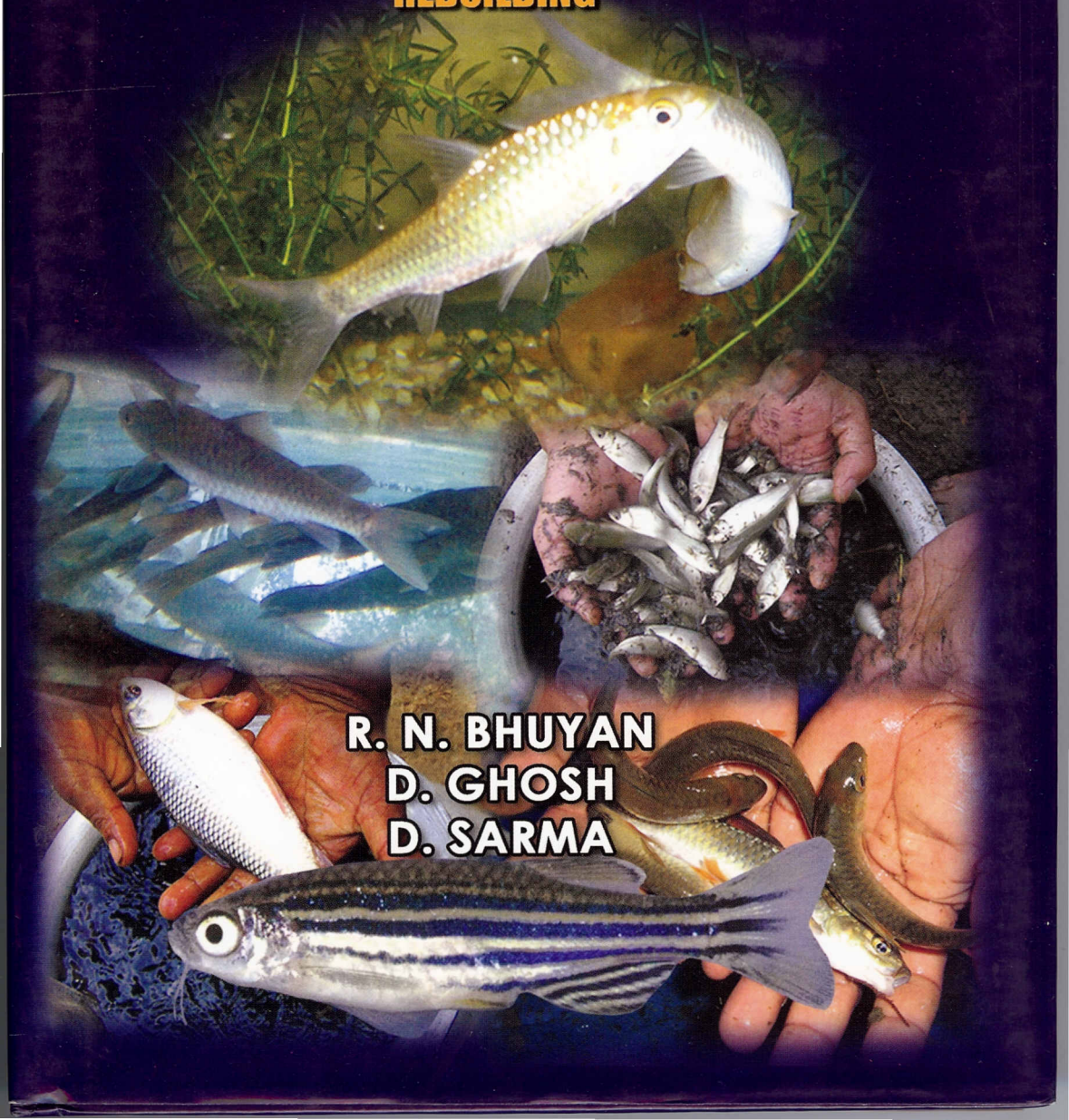
FISH AND FISHERIES IN NORTH EAST INDIA

RECENT ADVANCES AND REBUILDING



R. N. BHUYAN
D. GHOSH
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R.N. Bhuyan

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FOREWORD

The book ***"Fish And Fisheries In North East India, Recent Advances & Rebuilding"*** collection of scholarly papers edited by Dr. R. N. Bhuyan, Dr. D. Ghosh and Dr. Sarma, is the result of the hard work of the learned scientists who shared their knowledge in the seminar organised by the ***Department of Pisciculture*** of ***St. Anthony's College.*** The suggestions and findings of the Seminar which are the results of sharing and discussion on the researches of the scientists who have done a lot f surveys, experiments, thinking, coupled with their innovative initiative are of immense value. I am sure that the book will be a treasure for all scholars and lay persons, students and farmers.

One aspects of the book, which can be easily seen, is the application possibility. The gems of knowledge are not and will not be confined only to the library but they will be translated into practical realities. It is clear examples of the possibility of fast transformation and application of scientific findings. Even a lay person can see that it is possible to journey *"from idea to reality, from theory to practice, from seminar to the fields, from paper to farm tools."*

I congratulate the *Department of Pisciculture, St. Anthony's College*, for publishing the book. I thank the scientist and scholars for their contributions. I thank North Eastern Council for financial support.

God bless

24.06.2008

Fr. I. Warpakma SDB
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PREFACE

The importance of freshwater aquaculture has assumed the status of a fast expanding industry not only in India, but also in several Asiatic Countries over the recent years. Various research organizations and NGOs have contributed to a great extent to this development process, blending the traditional practices with scientific data bases and techniques. In recent years, however, the main problem faced by the fisheries sector is not the culture of fishes for food purposes, but the destruction of the natural ecosystems and thereby loss of precious gene pool. In this context it may be mentioned that the North Eastern States of the country have a unique ecology and is considered as one of the mega biodiversity hot spots of the world, for both flora and fauna, among which fishes have an important place. The North Eastern States of India have bountiful aquatic resources like many species of indigenous ornamental fishes and game fishes especially the Chocolate Mahseer.

In recent years the country has been witnessing a great spurt in research activities in different disciplines of the fisheries sector. There is need for documentations of these new developments and recent advances that have taken place in the fisheries sector, particularly in North Eastern India. It is with this view that the Department of Pisciculture had organized a National Seminar on "Recent Advances and Rebuilding of Fish and Fisheries in North East India" to bring together all the researchers, NGOs, Institutes working in different fields and establish close linkage and exchange methodologies and other recent developments made so far in the country, particularly in N E region. The objective of this seminar

was a comprehensive documentation of recent fishery technology, conservation and sustainable use of resources available, which is yet to be carried out for the development and food security of the region in particular, and the country in general. Secondly, it was felt that the seminar would open new avenues to needy fisheries entrepreneurs and educate rural fish farmers on the recent technologies involved in fish culture, breeding and training so that more and more people can start fish culture as their vocation and join the blue revolution of the country. We hoped that the seminar would also identify the scientists working for the development of fisheries in the region and form a common platform for the further development of the fisheries sector.

The North-Eastern part of India, comprising of seven sisters and one brother, is a part of Eastern Himalayan region and is one of the global hotspots for flora and fauna biodiversity. Each state has its unique topography, diverse geographical features and varied watershed patterns. The region is fed by the Brahmaputra river system and its various tributaries crisscross different states and ultimately join the Brahmaputra. These make the area a favourable site for development of fisheries and fish biology studies. Innumerable hill streams, rivers, wetlands and perennial water sources abound in the area. In Meghalaya, diverse climatic conditions make it conducive for its floral and faunal wealth. The entire region mostly depends upon capture fisheries. The state abounds in numerous man-made water bodies, which have not been adequately exploited for its fishery potential. Vast lentic water bodies can be utilized for fish production catering to the needs of increasing demand of fish protein in the state.

The seminar was inaugurated by Smti. M.K.H. Marak, Secretary, Fisheries, Government of Meghalaya and attended by many other dignitaries including Principals of different colleges of Meghalaya. The seminar was a great success with more than eighty participants taking part in the deliberations. The papers presented in the seminar were discussed in four different sessions and there

were two theme papers in each session presented by distinguished scientists in each field. At the end of the deliberations in the technical sessions, there was a panel discussions and a 12 (Twelve) point recommendations are presented for reference in research and development of the aquaculture, implementation of recent advances in the field and conservation of threatened fish species from the N E Region of India. The valedictory function of the seminar was graced as Chief Guest by Mr. P. Kharkongor, Commissioner of Fisheries, Government of Meghalaya.

Admittedly, bringing out of the proceedings has been delayed, as great efforts had to be made get the contributed papers and regrettably some papers have been omitted and some could not be collected.

We hope, the proceedings will help all concerned in the development of fisheries, research in various fields and in the conservation of many important fish species of the country particularly from the North East India.

We take this opportunity to thank NEC for their sponsorship without which it would not have been possible to organize this important event. We wish to place on record our appreciation to the Principal and Vice-Principal of St. Anthony's College, Shillong and the national advisory committee as well as others who directly or in ay other way helped to make the seminar a success.

R. N. Bhuyan
Devjani Ghosh
Debajit Sarma

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Genetic Variation Detectable by Electrophoretic Separation of Enzyme Variants in Three Species of the Genus *Channa*

Q. Kharbuli and K. Chatterjee

Abstract

Channa, one of the commercially important air-breathing fishes of India are endowed with remarkable powers of respiration that enable them to lead an amphibious life and to survive the adverse conditions in swampy areas. The importance of the problem of culturing and propagating these fishes in the swamps need to be recognized and subsequently, techniques developed for the proper utilization of swamps for these purposes. Realization of these objectives would have considerable significance in the augmentation of fish production especially in rural areas. All the representatives are very much alike in colour and shape and therefore, are often difficult to distinguish when they are of the same size. Tissue extracts of brain, eye, heart, kidney, liver and muscle were screened by polyacrylamide gel electrophoreses (PAGE) for eleven enzymes. The results expressed as relative mobilities of the observed isozyme bands using specific histochemical techniques, allow direct comparison between the three species studied. The genetic variability patterns in loci encoding for the eleven enzymes was found to differentiate the three species viz., *Channa orientalis, Channa punctatus* and *Channa striatus.* The tissue specificity of these enzymes was also studied. The implications of these findings are discussed in relation to different strategies for identification and utilization of existing genetic resources.

Introduction

The information encoded in the sequence of a gene specifies the sequence and therefore, the structure and function of every protein molecule in a cell. Isozymes or isoenzymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. First described by Hunter and Markert (1957) they defined isozymes as different variants of the same enzyme having identical functions and present in the same individual. This definition encompasses enzyme variants that are the product of different genes and thus represent different loci and enzymes that are the product of different alleles of the same gene (described as allozymes). The term is sometimes used to refer to the quaternary structure of a multi-subunit protein where there are different possible combinations of isozymes subunits. Isozymes have been widely used as molecular markers in population genetics since the 1960's, i.e., to study the causes and effects of genetic variations within and between populations. The usefulness of isozymes as molecular markers is based on the fact that any change in the structure of an enzyme is directly related to a change in DNA.

Polyacrylamide gel electrophoresis is a powerful technique for detecting the differences in proteins (mostly enzymes) between species. A minor difference in the molecular structure of a protein may result in it having a different net charge and mobility leading to an altered position on the gel. The proteins thus identified serve as valuable tools for comparison of the genetic constitution of related and unrelated species.

The murrels commonly known as snakeheads are represented by a single genus the *Channa* (Scopoli, 1777; Greenwood et al., 1966). While considering the development and standardization of the fishery management techniques of these fish in this region many gaps in information can be experienced in their biology, systematics and genetics. Therefore, we have undertaken a detailed study of electrophoretic analysis for eleven enzymes in our laboratory. This paper reports the genetic variation detected by polyacrylamide gel electrophoresis in three species i.e., *C. orientalis, C. punctatus* and *C. striatus.*

Materials and Methods

C. orientalis, (Bloch and Schneider, 1801) *C. punctatus* (Bloch, 1793) and *C. striatus* (Bloch, 1793) were collected from natural populations from streams in and around Shillong and from some fresh water bodies near Guwahati. Live specimens were transported to the laboratory; some were acclimatized for 10-20 days while the rest were stored at –40°C in the ultra freezer (Scien Temp 2000, USA) until use. To avoid ontogenic problems only adult specimens were used for tissue extraction.

Tissues such as brain, eye, heart, kidney, liver and muscle were excised, blotted dry and weighed accurately. The tissues were then homogenized in 0.25M sucrose solutions using the Umetrex electric homogenizer. The extracts were then centrifuged at 14,000 X g for 20 minutes and the supernatant was collected. For mitochondrial extract 0.1 (M) phosphate buffer pH 7.4 was used and the homogenate subjected to centrifugation at 600 X g for 10 minutes. To the clear supernatant equal volume of triton X was added and mixed thoroughly. It was then left for 30 minutes after which each extract was sonicated for 30 seconds and again subjected to centrifugation at 14,000 X g for 30 minutes. The samples were then subjected to vertical polyacrylamide gel electrophoresis (Davis, 1964). The staining procedure as described by Shaw and Prasad (1970) and Pasteur et al. (1988) was followed. All the above procedures were carried at 4°C in ice to prevent denaturing of the enzymes. To avoid ontogenic problems only adult specimens were used for tissue extraction.

Results and Discussion

The most widely studied enzyme in vertebrates is lactate dehydrogenase and in all it is a tetramer with two major isozymes. These are *LDH A* and *LDH B*; the former is predominant in the skeletal muscle (Nadal-Ginard and Markert, 1975) and the latter in the heart (Wilson et. al., 1963; Everse and Kaplan, 1975). Isozymes A and B are encoded by two gene loci *ldh-A* and *ldh-B*. The two subunits can associate in the cytoplasm (Kaplan, et al., 1956; Markert, 1963) to produce as many as five bands i.e., the two homotetramers A_4 and B_4

and the three heterotetramers A_3B_1, A_2B_2 and A_1B_3 as in the case of *C. punctatus*. In *C. orientalis* and *C. striatus* only three bands are exhibited, the A_4 and B_4 homopolymers with an A_3B_1 heteropolymer in *C. orientalis* and A_2B_2 heteropolymer in *C. striatus*. A_4 (with normally low anodic speed) and B_4 (with a usually greater negative charge), exhibits reverse mobility in the genus *Channa*. This phenomenon has been reported in one-thirds of the teleosts examined (Markert and Faulhaber, 1965; Chatterjee and Dhar, 1985; Coppes et al., 1987). A third isozyme prevalent in most teleosts was observed only in *C. orientalis*, with its function restricted to the eye tissue. This tetramer with a high anodic rate is designated as *LDH C* (Markert et al., 1975) and is encoded by the *ldh-C* locus. Heteropolymers between A_4 and C_4 was also observed.

Malate dehydrogenase and Malic enzyme exhibit two isozymatic systems in all vertebrates, the cytosolic (cMDH) and mitochondrial (mMDH) forms. These two forms have the same molecular weight but differ in amino acid composition and electrophoretic mobility and are controlled by separate gene loci as well (Whitt, 1970b, 1971). Moreover the two forms do not combine with each other to form heteropolymer (Manwell and Baker, 1970); a characteristic that distinguishes the two forms beyond doubt.

In the majority of fish species duplicated cytosolic MDH loci appears to code for the two MDH isozymes i.e., *MDH A* and *MDH B*. Random association of the two dimers generate three bands A_2 and B_2 homodimers and A_1B_1 heterodimer. The B_2 homodimer with low activity is predominant in skeletal muscle (Bailey et al., 1970; Papasotiropoulos et al., 2001; Yang et al., 2001) while the A_2 homodimer with high activity is equally distributed in all tissues (Fisher et al., 1980; Basaglia, 1989). We also report the same observations in all three species. Mitochondrial malate dehydrogenase (mMDH) appears as a single cathodal band in *C. orientalis* and *C. striatus*. In *C. punctatus* a reverse mobility is exhibited with mMDH migrating more anodally than cMDH a phenomenon also observed in *Fundulus heteroclitus* (Whitt, 1970b) though rare in most fishes. In fishes a single gene is suggested to be involved in the production of mMDH.

Mitochondrial malic enzyme is expressed cathodally to cytosolic malic enzyme in all three species. A single gene codes for mME in *C. orientalis*, while 2 loci for *C. punctatus* and *C. striatus*. The duplicate status of mME has been reported for many salmonids (Allendorf et al., 1977; Stoneking et al., 1979). May (1980) however, stated that only one locus, is involved in three North American Coregonids. The bands in the anodal zone represent the cytosolic malic enzyme, which shows a relatively high amount of variation in *C. orientalis* and *C. punctatus*, in which five irregularly spaced bands are usually observed. The most plausible explanation for this variation is the involvement of two loci, with one fixed and one variable locus. This has been reported in *Vendace* (Vourinen, 1984). In *C. striatus* a single locus appears to code for cME.

The banding patterns reveal a tetrameric structure of the enzyme, which has been reported in all fish species, examined (Nevaldine et al., 1974).

Glucose 6-phosphate dehydrogenase is among the most thoroughly studied of the enzymes in mammals. G6PD was found to exist in two forms in deer mouse (*Peromyscus maniculatus*), which were arbitrarily, designated as the A and the B forms (Shaw and Barto, 1965). These two forms have also been the subjects of several reports, many of which have noted with interest the nonhomology between the two forms. The A form is specific for glucose 6-phosphate and $NADP^+$ (Noltman and Kuby, 1963) while the B form shows a broader spectrum of substrate specificity. It is able to catalyze the oxidation of glucose 6-phosphate, as well as galactose 6-phosphate, 2-deoxyglucose 6-phosphate and glucose, with either NAD^+ or $NADP^+$ serving as coenzyme (Beutler and Morrisson, 1967; Shaw and Koen, 1968). Although these two enzymes fit the criteria of Shaw (1969) for primary isozymes, certain investigators (Ohno et. al., 1966) designated the B form of G6PD enzyme as hexose 6-phosphate dehydrogenase. The evidence of a homology between glucose 6-phosphate dehydrogenase with hexose 6-phosphate dehydrogenase has been reported by Matsuoka et al., (1983) by comparison of the amino acid sequence. Another enzyme glucose dehydrogenase, was suggested by Strecker

and Korkes (1952), Metgzer et al., (1965) and Beutler and Morrison (1967) to be identical to hexose 6-phosphate dehydrogenase based on properties other than substrate specificity. In addition, Stegeman and Goldberg (1971) found a commercially available GD (stigma) that exhibited catalytic activity with the various substrates and coenzymes acted upon by H6PD. Further investigations would enlighten the result obtained by us though our report suggested that these two enzymes are encoded in different genes.

The observed pattern for G6PD suggests the expression of a single locus in *C. punctatus* and two loci for *C. orientalis* and *C. striatus*. In *C. orientalis G6PD-2* isozyme was found to be stable in heart, kidney and liver, but unstable in brain, eye and muscle tissues. Moreover, an allele for *g6pd-2* locus that appeared to be stable was detected in liver but unstable in heart and kidney. *C. punctatus* exhibited two alleles for *g6pd-1* locus, the wild type was found to be expressed in all the six tissues, while the mutant allele was highly unstable. It appeared to be fixed in kidney but unstable in the rest of the tissues. In *C. striatus* on the other hand *G6PD-1* isozyme was predictable in brain and eye and *G6PD-2* isozyme in liver, while heart and kidney showed a flexible expression for both isozymes. In some individuals *G6PD-1* isozyme is active and in others *G6PD-2* but both were never expressed together at the same time in an individual in these two tissues. The pattern of phenotypic expression of these isozymes strongly revealed that both loci are never required for the synthesis of G6PD enzyme, either one is sufficient to produce a functional G6PD. It is also tempting to conclude that the second locus of G6PD arose as a result of gene duplication.

Investigation on the H6PD isozyme system is scarce. Two loci appear to code for H6PD in *C. orientalis* and *C. striatus*. In *C. orientalis* the bands at the cathodal zone are designated as *H6PD-1* and the two anodal bands observed only in liver arising as a result of the expression of two codominant alleles as *H6PD-2*. In *C. striatus* the two loci are polymorphic. In liver some individuals shows the activity of *H6PD-1* and others *H6PD-2*. The appearance of two bands for *H6PD-1* in eye and heart suggest two alleles for this locus. Stegeman and Goldberg (1971) also observed polymorphism of H6PD in lake, brook and rainbow trout. Ropson and Powers (1989) reported the allelic isozymes of H6PD for *Fundulus heteroclitus*. In *C. punctatus* the presence of a single locus is explicit whose expression is highest in eye and liver.

Review of literature shows limited data on biochemical genetic studies of glucose dehydrogenase (GD) enzyme systems. Peres et al., (2002) reported a single locus for *Hoplias malabaricus* whose expression is predominant in liver. This was also observed by us in *C. orientalis* and *C. striatus* where GD was observed as a single invariant band in the liver tissue which was slow migrating in the former and fast migrating in the latter. No GD activity was resolved in *C. punctatus*.

Alcohol dehydrogenase has been examined in a wide variety of teleostean species and in a majority of vertebrates it is encoded in a single gene locus presumed to be controlled by two alleles. Homozygous individuals show a single-banded phenotype and heterozygotes a three-banded phenotype indicating a probable dimeric structure of the enzyme. The ADH locus was designated as *adh-A* and its corresponding allele as *adh-A'*. (Hitzeroth et. al., 1968; Shaklee et. al., 1977; Vuorinen, 1984; Ramirez et. al, 1998; Leesa-Nga et. al., 2000; Perez et. al., 2002). The liver extracts of all the three species of *Channa* showed both the single and three-banded phenotype, as observed in a majority of fishes. We, therefore, consider that ADH is encoded in a single locus in the presently studied fishes and both homozygous and heterozygous individuals are prevalent in natural populations. The additional bands observed in the brain and eye tissues in *C. punctatus* are indicative of the presence of three loci in these tissues.

á-Glycerophosphate dehydrogenase is a dimeric enzyme and has been widely studied in a number of fishes. A single locus has been reported in *Salmo trutta* (Engel et. al., 1971), *Mystus nemurus* (Leesa-Nga et. al., 2000), two loci in *Oncorhynchus nerka* (Grant et. al., 1980), *Platycephalidae* (Keenan, 1991), grey mullets from Spain (Papasotiropoulos et. al., 2001) and three loci in *Tilapia zilli* (Cruz et. al., 1982) and *Coregonus albula* (Vuorinen et. al., 1986). The appearance of a single invariant band in *C. orientalis* and *C. striatus* suggests a single locus for this enzyme. The five bands observed in

brain, eye, heart and kidney of *C. punctatus* is perhaps due to multiple loci arising due to gene duplication.

A review of literature shows scarce data on Sorbitol dehydrogenase, Xanthine dehydrogenase, and Glutamate dehydrogenase enzymes.

Sorbitol dehydrogenase is a tetrameric enzyme (Ward, 1978). The presence of two forms of SDH is clearly indicated from the banding patterns exhibited in all three species. These are designated as *SDH-1* and *SDH-2*. This has been observed in a number of fishes like *Chanos chanos* (Winans, 1980) *Barbus brevipinnis* (Engelbrecht and Van Der Bank, 1994). Indications of the tetrameric structure of the isozyme are pronounced in *C. punctatus* with the presence of five equally spaced bands. In *C. orientalis* SDH appears to be liver specific as was found in a number of fishes such as sea lamprey (Krueger, 1980).

Xanthine dehydrogenase is a dimer with multiple alleles (Smith and Jamieson, 1978). In most fishes XDH is monomorphic and liver specific. A single locus is assumed to code for XDH in *C. orientalis* and *C. punctatus* while two loci in C. striatus. Both loci do not appear together in the same tissue in an individual. Brain, eye and heart appear to be encoded by *xdh-1* locus, while kidney and liver in some individuals showed the expression of *xdh-1* and in others by *xdh-2* locus.

Glutamate dehydrogenase enzyme is encoded by two loci in *Chanos chanos* (Winans, 1980), *Tilapia zillii* (Cruz et al., (1982) and a single locus in *Theregra chalcogramma* (Grant et al., 1980), *Salmo salar* (Ståhl, 1981) and *Gadus morhua* (Mork et al., 1982). The banding pattern suggests the presence of two loci (designated as *gdh-1* and *gdh-2*) in *C. oreintalis* and a single locus in *C. punctatus* and *C. striatus*. Absence of GDH activity in all the males investigated for the three species tempts us to suggest the non-expression of the gene at this particular season of the year, or it maybe due to very low expression hence, its activity was not detectable on the gel. Works on this enzyme reported absence of GDH activity in Arctic charr (Andersson et al., 1983), and *Petromyzon marinus* showed insufficient staining activity (Krueger, 1980).

Conclusion

The prime purpose of this work was to identify electrophoretically detectable loci that could be used in routine population genetic surveys. We have detected a minimum of forty-six loci (46) coding for the eleven enzymes in the three species of *Channa*. We observed that the patterns and distributions of the eleven enzymes under investigation are species-specific and the occurrence of isozymes has enabled us to separate the three species of *Channa* without controversy. The unique banding pattern in *C. punctatus*, in which five isozymes were displayed for most of the enzymes, has specially marked this species from the rest two. This has proved the utility of isozymes as a powerful tool for identifying morphologically indistinguishable individuals, hence making the study of genetic variation in a population interesting.

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