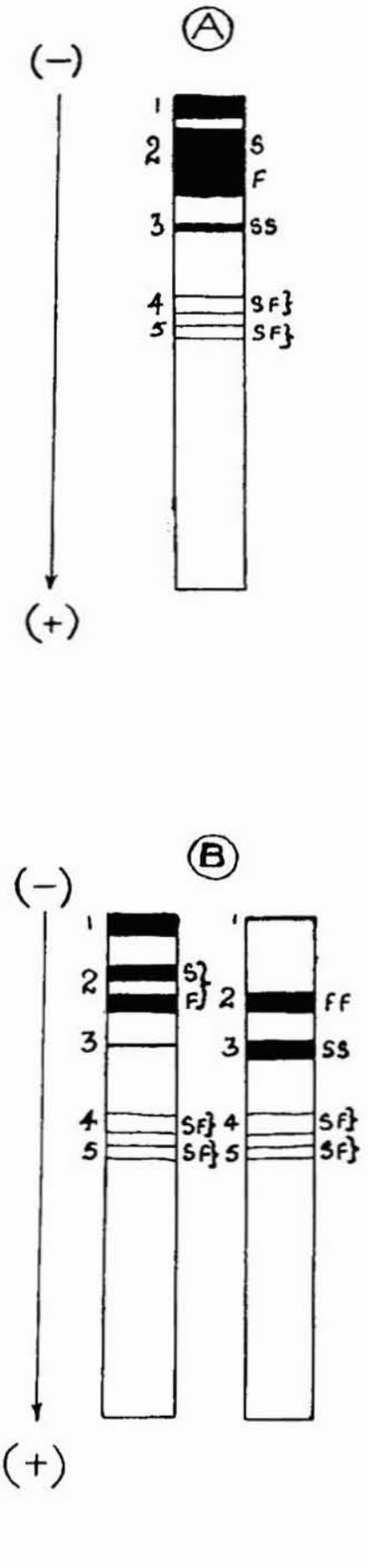


PLATE 8. Disc gel electrophoretic pattern of muscle protein of S. longiceps collected from Mangalore and Mandapam.

Fig. 6. Zymogram patterns of muscle proteins in S. longiceps from Mangalore and Mandapam.

PLATE-8

A

B

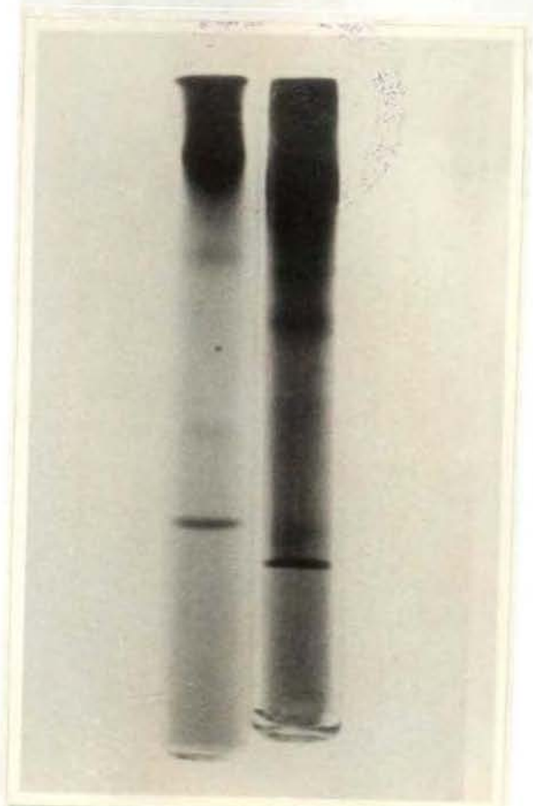
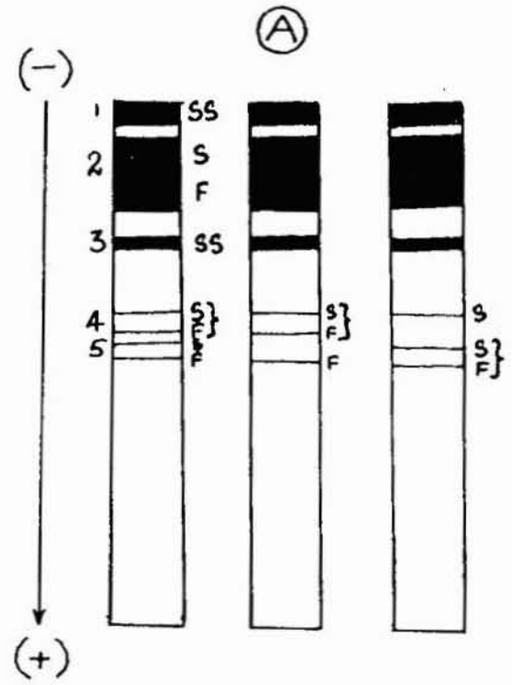


Figure.6.



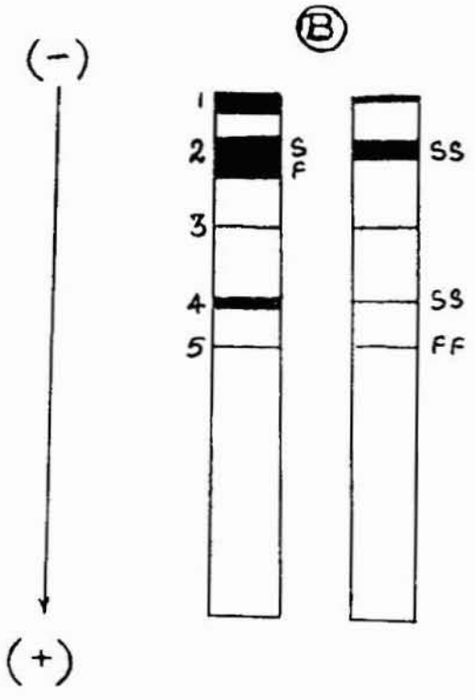


Table 6: The estimated allelic frequencies at the assumed muscle protein loci in S. longiceps

LOCUS	ALLELES	CENTRES			
		COCHIN	CALICUT	MANGALORE	MANDAPAM
1	S	1.00	1.00	1.00	1.00
2	S	0.500	0.180	0.500	0.813
	F	0.500	0.82	0.500	0.188
3	S	1.00	1.00	1.00	1.00
4	S	0.500	0.500	0.722	1.08
	F	0.500	0.500	0.278	0
5	S	0.500	0.500	0.264	0
	F	0.500	0.500	0.736	1.00

Table 7: **The observed and the expected genotype frequencies (in paranthesis) and the chisquare value at the muscle protein loci in S. longiceps.**

LOCUS	PHENO TYPES	COCHIN		CALICUT		MANGALORE		MANDAPAM	
		GENOTYES	CHISQUARE VALUE	GENOTYES	CHISQUARE VALUE	GENOTYPES	CHISQUARE VALUE	GENOTYPES	CHISQUARE VALUE
1	SS	48 (48)		72 (72)		36 (36)		72 (72)	
2	SS	0 (12)	12)	0 (2.33)	2.33	0 (9)	9)	45 (47.44)	0.125
	FF	0 (12)	12)*	46 (48.41)	0.0995	0 (9)	9)*	0 (2.52)	2.520
	SF	48 (24)	24)	26 (21.25)	0.4470	36 (18)	18)	27 (21.88)	1.198
3	SS	48 (48)		72 (72)		36 (36)		72 (72)	
4	SS	0 (12)	12)	0 (18)	18)	16 (18.75)	0.4033	72 (72)	0.000
	FF	0 (12)	12)*	0 (18)	18)*	0 (2.77)	2.7700	0	0.000
	SF	48 (24)	24)	72 (36)	36)	20 (14.40)	2.178	0	0.000
5	SS	0 (12)	12)	0 (18)	18)	0 (25)	1.811	0 (0.00)	0.000
	FF	0 (12)	24)*	0 (18)	36)*	17 (19.51)	0.323	0 (0.00)	0.000
	SF	48 (24)	12)	72 (36)	18)	19 (13.97)	1.811	72(72.00)	0.000

3.4. DISCUSSION

3.4.1. General Protein

This is for the first time two tissue protein types of oil sardine populations from Cochin, Calicut, Mangalore and Mandapam have been compared in detail. The only previous report on the electrophoretic patterns of general proteins in Sardinella longiceps was concerned with simple differences in the number of eyelens and serum protein bands from a particular region alone, (Rao and Dhulkhed 1976; Dhulkhed and Rao 1976 and Menezes 1976, 1980). Rao and Dhulkhed (1976) observed three and four banded eye lens protein phenotypes in just 10 specimens collected from the region between Malpe and Cannanore. Dhulked and Rao (1976) reported similar four, five and six banded serum protein phenotypes in 23 specimens of S. longiceps collected from a single region, about 15 km. north of Mangalore. Though these phenotype variations in eyelens and serum proteins indicated intraspecies polymorphism, Rao and Dhulkhed (1976) and Dhulkhed and Rao (1976) concluded that these simple variations are indicative of population genetic differences. Menezes (1976) reported 10 eyelens protein components in S. longiceps whereas Menezes (1976) reported a considerably different eye lens protein patterns in S. longiceps from Panaji Goa, where pattern A with seven fractions and pattern B with nine fractions were observed. The details of the number of specimens tested or the number of specimens under each pattern were not given in the report. Menezes (1976) also concluded that seven and nine banded

variation specimens of S. longiceps belonged to genetically different populations. On the otherhand, Menezes (1976) observed seven banded serum protein patterns in all the specimens tested from Panaji Goa on the observations of mere phenotype variations. Their conclusions without proof for genetic variations were misleading (George, 1980).

Inspite of the apparent regional differences in the overall eyelens protein characteristics mentioned in the above discussion, there appears to be certain comparison between the eye lens protein patterns reported by Menezes (1976) and that of the present investigation. The total number of eye lens protein fractions observed in the present investigation were eight and this is closely comparable to seven and nine banded patterns observed by Menezes (1976). On the otherhand, the report of four and five banded eye lens protein patterns in S. longiceps by Rao and Dhulkhed (1976) is significantly different from the patterns reported by Menezes (1976, 1980) and that of the present investigation. These contrasting differences are more pronouncing between the reports of Rao and Dhulkhed (1976) and Menezes (1976), when considering their protein separation medium were identical cellulose. Hence, the first reasonable explanation for the reported differences in the eye lens protein patterns in S. longiceps tested by these different authors may be that these regional populations are only phenotypically heterogenous stocks.

In this context, a comparison of eight banded eye lens protein patterns in S. longiceps tested in the present investigation from Cochin, Calicut, Mangalore and Mandapam

produced very interesting informations. It is surprising to observe the lack of polymorphism at the eye lens protein locus in all the four regions of the present study whereas Dhulkhed and Rao (1976) and Menezes (1976) reported polymorphic patterns even from a single local region. As discussed earlier, a comparison of eyelens proteins from all these different centres using a single standard method can alone solve the problems associated with the contrasting results produced by different authors.

The phenomenon of monomorphism or absence of eye lens protein variations is not uncommon in fishes. Absence of intraspecies eye lens protein variations were also reported in Atlantic mackeral Scomber scombrus L. (Smith and Jamieson, 1978) and Indian mackeral (Menezes 1986). On the otherhand, intraspecies variation in eye lens proteins were reported in some fishes like brook trout Salvelinus fontinalis (Eckroat and Wright 1969), and Mugil cephalus (Vijayakumar 1992).

3.4.2. Muscle protein

The species specific electrophoretic patterns of muscle protein were reported as early as 1953 by Cornell in several fishes. Later the species specificity and biochemical genetic

nature of electrophoretic patterns of muscle protein were reported in many fishes (Tsuyuki et al., 1966).

The genetic inheritance of muscle protein patterns were also reported by interspecific hybridisation experiments (Tsuyuki and Roberts, 1965). The first report of intraspecies muscle protein variation in a marine fish species may be that of Anoplomoma fimbria (Tsuyuki et al., 1965a). In marine fishes like Atlantic Salmon, Salmo salar (Nyman, 1967) and Walleye Pollock (Uthe and Ryder, 1970).

The recent report of intraspecies muscle protein variation in marine fish species were that of Chanos chanos (Winans, 1980) Atlantic eel (Jamieson & Turner 1980) Lates calcarifer (Salini and Shaklee, 1988) and Mugil cephalus (Vijayakumar, 1992).

The present observation and report of intraspecies muscle protein polymorphism in S. longiceps collected from Cochin, Calicut, Mangalore and Mandapam is the first of its kind for the species. The allelic frequencies estimated at three assumed polymorphic loci, namely Pro (M) II, Pro (M) IV and Pro (M) V showed significant differences among the three populations from

the west coast (Cochin, Calicut, Mangalore) as well as between west coast and the east coast (Mandapam) at one or more loci examined. Mandapam population was distinct in having monomorphic phenotypes at Pro (M) IV and V loci whereas the west coast populations were polymorphic at the same loci (Table 6). The presence of one or more fixed alleles in one population and polymorphic nature at the same loci in another population can be considered as a sure sign of their distinct stock structure (Jamieson, 1974). The heterogenous are Cochin and Calicut populations, having allelic frequency differences only at one locus. The above conclusion is also supported by data on enzyme patterns studied by Venkitakrishnan (1995).

Published reports on the application of electrophoretic patterns of muscle protein variation in genetic stock structure analysis are only few. Significant differences in the frequencies of muscle protein alleles were reported in different populations of the pacific hake (Merluccius productus (Utter, 1971) Chanos chanos (Winans, 1980) Lates calcarifer (Salini & Shaklee, 1988). The range of significant differences reported in these populations for the major allele was 0.730 to 0.982, (M. Productus), 0.68 to 1.00 (Chanos chanos) and 0.821 to 1.00 (Lates

calcarifer). The range of allele frequency considered to be significantly different in all these cases which was about 0.25 to 0.30 between the populations to separate them as genetically different stocks. In the present investigation, the range of differences of major alleles in four populations were 0.180 (Calicut) to 0.813 (Mandapam) considering F allele and 0.5 (Mangalore/Cochin) to 0.82 (Calicut) of the second locus considering F allele , 0.5 (Cochin) to 1.0 (Mandapam) considering S allele of the fourth locus and 0.5 (Cochin) to 1.0 (Mandapam) considering F allele of the fifth locus. Compared to the significant allele frequency ranges discussed above in European hake (Utter 1971), Chanos chanos (Winans, 1980) and Lates calcarifer (Salini and Shaklee, 1988) some of the range of allele frequency differences between certain populations of S. longiceps examined here are highly significant (Table 6). However, the question is whether the observed and assumed allelic frequency differences are due to genetic stock differences.

In population genetic analysis, a comparison of observed and expected genotype frequency distribution for the polymorphic loci is usually done for estimating the genetic nature of the polymorphism and also to evaluate the degree of chi-square values and their significance (Ayala and Kiger, 1980). The chi-square values obtained in the present case was highly significant at the second locus in Cochin and Mangalore at fourth and fifth loci in Cochin and Calicut. In all these regions excess of heterozygotes caused highly significant chi-square values. The major reason

for excess of heterozygotes was due to the presence of cent percent heterozygotes in Cochin, Calicut and Mangalore. However, it is interesting to observe non-significant chi-square values for the second locus in Calicut and Mandapam.

It is well known that Hardy-Weinberg equilibrium condition between observed and expected genotype frequencies is expected to occur in a particular population which is not affected by the effects of selection, mutation, migration and genetic drift etc. The occurrence of non equilibrium condition due to excess of homozygotes or heterozygotes is not a rare phenomenon in fishes, crustaceans, and molluscs as reported by many investigators. For example, excess of homozygotes were reported in fishes like anchovies (Dally and Richardson, 1980), milk fish (Winans, 1980), European mackeral (Smith et al., 1981), Sharks (Laveri and Shaklee, 1989), Marine prawns (Philipsamuel, 1987) and marine molluscs (Singh and Green, 1984). Mugil cephalus (Peterson and Shehason, 1971), Newzealand snapper (Smith et al., 1980) lake white fish (Imhoff et al., 1980) Lake trout (Dehring et al., 1981) Atlantic Salmon Salmo salar (Sthal, 1987) and Mugil cephalus (Vijayakumar, 1992). Though it is impossible to point out the exact mechanism that leads to the occurrence of excess of heterozygotes in a population sample, possible reasons for the presence of excess of heterozygotes may be suggested. The phenomenon of heterosis particularly produced by two different strains can lead to the formation of excess of heterozygotes of a particular locus. A comparable heterozygotes advantage present in a population at a biochemical genetic locus

like muscle protein can cause excess of heterozygotes production as observed in the present investigation. It may be due to other reasons like small number of parents during the breeding process (Sthal, 1987) duplication of locus (Stoneking et al., 1981) as referred by Dherring et al., (1981). The role of environmental factors probably affecting genotype of frequency distribution was also suspected. A typical example was the excess of heterozygotes in abyssal populations of the fish Sebastodes alutes at two loci while normal heterozygosity in shallow water populations (Ponniah, 1989). As majority of sixteen polymorphic loci in the same populations of S. longiceps (Venkitakrishnan, 1995) were in Hardy-Weinberg equilibrium condition, the question of small number of parents (Sthal, 1987) or abyssal population effect does not arise in the case of S. longiceps investigated here. Moreover, these loci which produced excess of heterozygotes in a particular population sample also showed normal distribution of different genotypes in another population (Table 6). Therefore, it may be difficult to isolate the probable causes that produced excess of heterozygotes at a particular muscle protein locus in S. longiceps.

The significant deviation from the Hardy-Weinberg equilibrium condition shown by the expected phenotype or the assumed genotypes at the second locus may indicate either a non-Mendalian genotype distribution or some other unknown genetic pattern inherent at this second locus.

Though the sex and maturity of the specimen tested for the general protein phenotype pattern in different populations were not recorded and therefore, the patterns could not be correlated to these two parameters, all the specimens tested were of uniform size range 15.5 to 18.9 cm. and therefore, there is every probability for the occurrence of both sexes in the sample. If the observed two banded patterns were correlated to either sex or size variations, only two banded patterns were expected in Mandapam and Calicut samples. On the other hand, both one and two banded phenotypes were present in these two areas at the second locus. Therefore, the observed differences in the phenotype patterns may not be due to sex or size dependent effects.

In biochemical genetic analysis of population structure, it is mandatory that as many loci as possible are to be compared before genetic discreteness of two populations is considered. In the present investigation the biochemical genetic analysis of S. longiceps was restricted to loci represented by general proteins tested of muscle and eyelens proteins. However, out of these two protein systems muscle protein alone showed polymorphism. Again out of the five loci possibly present in muscle tissue, only three showed polymorphism. The sole reason for restricting the analysis only to two protein system was due to a stricture to be accepted during the allocation of the research problem.

The present biochemical genetic interpretation on the population characteristics of S. longiceps populations compared

from Cochin, Calicut, Mangalore and Mandapam is interestingly comparable with that of morphometric analysis done for the same populations. The principal component analysis indicated the presence of populations with statistically significant morphometric differences of Calicut, Mangalore and Mandapam regions. These details are further discussed in the chapter concerned and general discussion section of the present report.

4. CHAPTER - III

MORPHOMETRICS

4.1. REVIEW OF LITERATURE

The earliest report on study of morphomeristic variability among fish populations was that of Heincke, (1898). He discovered significantly different vertebral counts among the European herring populations examined. Then, studies of Schmidt (1917) suspected the role of the environmental factors on the vertebrate variability as reported by Heincke (1898). Later morphometric variability based on body parts such as length, depth, and width of fish, body and that of head and tail regions were described by Hubbs and Lagler (1947). Meanwhile it was suspected that these measurements were related to the overall growth history of the fish because there appears to be distinct stanzas in the growth history of fishes, hence any comparison should be made within the same life history stanza (Martin, 1949).

Several authors felt that all morphometric and meristic characters also may be influenced by the environment (Dannevig, 1932; 1950; Martin, 1949; Barlow, 1961; Orsaka, 1963; Villwock, 1963; Gross, 1977; Fowler, 1979; Kirpichnikov, 1981; Winans, 1984; Vanderbank and Ferreira, 1986; Meyer, 1987; Currens et al., 1989; Beacham, 1990).

On the other hand the effect of physiological and epigenetic constraints on morphology of fishes of response to certain environmental parameters such as temperature and oxygen are poorly understood (Martin, 1949; Gould, 1977; Reide, 1978; Stanley, 1979; Bock, 1980; Todd et al., 1981).

It was argued that phenotypic variation in these characters has not been directly related to particular differences in the genome (Clayton, 1981). Moreover, morphological characters represent the synergism between shape and size and the level of differentiation between stocks may be more subtle than that commonly seen between most species of fish and is also strongly affected by allometry (Huxley, 1924; Gould, 1966; Sweet, 1980).

Hubbs (1928) felt that some characteristics of the species that lived in a new area would be altered by the environment and adaptive changes would become genetically fixed. On the contrary genetically influenced morphological differences between populations were also reported in non-salmonoids (Barlow, 1961) and salmonoids (Ricker, 1972; Riddell et al., 1981, Taylor and McPhail, 1985; Swain et al., 1991).

The proof of the genetical basis of differentiation of races is based on the so called "quetelets law" which assumes that race is genetically homogenous (Heincke, 1898).

Meanwhile, many morphometric characters like pectoral fin width, head length, pelvic fin height, peduncle height and position of fins were found useful in racial studies of many fish species like Stripped bass Roccus saxatilis (Lund, 1957) Pacific herring. Clupea harengus pallasi (Meng and Stocker, 1984) Coho salmon, Oncorhynchus kisutch (Taylor and McPhail, 1985) Arctic charr Salvelinus alpinus (Pattington and Mills, 1988) Common carp Cyprinus caprio (Corti et al., 1988) Yellow fin tuna Thunnus albacares (Schaefer, 1989) Lake white fish Coregonus clupea

formis (Kristofferson and Clayton, 1990) and Brown trout Salmo trutta (Karakousis et al., 1991). According to Meng and Stocker (1984), the important morphometric characters are pectoralfin width, head measures, pelvic fin height, peduncle height and position of fins.

Though the controversy on the suitability of morphometric and meristic variability as phenotypic markers for racial studies exist, these characters have continued to be used by many workers as potential phenotypic markers for races (Casselman et al., 1981).

In the early period of morphometric studies, comparison of body parts were made on the basis of ratios and statistical techniques, like, least squares, regression analysis and covariance analysis (Saila and Jones, 1983). Mottley (1941) seems to have been among the first to utilize covariance analysis in the comparison of regressions based on morphometrics. One of the exhaustive bivariate morphometric comparisons was made by Hennemuth (1959) on skipjack tuna.

Natural variations in the body shape of fishes/shellfishes have become a strong base for morphometric analysis of populations (Saila and Jones, 1983). Multivariate analysis of the scale shape vectors was applied for stock identification of walleye in Lake Erie (Riley and Carline, 1982).

The truss network analysis of Strauss and Bookstein (1982) describes a geometric protocol for morphometric character selection and systematic coverage of the body form. Siegel (1982) and Siegel and Benson (1982) have described resistant fitting regression techniques better than least square regression.

According to Sokal and Rinkel (1963) geographic variation is not likely due to the adaptation of a few characters to single environmental variable but a multidimentional process involving the adaptation of many characters to a myriad of interdependent environmental factors. Hence a better understanding of morphological variations may be achieved by thoroughly examining the patterns of variance and co-variance among as many characters as possible in a data set using multivariate statistical analysis (Gould and Johnston, 1972; Reyment et al., 1981; Thorpe, 1976; 1983, 1987).

Multivariate analysis of morphometric data sets usually identify size and shape difference among individuals as well as groups. Size is defined not as single character but as a factor that can predict any distance measurement (Humphries et al., 1981). Shape is defined as a specific relationship among characters as described by specific correlations, +, -, or O, between the characters a measure of geometry (Winans, 1987). For most of the stock identification works, shape discriminators were preferred. There are three general approaches for removal of size influence in analysis of shape - ratios, regressions and

multivariate analysis. The division of a character by a measure of size, say fork length will produce a size free measure of that character (Thorpe, 1976). According to Humphries et al. (1981) that these ratios only remove the effect of the one variable, eg: fork length from the measurement. Another possibility for producing size free, shape components is through multivariate analysis such as discriminate function and principal component analysis. The recommendation of Humphries et al., (1981) is to apply principal component analysis, where in individuals are not assigned to prior groups, thus permitting group differences to be discovered.

The significant aspect of morphological characters are that they are polygenetically inherited but individually have low heritability and subjected to considerable environmental plasticity. Thus multivariate analysis of many sets of phenotypic characters are regarded as a more appropriate method than the use of single character for determining the extent to which populations or species may be genetically diverse (Smith and Chesser, 1981). The multivariate methods, like, principal component analysis have been proposed as an efficient tool for stock identification in fishery management programmes (Winans, 1985; McCrimmon and Claytor,1986).

According to Saila and Jones (1983) it is important to recognise that these morphometric measurements are related to the overall growth history of the fish. The morphometric measurements appear to be distinct stanzas in the growth history of fishes

(Martin 1949). According to Corti et al., (1988), the principal component analysis does not require a prior recognition of groups and if there are several groups, data is pooled irrespective of groups. Further the detection of variation between strains of common carp (Cyprinus carpio) suggested that multivariate morphometrics could represent an appropriate and convenient tool to detect variation between stocks. (Cataudella et al., 1987) The method allows the characterisation of samples spanning several life history stages based on a dynamic relation between size and shape. It is important to note that the optional procedure for size adjustment in morphometric analysis is currently a matter of debate (Rohlf and Bookstein 1987; Rohlf, 1990).

Principal component analysis computes a set of uncorrelated composite variables called principal components (PCs) from a variance - covariance (or correlation) matrix (Dunn and Everitt 1982). According to Campbell and Atchley (1981) and Green (1976), geometrically PC-I is thought to lie parallel with the largest axis in the hyperdimensional cloud of data. The PC-II is independent of PC-I and it lies perpendicular to the axis of PC-I. Each PC is linear combination of the variables and is defined by a vector of coefficients and an eigenvalue. Humphries et al., (1981), described and illustrated a multivariable method called shear analysis for removing size from PC scores and vectors. Swain et al., (1991) performed a PC analysis to find out morphological differences between hatchery and wild populations of Coho salmon Oncorhynchus kisutch using sheared principal

component of the correlation matrix of the unadjusted data and led to the same conclusions as the PC analysis of adjusted data. Copeman (1977), Saila and Jones (1983) suggested that the case in which growth differences were observed, only fish with the same age and life history can be used and no correction for length is required.

According to Meng and Stocker (1984) over lapping of nearby samples might be an expression of mixing of different subpopulations.

The application of new multivariate morphometrics for fish stock identification will probably undergo a new upsurge since many of the previous limitations of bivariate and even multivariate methodologies have been eliminated in the new techniques. In short, the improved method of morphometric studies and analysis should help one to draw a better conclusion than ever before on the population structure of the species (Saila and Jones, 1983).

Considering the efficiency of the multivariate methods in the analysis of the inherent polygenically expressed morphological variability, the method was also applied in the present investigation. The details of the morphometrics investigated and the results obtained are given in the following section of the thesis.

4.2. MATERIALS AND METHODS

The samples, of S. longiceps were collected from Cochin, Calicut, Mangalore and Mandapam (Fig. 1). The morphometric and meristic data were collected according to the guidelines given by Anon (1960). The twentyfive parameters measured are given below: (Fig.7).

1. Dorsal normal length LN
2. Ventral normal length LN^1
3. Midcaudal length LF
4. Standard length LS
5. Body length LB
6. Maxillary sheath length UJ
7. Mandibular length LJ^1
8. Snout length UO
9. Orbital diameter OO^1
10. Pre-anterial dorsal distance UD
11. Pre-pectoral distance UP
12. Pre-ventral distance UV
13. Dorsal fin base length DD^1
14. Pre-anal distance UA
15. Anal fin base length AA^1
16. Pectoral fin length Ph
17. Anal fin height Ah
18. Dorsal caudal fin length Ch
19. Ventral caudal fin length Ch^1
20. Length of the head LG

21. Pre-dorsal distance LD
22. Pre-pectoral distance LP
23. Pre-ventral distance LV
24. Pre-anal distance LA
25. Weight of fish WT.

The four morphomeristic characters studied were number of dorsal fin rays, number of anal fin rays, number of ventral fin rays and number of pectoral fin rays.

A total of 306 fishes representing the 4 sampling stations were measured and the morphometric data collected were analysed statistically using correlation coefficient and principal component analysis.

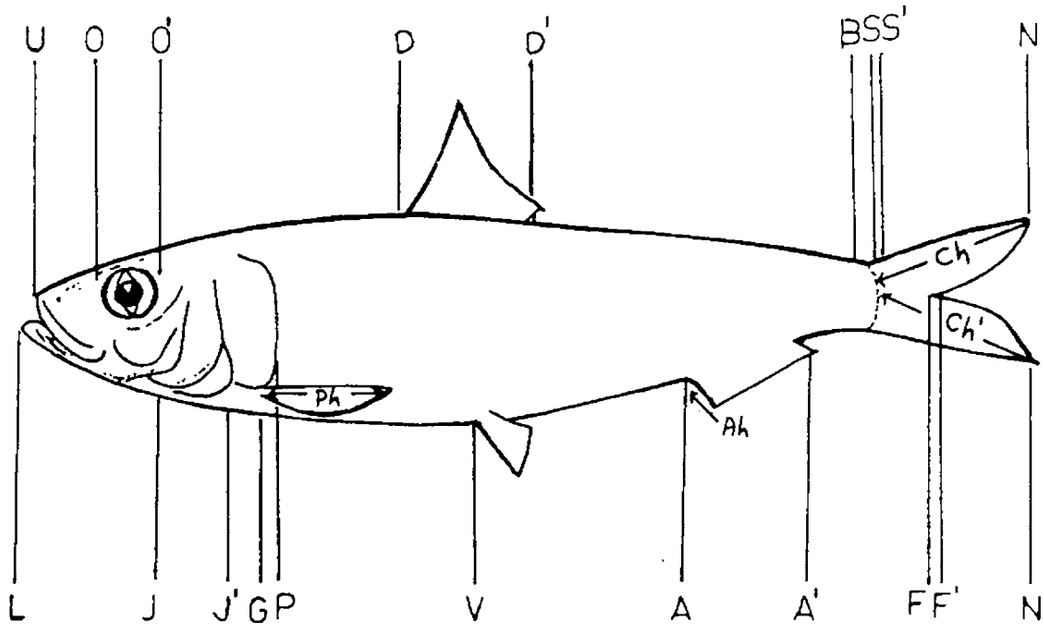
Principal component analysis was done according to the method described by Winans (1984). The analytical steps involved in the programme are as follows:

1. Correlation matrix
2. Principal component analysis
3. Principal scores
4. Plots of scores I Vs II
5. ANOVA for each components.

Correlations between the 25 characters were computed using the correlation matrix of the pooled data for all the centres, then principal component analysis was carried out. Since the first 3 PC's explain a major portion of the variations among

subjects with regard to the characters considered these 3 PC's were used for further analysis. The PC's scores were computed for each subject by summing the products of each character value with corresponding PC coefficients. To see the discrimination, the PCI score was plotted against the PC II score. Individual one way ANOVA was then carried out to see the differences between centres independently with each PC scores.

Fig.7. Morphometric characters and acronyms used for S. longiceps stock separation analysis.



1. Dorsal normal length LN
2. Ventral normal length LN^1
3. Mid-caudal length LF
4. Standard length LS
5. Body length LB
6. Maxillary sheath length UJ
7. Mandibular length LJ^1
8. Snout length UO
9. Orbital diameter OO^1
10. Pre-anterial dorsal distance UD
11. Pre-pectoral distance UD
12. Pre-ventral distance UV
13. Dorsal fin base length DD1
14. Pre-anal distance UA
15. Anal fin base length AA^1
16. Pectoral fin length Ph
17. Anal fin height Ah
18. Dorsal caudal fin length Ch
19. Ventral caudal fin length Ch^1
20. Length of the head LG
21. Pre-dorsal distance LD
22. pre-pectoral distance LP
23. Pre-ventral distance LV
24. Pre-anal distance LA
25. Weight of fish WT

4.3. RESULTS

4.3.1. Morphometrics

Table 8 shows the mean and standard deviations of morphometric characters in four sample populations of *S. longiceps* and Table 9 explains the correlation matrix of morphometric characters. These values were further transformed into three principal component values (PCs) (Table 10). Using the correlation matrix of the pooled data for all the four centres, Principal component analysis was carried out. Since the first three principal components explained about 85% of total variability existing between individuals and samples, further analyses were restricted to these 3 PCs. The PC scores were computed for each of the 25 character value with corresponding PC coefficient (Table 11). Scores of PC-I were then plotted against the PC-II to project the possible discrimination graphically (Fig. 8). No clear discrimination existed among the four centres as a whole. Therefore, individual one-way analysis of variance (ANOVA) was carried out to bring out inherent discrimination between particular centres like Cochin and Calicut, Cochin and Mangalore, Cochin and Mandapam, Calicut and Mangalore and also Calicut and Mandapam with reference to PC score. The results of the ANOVA are given in Tables 12, 13 and 14 .

The first one way ANOVA analysis explained 49% of the total variability of 25 morphometric characters existing among four sampling centres (Table 12). The results of the second one way ANOVA analysis explained another 20% of the total variability

(Table 13). Similarly, results of third one way ANOVA analysis explained another 15% of the total variability (Table 14). The degree of variability produced by these second and third ANOVA analysis were significant among all the four centres tested except between Calicut and Mandapam. In short, morphological characters of Cochin population differed significantly from that of other three populations whereas Calicut populations differed significantly only from that of Cochin and Mangalore population. Mangalore population also differed significantly from that of other three populations. On the contrary, it is interesting to note that the oil sardine populations from Calicut and Mandapam appeared to be morphologically similar. These considerable morphological differences as well as lack of difference between the centres were also reflected in the minimum - maximum PC scores computed for each centres as shown in the Table 11. The Minimum - maximum values of PC scores between Calicut and Mandapam were more closely comparable than between any other two centres.

4.3.2. **Meristics:**

The number of rays of fins were counted in samples of oil sardine from Cochin, Calicut, Mangalore and Mandapam. The number of rays were similar in all the four centres (Table 15).

Table 8. Mean and standard deviation of Morphometric characters of *Sardinella longiceps* collected from difrerent centres (in cms).

	Characters	Cochin (N=84)	Calicut (N=72)	Mangalore (N=79)	Mandapam (N=71)
1.	Dorsal normal length (LN)	14.43 (2.015)	15.69 (1.078)	16.73 (1.331)	15.82 (0.981)
2.	Ventral normal length (LN)	14.58 (1.998)	15.78 (1.062)	16.71 (1.364)	16.59 (1.680)
3.	Mid caudal length (LF)	12.65 (1.816)	14.11 (0.936)	15.13 (1.213)	14.18 (0.883)
4.	Standard length (LS)	12.42 (1.763)	13.40 (0.860)	14.23 (1.384)	13.86 (1.403)
5.	Body length (LB)	11.76 (1.672)	12.84 (0.859)	13.76 (1.108)	13.43 (1.262)
6.	Maxillary sheath length(UJ)	2.12 (0.256)	2.16 (0.117)	2.27 (0.171)	2.23 (0.511)
7.	Mandibular length (LJ)	3.59 (0.566)	3.92 (0.252)	4.13 (0.391)	4.01 (0.419)
8.	Snout length (UO)	1.53 (0.183)	1.25 (0.106)	1.41 (0.306)	1.21 (0.121)
9.	Orbital diameter (OO)	0.80 (0.115)	0.85 (0.597)	0.92 (0.125)	0.87 (0.062)
10.	Pre-anterior dorsal distance (UD)	5.83 (0.592)	6.27 (0.467)	6.76 (0.616)	6.42 (0.489)
11.	Pre-pectoral distance (UP	3.84 (0.592)	4.14 (0.301)	4.14 (0.672)	4.43 (0.390)
12.	Pre-ventral distance (UV)	6.97 (1.004)	7.53 (0.490)	8.01 (1.06)	8.15 (0.839)
13.	Dorsalfin base length (DD^1)	1.68 (0.259)	1.76 (0.176)	1.96 (0.207)	2.01 (0.175)
14.	Pre-anal distance (UA)	9.67 (1.411)	10.52 (0.772)	11.42 (1.039)	10.27 (0.705)
15.	Analfin base length (AA^1)	1.56 (0.250)	1.81 (0.210)	1.94 (0.309)	1.74 (0.179)
16.	Pectoral fin length (Ph)	1.99 (0.250)	2.14 (0.04)	2.23 (0.187)	2.15 (0.132)
17.	Anal fin height (Ah)	0.86 (0.195)	0.86 (0.914)	0.97 (0.894)	0.84 (0.059)
18.	Dorsal caudal fin length (Ch)	2.77 (0.481)	3.00 (0.310)	3.16 (0.346)	3.08 (0.236)
19.	Ventral caudal fin length (C^1)	3.26 (0.304)	3.17 (0.303)	3.48 (0.391)	3.30 (0.325)
20.	Length of the head (LG)	4.46 (0.406)	4.43 (0.249)	5.21 (0.878)	5.12 (0.998)
21.	Pre-dorsal distance(LD)	5.90 (0.926)	6.55 (0.419)	6.93 (0.684)	6.70 (0.497)
22.	Pre-pectoral distance (LP)	4.02 (0.679)	4.30 (0.254)	4.61 (0.584)	5.07 (0.461)
23.	Pre-ventral distance(LV)	7.14 (0.989)	7.66 (0.492)	8.27 (0.700)	7.98 (0.541)
24.	Pre-anal distance (LA)	10.63 (0.921)	10.70 (0.649)	11.16 (1.893)	10.23 (0.677)
25.	Weight of fish (Wt)(gms)	28.79 (2.762)	32.66 (7.209)	40.11 (8.666)	29.83 (4.527)

Table 9 Correlation Matrix of Morphometric Characters of Sardinella longiceps from 4 Centres - COCHIN, CALICUT, MANGALORE and MANDAPAM

Morphometric Characters	1 LN	2 LN'	3 LF	4 LS	5 LB	6 UJ	7 LJ'	8 UO	9 OO'	10 UD	11 UP	12 UV	13 DD'	14 UA	15 AA'	16 Ph	17 Ah	18 Ch'	19 Ch	20 LG	21 LD	22 LP	23 LV	24 LA	25 Wt
1. LN	1.000	0.998*	0.970*	0.994*	0.924	-0.612*	-0.182	-0.748*	-0.806*	0.164	-0.376*	-0.579*	-0.801*	-0.079	-0.804*	-0.565*	-0.651*	-0.278*	0.838*	0.369*	0.609*	-0.118	0.679*	0.630*	-0.183
2. LN'		1.000	0.967*	0.992*	0.918*	-0.641*	-0.170	-0.778*	-0.825*	0.199	-0.428*	-0.591*	-0.806*	-0.086	-0.813*	-0.531*	-0.680*	0.301*	0.838*	0.324*	0.616*	-0.156	0.703*	0.598*	-0.164
3. LF			1.000	0.967*	0.905*	-0.688*	-0.148	-0.725*	-0.765*	0.178	-0.320*	-0.525*	-0.795*	-0.005	-0.806*	-0.581*	-0.626*	0.280*	0.815*	0.428*	0.609*	-0.099	0.659*	0.664*	-0.248
4. LS				1.000	0.932*	-0.577*	-0.126	-0.712*	-0.775*	0.209	-0.351*	-0.524*	-0.768*	-0.022	-0.766*	-0.528*	-0.619*	0.293*	0.842*	0.382*	0.649*	-0.081	0.699*	0.646*	-0.170
5. LB					1.000	-0.551*	-0.022	-0.616*	-0.733*	0.220	-0.299*	-0.407*	-0.737*	0.089	-0.690*	-0.486*	-0.585*	0.268*	0.753*	0.363*	0.658*	-0.090	0.610*	0.690*	-0.268*
6. UJ						1.000	0.242	0.864*	0.800*	-0.155	0.699*	0.646*	0.782*	0.209	-0.818*	0.340*	0.809*	-0.304*	-0.468*	0.070	-0.258*	-0.580	-0.437*	-0.189	0.244*
7. LJ'							1.000	0.394*	0.416*	0.756*	0.159	0.741*	0.481*	0.704*	0.532*	0.698*	0.300*	0.373*	-0.069	-0.069	0.469*	0.339	0.283*	-0.026	0.267*
8. UO								1.000	0.949*	-0.093	0.826*	0.846*	0.860*	0.429*	0.902*	0.404*	0.899*	-0.259*	-0.593*	0.129	-0.263*	0.618	-0.525*	-0.161	0.125
9. OO									1.000	0.014	0.735*	0.852*	0.929*	0.418*	0.918*	0.539*	0.890*	-0.185	-0.604*	0.037	-0.277*	0.572	-0.447*	-0.311*	9.248*
10. UD										1.000	-0.305*	0.382*	0.164	0.540*	0.165	0.670*	-0.097	0.516*	0.244*	-0.209	0.680*	0.016	0.663*	0.030	0.342*
11. UP											1.000	0.612	0.557*	0.342*	0.592*	-0.092	0.808*	-0.336*	-0.296*	0.550*	-0.168	0.793	-0.449*	0.230	-0.064
12. UV												1.000	0.800*	0.762*	0.837*	0.660*	0.735*	0.060	-0.409*	0.066	0.140	0.548	-0.137	-0.083	0.172
13. DD'													1.000	0.337*	0.950*	0.686*	0.814*	-0.135	-0.594*	-0.153	-0.254*	0.476	-0.366*	-0.441*	0.399*
14. UA														1.000	0.355*	0.443*	0.250*	0.298*	-0.028	0 193	0.568*	0.315	0.219*	0.324*	-0.149
15. AA'															1.000	0.680*	0.840*	-0.157	-0.610*	-0.152	-0.250*	0.499	-0.417*	-0.400*	0.366*
16. Ph																1.000	0.319*	0.263*	-0.382*	-0.533*	0.107	0.012	0.088	-0.543*	0.456*
17. Ah																	1.000	-0.373*	-0.467*	0 213	-0.296*	0.705	-0.420*	-0.209	0.275*
18. Ch																		1.000	0.331*	-0.155	0.394*	-0.010	0.429*	0.013	0.282*
19. Ch'																			1.000	0.348*	0.584*	-0.031	0.685*	0.497*	-0.058
20. LG																				1.000	0.310*	0.471	0.186	0.645*	-0.349*
21. LD																					1.000	0.013	0.827*	0.547*	-0.159
22. LP																						1.000	-0.131	0.162	-0.355*
23. LV																							1.000	0.303*	0.066
24. LA																								1.000	-0.505*
25. Wt																									1.000

0.01247 Significant at 5% level; 0.2414 Significant 1%(*)

1. LN : Dorsal normal length; 2. LN': Ventral normal length; 3. LF : Mid caudal length; 4. LS : Standard Length; 5. LB : Body length;
6. UJ : Maxillary sheath length; 7. LJ' : Mandibular length; 8. UO : Snout length; 9. OO' :Orbital diameter; 10. UD : Pre-anterior dorsal distance;
11. UP : Pre-pectoral distance; 12. UV : Pre-ventral distance; 13. DD' : Dorsal Fin base length; 14. UA : Pre-anal distance; 15. AA' : Anal fin height;
16. Ph : Pectoral fin length; 17. Ah : Anal fin height; 18. Ch : Dorsal Caudal finlength; 19. Ch' : Ventral Caudal fin length; 20. LG : Length of the head;
21. LD : Pre-dorsal distance; 22. LP : Pre-pectoral distance; 23. LV : Pre-ventral distance; 24. LA : Pre-anal distance; 25. Wt : Weight of fish

Table 10. **Principal component values (PC I, PC II and PC III) of morphometric characters of Sardinella longiceps collected from 4 centres (1. Cochin, 2. Calicut; 3. Mangalore; 4. Mandapam)**

	Characters	PC I	PC II	PC III
1.	Dorsal normal length	0.268	0.107	0.084
2.	Ventral normal length	0.271	0.105	0.053
3.	Mid caudal length	0.264	0.119	0.103
4.	Standard length	0.262	0.135	0.085
5.	Body length	0.244	0.157	0.094
6.	Maxillary sheath length	0.229	0.064	0.151
7.	Mandibular length	0.093	0.361	0.155
8.	Snout length	0.259	0.105	0.162
9.	Orbital diameter	0.269	0.107	0.076
10.	Pre-anterior dorsal distance	0.023	0.343	0.302
11.	Pre-pectoral distance	0.171	0.106	0.385
12.	Pre-ventral distance	0.214	0.279	0.019
13.	Dorsal fin base length	0.265	0.108	0.038
14.	Pre-anal distance	0.065	0.362	0.021
15.	Anal fin base length	0.268	0.116	0.020
16.	Pectoral fin length	0.166	0.180	0.354
17.	Anal fin height	0.240	0.089	0.187
18.	Dorsal caudal fin length	0.076	0.202	0.239
19.	Ventral caudal fin length	0.220	0.150	0.054
20.	Length of the head	0.057	0.143	0.422
21.	Pre-dorsal distance	0.139	0.357	0.028
22.	Pre-pectoral distance	0.119	0.212	0.272
23.	Pre-ventral distance	0.176	0.264	0.136
24.	Pre-anal distance	0.146	0.172	0.320
25.	Weight of fish	0.084	0.068	0.221

Table 11. The minimum-maximum Principal components scores of morphometric Characters of Sardinella longicips collected from different centres

Characters		PC I	PC II	PC III
COCHIN	Min.	19.33	17.90	14.23
	Max.	42.60	36.79	32.55
CALICUT	Min.	26.53	23.84	22.49
	Max.	38.21	34.33	31.63
MANGALORE	Min.	25.89	23.27	21.44
	Max.	42.24	37.96	34.97
MANDAPAM	Min.	26.04	24.99	23.03
	Max.	37.32	34.33	31.76

Figure 8. Scatter plots from principal component analysis on morphometric measurements from four samples of *Sardinella longiceps*

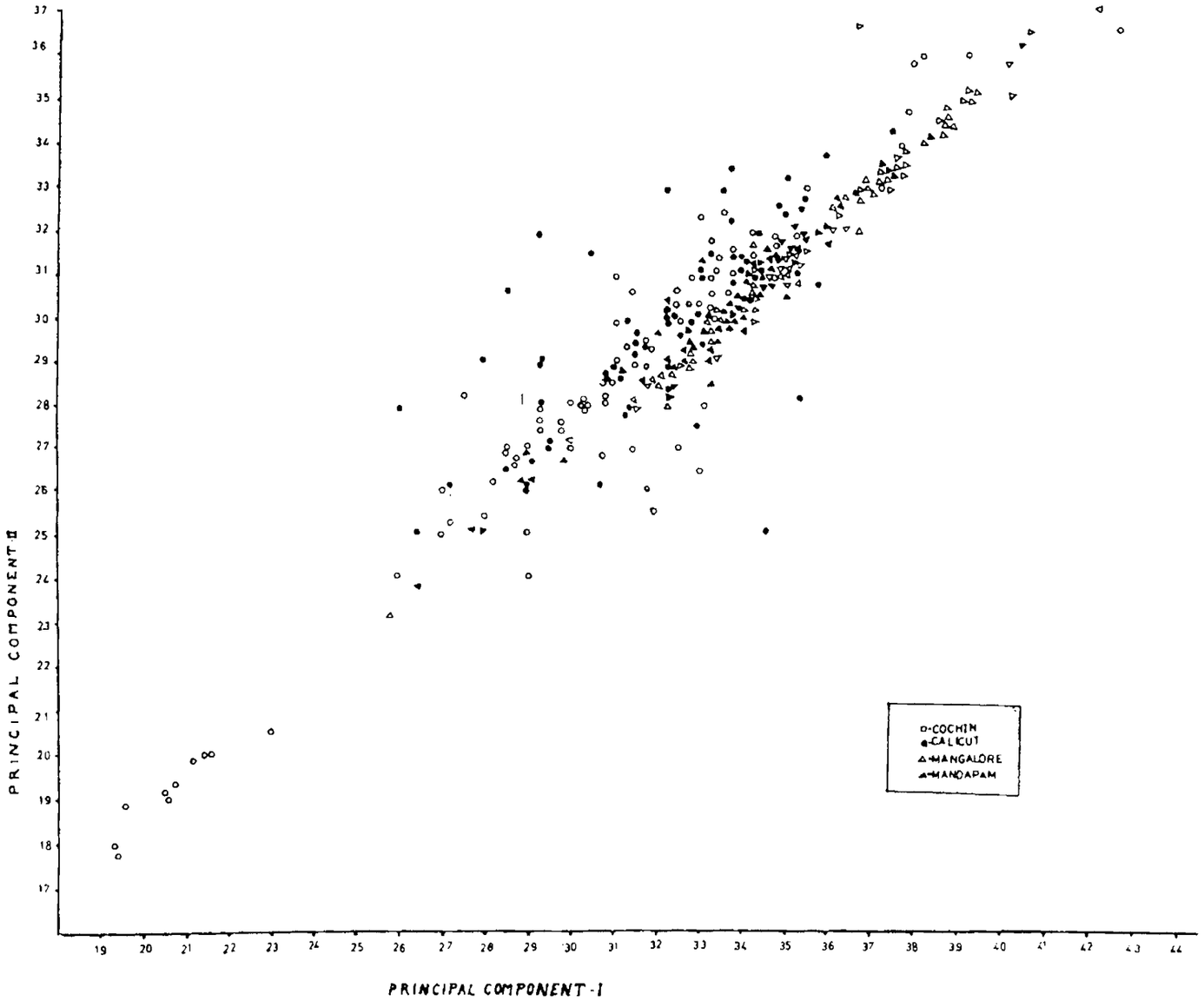


Table 12: First Principal component scores of 4 centres computed based on 25 morphometric characters. Centres are 1. COCHIN 2. CALICUT 3. MANGALORE and 4. MANDAPAM respectively. Total variance explained by this PC component is 49% of the total variability.

RESULTS OF ONE-WAY ANOVA WITH EQUAL/UNEQUAL REPLICATIONS

Grand mean 32.832
No. of treatments 4

Treatment means:

Tr.No.	Mean
1.	30.586
2.	31.939
3.	35.427
4.	33.499

ANOVA

SOURCE	DF	SS	MS	F
TREATMENTS	3	1045.344	348.448	33.45
ERROR	303	3156.500	10.417	
TOTAL	306	4201.844		

Treatments 1 and 3 differ significantly (SE = 0.506)

Treatments 1 and 4 differ significantly (SE = 0.518)

Treatments 2 and 3 differ significantly (SE = 0.526)

Table 13: **Second Principal component scores of 4 centres computed based on 25 morphometric characters. Centres are 1. COCHIN 2. CALICUT 3. MANGALORE and 4. MANDAPAM respectively. Total variance explained by this PC component is 20% of the total variability.**

RESULTS OF ONE-WAY ANOVA WITH EQUAL/UNEQUAL REPLICATIONS

Grand mean : 29.950
No. of treatments: 4

Treatment means:

Tr.No.	Mean
1	28.136
2	29.721
3	31.904
4	30.149

ANOVA

SOURCE	DF	SS	MS	F
TREATMENTS	3	584.500	194.833	21.46
ERROR	303	2751.000	9.079	
TOTAL	306	3335.500		

Treatments 1 and 2 differ significantly (SE = 0.484)

Treatments 1 and 3 differ significantly (SE = 0.472)

Treatments 1 and 4 differ significantly (SE = 0.484)

Treatments 2 and 3 differ significantly (SE = 0.491)

Treatments 3 and 4 differ significantly (SE = 0.491)

Table 14: Third Principal component scores of 4 centres computed based on 25 morphometric characters. Centres are 1. COCHIN 2. CALICUT 3. MANGALORE and 4. MANDAPAM respectively. Total variance explained by this PC component is 15% of the total variability.

RESULTS OF ONE-WAY ANOVA WITH EQUAL/UNEQUAL

Grand mean : 27.390
No. of treatments: 4

Treatment means:

Tr.No.	Mean
1	25.334
2	27.508
3	29.264
4	27.612

ANOVA

SOURCE	DF	SS	MS	F
TREATMENTS	3	636.891	212.297	21.93
ERROR	303	2933.641	9.682	
TOTAL	306	3570.531		

Treatments 1 and 2 differ significantly (SE = 0.500)

Treatments 1 and 3 differ significantly (SE = 0.488)

Treatments 1 and 4 differ significantly (SE = 0.500)

Treatments 2 and 3 differ significantly (SE = 0.507)

Treatments 3 and 4 differ significantly (SE = 0.507)

Table 15: Morphomeristic details of S. longiceps collected from Cochin, Calicut, Mangalore and Mandapam

Meristic characters	COCHIN	CALICUT	MANGALORE	MANDAPAM
Dorsal fin rays	11	11	11	11
Ventral fin rays	9	9	9	9
Pectoral fin rays	17	17	17	17
Anal fin rays	16	16	16	16

4.4. DISCUSSION

The application of intraspecific morphomeristic, phenotypic variability in the identification of races, stocks or genetically meaningful management units in fish populations was started as early as in the late 19th century when Heincke (1898) reported different local races of European herring on the basis of differences in vertebral count. Similarly, the hypothesis that fish species may be naturally broken up into genetically isolated groups or races was put forward at the very early stage of the Counsel Permanent International Pour Exploration de la Mer Meeting (Heincke, 1902).

However, studies of Schmidt (1917) on herrings suspected that the local variation in vertebral number was induced by the environment. He suggested that the appropriate tool would have to be one that was not affected by the environment, and that the idea of local phenotypic races may be abandoned in favour of a much larger genetic unit. Several authors felt that morphometric and meristic characters in fishes seem to be influenced by the environment (Dannevig, 1932; 1950; Martin, 1949; Barlow, 1961; Orska, 1963; Villwock, 1963; Gross, 1977; Fowler, 1979; Kirpichnikov, 1981; Winans, 1984; Vanderbank and Ferreira, 1986; Meyer 1987; Currens et al., 1989; Beacham, 1990). Others favoured the usefulness of statistical properties of phenotypic frequencies as a reliable guide to understand the biology, particularly, about population genetics of organisms. Thus, an unsettled difference was generated between biologists and

statisticians (Province, 1971). Proof of the genetical basis of different races was based on the so called "Quetelets law" which assumed that (Heincke, 1898) a race is a genetically homogenous group of populations of the species. On the contrary several marine species were divided into heterogenous groups that spawn at different times and places and which also showed morphological and ecological differences.

However, the merit of morphometric and meristic characters in stock identification, was doubted by the fact that phenotypic variation is not directly related to particular differences in the genome (Clayton, 1981). Moreover, the effects of physiological and epigenetic constraints on morphology in response to certain environmental parametre such as temperature and oxygen are poorly understood (Martin, 1949, Gould, 1977, Reide, 1978, Stanley, 1979, Bock, 1980, Todd et al., 1981). Though the controversy on the suitability of morphometric and meristic variability as phenotypic markers for racial studies existed, these characters continued to be used by many works as phenotypic markers for races (Casselman et al., 1981). In this background the opinion of Hubbs (1928) is worth considering. As an extension of the idea of Baldwin (1896), Hubbs suggested that species new to an area would be altered by the environment and that differences being associated with adaptive changes would become genetically fixed. Meanwhile genetically influenced morphological differences between populations were reported in salmonoids (Ricker, 1972; Riddell et al., 1981; Tayler and

Mc Phail, 1985, Swain et al., 1991) and nonsalmanoids (Barlow, 1961; McPhail, 1984).

According to the perspective of Sokal and Rinkel (1963) geographic variation is not likely due to adaptation of a few characters to a single environmental variable but a multidimentional process involving the adaptation of many characters to a myriad of interdependent environmental factors. Thus a better understanding of morphological variations may be achieved by thoroughly examining the patterns of variance and co-variance among as many characters as possible in a data set using multivariate statistics (Gould and Johnston, 1972; Reyment et al., 1981; Thorpe, 1976, 1983,1987). Multivariate analysis of morphometric data sets usually identify size and shape difference among individuals as well as groups. Size is defined not as single character but as a factor that can predict any distance measurement (Humphries et al., 1981). Shape is defined as a specific relationship among characters as described by specific correlations, +,-, or o, between the characters-a measure of geometry (Winans 1987). For most of the stock identification works, shape discriminators are preferred. There are three general approaches for removal of size influence in analysis of shape, ratios, regressions and multivariate analysis. The division of a character by a measure of size, say fork length will produce a size free measure of that character.

According to Humphries et al., (1981) that these ratios only remove the effect of the one variable, eg: fork length from the

measurement. Another possibility for producing size free shape components is through multivariate analysis such as discriminate function and principal component analysis. Moreover,principal component coefficients are essentially the co-variance of the measurement on the component axis, and are thus amenable to biological interpretation. Thus multivariate analyses of sets of phenotypic characters are regarded as a more appropriate method than the use of single character for determining the extent to which populations or species may be genetically diverse (Smith and Chesser, 1981). The multivariate methods like principal component analysis was proposed as an efficient tool for stock identification in management programmes (Winans 1985, McCrimmon and Claytor, 1986).

In the present study twenty five morphometric measurements were statistically compared for discriminating heterogenous populations of Sardinella longiceps distributed in the east and west coast of India. In the past, most of the morphometric investigations were based on the selection of characters as described primarily by Hubbs and Lagler (1947). In the recent past few morphometric characters have been described that are useful to racial studies in clupeoids (Meng and Stocker, 1984). In these traditional morphometric methods length, depth and width of fish shape, primarily, of the head and tail regions are measured. According to Meng and Stocker (1984) the important characters are pectoral width, head measures, pelvic fin height, peduncle height and position of fins. Hence, in the present

study sufficient number of such desirable characters were included to derive maximum shape differences between populations.

The positive aspect of morphometrics is that the basic mechanism of the expression of the phenotypes is controlled by polygenes (Ayala and Kiger, 1980). Hence, depending on the degree of variability and its statistical significance the observable phenotypic variations between individual populations can be interpreted as due to genetic factors to considerable extent. Thus a large number of investigators have utilised morphometric and meristic variability of different species including fishes for identification of racial and sub species differences within a species. Major examples are fish species like Arctic charr, Salvelinus alpinus (Partington and Mills, 1988), stripped bass, Roccus saxatilis (Lund, 1957), Pacific herring, Clupea harengus pallasi (Meng and Stocker 1984) coho salmon Oncorhynchus kistich (Taylor and Mcphail 1985) common carp, Cyprinus carpio (Corti et al., 1988).

Harris (1975) suggested that if the number of the individuals minus the number of the variables used is greater than thirty then the sample can be considered as large. In this respect the number of specimens (71-84) used in this study for each sample was adequate for stock identification purpose.

According to Saila and Jones (1983) it is important to recognise that these measurements are related to the overall growth history of the fish, because there appears to be distinct stanzas in the growth history of fishes (Martin, 1949). In

addition to this morphometric measurements taken from a sample of fish are strongly dependent on size fluctuations due to the age distributions of the sample. Taking all these into consideration,as recommended by Saila and Jones(1983) and Winans (1987) multivariate morphometric analysis specifically the principal component analysis used in the present investigation was the appropriate method to be followed to achieve the objective fully.

According to Corti et al. (1988) principal component analysis does not require a prior recognition of groups and if there are several groups, data are pooled irrespective of groups. As a result of their detection of variation between strains of common carp Cyprinus carpio they suggested that multivariate morphometrics could represent an appropriate and convenient tool to detect variation between stocks as this method allows the characterisation of samples spanning several life history stages based on a dynamic relation between size and shape.

In this context, it is important to note that the optimal procedure for size adjustment in morphometric analysis is currently a matter of debate (Rohlf and Bookstein, 1987, Rohlf, 1990) Swain et al.,(1991) performed a PC analysis to find out morphological differences between hatchery and wild populations of coho salmon (Oncorhynchus kisutch) using sheared principal component of the correlation matrix of the unadjusted data and led to the same conclusions as the PC analysis of the adjusted data.

The PC analysis computes a set of uncorrelated composite variables called principal components from a variance, covariance or correlation matrix (Dunn and Everitt 1982). In the present investigation 84% of the total variability was explained. Thus the Ist, IInd and IIIrd PC's explained 49%, 20% and 15% respectively giving the total 84% variability. PC I is a size related component where the ventral normal length, orbital diameter, dorsal normal length, anal fin base length, dorsal fin base length and mid caudal length largely contributed to the variability (sequence according to significance Table - 10). In PC II, characters like preanal distance, mandibular length, predorsal distance, pre-anterior dorsal distance and pre ventral distance had largest coefficients. In PC III, characters like length of head, pre pectoral distance, pectoral fin length and pre anal distance had largest coefficients. These characters heavily contributed to the distinctive fusiform morphology of S.longiceps and may represent an adaptation to prolonged swimming and thus it is genetically determined and inherited. Several studies involving salmonoids, have also noted the correlation between a fusiform body and either prolonged swimming performance or migration distance (Fessler and Wagner, 1969; Vanstone and Markert, 1968, Thomas and Dohahoo, 1977, Swain et al., 1991). Using these three PC coefficient values PC scores were calculated for each individual by summing up the products of each character value with corresponding PC coefficients and this helped to discriminate the samples in an efficient way (Winans

1987). Scatter plots of PC I, PC II, and PC III scores showed varying amounts of overlapping between samples (Fig. 8).

According to Meng and Stocker (1984) overlapping of nearby samples might be an expression of mixing of different sub populations. However for finer resolution between the samples, individual one way ANOVA analysis was carried out to see the difference between centres independently with each PC score. Results showed significant differences between the centres (Table, 12, 13, 14). Morphologically Cochin population differed from Calicut, Mangalore and Mandapam. Calicut differed significantly from Cochin and Mangalore. Mangalore sample also differed significantly from that of Calicut, Cochin and Mandapam.

The present findings strongly support the earlier view of Devanesan and Chidambaram (1943), that oil sardine populations from Karwar and Malabar are morphologically different stocks. Later comparable conclusions were drawn based on vertebral counts (Annigeri, 1978) and head/body length (Prabhu and Dhulkhed, 1972). On the contrary, what is unusual about the results is that the most distinct populations (Mangalore and Mandapam or Calicut and Mandapam) were morphologically similar while closest places like Calicut and Mangalore were morphologically dissimilar. Similar phenomenon in the threadfin Nemipterus japonicus (Rao and Rao, 1983) and in other marine teleosts (Richardson, 1983; Grant, 1984; and Smith, 1986) were reported. Whether these phenomena are consequences of comparable environmental selection pressure are not known. On the

otherhand, the principal component analysis method applied in the present investigation should have reduced the effect of such non-genetic factors to the minimum possible level (Winans, 1985). If the observed significant phenotype variation were due to environmental factors, similar effect at least to certain extent should have caused variations in the meristic counts (Fin rays) of the species. The present study showed identical meristies in the species (Table 15).

Though certain modern techniques like gel electrophoresis has become popular for discovering variation at molecular level, variability in large number of morphometric characteristics has its own important role in corroborating the results of the modern techniques and vice versa (Cushing 1981). The above conclusion of morphometric heterogenity was also supported by cytogenetic and biochemical genetic methods applied in the present investigation. On the contrary, the morphometric (Mangalore/ Mandapam or Calicut/Mandapam) cytogenetic (Cochin/Mangalore or Calicut/Mandapam) homogenity of oil sardine populations could not be supported by the results of biochemical genetic techniques applied by Venkitakrishnan (1995) where the same populations were found to be heterogenous in nature.

The present findings of morphometric heterogenity/homogenity based on 25 morphometrics and PC analysis on the four oil sardine populations are the first of its kind.

5. GENERAL DISCUSSION

The species, Sardinella longiceps, popularly known as the Indian oil sardine contributes significantly to the commercial marine fish catches of India. A major management problem of its fishery is the short and long term fluctuations in its annual abundance. Though its biology and fishery aspects have been thoroughly investigated, the basic reason for the fluctuations in its abundance has not been established. The fishery is exploited as a single stock fishery, vis., it is composed of interbreeding populations distributed over south west-east coast of India. On the other hand, it may be assumed that the fishery is naturally composed of regionally or genetically heterogenous populations. In fact, detailed knowledge on the population genetic composition of the species is essential to identify the natural management units of its fishery. None of the previous investigations had attempted to study the population genetic structure of the species except the recent study by Venkitakrishnan, (1995), which was restricted to biochemical genetic variations of enzymes. In this context, the present investigation on the population genetics of the species using three other different aspects such as cytogenetics, non-enzymatic proteins and morphometrics is a very valuable contribution towards understanding the current population genetic structure of the species. However, a general discussion on the available informations on the biology and fishery of the oil sardine is essential to draw appropriate conclusions from the present investigation.

The recent past history of the oil sardine catch statistics show wide fluctuations in its abundance. A comparison of the catches of S. longiceps in Kerala and Karnataka areas showed wide fluctuations during ten year period (1969-1978). The probable reason for the fluctuations of the catch in a particular year between these two centres may be interpreted in different ways. The first one is based on higher catch in one region correlated to lower catch in the other region or vice versa, particularly when the fishery is supported by a single stock. This interpretation is purely based on the possibility of migration of S. longiceps from one region to other on account of feeding habit or some other environmental or other unknown factors influencing its populations for the inter-regional migration. (Hornell, 1910; Banerjee 1967). According to Nair (1953), the suspected causes for regional fluctuations in its abundance may be much stronger than the annual migration and fishing effort. If it is due to migration, the catch of one region towards which the population had migrated will naturally show much higher catch whereas, correspondingly lower catch at the region where from it was thought to have migrated. For example, the table I of MFIS No. 14 (1979) showed a catch of 1,91,683 Mt. from Kerala in 1971 while 33, 834 from Karnataka during the same year In the following year (1972) the catch from Kerala was 1,94,977 Mt. whereas it was only, 11,836 Mt. from Karnataka. This marginal increase in the catch in Kerala may be thought to be related to considerable decrease in the catch in Karnataka region for the same period. Similarly, there was a considerable reduction in the catch from

Kerala (1,04,426) during 1972, while a marginal increase in the catch from Karnataka (15,610) for the same period. Again, during 1973-74 (1,22,783 Mt to 1,02,135 Mt) marginal increase was noticed in the Karnataka catch for the corresponding two year (15,945 to 20,784). Then during 1975 there was only a slight reduction of about 5,000 tons in Kerala (97,183), whereas more than 100 percent increase in the catch from Karnataka (92,701) occurred during the same period. Thus a close analysis of these catch figures of S. longiceps from Kerala and Karnataka during particular years infact shows the fluctuation of S. longiceps catch are not directly or sufficiently related to increase in one region to decrease in the other region.

The above analysis leads to the conclusion that the observed fluctuations or its abundance in the annual catches of S. longceps from Kerala and Karnataka may be correlated to one or more major independent factors. In this respect, it may be hypothesised that S. longiceps population in Kerala and Karnataka are two independent unit or stocks. In fact, the present investigation of the two populations from Kerala and Karnataka strongly supports the above hypothesis of considerable stock differences in these two regions. The findings of many earlier workers on the general variability of S. longiceps populations in different regions such as Malabar and Karnataka indicated racial differences on the basis of morphometric analysis (Devanesan and Chidambaram 1943).

Density-independent fluctuations in the abundance of pelagic clupeoids, namely, the pacific sardine (Sardinops sagax) and the northern anchovy (Engrualis mordax) have been observed on temporal scales of years, decades and centuries (Smith and Mosar, 1988). Very poor recruitment in the late 1940's, for example, precipitated the collapse during the 1950's and 1960's of the pacific sardine fishery (Radovich, 1982). Abundance of northern anchovy increased from 1950 to 1965, levelled off for a decade and declined in the late 1970's and early 1980's (Mac Call and Prager, 1988). Superimposed on this trend, however, were wide ranging fluctuations in abundance between years (Mac Call et al., 1983) and which have been attributed to climatically and oceanographically driven fluctuations in phytoplankton abundance, larval mortality and recruitment (Lasker, 1975; Peterman and Bradford, 1987).

The second apparent reason to be considered is a situation where no fluctuations in the catch for these two regions (Karnataka and Kerala) or increase in one region without affecting the catch in the other region or even an increase of catch in both regions at the same time. For instance, the tonnage of S. longiceps catch from Kerala during 1969 was 1,39,983 while it was 33,580 from Karnataka for the same period. The catch during 1970 showed 1,91,683 from Kerala and 33,834 from Karnataka indicating considerable increase of catch in Kerala while only marginal increase in the catch from Karnataka. Similarly, there was only a marginal increase in the catch from Kerala 91,17,356 to 1,19,937 whereas there was a considerable

increase in that of Karnataka (31,145 to 46,707) for the corresponding period 1977 to 1978.

Various reports on the oil sardine annual landings from different regions of west coast also indicated wide ranging fluctuations throughout its recorded period of landings. It may be hypothesized that if all the west coast regional oil sardine fishery is supported by a single breeding unit, (genetic stock) any positive or negative changes in the reproduction, recruitment and growth of the unit stock should have uniformly affected the quantity of its landing in all the regions. This hypothesis is again based on the assumption that there is no drastic reduction in the fishing effort on a particular year in any of these regions. Moreover, any such reduction in the fishing effort may not affect the above hypothesis when it is tested using the data for long period. For example, a comparison of seasonal landings of oil sardine in different regions for about 7 years (1960-1966) and ten years period (1969-1978), in fact, does not support the above hypothesis.

An alternate hypothesis that can be put forward to explain the short and long term fluctuations of S. longiceps landings throughout its range of distribution in Indian Waters is that the fishery is, infact, composed of more than one independent (isolated) genetic stocks. As a result, the fluctuations in one region supported by a particular independent stock is not expected to affect that of another regional stock, unless, similar non-genetic factors affected these naturally different

genetic stocks at the same time and at the same rate. The probability of which may be too small to be realistic. The third hypothesis, that can be logically considered is that there exists a single unit stock of S. longiceps fishery and the wide fluctuations in the regional landings of the fishery are mainly due to the fluctuations in the usual feeding, migratory behaviour of the species and or the transportation of its eggs and larvae through natural current patterns existing between the regions supported by the unit stocks fishery. A longterm investigation covering all these parameters alone could test the above hypothesis.

Kerala and Karnataka appeared to contributing 66 and 30 per cent respectively to the total all India oil sardine catch. During 1982-83, the landings of oil sardine declined by about 54,000 tonns. This was due to reduced landings in the states of Karnataka, Kerala and Goa, the catch of oil Sardine in the above states being about 37,800; 12,700 and 4,200 respectively. It is traditionally believed that the fluctuations in the abundance of the resources of the oil sardine depend on the success or failure of its spawning (Chidambaram, 1950, Raja 1969), changes in the availability of larval food or hydrobiological conditions (Hornell, 1910). Yet another cause of fluctuations may be due to overfishing, (Devanesan, 1943) and heavy fishing of the immature fish and the periodic off shore migrations (Sundara Raja 1934 and 1937) and destructive fishing of immature fish (Devanesan and Chidambaram 1943).

Various non-uniform reports on the spawning seasons and the appearance of juveniles in different regional fisheries also suggest that certain fishery and biology independent factors are responsible for wide annual fluctuations in the fisheries of S. longiceps. Such a suggestion is to emerge while analyzing, confusing or contradictory observations on the spawning season made by different authors.

As explained by Nair (1953) different spawning periods appeared to exist for the species, because S. longiceps spawners appeared first off the Kanara coast then later in the Calicut and Cochin region. Similarly reports in the peak spawning period also were not uniform, it varied from June-July (Hornell and Nayudu, 1924) June to October (Devanesan, 1943) and August-September (Nair 1953, 1959). Actual observations regarding the spawning grounds of S. longiceps were not made by anyone. There are different opinions on the possible spawning regions of S. longiceps. It may be near the coastal region (Prabhu, 1967) or beyond the present fishing zones (Nair, 1959).

Similarly reports on the fecundity of the species also ranged from 37,000 - 90,000 on regional basis. The fecundity ranges may be regionally differentiated as 37,000 - 38,000 at Cochin (Balan, 1966), 70,000 at Arabian sea (Devanesan and Chidambaram 1943) 70,000 to 90,000 at Malabar (Devanesan, 1943). Naturally the important question is whether such wide ranging fluctuations in the landings, appearance of spawners and rate of

fecundity etc. are the consequences of genetic heterogenity of the regional populations?

The above general discussion on the fishery and various aspects of biology of the Indian Oil Sardine Sardinella longiceps clearly revealed that the wide ranging periodical fluctuations in the abundance of the species cannot be explained on the basis of the assumption that the fishery is supported by a single unit stock of the species. On the other hand, the region oriented characteristics of the populations, significant differences of selected morphometrics, heterogenous nature of spawning periods and fecundity closely suggest that the oil sardine fishery, in fact may be composed of regionally heterogenous populations.

Interestingly, the results of three independent methods of the present investigation strongly corroborate the hypothesis that the oil sardine fishery of India tested from Mangalore, Calicut, Cochin and Mandapam are cytogenetically, biochemically and morphometrically heterogenous populations.

6. CONCLUSIONS

1. The population genetics of the oil sardine, Sardinella longiceps were studied by analysing cytogenetic, biochemical genetic (protein) and twenty five morphological characteristics of its sample populations from Cochin, Calicut, Mangalore and Mandapam.

2. The cytogenetic study revealed that the species is characterized by 48 acrocentric chromosomes as its diploid number (2n). All the four populations showed only 48 chromosomes. However, the total length values (μm) of the 24 paired chromosomes varied significantly between two groups of its populations. The total length of the population groups from Mandapam (61.3698) and Calicut (61.4125) significantly differed from that of Cochin (68.3986) and Mangalore (68.5455). Besides, the presence of a cytogenetically heterogenous population (Calicut) between cytogenetically homogenous populations (Cochin and Mangalore) suggested that these three populations are kept isolated. The same phenomenon occurred among Calicut, Cochin and Mandapam populations. Thus all the four populations are cytogenetically heterogenous in nature.

3. The heterogenous nature of the four populations were also indicated by the electrophoretic banding patterns of the muscle proteins of the species. The Mandapam and Calicut populations showed their characteristic protein phenotypes. These consisted of slow moving SS phenotype in Mandapam and faster moving FF phenotypes in Calicut at the second zone of

the protein zymogram. The Mandapam population was also distinctly different from other three populations in having centpercent SS phenotype at the fourth zone and FF phenotype at the fifth zone. The Cochin, Calicut and Mangalore populations differed considerably in having significantly different phenotype frequencies at the second, fourth and fifth zones.

4. The morphometric heterogenity of the four populations was also indicated by the results of the principal component/ANOVA analysis of the data collected on the twentyfive morphological parameters of the species. However, all these four populations were found to be homogenous in the number of fin rays.

7. SUGGESTIONS

1. The present discovery of considerable differences in the total length of the chromosomes among the four populations of Sardinella longiceps may be verified by a detailed investigation. The cytogenetic heterogenity implied by the above phenomenon may also be reinforced by applying modern chromosome banding techniques.

2. The present report on the phenotypic variability in the muscle protein of the species may be verified by modern flat gel electrophoresis and its genetic implications may be examined thoroughly.

3. The present findings of morphometric heterogenity among the four populations of the species may be reinforced by the truss-net work study on the body shape of the species.

8. SUMMARY

1. The thesis contains the results of the investigation entitled "Population genetic studies on the oil sardine, (Sardinella longiceps).

2. After a common introduction it is presented in three chapters, named, Cytogenetics, Biochemical genetics (Proteins) and Morphometrics.

3. Each chapter contains a review on the relevant literature, materials and methods, results and discussion. A section on general discussion critically analyses the relevant facts and figures available on the fishery and biology of the species S. longiceps to help to arrive at a proper conclusion on the study. Then three conclusions drawn from the investigation is followed by the three suggestions for future line of research on the species form the last part of the thesis.

4. The materials used for cytogenetic studies were live S. longiceps collected from Cochin, Calicut, Mangalore and Mandapam. The materials used for electrophoresis of proteins and morphometric measurements were collected from local centres at Cochin, Calicut, Mangalore and Mandapam.

   The samples from distant places were first got frozen and then transported to Cochin in ice cold condition. Then the samples were kept stored in deep freezer until used for analysis/experiment.

5. Six known methods were tried to standardise the procedures for chromosome preparation in the species S. longiceps and the method followed mainly was that of Kligerman and Bloom (1977).

6. The procedures followed for separation of general proteins present in the muscle and eye lens tissues were that of Davis (1964) for disc gel electrophoresis. It consisted of Tris-HCl gel buffer (pH. 8.9), Tris glycine (pH 8.3) tank buffer and 7.5% acrylamide gel. The proteins were detected by amido black stain.

7. The methods followed for collection of morphometric/meristic data from individual fish were the guidelines described in Anon (1960). Twentyfive morphological parameters including the weight were considered for morphometric measurements. A total number of 306 specimens consisting of 84 from Cochin, 72 from Calicut, 79 from Mangalore and 71 from Mandapam were used for morphometrics. The methods of Winans (1984) were followed for principal component analysis of morphometric data.

8. A total of 1660 metaphase plates were prepared from a total of 415 specimens collected from four centres. The modal diploid chromosome number (2n) for the species was found to be 48. All the chromosomes were acrocentric in structure/shape with NF value 48. All the four populations also had identical chromosome number and NF value.

9. However, the total mean length (um) of the chromosomes differed significantly between the Mandapam (61.3698) and Calicut (61.4125) group and Cochin (68.3986) and Mangalore (68.5455) group indicating the presence of population cytogenetic heterogenity in the species.

10. The electrophoretic patterns of eyelens consisted of eight identical fractions in all the four populations compared, indicating lack of polymorphism of eyelens protein loci in the species.

11. The electrophoretic patterns of muscle proteins, on the other hand, showed polymorphic forms at the second, fourth and fifth assumed loci, out of five. A comparison of the polymorphic patterns and their allele frequencies showed only the presence of significant protein phenotype heterogenity among the four populations of the species. The highest heterogenity was shown by the Mandapam populations having apparently fixed alleles at the fourth and fifth assumed loci.

12. The meristic counts of fin rays showed identical number in all the four populations examined.

13. The results of the three levels of principal component analysis revealed that all the four populations (except between Calicut and Mandapam) are having significantly different morphometrics.

14. Though cytogenetic homogenity between Calicut and Mandapam was also indicated by closely similar values of total chromosome lengths, their protein phenotypes were distinctly different. Thus a combined results of the three independent methods (cytogenetic, biochemical genetics and morphometrics) helped to reveal that all the four populations examined are heterogeneous in one or more population genetic aspects examined.

15. The results of the independent studies on the Cytogenetics, Biochemical genetics and Morphometrics were critically analysed and discussed at the end of respective chapters. In each case direct evidence of population genetic heterogenity/homogenity was emphasised and marked out. Additionally, indirect evidence of isolation of two homogenous populations because of the presence of heterogenous population in between, was also pointed out.

16. A general discussion on the facts and figures on the fishery and biology of the species reported earlier was enabled to reinforce the conclusions drawn from the results of the present study.

17. Three relevant and important conclusions were drawn from the present investigation on the population genetic studies on the oil sardine (Sardinella longiceps).

18. Three suggestions were also listed for future line of research on the species.

9. REFERENCES

ALLENDORF, F.W., RYMAN, N., STENNEK, A., AND STAHL, G., 1976. Genetic variation in Scandinarian trout (Salmo trutta, L.) evidence of sympatric populations. Hereditas 83: 73-82.

ALLENDORF, F.W., AND THORGAARD, G.H., 1984. Tetraploidy and the evoluation of salmonid fishes. P. 1-53 in B.J. Turner, Editor. "Evolutionary genetics of fishes". Plenum New York.

ALLENDORF, F.W., AND UTTER, F.M., 1979. Population genetics. In W.S.Hoar and D.J. Randall (Eds). Fish Physiology, Vol. 8. Academic Press, New York.

AMEMIYA, C.T., AND GOLD, J.R., 1988. Chromosomal NOR's as taxonomic and systematic characters in North American cyprinid fish. Genetica., 76: 81-90.

ANDERSSON, L., RYMAN, N. AND STAHL, G., 1983. Protein loci in Arctic charr, Salvelinus alpinus L.:Electrophoretic expression and genetic variability patterns. J.Fish.Biol.23: 75-94.

ANNIGERI, G.G., 1978. Studies on variations in the vertebral counts of oil sardine along the North Kanara coast. Indian J. Fish;., 25(1&2) 14-22.

ANON., 1960. Manual of field methods in fisheries biology, FAO manuals in fisheries science, No.1 FAO Rome.

ANON., 1979. The Indian oil sardine. Mar. Fish. Infor. Ser. T & E Ser No.14 1-13.

ANTONY RAJA B.T., 1969. The Indian oil sardine. Bull Cent. Mar. Fish. Res. Inst. No.16, 128p (Mimeograph).

ANTONY RAJA B.T., 1973. The Indian oil sardine fishery: problems in perspective. J. Mar. Biol. Assoc. India, 15(2) 735-749.

ASPINWALL, N., 1973. Inheritance of alpha-glycerophosphate dehydrogenase in the pink salmon, Oncorhynchus gorbuscha Walbaum, Genetics, 73: 693-643.

AVISE, J.C., 1974. Systematic value of electrophoretic data, Syst. Zool. 23: 465-481.

AVISE, J.C., SMITH, J.J., AND AYALA, F.J. 1975. Adaptive differentiation with little genic change between two native California minnows. Evolution 29: 411-426.

AVTALION, R.R., AND WOJDANI A. 1971. Electrophoresis and immuno-electrophoresis of sera from some known F1 hybrids of Tilapia. Bamidgeh 23: 117-124.

AYALA, F.J. AND KIGER,J.A.Jr., 1980. In: Modern genetics. The Benjamin cummings publishing company, Inc. 2727 Sand Hill Road, Menio Park, California - 94025. 844 p.

BAILEY, G.S, TSUYUKI, H., AND WILSON, A.C. 1976. The number of genes for lactate dehydrogenase in Salmonid fishes. J. Fish. Res. Bd. Canada, 33: 760-767.

BALAN V., 1966. The fecundity and sex composition of Sardinella longiceps Val. along the Cochin coast. Indian J. Fish., 12(2): 473-491.

BALDWIN, J.M., 1896. A new factor in evolution, Amer. Natura list, 30: 441-475, 536-553.

BANERJI, S.K., 1967. Fish population studies, Souvenir, 20th Anniversary, Cent. Mar. Fish. Res. Inst. 37-40.

BARLOW, G.W. 1961. The causes and significance of morphological variation in fishes. Syst. Zool. 10: 105-117.

BARSHIENE, J.V., 1978. Ontogenetic variability of chromosome number in the Atlantic salmon (Salmo salar. L.). Genetika (U.S.S.R.) 14: 2029-2036.

BARSHIENE, J.V., 1981. Intercellular polymorphism of chromosome sets in the Atlantic salmon. Tsitologiya (U.S.S.R.) 23: 1053-1059.

BEACHAM, T.D., 1990. A genetic analysis of meristic and morphometric variation in chum salmon (Oncorhynchus keta) at three different temperatures. Can. J. Zool. 68:225-229.

BEACHAM, T.D., GOULD A.P., WITHLER, R.E., MURRAY, C.B. AND BARNER, L.W., 1987. Biochemical genetic survey and stock identification of chum salmon (Oncorhynchus keta) in British columbia. Can. J. Fish and Aquat. Sci. 44: 1702-1713.

BLAXHALL, P.C., 1983. Lymphocyte culture for chromosome preparation. Fish. Biol. 22: 279-282.

BOCK, W.J. 1980. The definition and recognition of biological adaptation. Am. Zool. 20: 217-227.

BOOKE, H.E., 1968. Cytotaxonomic studies of the coregonine fishes of the Great Lakes, U.S.A.: DNA and karyotype analysis. J. Fish Res. Board Can. 25: 1667-1687.

BOOTHROYD, E.R., 1959. Chromosome studies on three Canadian populations of Atlantic salmon Salmo salar L. Can. J. Genet. Cytol. 1: 161-172.

BREWER, G.J., 1970. An. Introduction to isozyme techniques. Academic Press, New York, 186 p.

BUSACK, C.A., AND GALL, G.A.E., 1981. Introgressive hybridization in populations of paiute cutthroat trout (Salmo clarki seleniris). Can. J. Fish. Aquat. Sci. 38: 939-951.

BUSACK, C.A., THORGAARD, G.H., BANNON,M.P., AND GALL, G.A.E. 1980. An electrophoretic, karyotypic and meristic characterization of the eagle lake trout. Salmo gairdneri aquilarum. Copeia, 3: 418-424.

CAMPBELL, N.A., AND ATCHLEY, W.R., 1981. The geometry of canonical variate analysis. Syst. Zool. 30: 268-280.

CASSELMAN, J.M., COLLINS, J.J., CROSSMAN, E.J., IHSSEN, P.E., AND SPANGLER, G.R., 1981. Lake whitefish (Coregonus clupeaformis) stocks of the Ontario waters of lake Huron. Can. J. Fish. Aquat. Sci., 38: 1772-1789.

CATAUDELLA, S., SOLA, L., CORTI, M., ARCANGELI, R., ROSA,G.L., MATTOCCIA. M., SBORDONI, M.C. AND SBORDONI V., 1987. Cyto-genetic, genic and morphometric characterisation of groups of common carp. Cyprinus carpio, Proc. World. Symp. on Selection Hybridisation and Genetic engineering in Aquaculture-I Berlin.

CATTON, W.T., 1951. Blood cell formation in certain teleost fishes. Blood 6: 39-60.

CHAKRABORTY, S.K. AND KAGWADE, P.V., 1989. Somatic chromosomes of two marine teleosts. Fish Genetics in India, editors. Das & Jhingran. Today and Tomorrow's printers and publishers, New Delhi. 63-68.

CHATTERJEE, K., AND MAJHI, A., 1973. Chromosomes of Mugil parsia. Hamilton. Teleosteii, Mugiliformes. Genen phaenen 16(2): 51-54.

CHEN, T.R., 1969. Karyological heterogamety of deep-sea fishes, Postilla. 130: 1-29.

CHIARELLI, A.B., AND CAPANNA, E., 1973. Checklist of fish chromosomes. In cytotaxonomy and vertebrate evolution 205 New York Academic Press.

CHIDAMBARAM, K., 1950. Studies on length frequency of the oil sardine, Sardinella longiceps Cuv. & Val. and certain factors influencing their appearance on the Calicut coast of the Madras presidency. Proc. Indian. Acad. Sci. 31B(5): 252-286.

CHOURROUT, D., AND HAPPE, A., 1986. Improved methods of direct chromosome preparation in rainbow trout, Salmo gairdneri. Aquaculture, 52: 255-261.

CLAYTON, J.W, 1981. The stock concept and the uncoupling of organismal and molecular evolution. Can. J. Fish. Aquat. Sci. 38: 1515-1522.

CONNELL, J.J., 1953. Studies on the proteins of fish skeletal muscle. 1. Electrophoretic analysis of codling extract of low ionic strength. Biochemical J. 54: 119-126.

COPEMAN, D.G., 1977. Population differences in rainbow smelt, Osmerus mordax: multivariate analysis of mensural and meristic data. J. Fish. Res. Board Can: 34: 1220-1229.

CORTI,M., THORPE,R.S., SOLA,L., SBORDONI, V. AND CATAUDELLA, A.S. 1988. Multivariate morphometrics in Aquaculture: A case study of six stocks of the common carp (Cyprinus carpio) from Italy. Can. J. of Fish. Aquat. Sci. 45, 1548-1554.

CROW, J.F., AND KIMURA, M. 1970. An introduction to population genetics theory. Harper and Row, New York. 589 p

CUCCHI, C. AND BARUFFALDI, A., 1989. A simple invivo method for increasing mitoses in teleost fishes. Cytobios 60, 165-169.

CUCCHI, C. AND BARUFFALDI, A., 1990. A new method for karyological studies in teleost fishes. J. Fish Biol. 37: 71-75.

CURRENS, K.P., SHARPE, C.S., HJORT, R., SCHRECK, C.B. AND LI, H.W., 1989. Effect of different feeding regimes on the morphometrics of chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (O. mykiss) Copeia: 689-695.

CUSHING, D.H. 1981. "Fisheris Biology: A study in Population Dynamics". Univ. of Wisconsin Press. Madison.

DALY, J.C, AND RICHARDSON, B.J. 1980. Allozyme variation between populations of bait fish species. Stolephorus heterolobus and S. devisi (Pisces; Dussumieriidae) from Papua New Guinea Waters. Aust. J. Mar. Fresh water Res., 31: 701-711.

DANNEVIG, A., 1932. Is the number of vertebrae in the cod influenced by light or high temperature during early stages? J. Cons. 7: 60-62.

DANNEVIG, A. 1950. The influence of temperature on number of vertebrae in plaice. Rep. Norw. Fish. Invest. 9: 1-6.

DAS, C.C. 1983. Chromosomes in marine fishes. Proc. 70th Indian Science congress. 2: 1-18.

DAVIS, B.J., 1964. Disc electrophoresis II. Methods and application to human serum proteins. Annals. Yew York Acad. Sci., 121: 404-429.

DAVISSON, M.T., WRIGHT, J.E. AND ATHERTN, L.M., 1972. Centric fusion and trisony for the LDH-B locus in brook trout, Salvelinus fontinalis, Science 178: 992-994.

DEHRING, T.R, BROWN, A.F., DAUGHERTY, C.H. AND PHELPS, S.R. 1981. Survey of the genetic variation among eastern lake superior lake trout (Salvelinus namaycush). Can. J. Fish. Aquat. Sci., 38: 1738-1746.

DENTON, T.E., 1973. "Fish chromosome methodology". Thomas, C.C. (Publ.) Springerfield, U.S.A. pp. 166.

DENTON, T.E. AND HOWELL, W.F., 1969. A technique for obtaining chromosomes from scale epithelium of teleost fishes. Copeia No.2, 392-393.

DEVANESAN, D.W. 1943. A brief investigation into the causes of the fluctuation of the annual fishery of the oil sardine of Malabar Sardinella longiceps: determination of its age and an account of the discovery of its eggs and spawning grounds. Madras, Fish Bull. 28(1): 1-38.

DEVANESAN, D.W. AND CHIDAMBARAM, K., 1943. On the alleged existence of races in the population of the oil sardines, Sardinella longiceps, in the Arabian sea. Proc. 30th Indian Sci. Congr. Calcutta (Abstr).

DHAR, N.J. AND CHATTERJEE K. 1984. Chromosomal evolution in Indian murrels (Channiformes Channidae). Caryologia., 37(4): 359-371.

DHULKHED, M.H., AND RAO. S.N., 1976. Electrophoretic studies on serum proteins of oil Sardine (Sardinella longiceps) and Mackerel (Rastrelliger kanagurta). Fish, Technol., 13(1): 16-19.

DOUCETTE, J.A., AND FITZSIMONS J.M., 1988. Karyology of Elopiform and Clupeiform fishes. Copeia., 1988: 124-130.

DREWRY, G., 1964. Appendix I, Chromosome number. Bull. Tex. Mem. Mus. No. 8: 5-72.

DUNN, G., AND EVERITT, B.S. 1982. An introduction to mathematical taxonomy. Cambridge Univ. Press, Cambridge, MA, 152 P.

EBELING, A.W. AND CHEN, T.R., 1970. Heterogamety in teleostean fishes. Trans. Am. Fish. Soc. 99: 131-138.

EVONS, E.P., BURTEN SHAW, M.D., AND FORD, C.E., 1972. Chromosomes of mouse embryos and new born young: Preparation from membrane and tail tips. Stain tech. 47: 229-234.

ECKROAT, L.R., AND WRIGHT, J.E., 1969. Genetic analysis of soluble lens protein polymorphism in brook trout, Salvelinus fontinalis, Copeia, 3: 466-473.

FAN.Z., AND FOX D.P., 1990. A new method of fish chromosome preparation. J. Fish. Biol. 37. 553-561.

FERGUSON, A., AND MASON, F.M., 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout Salmo trutta L. in Cough Melvin Ireland. J. Fish. Biol. 18: 629-642.

FITZSIMONS, J.M. 1972. A revision of two genera of Goodeid fishes (Cyprindontiformes, Osteichthyes) from Mexican plateau. Copeia., 1972 (4): 728-755.

FESSLER J.L. AND WAGNER, H.F., 1969. Some morphological and biochemical changes in steelhead trout during the parr smolt transformation J. Fish. Res. Board. Can. 26: 2823-2841.

FOWLER, J.A., 1979. Control of vertebrae number in teleosts - an embroyological problem. Q. Rev. Biol. 45: 148-167.

FUJINO, K., 1967. Instructions of collecting blood and serum samples from Tuna fishes. FAO Fisheries Circular No. 26.

FUJINO, K., AND KANG, T., 1968a. Serum esterase groups of pacific and Atlantic tunas. Copeia (1): 56-63.

FUJINO, K., AND KANG, T. 1968b. Transferrin groups of Tunas. Genetics 59: 79-91.

GALETTI,P.M., FORESTI,F., BERTOLLO,L.A.C., AND MORERIA, O., 1984. Characterisation of eight species of Anostomidae (Cypriniformes) fish on the basis of nucleolar organiser region. Caryologia, 37(4) 401-406.

GALL, G.A.E., BUSAK, C.A., SMITH, R.C. GOLD, J.R. AND KORNBLATT, B.J., 1976. Biochemical genetic variation in population of golden trout, Salmo aquabonita. Evidence of the threatened little kern River golden trout, S. aquabonita. Whitei. J.of Heredity. 67: 330-335.

GARCIA-VAZQUEZ, E., LINDE, A.R., BLANCO, G., SANCHEZ, J.A., VAZQUEZ, E AND RUBIO, J. 1988. Chromosome polymorphism in farm fry stocks of Atlantic salmon from Austurias. Fish Biol. 33: 581-587.

GEORGE, K.C. 1980. Studies on the distribution and abundance of fish eggs and larvae off the South-west coast of India with special reference to Scombroids (Ph.D. thesis) Cochin University of Science and Technology Abstr. No. M.S.19: p. 222-223.

GJEDREM, J., EGGUM AND REJSTIE, T., 1977. Chromosome of some salmonids and salmonid hybrids. Aquaculture 11, 335-348.

GOLD, J.R., 1974. A fast and easy method for chromosome karyotyping in adult teleosts. Prog. Fish Cult. 36, 169-171.

GOLD J.R., 1979. Cytogenetics, Pages 353-405 in W.S. HOAR, D.J. RANDALL AND J.R. BRETT, editors, Fish Physiology Volume 8. Academic Press, New York.

GOLD,J.R., AND AMEMIYA, C.T. 1986. Cytogenetic studies in North American minnows (Cyprinidae) XII. Patterns of chromosomal nucleolus organiser region variation among 14 species. Can. J. Zool. 64: 1869-1877.

GOLD, J.R., 1984. Silver staining and heteromorphism of chromosomal nucleolus organiser regions in North American Cyprinid fishes. Copeia: 133-139.

GOLD J.R., AND AVISE, J.C., 1977. Cytogenetic studies in North American minnows (Cyprinidae). I Karylogy of nine California genera. Copeia. No.3: 541-549.

GOLD, J.R. AND GALL, G.A.E. 1975. Chromosome cytology and Polymorphism in the California high sierra golden trout, (Salmo aquabonita). Can. J. of Genetics and Cytology, 17: 41-53.

GOLD J.R., LI, Y.C., SHIPLEY, N.S. AND POWERS, P.K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. The Fish. Soc. of the British Isles; 563-575.

GORDON, M., 1947. Speciation in fishes: distribution in time and space of seven dominant multiple alleles in Platypoecilus maculatus. In: Advances in genetics. Vol. 1 pp. 95-132.

GOULD, S.J. 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. Cambridge Philos. Soc. 41: 587-640.

GOULD, S.J. 1977. Ontogeny and phylogeny. Belknap Press, Howard University, Cambridge, 510 p.

GOULD, S.J., AND JOHNSTON, R.F. 1972. Geographic variation. Annu Rev. Ecol. Syst. 3: 457-498.

GRAMMELTVEDT, A.F., 1974. A method of obtaining chromosome Preparations from rainbow trout (Salmo gairdneri) by lukocyte culture. Norw. J. 3001, 22: 129-134.

GRANT, W.S., 1984. Biochemical population genetics of Atlantic herring, Clupea harengus. Copeia, 2: 357-364.

GRANT W.S., MILNER, G.B., KRASNOWSKI, P. AND UTTER, F.M. 1980. Use of biochemical genetic variants for identification of sockeye salmon (Oncorhynchus nerka) stocks in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci., 37: 1236-1247.

GRANT, W.S. AND UTTER, F.M. 1980. Biochemical genetic variation in walleye pollock, Theragra chalcogramma population structure in the south-eastern Bering sea and the Gulf of Alaska. Can. J. Fish. Aquat. Sci. 37: 1093-1100.

GRANT, W.S., AND UTTER, F.M. 1984. Biochemical population genetics of pacific herring (Clupea pallasi). Can. J. Fish. Aquat. Sci., 41: 856-864.

GREEN, P.E. (ed) 1976. Mathematical tools for applied multivariate analysis. Academic Press, N.Y., 376 p.

GROSS, H.P. 1977. Adaptive trends of environmentally sensitive traits in the three-spined stickleback Gasterosteus aculeatus L. Zool. Syst. Evolution. Forsch. 15: 252-278.

HARRIS, R.J. 1975. A primer of multivariate statistics. Academic Press, New York, NY 332 P.

HARTLEY, S.E. 1987. The Chromosomes of salmonid fishes. Biol. Rev., 62: 197-214.

HARTLEY, S.E. AND HORNE, M.T., 1982. Chromosome polymorphism in the rainbow trout (Salmo gairdneri Richardson) Chromosoma, 87: 461-468.

HARTLEY, S.E. AND HORNE, M.T., 1983. A method for obtaining mitotic figures from blood leucocyte cultures of rainbow trout, Salmo gairdneri, Fish Biol. 22: 77-82.

HARTLEY, S.L. AND HORNE M.T. 1984a. Chromosome polymorphism and constitutive heterochromatin in Atlantic salmon, Salmo salar Chromosoma (Berl) 89: 377-380.

HARTLEY, S.E. AND HORNE, M.T., 1984b. Chromosome relationship in the genus Salmo. Chromosoma (Berl) 90: 229-237.

HECKMAN,J.R., ALLENDORF,F.W. AND WRIGHT, J.E., 1971. Trout leukocytes: Growth in oxygenated cultures. Science 173, 246-247.

HECKMAN, J.R. AND BRUBAKER, P.E., 1970. Chromosome preparation from fish blood leukocytes. Prog. Fish Cult. 32, 206-208.

HEINCKE, F., 1898. Naturgeschichte de Herings, Teil 1, Abh. dt. Seefish Ver., Band 2, CXXXVI, 128 pp. text, 223 p.

HEINCKE, F., 1902. Meeting of committe. A. Rapp. P.V. Renm. Cons. Perm. Int. Explor. Mer 1:95.

HENNEMUTH, R.C., 1959. Morphometric comparisons of skipjack from the central and eastern tropical pacific ocean. Bull. Inter-Amer. trop. Tuna Comm., 3(6): 241-304.

HERZBERG, A., AND PASTEUR, R. 1975. The identification of grey mullet species by disc electrophoresis. Aquaculture 5:99-106

HODGINS, H.O., AMES, W.E., AND UTTER, F.M., 1969. Variants of lactate dehydrogenase isozymes in sera of sockeye salmon (Oncorhynchus nerka) J. Fish. Res. Bd. Canada 26: 15-19.

HORNELL, J. 1910. Report of the results of a fishery cruise along the Malabar coast and to the Laccadive islands. Madras Fish. Bull. 4' 71-126.

HORNELL, J., AND NAYUDU, M.R., 1924. A contribution to the life history of the Indian sardine with notes on the plankton of the Malabar coast. Madras Fish. Bull. 17:129-197.

HUBBS, C.L., 1928. An hypothesis on the origin of graded series of local races in fishes. Anat. Record, 51:91 (Abstract)

HUBBS, C.L., AND LAGLER, K.F., 1947. Fishes of the Great lakes region. Cranbrook institute of Science, Bull. 26: 186p.

HUMASON, G.L., 1979. Animal tissue techniques. San Francisco: W.H. Freeman (ed).

HUMPHRIES,J.M., BOOKSTEIN,F.L., CHERNOFF, B., SMITH, G.R., ELDER, R.L., AND POSS, S.G. 1981. Multivariate discrimination by shape in relation to size, Syst. Zool. 30: 291-308.

HUXLEY, J.S. 1924. Constant differential growth ratios and their significance. Nature (London) 11:895-896.

IMHOF, M., LEARY, R., AND BOOKE, H.E., 1980. Population or stock structure of lake whitefish, Coregonus clupeformis, in northern Lake Michigan as assessed by isozyme electrophoresis Can. J. Fish. Aquat. Sci. 37: 783-793.

JAMIESON, A., 1967. New genotypes in greenland Cod. Nature (London) 215: 661-662.

JAMIESON, A., 1974, Genetic tags for marine fish stocks. In: F.R. Harden Jones (Ed) Sea fisheries Science London. EIK Science 5 p. 91-99.

JAMIESON, A. AND BIRLEY A.J., 1989. The demography of a haemoglobin polymorphism in the Atlantic cod Gadus morhua L. J. Fish. Biol. 35(A): 193-204.

JAMIESON, A. AND TURNER, R.J., 1980. Muscle protein differences in two eels, Anguilla anguilla (Linnaeus) and Anguilla rostrata Le (sever). Biological Journal of the Linnean society, 13: 41-45.

KANG, Y.S. AND PARK, E.H. 1975. Leukocyte culture of the eel without autologous serum. Jap. J. Genet. 50: 159-161.

KARAKOUSIS. Y., TRIANTAPHYLLIDIS. C., AND ECONOMIDU P.S., 1991. Morphological variability among seven populations of brown trout, Salmo trutta L., in Greece. J.Fish.Biol. 38: 807-817.

KASTSCHENKO, 1890. Zeit Mikros Anatomische Forschung, 50 (Quoted from Makino 1951).

KHUDA-BUKHSH, A.R. 1975. Somatic chromosomes of an exotic fish Puntis japonicus. Proc. Second All India Congr. Cytol Genet. J. Cyto. Genet. Congs. Suppl. 118-120.

KHUDA-BUKHSH, A.R., AND BARAT, A., 1987. Chromosomes in fifteen species of teleosts (Pisces). Caryologia 40: 131-144.

KHUDA-BUKHSH, A.R. AND MANNA, G.K., 1977. Somatic and germinal chromosomes of the aquarium fish, Molliensia latipinna Geobios 4:49.

KIRPICHNIKOV, V.S. 1981. Genetic basis of fish selection. Springer-verlag, N.Y. 430 p.

KLIGERMAN, A.D. AND BLOOM, S.E., 1977. Rapid chromosome preparations from solid tissues of fishes, J. Fish. Res. Board of Canada 34. 266-269.

KOLJONEN, M.L., 1986. The enzyme gene variation of ten Finnish rainbow trout strains and the relation between growth rate and mean heterozygosity. Aquacultre. 57: 253-260.

KRISTOFFERSON, A.H., AND CLAYTON, J.W. 1990. Subpopulation status of lake white fish (Coregonus clupeaformis) in lake Winnipeg. Can. J. Fish. Aquat. Sci. 47: 1484-1494.

LABAT, R., LARROUY, G. AND MALASPINA, L., 1967. Technique de culture des leucocytes de Cyprinus carpio, L.C.R.Acad. Sci., Ser. D 264: 2473-2475.

LASKER, R., 1975. Field criteria for survival of anchovy larvae: The relation between inshore chlorophyll maximum layers and successful first feeding. Fish. Bull. S. 73: 453-462.

LAVERY, S. AND SHAKLEE, J.B. 1989. Population genetics of two tropical sharks, Carcharhinus teleostomi and C. sorrah in northern Australia. Aust.J.Mar.Freshwater Res., 40: 541-557.

LEGENDRE, P., 1975. A field-trip microtechnique for studying fish leucocyte chromosomes. Can. J. Zool. 53, 1443-1446.

LEGRANDE, W.H., AND FITZSIMONS, J.M. 1976. Karyology of the mullets (Mugil curema and M. cephalus (Perciformes: Mugilidae) from Lousiana. Copeia: 388-391.

LEVAN, A., FREDGA, K. AND SANDBERG. A.A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52, 201-220.

LEIDER, U., 1963. On presumptive sex chromosomes in Perca, Acernia and Anguilla. Biol. Zbl. 82:296.

LIEPPMAN, M., HUBBS, C., 1969. A Karyological analysis of two cyprinid fishes, Notemigonus crysoleucas and Notropis lutrensis. Tex. Rep. Biol. Med. 27: 427-435.

LUND, W.A., 1957. Morphometric study of the striped bass, (Roccus saxatilis) U.S. Fish and Wildlife service special scientific Report - Fisheries 216. 24 p.

MAC CALL, A.D., METHOT, R.D., HUPERT, D.D. AND KLINGBEIL, R., 1983. Northern Anchovy fishery management Plan (Amendment No.5). Pacific fishery management council Portland.

MAC CALL, A.D. AND PRAGER, M.H. 1988. Historical changes in abundance of six species off Southern California, based on egg and larval samples. Calif. Coop. Ocean Fish. Invest. Rep. 28: 81-84.

MAC CRIMMON, H. AND CLAYTOR, R. 1986. Possible use of taxonomic characters to identify Newfoundland and Scottish stocks of Atlantic Salmon, (Salmo salar L.) Aquaculture and Fisheries Management 17: 1-17.

MANGALY, G. AND JAMIESON, A. 1978. Genetic tags applied to the European hake, Merluccius merluccius (L). Anim. Blood, groups. biochem. Genet., 9: 39-48.

MANNA, G.K., 1983. Cytogenetic studies on fishes and amphibia, In Genetical Researches in India XV Int Cong. Genet, Delhi, ICAR publication.

MANNA,G.K.,1987. Fish cytogenetics related to taxonomy, evolution and monitoring aquatic genotoxic agents Proc. Symp. on Conservation and Management of Fish Genetic Resources of India. Today and Tommorrow's Printers, New Delhi: 21-46.

MANNA G.K., AND KHUDA-BUKHSH, A.R., 1977. Karyomorphology of cyprinid fishes and cytological evolution of the family. Nucleus, 20; 119-127.

MANNA, G.K. AND KHUDA-BUKHSH, A.R., 1978. Karyomorphological studies in three species of teleostean fishes Cytologia 43:69.

MARTIN, W.R. 1949. The mechanics of environmental control of body form in fishes. Univ. Toronto Stud. Biol. Ser. 58 Pub. Ont-Fish Res. lab. 70:1-72.

McANDREW, B.J. AND MAJUMDAR, K.C., 1983. Tilapia stock identification using electrophoretic markers. Aquaculture, 30: 249-261.

McPHAIL, J.D., 1984. Ecology and evolution of sympatric sticklebacks (Gasterosteus): morphological and genetic evidence for a species pair in Enos lake, British Columbia. Can. J. Zool. 62: 1402-1408.

McPHAIL, J.D. AND JONES, R.L., 1966. A simple technique for obtaining chromosomes from teleost fishes. J. Fish. Res. Bd. Can 23: 767-768.

MENEZES, M.R., 1976. Electrophoretic studies of eyelens and serum proteins of Sardinells fimbriata (Val) and Sardinella longiceps (Val). Mahasagar, 8(3 and 4): 117-121.

MENEZES, M.R., 1980. Soluble lens protein polymorphism in the oil sardine, Sardinella longiceps Val. Mahasagar, 13(2): 183-185.

MENEZES, M.R., 1986. An electrophoretic study of the soluble lens proteins from the Indian mackerel, Rastrelliger kanagurta (Cuv) Mahasagar, 19(1): 69-71.

MENEZES, M.R., 1990. Biochemical genetic divergence in three carangids from the Andaman sea. Curr. Sci., 59(4): 209-212.

MENG, H.J. AND STOCKER, M. 1984. An evaluation of morphometrics and meristics for stock separation of pacific herring (Clupea herengus Pallasi). Can. J. Fish. Aquat. Sci. 41: 414-422.

MEREDITH, R., 1969. A simple method for preparing meiotic chromosomes from mammalian testis. Chromosoma (Berlin) 26: 254-258.

MEYER, A. 1987. Phenotypic plasticity and heterochrony in Cichlasoma managuense (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution 41: 1357-1369.

MOLLER, D., 1966. Genetic differences between cod groups of the Lofoten area. Nature (London). 212: 824-826.

MOLLER, D., 1968. Genetic diversity in spawning cod along the Norwegian coast. Hereditas. 60: 1-32.

MOLLER, D., 1970. Genetic diversity in Atlantic salmon and salmon management in relation to genetic factors. In: The International Atlantic Salmon Foundation. Spec. Publ. Ser. 1(1): 1-30.

MORAN, P., PENDAS, A.M., GARCIA - VAZQUEZ, E. AND LINDE, A.R. 1989. Chromosomal and morphological analysis of two populations of Salmo trutta sbp. fario employed in repopulation. J. Fish. Biol., 35: 839-843.

MOTTLEY, C.M., 1941. The covariance method of comparing head lengths of trout from different environments. Copeia (3): 154-159.

NAIR, R.V., 1953. Studies on the revival of the Indian oil sardine fishery. Proc. Indo-Pac. Fish. Coun. (2): 115-129.

NAIR, R.V. 1959. Notes on the spawning habits and early life history of the oil sardine, sardinella longiceps (Cuv & Val.) Indian J. Fish 6: 342-359.

NATARAJAN, R., 1969. Cytological studies in Indian molluscs (Archaegastropoda. Neritidae). Malacologia 9(1): 279-281.

NATARAJAN, R., 1970. Cytological studies in Indian Molluscs. Chromosomes of some opisthobranchus from Portnova. Malacological Review 3: 19-23.

NATARAJAN, R. AND SUBRAHMANYAM, K., 1974. A karyotype study of some teleosts from Portnovo waters. Proc. Ind. Acad. Sci. (B) 5: 173-196.

NEI, M., 1972. Genetic distance between populations. Am. Nat., 106-(999): 283-232.

NELSON-REES, W.A., KNIAZEFF, A.J. AND DARBY, W.B., Jr. 1967. Debut and accumulation of centric fussion products. An index to age of certain cell lines. Cytogenetics 6: 436-450.

NOGUSA, S., 1960. A comparative study of the chromosomes in fishes with particular considerations on the taxonomy and evolution. Mem. Hyogo. Univ. Agric. Biol. Ser. 3(1): 1-62.

NORTHCOTE, T.G., WILLISCROFT, S.N. AND TSUYUKI, H., 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a water fall. J. Fish. Res. Bd. Canada, 27:1987-1996.

NYGREN, A., NILSSON, B. AND JAHNKE, M. 1968. Cytological studies in Atlantic salmon (Salmo salar). K. Vetenksamk Upps. Arsb. 11: 21-52.

NYGREN, A., NILSSON, B., JAHNKE, M. 1972. Cytological studies in Atlantic Salmon from Canada, in hybrids between Atlantic salmon from Canada and Sweden and in hybrids between Atlantic Salmon and sea trout. Hereditas 70: 295-306.

NYMAN, L. 1967. Protein variations in salmonidae. Rep. Inst. Freshwat. Res. Drottningholm 47: 5-38.

ODENSE, P.H., ALLEN, T.M. AND LEVENG, T.C., 1966. Multiple forms of lactate dehydrogenase and aspartate aminotransferase in herring (Clupca harengus L.) Can. J. Biochem., 44: 1319-1326

OHNO S., 1970. "Evolution by Gene duplication". Springer-Verlag, Berlin and New York.

OHNO, S., 1974. Protochordata, cyclostomata and pisces. In "Animal cytogenetics (B. John ed.) Vol.4, Chordata 1: pp 1-91. Borntraeger-Berlin.

OHNO, S., STENIUS, C., FAISST, E. AND ZENZES,M.T., 1965. Post-zygotic chromosomal rearrangements in rainbow trout (Salmo irideus Gibbins). Cytogenetics 4; 117-129.

OJIMA Y., 1982. Methods in fish cytogenetics. The nucleus, 25:1-7

OJIMA, Y. AND HITOTSUMACHI.S., 1967. Cytogenetic studies in lower vertebrates IV. Japan. J. Genet. 42: 163.

OJIMA, Y., HITOTSUMACHI,S. AND HAYASHI, M., 1970. A blood culture method for fish chromosomes. Jap. J. Genet. 45: 161-162.

OKAZAKI, T., 1986. Genetic and population structure in masu salmon. Oncorhynchus masou of Japan. Bull. Jap. Soc. Sci. Fis., 52(8): 1365-1376.

ORSKA, I., 1963. The influence of temperature on the development of meristic characters of the skeleton in salmonidae. I. Temperature controlled variations of the number of vertebrae in Salmo virideus Gibb. Zool. Pol. 12(3): 309-339.

PARTINGTON, J.D. AND MILLS, C.A. 1988. An electrophoretic and biometric study of Arctic charr, Salvelinus alpinus (L), from ten British lakes. J. Fish. Biol. 33: 791-814.

PETERMAN, R.M. AND BRADFORD, M.J. 1987. Wind speed and mortality rate of a marine fish, the northern anchovy (Engraulis mordax). Science 235: 354-356.

PETERSON, G.L. AND SHEHADEH, Z.H., 1971. Subpopulations of the Hawaiian striped mullet, Mugil cephalus: Analysis of variations of nuclear eyelens protein electropherograms and nuclear eyelens weights. Mar. Biol., 11: 52-60.

PHELPS, S.R. AND ALLENDORF, F.W., 1983. Genetic identity of palliid and shovelnose sturgeon (Scaphirynchus albus and S. platorynchus). Copeia: 696-700.

PHILIP SAMUEL, P., 1987. Biochemical genetics of selected commercially important penaeid prawns. Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala, India.

PHILLIPS, R.B. AND IHSSEN, P.E., 1985. Chromosome banding in salmonid fishes; Nucleolar organiser regions in Salmo and Salvelinus Can. J. of Genetics and Cytology, 27: 433-440.

PHILLIPS, R.B. AND IHSSEN, P.E., 1986. Inheritance of Q-band Chromosomal polymorphisms in lake trout. J. of Heredity, 77: 93-97.

PHILLIPS, R.B. AND ZAJICEK, K.D, 1982. Q-band chromosomal banding polymorphisms in lake trout (Salvelinus namaycush). Genetics, 1091: 222-234.

PHILLIPS, R.B., ZAJICEK K.D. AND IHSSEN, P.E., 1989. Population differences in chromosome banding Polymorphisms in lake trout. Transactions of the American Fisheries Society 118: 64-73.

PONNAIAH, A.G., 1989. Functional relationship between biochemical genetic polymorphism and physiological variation in aquatic animals. In: P. Das and A.G.Jhingran (Eds). Fish genetics in India. Proceedings of the symposium on conservation and management of fish genetic resources of

India, held on 11-13 April, 1986. Today and Tommorow's. Printers and Publishers 24 B/15, Deshbandhu Gupta Road, New Delhi, 266 pp.

PRABHU M.S., 1967. The oil - sardine. Souvenir 20th Anniversary, Central Marine Fisheries. Res. Ins. Mandapam camp. pp.41-43.

PRABHU M.S., DHULKHED, M.H. 1972. On two new varieties of the Indian Oil Sardine, Sardinella longiceps val., Mahasagar - Bull. Nat. Inst. Oceanogr. 5(1): 27-30.

PROVINE, W.B., 1971. The origins of theoretical population genetics. In A.G. Debus (ed). The Chicago History of science and medicine. University of Chicago Press, Chicago and London 201 p.

RADOVICH, J., 1982. The collapse of the California Sardine fishery. What have we learned? Calif. Coop. Oceanic Fish. Invest. Rep. 28: 56-78.

RAO, S.N. AND DHULKHED, M.H., 1976. Electrophoretic characteristics of oil sardine (Sardinella longiceps) and mackerel (Rastrelliger kanagurta) eyelens proteins. Fish Technol., 13(1): 13-15.

RAO, D.M. AND RAO, K.S.,1983. Biometric comparison of Nemipterus japonicus (Bloch) populations along the east coast of India Proc. Indian Acad. Sci. (Anim. Sci.)., 92(5): 351-360.

RAJA B.T.A. 1969. Possible explanation for the fluctuation in abundance of the Indian oil sardine. Proc. Indo-Pac Fish. Coune. 15: 242-250.

REDDY, P.V.G.K., AND JOHN,G., 1986. A method to increase mitotic metaphase spreads and permanent chromosome preparation for karyotype studies in fishes. Aquaculture Hungarica (Szrvas), Vol. 5: 31-36.

REES, H., 1957. The chromosomes of Salmo salar. Chromosoma (Berlin) 15: 472-474.

REIDE, R., 1978. Order of living organisms. John Wiley and Sons, New York, 313 p.

RETZIAT, 1890. Biol Foren stockholm (Quoted from Makino, 1951).

REYMENT, R.A., BLACKITH, R.E. AND CAMPBELL, N.A. 1981. Multivariate morphometrics.Academic Press New York. NY 233p.

RICHARDSON, R.J., 1983. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. I. The jack mackerel, Trachurus declivis Jenyns. Aust. J. Mar. Freshwater Res., 33: 917-926.

RICKER, W.E., 1972. Hereditary and environmental factors affecting certain salmonid populations, P. 19-160. In R.C. Simon and P.A.Larkin (ed). The stock concept in pacific salmon. H.R. Mac Millan lectures in Fisheries, University of British Columbia, Vancouver, B.C. 231 p.

RIDDELL, B.E., LEGGETT, W.C. AND SAUNDERS, R.L., 1981. Evidence of adaptive polygenic variation between two populations of Atlantic salmon (Salmo salar) native to tributaries of the S.W. Miramachi River, N.B. Can. J. Fish. Aquat. Sci. 38: 321-333.

RILEY, L.M. AND CARLINE, R.F., 1982. Evaluation of scale shape for the identification of walleye stocks from western lake Erie. Trans. Amer. Fish. Soc. 11: 736-741.

RISHI, K.K., 1973. A preliminary report on the karyotypes of 18 marine fishes. Res. Bull. (NS) Punjab Univ. 24: 161.

RISHI, K.K. 1975. Somatic and meiotic chromosomes in Trichogaster fasciatus (Bloch & Sch) (Teleostei, Perciformes, Osphronemidae) Genen Phaenen 18:49.

RISHI, K.K. 1976. Mitotic and meiotic chromosomes of Callichorus bimacultus (Bloch) with indications of male heterogamety, Ciencia Cult. 28: 1171.

RISHI, K.K., 1978. Giemsa banding in fish chromosomes. Curr. Sci. 47(11): 393-394.

RISHI, K.K., 1979. Somatic G-banded chromosomes of Colisa fasciatus (perciformes, Belontidae) and confirmation of female heterogamety copeia 1:146.

RISHI, K.K. AND GAUR, P., 1976. Cytological female hetorogamety in jet black molly Molliensia sphenops. Curr. Sci. 45: 660.

RISHI, K.K. AND SINGH J., 1982. Karyological studies on five estuarine fishes. Nucleus 25: 178-180.

ROBERTS, F.L., 1964. A chromosome study of twenty species of Centrachidae. J. Morph., 115: 401-418.

ROBERTS, F.L., 1966. Cell culture of fibroblasts from Clupea harengus gonads. Nature, Lond., 212: 1592-1593.

ROBERTS, F.L., 1967. Chromosome cytology of the Osteichthyes. Prog. Fish Cult., 29: 75-83.

ROBERTS, F.L., 1968. Chromosomal polymorphisms in North American landlocked Salmo salar. Can. J. Genet. Cytol. 10: 865-875.

ROBERTS, F.L., 1970. Atlantic salmon (Salmo salar) chromosomes and speciation. Trans. Am. Fish. Soc. 99: 105-111.

ROHLF, F.J., 1990. Morphometrics. Annu. Rev. Evol. Syst. 21: 299-316.

ROHLF, F.J. AND BOOKSTEIN, F.L., 1987. A comment on shearing as a method for "Size correction". Syst. Zool. 36: 356-367.

RYMAN, N.1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture: 33:1-21.

RYMAN, N., ALLENDORF, F.W. AND STAHL, G. 1979. Reproductive isolation with little genetic difergence in sympartic populations of brown trout (Salmo trutta). Genetics, 92: 247-262.

RYMAN, N., LAGERCRANTZ, U., ANDERSSON, L., CHAKRABORTY, R. AND ROSENBERG, R., 1984. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (Clupea harengus) Heredity 53: 687-704.

SAGE, R.D. AND SELENDER, R.K., 1975. Trophic radiation through polymorphism in cichlid fishes. Proc. Nati. Acad. Sci. USA. 72: 4669-4673.

SAILA, S.B. AND JONES, C., 1983. Fishery science and the stock concept. Final report P.O. NA83-B-A-0078 (MS) NMFS, Northeast Fisheries Centre, Woods Hole, MA 02543 F.L.

SALINI, J. AND SHAKELEE, J.B., 1988. Genetic structure of Barramundi(Lates calcarifer) stocks from Northern Australia. Aust. J. Mar. Fresh Water. Res. 39: 317-329.

SCHAEFER, K.M., 1989. Morphometric analysis of Yellowfin tuna, Thunnus albacares, from the eastern pacific Ocean. Inter - Am. Trop. Tuna Comm., Bull. 19: 387-427.

SCHMIDT, J., 1917. Racial investigations I. Zoarces viviparus L. and local races of the same. C.R. Trav. Lab. Carlsberg, 13: 279-397.

SHAKLEE, J.B., 1983. The utilization of isozymes as gene markers in fisheries management and conservation. In M.C. Rattazzi, J.G. Scandalios and G.S. Whitt (editors), Isozymes. Current topis in biological and medical research, Vol. II, p. 213-247.

SHAKLEE, J.B., 1984. Genetic variation and population structure in the demersal fish, Stegastes fasciolatus throughout the Hawaiian Archipelago. Copeia, 3: 629-640.

SHAKLEE, J.B.,ALLENDORF, F.W., MORIZOT, D.C. AND WHITT, G.S., 1989. Genetic nomenclature for protein coding loci in fish: Proposed guidelines. Trans. AM.Fish. Soc. 118:218-227.

SHAKLEE, J.B., AND SALINI J.P., 1985. Genetic variation and population subdivision in Australian barramundi, Lates calcarifer (Bloch). Aust.J.Mar.Freshwater Res., 36: 203-218.

SHAKLEE, J.V. AND TAMARU, C.S. 1981. Biochemical and morphological evolution of Hawaiian bonefishes. (Albula) Syst. Zool., 30: 125-146.

SHAW, C.R. AND PRASAD, R. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem.Genet.,4:297-320.

SICK, K., 1961. Haemoglobin polymorhism of cod in the Baltic and the Danish Belt Sea. Hereditas. 54: 19-48.

SICK, K., WESTERGUARD, M. AND FRYDENBERG O., 1962. Haemoglobin pattern and chromosome number of American, European, and Japanese eels. Nature, London. 193: 1001-1002.

SIEGEL, A.F. 1982. Geometric data analysis: an interactive graphics programme for shape comparison. In: Modern data analysis. R.L. Lanner and A.F.Siegel (eds) PP 101-122. New York Academic Press.

SIEGEL A.F., AND BENSON, R.H. 1982. A robust comparison of biological shapes, Biometrics 38: 341-350.

SIMON, R.C., 1963. Chromosome morphology and species evolution in the five North American species of pacific salmon (Oncorhynchus) J. Morphol. 112, 77-95.

SIMON, R.C., AND DOLLAR, A.M., 1963. Cytological aspects of speciation in two North American teleosts, Salmo gairdneri and S. clarki lewisi, Can J. Genet. Cytol 5: 43-49.

SINGH, S.M., AND GREEN, R.H., 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. Malacologia, 25: 569-581.

SMITH, I., 1968. In: Chromotographic and electrophoretic techniques, 2nd edition. Heinmann Medical Books Ltd., London, 2: 524 pp.

SMITH, P.J., 1986. Genetic similarity between samples of the organge roughy Hoplostethus atlanticas from the Tasman sea, South West Pacific Ocean and North East Atlantic Ocean.

SMITH, P.J., BIRLEY, A.J. AND JAMIESON, 1989. Electrophoretic studies and the stock concept in marine fishes. J. Fish. Biol. 35(A): 345-346.

SMITH, M.H. AND CHESSER, R.K. 1981. Rationale for conserving genetic variation of fish pools. In fish Gene pools (RYMAN, N., ed), Ecological Bulletin, 34.

SMITH, P.J., FRANCIS, R.I.C.C. AND PAUL, L.J., 1978. Genetic variation and population structure in the New Zealand snapper. N.Z.J. Mar. Freshwater Res., 12(4): 343-350.

SMITH P.J., FRANCIS, R.I.C.C. AND JAMIESON, A., 1981. An excess of homozygotes at a serum esterase locus in the Atlantic mackerel, Scomber scombrus. Anim. Blood Grps. Biochem. Genet., 12: 171-180.

SMITH, P.J., AND JAMIESON, A., 1978. Enzyme polymorphisms in the Atlantic mackerel, Scomber scombrus L., Comp. Biochem. Physiol., 60B: 487-489.

SMITH, P., JAMIESON, A. AND BIRLEY, A.J., 1990. Electrophoretic studies and stock concept in marine teleosts. Journal du conseil, 47: 231-245.

SMITH, P.J., MCKOY, J.L., AND MACHIN, P.J., 1980. Genetic variation in the rock lobsters, Jasus wardsii and Jasus novachollandiae, N.Z.J. Mar. Freshwater Res., 14(1): 55-63.

SMITH, P.F. AND MOSER, H.C., 1988. Calco Fi times: an overview of fishes. Calif. Coop. Oceanic fish. Invest. Rep. 29:66-77.

SMITH, J. AND SHAKLEE, J.B., 1988. Genetic structure of barramundi (Lates calcarifer) stocks from Northern Australia, Aust. J. Mar. Freshwater. Res., 39: 317-329.

SOKAL, R.R., AND RINKEL, R.C. 1963. Geographic variation of alate Pemphigus populitransversus, in Eastern North America. Univ. Kans. Sci. Bull. 44: 467-507.

SOLA, L., CAMERINI, B. AND CATAUDELLA, S., 1984. Cytogenetics of Atlantic eels; G-banding, nucleolar organizer regions and DNA content. Cytogenet cell Genet 78(3): 206-210.

STAHL, G., 1987. Genetic population structure of Atlantic salmon. In: N. RYMAN AND UTTER F.M. (eds.). Population genetics and Fisheries management. University of Washington Press, Seattle, W.A., PP. 121-140.

STANLEY, J.B. 1979. Macroevolution: Pattern and process. W.H. Freeman and Company. San. Francisco CA 382. p.

STEWART, K.W. AND LEVIN, C.B., 1968. A method of obtaining permanent dry mounted chromosome preparations from teleost fish. J. Fish Res. Board of Canada 25: 1091-1093.

STOCK, A.D., BURNHAM, D.B. AND HSV, T.C., 1972. Giemsa banding of meiotic chromosomes with description of a procedure for cytological preparations from solid tissues. Cytogenetics 11:534-539.

STONEKING, M., WAGNER, D.J. AND KIDDEBRAND, A.C., 1981. Genetic evidence suggesting subspecific differences between northern and southern population of brook trout(Salvinus fontinalis). Copeia, 4: 810-819.

STRAUSS, R.E. AND BOOKSTEIN, 1982. The truss: body form reconstructions in morphometrics. Syst. Zool. 31:113-135.

SUBRAHMANYAM, K. AND RAMAMURTHI K., 1971. A karyotype study in the esturine worm eel, Moringa linearis (Gray) Sci. Cut., 37(4): 201-202.

SUNDARA RAJ, B., 1934. Administration Report for the year 1932-33, Madras Fisheries Department, Madras.

SUNDARA RAJ, B, 1937. Administration Report for the year 1935-36, Madras Fisheries Department, Madras.

SVARDSON, G., 1945. Chromosome studies in Salmonidae. Rep. Inst. Fresh Water. Res. Drottningholm, 23: 1-151.

SWAIN D.P., RIDDEL, B.E. MURRAY B.C., AND MURRAY, 1991. Morphological differences between hatchery and wild populations of Coho salmon (Oncorhynchus kisutch): environmental versus genetic origin. Can. J. Fish. Aquat Sci. 48: 1783-1791.

SWARUP, H., 1959. Production of triploidy in Gasterosteus aculeatus. J. Genet. 56: 129-142.

SWEET, S.S., 1980. Allometric inference in morphology. Am: Zool. 28: 643-652.

TAYLOR, E.B. AND McPHAIL J.D., 1985. Variation in body morphology among British Columbia populations of coho salmon (Oncorhynchus kisutch). Can.J.Fish.Aquat.Sci. 42: 2020-2028.

THOMAS, A.E. AND DOHAHOO, M.J., 1977 Differences in swimming performance among strains of rainbow trout (Salmo gairdneri) J. Fish. Res. Board. Can. 34: 304-306.

THORGAARD, G.H., 1976. Robertsonian polymorphism and constitutive heterochromatin distribution in chromosomes of the rainbow trout (Salmo gairdneri), Cytogenetics and cell genetics 17: 174-184.

THORGAARD, G.H., 1977. Heteromorphic sex chromosomes in male rainbow trout, Science 196: 900-902.

THORGAARD, G.H., 1983. Chromosomal differences among rainbow trout populations Copeia: 650-662.

THORGAARD, G.H. AND ALLEN, S.K., 1987. Chromosome manipulations and markers in fishery management. P. 319-332 in Ryman and Utter (1987).

THORPE, R.S, 1976. Biometric analysis of geographic variation and racial affinities. Biol. Rev. 51: 407-452.

THORPE, R.S., 1983. A review of the numerical methods for rec ognising and analysing racial differentiation. In numerical Taxonomy (Felsestein, J., ed.). PP. 404-423. Berlin: Springer - Verlag.

THORPE, R.S., 1987. Georgraphic variation: a synthesis of cause, data pattern and congruence in relation to sub species, a multivariate analysis of size and shape. Experientia 38: 920-923.

TJIO, J.H. AND LEVAN, A., 1956. The chromosome number of man. Hereditas: 42: 1-6.

TODD, T.N., SMITH, G.R. AND CABLE. L.E., 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (Coregoninae) of the Great lakes. Can. J. Fish. Aquat. Sci. 38: 59-67.

TSUYUKI, H., AND ROBERTS, E., 1965. Zone electrophoretic comparison of Muscle myogens and blood proteins of artificial hybrids of Salmonidae with their parental species. J. Fish. Res. Bd. Canada. 22: 767-773.

TSUYUKI, H., KERR, R.H., UTHE, J.F. AND CLARKE, L.W., 1967. Comparative electropherograms of the family Catostomidae. J. Fish. Res. Bd. Canada 24: 299-304.

TSUYUKI, H., ROBERTS, E., KERR, R.H. AND RONALD, A.P., 1966. Microstarch gel electrophoresis. J. Fish. Res. Brand Can. 23: 929-933.

TSUYUKI, H., ROBERTS, H. AND VANSTONE, W.E., 1965a. Comparative zone electropherograms of muscle myogens and blood haemoglobins of marine and fresh water vertebrates and their application to biochemical systematics. F. Fish. Res. Bd. Canada, 22(1): 203-213.

TSUYUKI, H., ROBERTS, H., VANSTONE,W.E. AND MARKERT, J.R., 1965b. The species specificity and constancy of muscle myogen and hemoglobin electropherograms of Oncorhynchus. J. Fish. Res. Bd. Canada, 22(1): 215-217.

TURNER, B.J., 1974. Genetic divergence of death valley pupfish species; Biochemical versus morphological evidence. Evolution 28: 281-294.

UEDA, T., OJIMA, Y., KATO, T. AND FUKUDA, Y., 1983. Chromosomal polymorphisms in the rainbow trout (Salmo gairdneri) Proc. Jpn. Acad., B 59: 168-171.

UTHE, J.F. AND RYDER, R.A, 1970. Regional variation in muscle myogen polymorphism in walleye (Stizostedion vitreum Vitreum) as related to morphology. J. Fish. Res. Bd. Canada, 27: 923-927.

UTTER, F.M., 1987. Protein electrophoresis and stock identification in fishes. In: H.E. KUMPF., R.N. VAUGHI, C.B. GRIMES, A.G. JOHNSON AND E.L. NAKAMURA (Eds.) Proceedings of the stock identification workshop. NOAA Technical report.

UTTER, F.M., AEBERSOLD, P., AND WINANS, G., 1987. Interpreting genetic variation detected by electrophoresis. In: N. Ryman and F.M. Utter (Eds.). Population genetics and Fishery management. Washington sea Grant program, University of Washington Press, Seattle and London, PP. 21-45.

UTTER, F.M., ALLENDORF, F.W., AND HODGINS, H.O., 1973. Genetic variability and relationships in pacific salmon and related trout, based on protein variations. Sys.Zool.,22(3):257-270.

UTTER, F.M. AND HODGINS, H.O., 1972. Biochemical genetic variation at six loci in four stocks of rainbow trout. Trans. A.M. Fish. Soc., 3: 494-502.

UTTER, F.M., HODGINS H.O. AND ALLENDORF, F.W., 1974. Biochemical genetic studies of fishes: Potentialities and limitations. In: Biochemical and Biophysical perspectives in marine biology, Vol. 1, P. 213-237. Academic Press, San Francisco.

UTTER, F.M., MILNER, G.B., STAHL, G., AND TEEL, D.J., 1989. Genetic population structure of chinook salmon. Oncorhynchus tshawytscha in the pacific northwest. Fish.Bull., 87:239-264.

UTTER, F.M., RIDGWAY, G.J. AND WARREN. J.W., 1966. Serological evidence for inbreeding of Lahontan cutthroat trout, Salmo clarki in summit lake Nevada. Calif. Fish. Game 52: 180-184.

UTTER, F.M., WAPLES, R.S. AND TEEL, D.J., 1992. Genetic isolation of previously indistinguishable chinook salmon populations of the Snake and Klamath rivers: Limitations of negative data. Fish. Bull., 90(4): 770-777.

UYENO, T. AND MILLER, R.R. 1971. Multiple sex chromosomes in a Mexican cyprinodontid fish. Nature (London) 231: 452-453.

VANDERBANK, F.H., AND FERREIRA, J.T. 1986. Morphometric and electrophoreptic analyses of three wild populations of Oreochromis mossambicus (Peters). Proc. World. Symp. on Selection. Hybridisation and Genetic engineering in Aquaculture: Bordeaux, 103-112.

VANSTONE, W.E. AND MARKERT, J.E, 1968 Some morphological and biochemical changes in coho salmo (Oncorhynchus kisutch) during parr-smott transformation. J. Fish. Res. Board. Can. 25: 2403-2418.

VENKITAKRISHNAN, P., 1994. "Biochemical genetic studies on the oil sardine, Sardinella longiceps (CUVIER AND VALENCIENNES 1847) from selected centres of the West Coast of India.Ph.D. Thesis Cochin University of Science and Technology, Kerala, India.

VERSPOOR, E., 1988. Reduced genetic variability in first generation hatchery populations of Atlantic salmon (Salmo salar) Can. J. Fish. Aquaqt. Sci., 45: 1686-1690.

VIJAYAKUMAR, S., 1992. Studies on biochemical genetics of the grey mullet Mugil cephalus (Linnaeus). Ph.D. Thesis, Cochin University of Science and Technology, Cochin, Kerala, India, 113 pp.

VILLWOCK, W., 1963. Genetische analyse des merkmals "Bschuppung" beig anatolischan Zahnkarpfen (Pisces: Cyprinadontidae) in Aufloserver-auch. Zool. Anz. 170. (1-2): 23-45.

WALMAN, 1988. Review of stock discrimination techniques for stripped Bass. North American J. Fish Management 8: 410-425.

WHITE, M.J.D., 1973. "Animal cytology and Evolution" Cambridge Univ. Press. London.

WILLISCROFT, S.N. AND TSUYUKI, H., 1970. Lactate dehydrogenase systems of rainbow trout-evidence of polymorphism in liver and additional subunits in gills. J. Fish., Res. Bd., Canada. 27: 1563-1507.

WILSON, G.M., THOMAS, W.K., BECKENBACH A.J., 1985. Intra and inter-specific mitochondrial DNA sequence divergence in Salmo: rainbow, steelhead and cutthroat trouts. Can. J. Zool. 63: 2088-2094.

WINANS, G.A., 1980. Geographic variation in the milkfish, Chanos chanos I. Biochemical evidence. Evolution, 34(3): 558-574.

WINANS, 1984. Multivariate morphometric variability in Pacific salmon: Technical demonstration. Can. J. Fish. Aquat. Sci. 41: 1150-1159.

WINANS, 1985. Geographic variation in the milk fish Chanos chanos II Multivariate morphological evidence. Copeia 1985: 890-898.

WINANS, G.A. 1987. Using morphometric and meristic characters for identifying stocks of fish. Pages 25-62 in H.E. KUMPF, R.N. VAUGHT, C.B.G. RIMES, A.G. JOHNSON, AND E.L. NAKAMURA, editors. Proceedings of the stock identification workshop, November 5-7, 1985. PANAMA CITY, FLORIDA. NOAA Technical Memorandum NMFS, SEFC. 199.

WISHARD-SEEB, L., AND GUNDERSON, D.R., 1988. Genetic variation and population structure of Pacific ocean perch Sebastes alutus. Can. J. Fish. Aquat. Sci. 45: 78-88.

WISHARD, L.N., SEEB, J.E., UTTER, F.M., 1980. Biochemical genetic characteristic of native trout populations of owyhee country, Idaho. Final report, Bureau of land management.

Bois district office, pacific fisheries Research, 509, East 12th/10, Olympia, Washington, 98501.

WITHLER, R.E., 1985. Ldh-4 allozyme variability in North American sockeye salmon (Onchorhynchus nerka) population. Can. J. Zool., 653: 2924-2932.

WOLF, K. AND QUIMBY, M.C., 1969. Fish cell and tissue culture. In Fish Physiology, Vol. 3: 252. New York.

WOLF, K., QUIMBY, M.C., PYLE, E.A. AND DEXTER R.P., 1960. Preparation of monolayer cell cultures from tissues of some lower vertebrates. Science 132, 1890-1891.

WRIGHT, J.. (1955) Chromosome numbers in trout. Prog. Fish Cult. 17: 172-176.

WRIGHT, J.E. AND ATHETON, L.M., 1970. Polymorphisms for LDH and transferin loci in brook trout populations. Trans. AM. Fish. Soc., 1: 179-192.

YAMAMOTO, K. AND OJIMA, Y., 1973. A PHA-culture method for cells from the renal tissues of teleosts. Jap.J.Genet. 48:235-238.

ZHANG, S.I., 1990. Somatic chromosomes of two air-breathing fishes Channa punctatus (Bloch) and Heteropneustes fossilis (Bloch) J. Appl. Zool. Res. 1(1): 7-10.