Taxonomy and biochemical genetics of some African freshwater fish species.

Abban, Edward Kofi

TAXONOMY AND BIOCHEMICAL GENETICS OF SOME AFRICAN FRESHWATER FISH SPECIES.

BY

EDWARD KOFI ABBAN

A Thesis submitted for the degree of Ph.D.

UNIVERSITY OF WALES.

1988

To My Family

C O N T E N T S

* * * * * * * * *

S U M M A R Y

Meristic and electrophoretic taxonomy of Marcusenius senegalensis (mormyridae), Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus (schilbeidae), Chrysichthys auratus and C. nigrodigitatus (bagridae), Oreochromis aureus, O. niloticus, Sarotherodon galilaeus, S. melanotheron, Tilapia busumana and T. zillii (cichlidae) were studied to contribute to systematics of the fishes. Genetic differentiation among related taxa and structure of populations were assessed to add to similar information on tropical freshwater fishes.

In all species, protein markers were found that were more precise for their identification compared to morphological or meristic characteristics. Based on 27 gene loci, systematic relationships among the schilbeids were in general agreement with that based on meristic characters. Results here support previous consideration of Schilbe and Eutropius as substantive genera. This does not support De Vos' (1984) proposal to consider the two as a single broad genus.

The genetic identity (I) between the two Eutropius species was 0.642. Among schilbeid genera, I ranged from 0.183 to 0.615 with a mean of 0.361±0.163 (S.E.). Among the cichlids, I between populations of Oreochromis species ranged from 0.904 to 0.962 ($\bar{I}$ = 0.939±0.025). Identity between populations of Sarotherodon galilaeus was 0.912. Between populations of Tilapia zillii, I was 0.829±0.044. Within Oreochromis, Sarotherodon and Tilapia, the mean Is were 0.691±0.094, 0.649±0.053 and 0.499±0.039 respectively. Between genera of the cichlids mean I was 0.582±0.089. These estimates support Trewavas' classification of Oreochromis, Sarotherodon and Tilapia as separate genera. They also suggest that among the cichlids, Tilapia would be ancestral to Sarotherodon and the latter to Oreochromis.

Mean observed heterozygosity of ten wild populations of ten species ranged from 0.004±0.003 to 0.054±0.042. However, what would have been expected of them as tropical fishes was an average of 0.067. The low genetic variability in these populations has been attributed to the evolutionary status of some species, range of distribution and the hydrological regime of the study area.

* * * * * * * * *

ACKNOWLEDGEMENTS

This work was made financially possible by a 36 months fellowship granted me by the World Health Organisation's African regional office (WHO/AFRO). I am therefore indebted to the organisation. I am grateful to the Council for Scientific and Industrial Research (CSIR), Ghana, my employers, for granting me paid study leave during the fellowship period.

I am deeply grateful to my supervisor Dr. D.O.F. Skibinski, School of Biological Science, University College of Swansea, for his guidance, encouragement and most of all, his personal interest in the investigations undertaken. During the study period four months were spent in preparation and collection of the fish from the study area (Ghana). The fish collection could not have been possible without the assistance of some institutions, colleagues and friends, all of whom I collectively acknowledge and thank. The Director and staff of the Institute of Aquatic Biology of the CSIR, Prof. Asare of the University of Science and Technology, Kumasi, Ghana; and Dr. C. Leveque, ORSTOM, Paris, merit special thanks for their contributions towards my fish collection. Materials studied also included tissue samples obtained from the Aquaculture Unit, University of Stirling, Scotland. My gratitude therefore goes to them.

The isoelectric focusing experiments and analysis of results obtained were performed in the laboratories of the Ministry of Agriculture, Food and Fisheries, Lowestoft (U.K.). This was made possible by the personal involvement and interest of Dr. A. Jameison and Mr. R.J. Turner of the Ministry's laboratories. I will remain indebted to them for exposing me to the technique and its possible applications.

In Swansea, Mrs. C. Fisher and Mrs. M. Warren were the technical 'gurus'. Their advice and help to overcome technical problems throughout the study had no limits. I am most grateful to them.

To all my colleagues in the electrophoretic laboratory I am thankful for all the time we spent arguing over the interpretation of gels, and propounding hypotheses. I only hope none will go away from the laboratories more confused than before entering it. Special mention must be made of Mr. P. Shaw, J. Gardner and Miss M. Whitehouse, among my colleagues for their practical and moral support.

Outside the university and the mainstream of the investigations, I received valuable advice from Dr. E. Trewavas, of the British Museum (Natural History). She and Dr. G. Howes of the museum, allowed and assisted me to make some observations on some fish collections of the museum. For every minute they spent on me, I am most grateful.

It would be impossible to sufficiently thank Mrs. G. Goodhead, who typed the work, for doing it not only as a job, but also as her contribution to the whole work.

Finally, I will always remain indebted to my family for their material and moral support during my absence from home.

* * * * * * * * *

CHAPTER 1

INTRODUCTION

African fishes and those of other tropical regions, are known to have large numbers of species with very complex inter-relationships. These two rather special characteristics of tropical fish fauna have generated several studies over the years. However, the primary stimulus for most studies has been the fact that fish remains an important source of protein in the diets of many indigenous populations in the tropics. The importance of fish as food and thus the need to conserve and develop fishery resources (to avoid damaging fish stocks by either over-exploitation or/and habitat destruction) have also provided reasons for many studies.

The extraordinary species richness of tropical fishes (see Lowe-McConnell, 1987) and the complex inter-relationships in fish communities remain to ecologists, webs of biotic and abiotic systems that attract investigation. To the evolutionary biologists, several tropical fish communities, especially those of the African Great Lakes (Victoria, Tanganyika and Malawi) provide significant opportunities to study the evolutionary process and appreciate mechanisms of speciation. For biological and economic reasons therefore, African fishes and their communities constitute rich resources.

Freshwater fish fauna of Africa, which on their own have attracted several studies, consist of primary, secondary and diadromous fishes (Lowe-McConnell, 1987). The primary freshwater fishes are those that evolved in freshwater and are unable to tolerate brackish waters. They include many primitive but important families such as the Protopteridae, Polypteridae, Osteoglossidae and Mormyridae. Most of the less advanced families are characteristically endemic to the African continent (Lowe-McConnell, 1987). The secondary freshwater fishes include species evolved in freshwater but of marine groups. Therefore, the fishes are generally capable of tolerating brackish waters. The most important family of this group in Africa is the Cichlidae, which includes the ubiquitous tilapias. The third major component of the African freshwater fishes, the diadromous fishes, are those which make regular migrations between freshwater and the sea. The main groups are complemented by marine elements which sporadically visit the freshwaters.

The total freshwater fish fauna of Africa contains over 2,000 known species, with many important families being remnants of archaic fauna (Lowe-McConnell, 1987). The present distribution of the fauna, reflects effects of geomorphology, hydrographic history and pleistocene climatic fluctuations (Roberts, 1975; Greenwood, 1983c). Both authors recognised ten ichthyofaunal provinces in Africa (Sub-Sahara mainly), based on present day drainage systems,

dominated by those of the Niger, Nile, Zaire and Zambezi (Lowe-McConnell, 1987). Smaller inland drainage systems which complement the four mentioned above, include those of Lake Chad, Turkana and the Volta.

The fishes studied here, originated mainly from the Volta system and therefore belong to the Sudanian ichthyofauna province (Greenwood, 1983c). The fauna, recently reviewed by Daget and Durand (1981), was considered rich but with many elements of archaic fauna which have very limited distribution.

Information on freshwater fishes and fisheries of Africa, has accumulated mainly from activities associated with some important commercial fisheries. Within the last three decades an appreciable body of knowledge has been added from investigations in man-made lakes and reservoirs created for other purposes. The impoundments have provided large-scale experimental opportunities to study changes associated with riverine fishes and their communities adapting to lacustrine environments. More recently, attempts to organize fish culture in tropical countries has also yielded information on the biology of some species (especially tilapias) in captivity. Fishery research invariably aims at determining the optimum economic yields of desirable species. However, identifying desirable fish species in a community requires knowledge of almost all species available and some idea of their population dynamics.

In all the above studies two special problems which complicate research, have remained largely unresolved with tropical freshwater fishes. First and most fundamental, is the problem of distinguishing species from close relatives. Secondly, a technique is yet to be developed to determine the age and growth rates of tropical fishes. The taxonomic problems of African fishes persist because species identification still relies mainly on morphological and meristic variability among species. Given the species richness of fish communities on the continent and the wide distribution of fish groups in varying habitats, the influence of ecology on fish morphometrics further complicates the taxonomy and systematics of African fishes. It also raises obvious questions about the genetic uniformity and relationship between and within species.

However, the primary importance of proper identification of species and systematics of organisms has been expressed by many eminent biologists (for example, Dobzhansky, 1973; Mayr, 1969; Tamaru, 1986). Ferguson (1980) emphasised that without proper identification, no other biological studies can be carried out in a rational manner, just as it is important for the rational exploitation and management of natural populations of food resources - animals and plants. Intraspecific systematics or population taxonomy has been found necessary for general conservation and that of identifying unique genotypes for potential use of economical, agricultural or medically desirable traits. Unfortunately,

morphologically based systematics provides no information on genotype variation unlike biochemical methods.

The use and advantages of using biochemical methods to refine aspects of systematics has a history going back to the beginning of this century, with the work of Nuttall (1901). Since then various techniques have been developed and used for the same purpose. Reviews of such methods have been provided by Avise (1974), Merrell (1981), Ferguson (1980) and Selander and Johnson (1973). Of all the methods however, the electrophoretic analysis of proteins (described in more detail in Chapter 2), has become the major approach which has contributed to systematics and evolutionary considerations of many organisms. The technique is effective and simple. It involves placing tissue homogenates (samples) in a porous, homogeneous matrix (Starch gel, Cellulose acetate, Polyacrylamide gel, etc.) and subjecting the gel to an appropriate electrical current. After a suitable length of time the gel is removed and stained according to a variety of available histochemical procedures. With experience, the proteins (or their products) stained on the gel, can be interpreted as biochemical phenotypes. Then, because of the relationship between the nucleotide sequence of the DNA molecule and the amino acid sequence of proteins (stained), the differential migrations can be interpreted as particular genotypes.

The electrophoretic approach has, in the last 30 years, been used to resolve taxonomic and evolutionary issues

involving several groups of organisms including fishes (e.g. Ferguson et al., 1978; Avise and Ayala, 1976; Avise, 1974; Ayala et al., 1975; Selander et al., 1971; Smith and Robertson, 1981; Webster et al., 1972). Among tropical fishes, the application of the electrophoretic technique to systematics and evolution is virtually non-existent. The few published works relate to a limited number of tilapia species and two schilbeids (Kornfield, 1978; McAndrew and Majumdar, 1983, 1984; Abban and Skibinski, 1988).

The work presented here uses starch gel electrophoresis and to a limited extent, isoelectric focusing in thin-layer polyacrylamide gels, to evaluate and contribute to the refinement of taxonomy based on morphology. Data obtained also permit a discussion of the genetic structure of populations and differentiation within major taxa.

* * * * * * * * *

CHAPTER 2

STUDY AREA, MATERIALS AND METHODS

2.1 Study Area:

Fishes studied were mainly samples of freshwater species and stocks from Ghana, a tropical environment in West Africa. Figure 1A shows the inland water system of Ghana. Sampling sites of wild populations studied are indicated. Although the country is inundated by rivers, the Mampong-Bisa range of mountains (Fig. 1A) divides the rivers into two groups with regards to their final drainage into the Atlantic ocean. Rivers east of the range, covering more than two-thirds of the country, drain into the Atlantic's Gulf of Guinea, through the Volta river, and constitute the major part of the Volta basin in West Africa. The second group of rivers, originating from the western front of the mountain range, drain into the Atlantic mostly as individual systems. These coastal rivers are thus greatly influenced by sea-water which seasonally influences the composition of their fish communities.

The Volta system, as at the time of this study, consisted of the man-made Volta lake and its tributary rivers. The main rivers of the system are Asukawkaw, Oti, White Volta, Black Volta, Pru, Sene and Afram. Major literature on the fishes of Ghana imply that fishes of the rivers and the lake form a single community (Trewavas

Fig. 1A; Drainage Map of Ghana.

Legend

Country border

Rivers

Volta Lake

Main fish sampling sites on a river.

Major bases used to hold live fish.

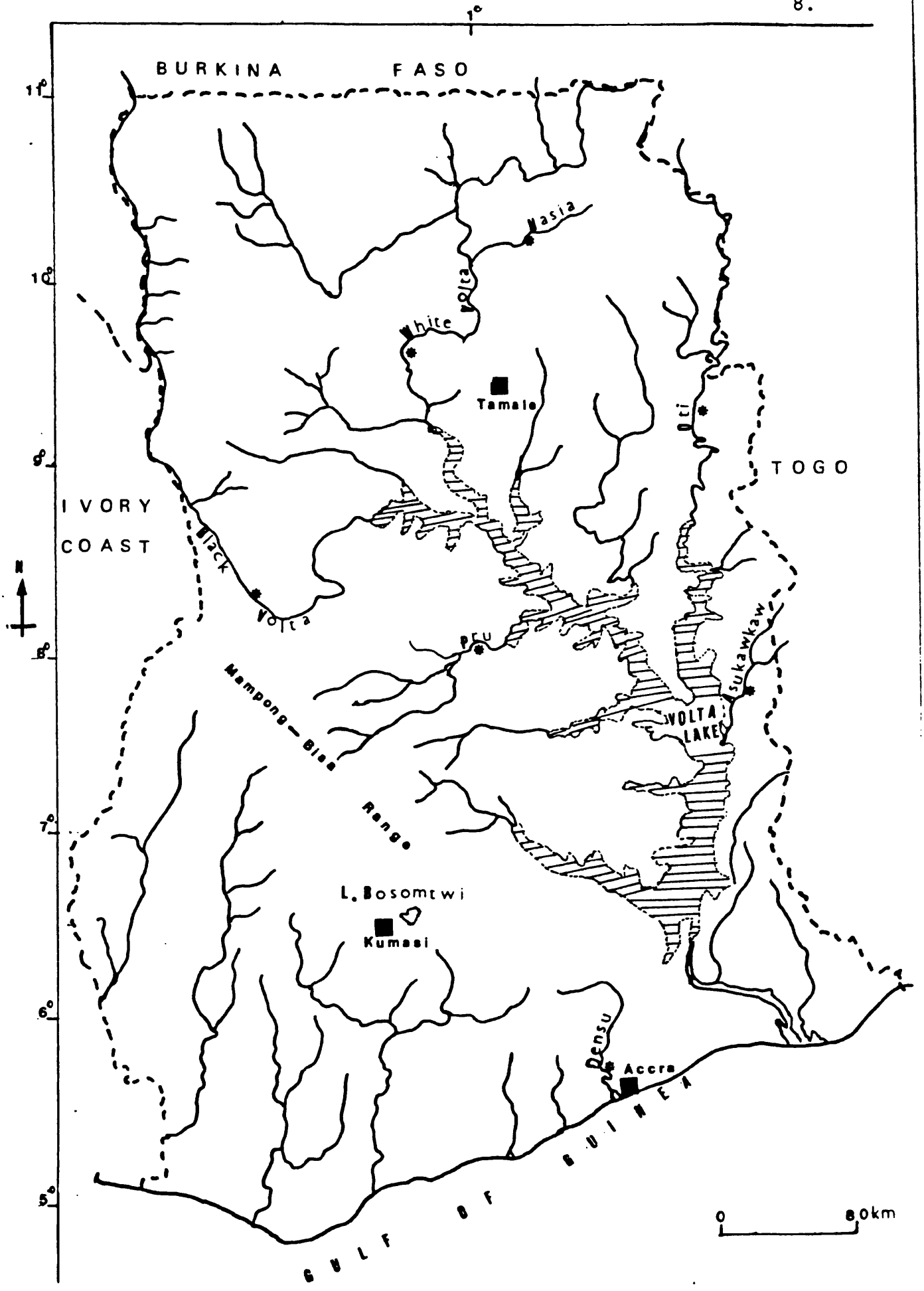


Figure 1A: Drainage Map of Ghana.

and Irvine, 1947; Lowe-McConnell, 1972; Leveque and Paugy, 1984; Abban, 1979). A hydrological feature of the rivers which undoubtedly affects the fish community of the basin is that all the rivers are tropical savanna types. Being so, they characteristically have a short flood period (May/June to September) followed by a relatively longer dry period (October to May) each year (Hopson, 1965; Holden, 1963; Abban, 1979; Abban and Samman, 1982). During the latter part of the dry period (about March - May) river sections, especially North of lat. $7^{o}N$ (Fig. 1A), become reduced to isolated and connected pools. The smaller tributaries of the system often dry out completely. Such a hydrological regime annually reduces habitable space for the fish populations and restricts movement of those left in the pools. Prior to the formation of the Volta lake in 1964, it is conceivable that the regular reduction of aquatic space exerted drastic bottle-neck effects on the fish populations of the Volta basin. This is a situation which cannot be overlooked in a discussion of the genetic structure of fish populations in the area.

Since the creation of the lake, it has provided a reservoir where an undetermined, but high proportion of fish populations in the basin take refuge during the dry period or for most of their lives. Thus, the Volta lake has become a macro-environment which unintentionally, could enhance the homogeneity of fish populations within the basin. However, the Volta lake is young and fishes living in it, being legacies of riverine populations, could not have

adapted to completing their life cycles in the lacustrine environment provided by the lake. A majority of the fishes, therefore engage in annual spawning migrations from the lake up the tributaries during the flood period (Abban, 1979).

It must be mentioned that, while evidence for the upstream movement has not been difficult to obtain, it remains a practical problem to estimate what proportions of fish which migrate upstream annually, return to the lake at the end of the floods. There is however, some evidence showing that fry, spawned on the flood plains drift with currents downstream into the lake (Abban, 1979). Available literature on tropical savanna freshwater fish communities, also indicate that generally, fish spawned on the flood plains move to the lower reaches of such seasonal rivers prior to the beginning of the dry period (Lowe-McConnell, 1987). A human activity in the study area which over the years could have greatly influenced the genetic structure of fish populations, is fishing. The fishing pressure exerted on the fish populations in the Volta basin in Ghana has been overwhelming for many years. The pressure on fishes in the study area involves the gear used, methods deployed and intensity of fishing. For example, the use of natural fish poisons to fish in dry season pools was first recorded by Irvine (1947). Although the practice has always been illegal, poison-fishing has been suspected at a number of seasonal

fishing sites in recent years (personal observation 1976 to 1983). Outside the Volta basin, wild populations studied included fish from the river Densu, a coastal river and lake Bosomtwi (Figs. 1A and 1B). Lake Bosomtwi, a crater lake, is perhaps the only natural lake in Ghana with historical fishery importance. Located at 6^{o} 30'N, 1^{o}24'W in the forest zone of Ghana, it is almost circular in shape (Fig. 1B) and considered to be meteoritic in origin (Whyte, 1975). Whyte's work provides further details of the lake's physical features. For the purpose of this work, perhaps the most important characteristic of lake Bosomtwi is that it has a self contained drainage (Fig. 1B).

Tributaries of the lake are small shallow streams which originate as springs from the rocky hills enclosing the lake. Thus fish populations of the lake are effectively isolated from that of other waters. The earliest record on fishes of lake Bosomtwi was made by Gunther (1902) when she described a collection made by Mr. R.B.N. Walker. Since then, there have been very few reports on the fishes of this lake (see Whyte, 1975; Owusu-Frimpog (1987), and references therein). These records indicate that cichlids, especially Tilapiine (Tilapia busumana being very prominent), constitute the major proportion of the lake's fish community.

Fig. 1B: Drainage System of Lake Bosomtwi.

Legend

Outer ring of hills. 

Water shed of Bosomtwi basin. 

Lowest point along rim. ▲

Highest point along rim. ▼

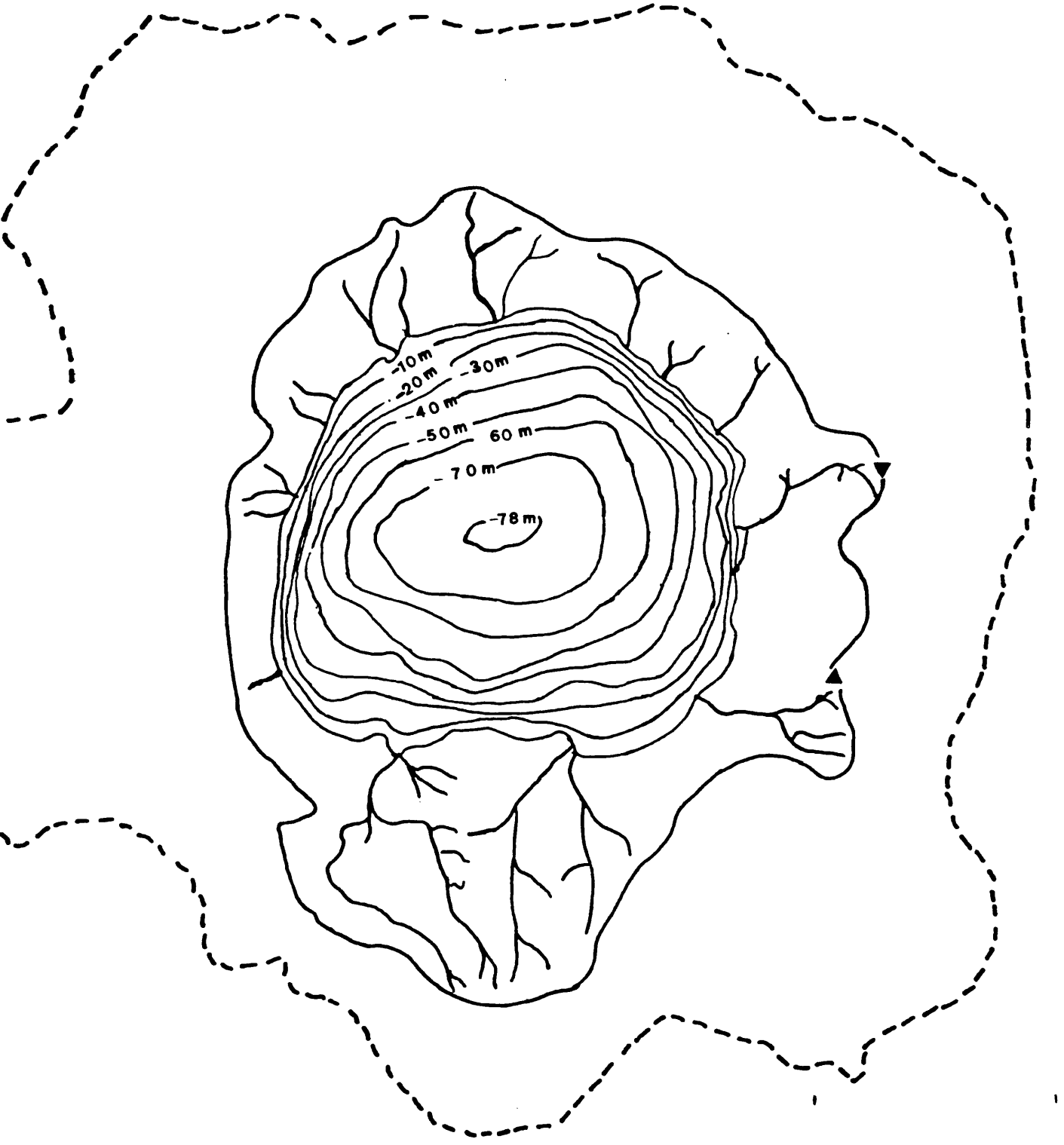


Figure 1B: Drainage system of Lake Bosomtwi.
(From Whyte, 1975)

2.2 Fishes Studied:

Fishes are known to be the most primitive and diversified group of vertebrates and contain numerous taxonomic groups as may be expected. All fishes studied belong to the class Osteichthys which constitute about 90% of all known fishes (Romer, 1950). They are also all of the sub-class teleostei, which contain some 30,000 living species which makes it difficult to have a simple definition to accurately encompass all teleosts (Romer, 1950, 1966). However, the teleosts are generally characterised by showing bone patterns, presence of skull with sutures, gill coverings and bony scales on their bodies.

Taxonomic literature of African, including West African teleosts is reasonably extensive and continues to be reviewed. For identification of materials studied here I have relied mainly on the works (keys) of Trewavas and Irvine (1947), Daget (1954), Roman (1966), Lowe-McConnell (1972), Trewavas (1983), and Leveque and Paugy (1984). These works, individually took account of earlier works.

Species investigated during this study are presented in Table 1, which also indicates populations (rivers and lakes) from which samples were derived. The table shows sixteen species belonging to eight genera and four families. All the species are clearly nominated fishes widely known to freshwater fish systematists and biologists, although tropical freshwaters (as in the study area) are suspected to have species yet unknown (Lowe-McConnell, 1972). The

Table 1: Fishes Studied and their Sources.

FISH	SOURCE (code)	R.White Volta	R.Black Volta	R. Oti	R.Asu-kawkaw	R. Pru	R. Nasia	R. Densu	Lake Bosomtwi	Ivory Coast	IAB	Private Farm	Stirling/ Swansea
Family (Fm.) Mormyridae													
Marcusenius senegalensis				+		+							
Fm. Schilbeidae													
Eutropius niloticus		+	+	+	+								
E. mandibularis										+			
Schilbe mystus			+	+	+	+							
Siluranodon auritus			+				+						
Fm. Bagridae													
Chrysichthys nigrodigitatus								+					
C. auratus							+						
Fm. Cichlidae													
Oreochromis aureus													*
O. mossambicus													*
O. niloticus											*	*	*
Sarotherodon galilaeus											*		*
S. melanotheron								+					
S. macrochir													*
Tilapia busumana									*				
T. rendalli													*
T. zillii								+			*	*	*

+ = Wild Population
* = Experimental or culture stock.

fishes also are all of economic importance as food and are familiar to consumers in perhaps all regions in Africa. A few (Schilbeidae, Marcusenius and Tilapia species) are also known to culturists and aquarium enthusiasts outside the endemic tropical regions. They therefore require only functional introductions here to specify them.

Figures 2 to 5 show diagramatic representations of the fishes together with their taxonomic diagnostic features as indicated by previous workers.

Fig. 2: Family Mormyridae

Marcusenius senegalensis (Steindachner, 1870).

Synonyms: *Gnathonemus gracilis* (Pellegrin, 1922).

Gnathonemus gambiensis (Svensson, 1933).

Gnathonemus elongatus (Pfaff, 1933).

Specific features: Dorsal 22-31 rays; Anal 28-38 rays; Lateral line scales 57-78; Number of scales around caudal peduncle 12.

From Leveque and Paugy, 1984, pp.73-74.

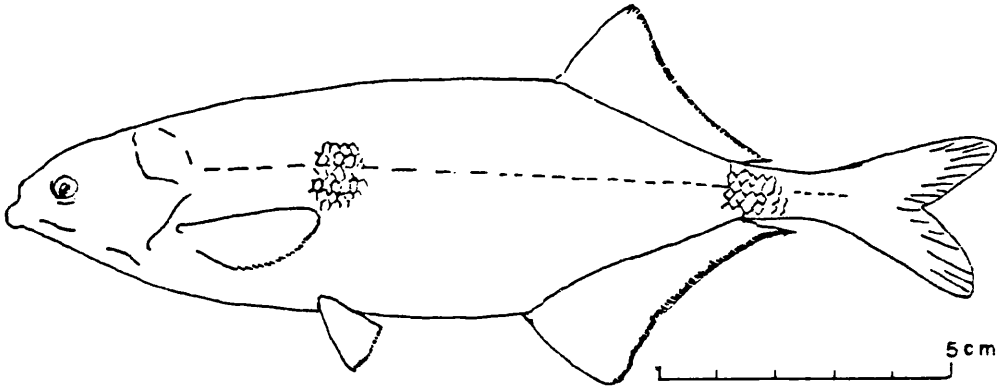


Fig. 2: Family: Mormyridae.

Species: Marcusenius senegalensis.

Figure 3: Family Schilbeidae

(i) Schilbe mystus (Linnaeus, 1762).

Only species of genus in West Africa.

Specific features: No adipose fin. Dorsal fin 5-6 branched rays. Pectoral fin 9-11 branched rays; pelvic 6 branched rays; Anal fin 45-65 branched rays.

(ii) Siluranodon auritus (Geoffroy Saint Hilaire, 1827).

Only species in genus Siluranodon (Bleeker, 1858).

Specific features: No adipose fin. Dorsal no spine; 5 branched rays; pectoral 9-10 rays; Anal fin 67-87 rays.

(iii) Eutropius niloticus (Ruppel, 1820).

Synonyms: Eutropius altipinnis (Steindachner, 1894).

Barbus adansoni (Cuvier and Valenciennes, 1839).

Eutropius liberiensis (Hubrecht, 1881).

Specific features: Adipose fin present. Dorsal 6 rays; pectoral 8-11 rays; pelvic 5 rays; Anal 47-62 rays.

(iv) Eutropius mandibularis (Gunther, 1865).

Synonym: Eutropius mentalis (Boulenger, 1901).

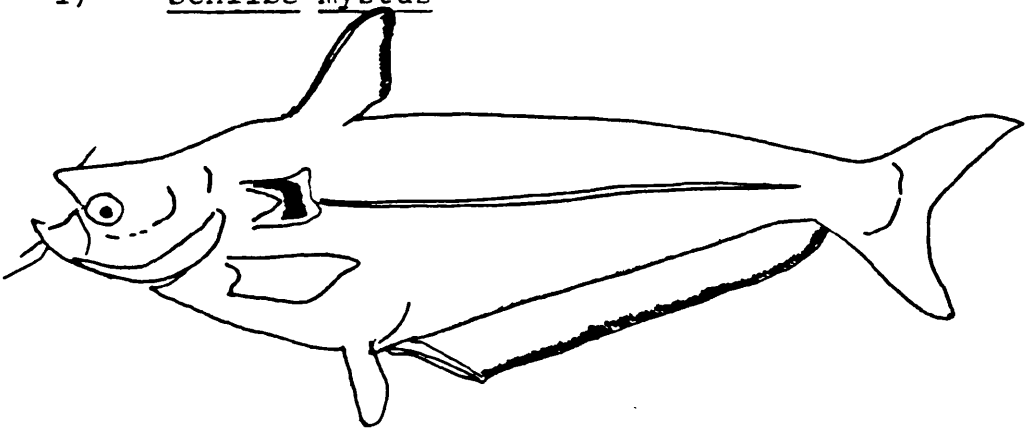
Specific features: Pectoral 8-12 rays.

Anal 39-70 rays.

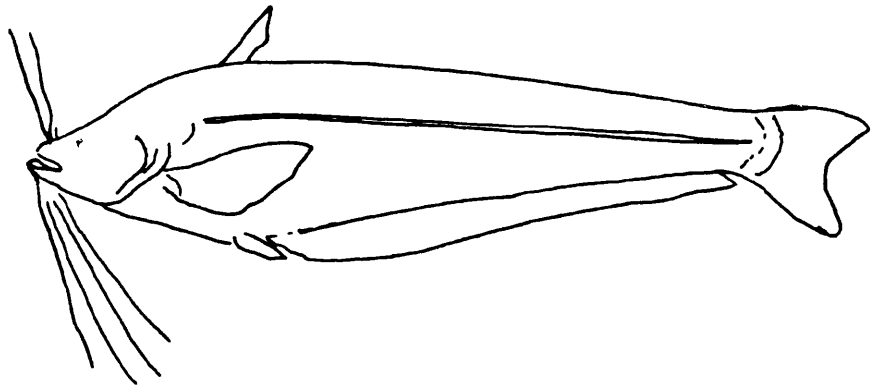
(Diagrams from Leveque and Paugy, 1984, pp. 205-215).

Figure 3: Family Schilbeidae.

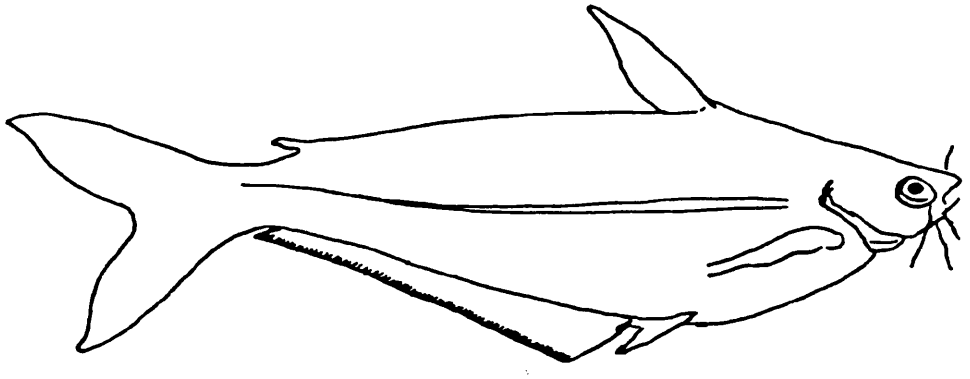
i) Schilbe mystus



ii) Siluranodon auritus



iii) Eutropius niloticus



iv) Eutropius mandibularis

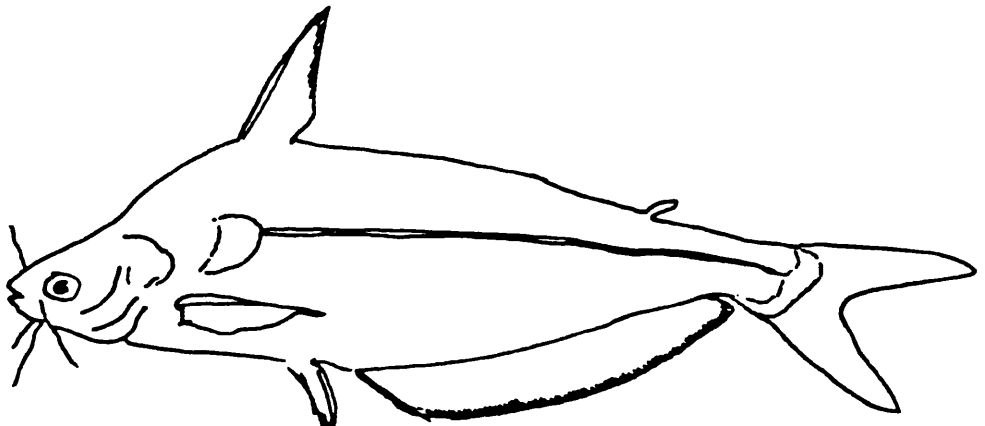


Figure 4: Family Bagridae.

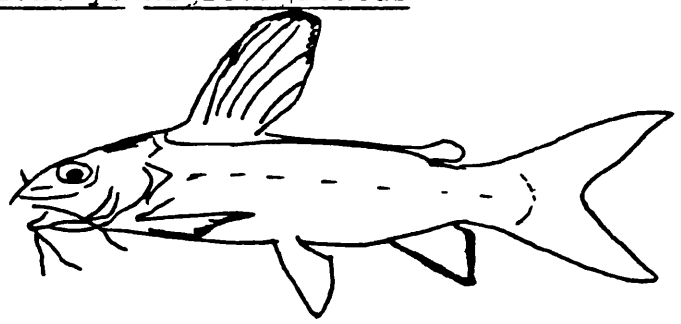
(i) Chrysichthys nigrodigitatus (Lacepede, 1803).
Specific features: Prominent snout. Dorsal fin 6 branched rays, the 2nd or 3rd being longest. Caudal fin deeply forked with a narrow black edge. Anal 7-9 rays.

(ii) Chrysichthys auratus (Geoffroy Saint Hilaire, 1809).
Specific features: Dorsal 6 branched rays, the first prolonged into a filament. Snout rounded. Anal 6-8 rays. Caudal fin forked with upper lobe longer.

(Diagrams from Leveque and Paugy, 1984).

Figure 4: Family Badridae

i) Chrysichthys nigrodigitatus



ii) C. auratus

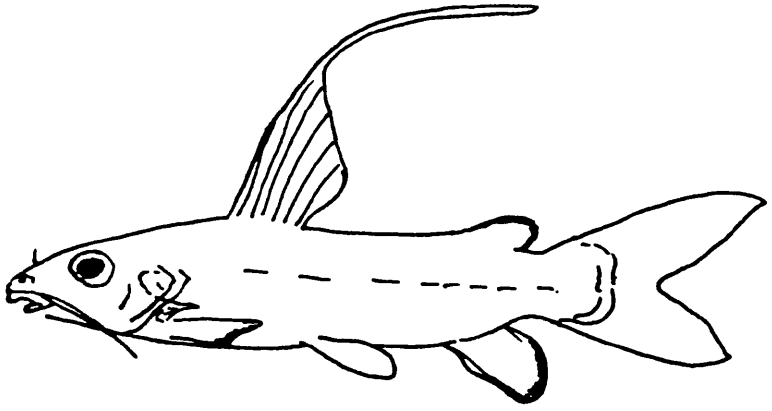


Figure 5: Family Cichlidae (Representatives)

(i) Oreochromis niloticus (Linnaeus).

Dorsal XVI-XVIII, 12-13; Gill rakers (lower) 19-26. Anal III, 8-10.

Pectoral 15.

Lateral line scales 31-35.

(ii) Sarotherodon melanotheron (Ruppel).

Dorsal XIV-XVI, 10-12; Gill rakers (lower) 12-17). Anal XV - 9;

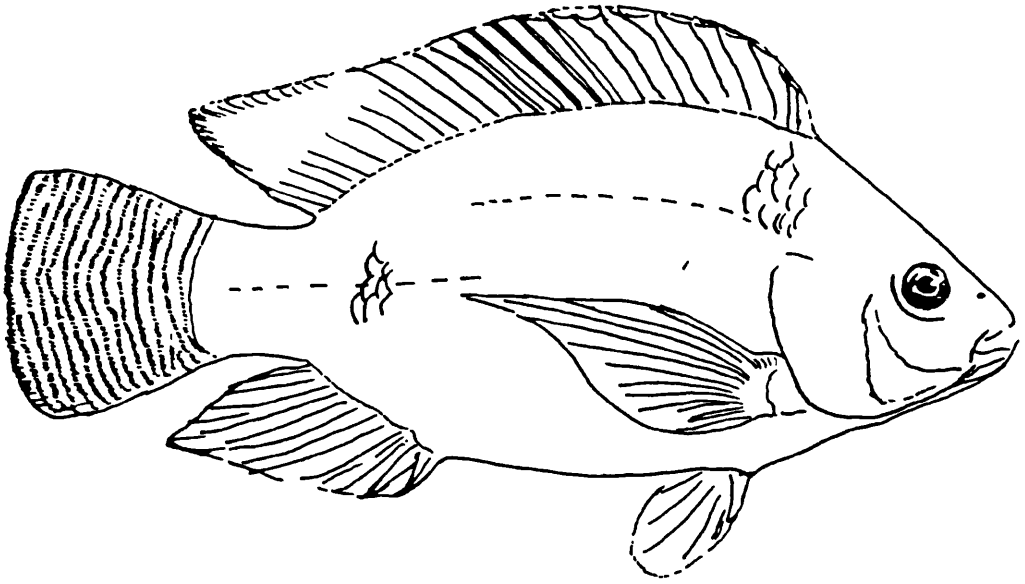
Pectoral 14.

Lateral line scales 27-29

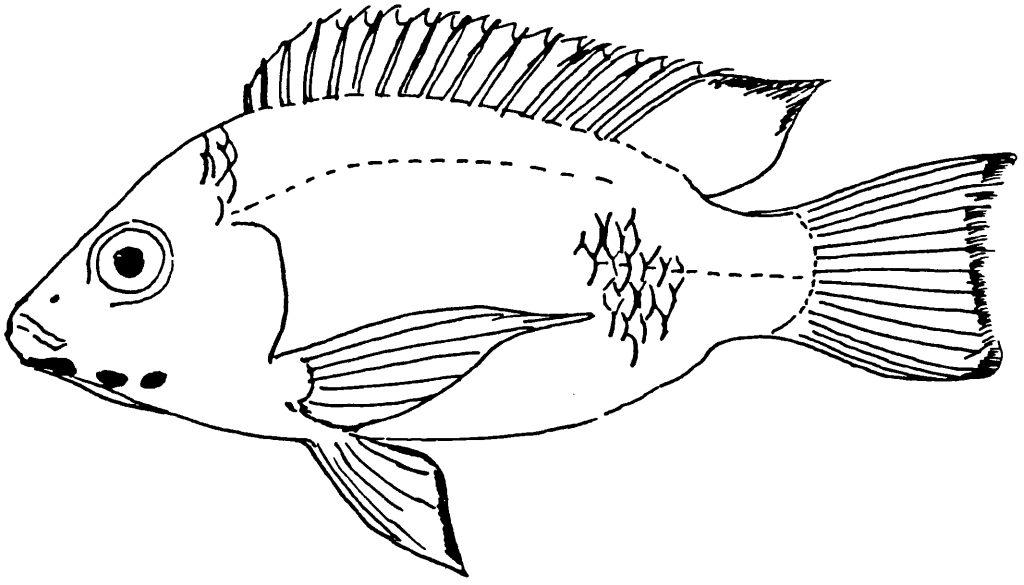
(Diagrams from Trewavas, 1983).

Figure 5: Family Cichlidae

i) Oreochromis niloticus



ii) Sarotherodon melanotheron



2.3 Methods:

2.3.1 Fish Sampling:

As earlier indicated, fishes studied were representatives of freshwater populations from mainly Ghana, West Africa. Table 1 shows the sources of the species and stocks. It also shows sources and origins of fish obtained from outside the main study area.

To obtain the fish from Ghana, a three month sampling trip was undertaken from Swansea, U.K. during June, July and August, 1986. The period was chosen to coincide with the annual migration of most species from the Volta lake upstream to the tributary rivers. It was anticipated that the timing would provide some advantages, among them, an increased probability of obtaining a desired species from more than one locality and thus increase the sampling area. It was also considered that less effort would generally be required to yield results. The period, being part of the major fishing season in the basin, the availability of local fishermen could be assumed for their help and advice. However, the situation (river hydrology) demanded parts of the basin (often more than 500 km. apart) to be sampled during very limited period. This was because the Schilbeidae, for example, were more available within the initial three weeks of the wet season in the Volta basin, as described earlier. The problem becomes clear if it is considered that all fish had to be obtained live and needed some understanding

of fish-life to 'hold' for a number of days prior to transportation to base. The impossible situation was reasonably solved with the help of staff of the Institute of Aquatic Biology, Accra, and other professional colleagues.

Fishing gear used were basket traps, seine and cast nets. All had the advantage of trapping fish live and allowed fish to be removed with minimum risk of causing wounds or loosing scales. Occasionally when fishing was done in deep waters (more than 3 m) gill nets were deployed. On such occasions the nets were constantly inspected to remove fish trapped before they struggled to death. Fish caught were held in a variety of temporary facilities prior to their transportation in insulated boxes with water to any of three bases previously set up. The bases were established at Accra, Tamale and Kumasi (Fig. 1A).

At any of the bases, fish were finally killed and immediately stored at -30 to -40^{o}C in individually labelled plastic bags. At the end of the sampling period, fish (frozen) were tightly packed in insulated boxes and flown from Accra to London, from where they were immediately transported with dry ice to the study laboratory in Swansea. In Swansea, all samples were stored as previously stated prior to study. All fish from Stirling, Scotland and the Ivory Coast, West Africa were received as fresh frozen tissue and were stored under similar conditions as the main sample from Ghana.

Three main approaches were used to study samples. These were morphological and meristic measurements, starch gel electrophoresis for screening of specific muscle enzymatic proteins and thin-layer polyacrylamide gel isoelectric focusing of general proteins from muscle and the eye lens tissues.

2.3.2 Body proportions and meristic measurements:

Figures 6A and 6B show demarcations of various body parts measured in all fishes. A pair of calipers with 0.1 mm graduations, magnifying glass and a binocular microscope were the major tools for the measurements.

a) Standard Length (SL): is the length from the anterior end of the upper lip to the base of the caudal fin. The base of the caudal fin being the line along which the vertebrae ends.

b) Body Depth (BD): is the greatest depth of the body excluding fins. For each species studied, a preliminary inspection of a number of fish helped to decide from the base of which dorsal fin ray the measurement was to be made for consistency.

c) Head Length (HL): This is measured from the anterior edge of the upper lip to the most posterior end of the bony operculum.

d) Snout Length (Sn.): Measured from the edge of the orbit to the anterior tip of the upper lip.

Figure 6(A & B): Demarcations of fish body parts (measured).

Legend

S.L.	=	Standard length.
H.L.	=	Head length
Sn.	=	Snout length
Sn.-D.	=	Snout length to dorsal fin.
Sn.-Ad.	=	Snout to adipose fin.
D.-Ad.	=	Dorsal fin to adipose fin.
B.D.	=	Body length.
An.Ba.	=	Anal base length.
E.D.	=	Eye diameter (Horizontal).
C.L.	=	Caudal peduncle length.
C.W.	=	Caudal peduncle width.
I.A.Sp.	=	First Anal Spine.
I.D.Sp.	=	First Dorsal Spine.

Figure 6A: Demarcations of main body parts.

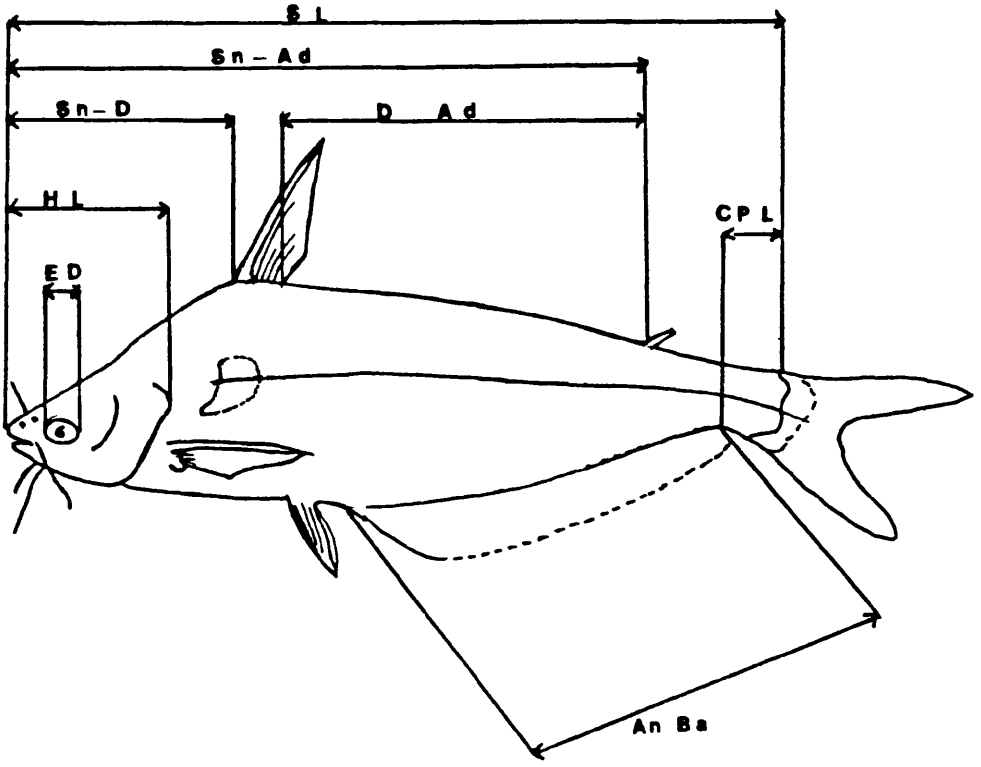
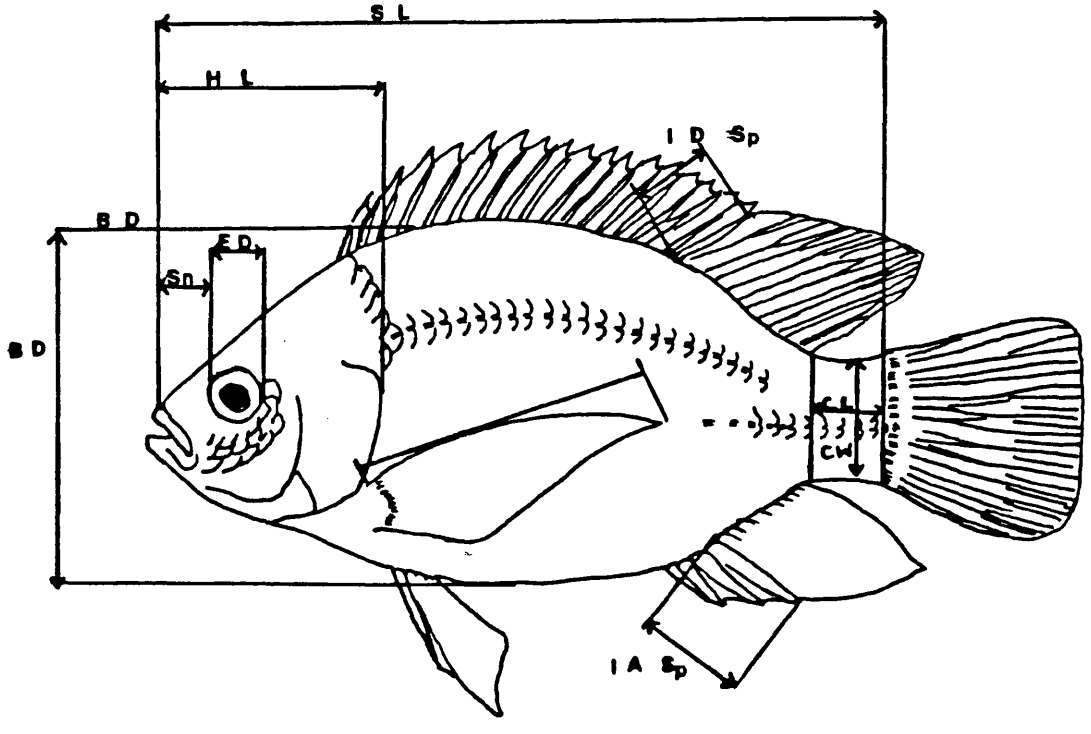


Figure 6B:



e) Eye Diameter (ED): The diameter of the iris horizontally.

f) Interorbital Width (Intob. W): Is the least width of the roof of the skull between the eyes with the calipers pressing lightly on the skinny orbit.

g) Caudal Peduncle Length (C.L.): The peduncle length is the horizontal distance from the base of the dorsal fin to the caudal base.

h) Caudal Peduncle Depth (C.W.): Is the least vertical width of the caudal peduncle.

Meristic counts:

a) Lateral Line Scales: These were counted from the end of the skull to the base of the caudal fin. In the Cichlids however, there is an upper and a lower lateral line series, both incomplete (Fig. 6B). The total is a total count of the upper series and the lower starting with the scale downwards obliquely from the last of the upper series. This may often have one more than the mid-lateral line series of other species.

b) Fin-Ray Counts: The notation used is that usually adopted by fish workers:- the number of spinous (unbranched) rays indicated in roman numerals while branched rays numbers are written in arabic numerals. The last dorsal or anal ray, which usually has no skeletal support is counted if it is distinct to the base.

c) Gill Raker Count: The rakers counted are the outer series of the first (anterior) gill arch. To obtain accurate counts, the gill arch was dissected out as completely as possible, cleaned under water using a camel hairbrush or tissue paper held by forceps, and the rakers counted under the microscope.

2.3.3 Starch Gel Electrophoresis and Analysis of Electrophoretic Data:

The general procedure adopted here was described as the zymogram technique by Hunter & Markert (1957) and as horizontal starch gel electrophoresis by other workers such as Smithies (1955), Beckman & Johnson (1964) and Ward & Beardmore (1977). For convenience, the procedure is described here in four stages: viz., (i) Preparation of starch gel. (ii) Sample(s) preparation and application to gel. (iii) Running of gel. (iv) Slicing and staining of gel for specific enzyme activity.

Preparation of Gel:- For a single gel (12.5%) 27 g of connaught starch was mixed with 220 mls of appropriate buffer solution (Table 2) in a Buchner flask. With constant rotation of the flask the mixture was heated to an almost translucent gelly state, quickly degassed using a vacuum water pump and poured into a Shandon starch former. The gel, covered with a glass plate, was allowed to set and cool overnight. Gels were cooled in a refrigerator prior to the application of samples to them.

Sample Preparation and Application:- As a general rule samples used in gel electrophoresis are homogenates of tissue, otherwise blood may be used. Frozen fish muscle was the tissue used in the present study. Small pieces of tissue were removed from fish into separate 0.5 cm diameter wells sunk into a perspex

Table 2: Proteins Investigated.

ENZYME (Protein)	Abbreviation	and EC No.	Buffer	Voltage (V) and Time
Adenosine deaminase	ADA	3.5.4.4	CTC	200V; 4½h.
Alcohol dehydrogenase	ADH	1.1.1.1.	CTC	"
Aminopeptidase	AP	—	CTC	"
Creatine phosphokinase	CK	2.7.3.2.	TEB	"
Esterases	EST	3.1.1.1; 3.1.1.2; 3.1.1.6; 3.1.1.7; 3.1.1.8; 3.1.1.10	Paulik	300V; 3h.
Fumarase	FUM	4.2.1.2.	CTC	200V; 4½h.
Alpha glycerophosphate dehydrogenase	αGPDH	1.1.1.8.	TEB	"
Glucose-6-phosphate dehydrogenase	6GPDH	1.1.1.49	TEB	"
Hexokinase	HEX or HK	2.7.1.1.	TEB	"
Isocitrate dehydrogenase	IDH	1.1.1.42.	CTC	"
Leucine aminopeptidase	LAP	3.4.1.1.1.	Paulik	300V; 3h.
Lactate dehydrogenase	LDH	1.1.1.27.	CTC	200V; 4½h.
Malate dehydrogenase	MDH	1.1.1.37.	CTC	"
Malic enzyme	ME	1.1.1.40.	CTC	"
Phosphomannose isomerase	MPI	5.3.1.8.	CTC	"
Octanol dehydrogenase	ODH	1.1.1.1.	CTC	"
6-Phosphogluconate dehydrogenase	6PGDH	1.1.1.43.	TEB	"
Peptidase	PEP	3.4.11.1.	CTC	"
Phosphogluconate isomerase	PGI	3.5.1.9.	CTC	"
Phosphoglucomutase	PGM	2.7.5.1.	CTC	"
Sorbital dehydrogenase	SDH	1.1.1.14.	CTC	"
Superoxide dismutase	SOD	1.15.1.1.	CTC	"
Xantanime dehydrogenase	XDH	1.2.3.2.	CTC	"

CTC Continuous Tris-Citrate, pH 8.0 (Ward and Beardmore, 1977).

TEB Continuous Tris-EDTA-Borate, pH 8.5 (Shaw and Prasad, 1970).

Paulik Discontinuous Tris-Citrate, pH 8.2 (Electrode pH 8.7 (Gel buffer) (Ward and Beardmore, 1977)

EC No. Enzyme Commission Number.

block and moistened with distilled water. Samples were manually ground with a glass rod into crude homogenates (diluted with distilled water as appropriate). Into the homogenates, strips of Whatman No. 3 filter paper (about 8 x 3 mm) were inserted to absorb tissue homogenate.

To apply samples (on filter paper) to gel, a part of the gel was cut to provide an inner surface on which samples were arranged. The cut part of the gel was then pushed into place, putting the samples between the two parts of the gel. A spacer was pushed into the gel former together with gel to ensure very close contact of samples to gel.

Running of Gel:- The gel, with samples arranged on it, was placed in a Shandon electrophoretic bath with appropriate buffer (Table 2). Gauze wicks soaked in the buffer were applied to both ends of the gel to permit even flow of current through gel during the procedure. The gel was then covered with polythene on which an ice tray was placed. Both the cover and tray of ice were used to minimise evaporation from and shrinkage of gel as a result of heating when the current is applied. The unit (i.e. - bath with electrode buffer connected to both ends of gel, gel covered with polythene and cooled by ice) was put in a refrigerator, leads connected from a power pack to

the tray and the gel 'run' using the appropriate voltage and time (Table 2).

Slicing and Staining:- After running, a gel was sliced horizontally into three or four slices and the inner cut surface stained for specific enzyme activity. To stain for an enzyme a slice of gel is incubated with an appropriate staining mixture (Appendix 1) until distinct bands become visible on the gel.

A staining mixture produces bands on the general principle that a substrate (in the mixture) forms a colourless product with the enzyme (in gel from the tissue sample). The initial product then couples with a salt (also in the mixture) giving out coloured bands at positions where the enzyme being stained for had migrated to during the running of gel.

Enzyme (from specimen)	+	Substrate (provided in mixture)	→	Colourless Product (Intermediate)	+	Salt (in mixture)	→	Coloured Product

Where the intermediate or final product is soluble, agar is used in the staining mixture to help confine the product to the site of enzyme activity.

Analysis of Electrophoretic Data:

The basis of the use of the electrophoretic approach in species and genera-level systematics is that, in almost all cases where sufficient proteins have been examined in a species, species-specific patterns have been identified. Secondly, the more closely related species are, the greater is the similarity in their electrophoretic patterns and the electropherograms resulting are likely to be closely similar only if they are derived from genetically similar organisms (Ferguson, 1980). To determine degrees of similarity between specimens studied, the number and relative mobilities of bands produced at individual loci were obtained. To measure homologies of bands on different gels, specimens of known mobilities in relation to others were included on new gels.

From the bands made at each locus allelic frequencies were estimated by the formula :

$$(2Hom + Het)/2N \quad \text{(Ferguson, 1980).}$$

Where Hom = number of homozygotes for an allele.

Het = number of heterozygotes for the allele.

N = number of individuals screened.

Heterozygosity: Observed heterozygosity (Ho) at a locus was the fraction of heterozygous individuals. Estimates of expected heterozygosity (He) per locus according to Hardy-Weinberg, was estimated by the formula :

$$He = 1 - \Sigma X_i^2 \quad \text{(Ferguson, 1980)}$$

Where X_i is the frequency of the i^{th} allele at a locus. Mean heterozygosity of a population was estimated as the sum of Ho or He over all loci divided by the total number of loci examined.

Genetic Identity (I): The definition of genetic identity for a locus is given by Nei (1972) as:

$$I = \Sigma X_i Y_i / \sqrt{(\Sigma X_i^2 \ \Sigma Y_i^2)}.$$

Where X_i and Y_i represent the frequencies of the i^{th} allele in populations X and Y, respectively. For all loci, the overall or mean genetic identity (I) of a population was estimated by the formula:

$$\Sigma I_k / n \qquad \text{(Hillis, 1984)}$$

Where I_k is the genetic identity at the k^{th} locus; and n, the number of loci screened.

Genetic Distance (D):

Having obtained the I values for various groups or units, the genetic distance between pairs of populations was estimated by :

$$D = -\ln I \text{ or } D = -\log_e I \qquad \text{(Nei, 1971)}$$

Where I is the identity between two taxonomic units. The approximate formula for the estimation of standard error of D (S_D) given by Nei (1971), as :

$$S_D = ((1 - I)/(In))^{\frac{1}{2}}$$

Where n is the number of loci examined, as adopted here.

2.3.4 Isoelectric Focusing of Proteins in Thin-Layer Polyacrylamide Gel (IFPAG):

Soluble general proteins from skeletal muscle and eye lens tissues of fish were subjected to the IFPAG technique to provide complimentary taxonomic data to the morphological, meristic and conventional electrophoretic approaches used. Equipments, sample preparation and general procedure for IFPAG are given below, and the sequence of experimental activity in Appendix I.

Equipment and materials used, with the major exception of specimens subjected to the technique, were obtained directly from manufacturers and are listed below. Solutions used were prepared according to LKB manual instructions except the buffer, which was obtained from manufacturers (LKB).

Materials and Equipment:

1. An electrophoresis unit with cooling plate ('Multiphor' base unit - LKB instruments 2117-301.
2. A regular power supply unit - LKB-2103.
3. A regular chiller thermocirculator - Conair Churchil 02/CTCV.
4. A refrigerated centrifuge - Beckman J-21.
5. A digital pH meter - Beckman 4500.
6. A flat bulb combination electrode - Beckman 39507.
7. A laser beam densitometer - Ultroscan, LKB 2202.
8. A recording intergrator - Hewlett-Packard, HP3390 A.
9. Thin-Layer Polyacrylamide gels for isoelectric focusing pH range 3.5 to 9.3 - LKB.
10. Ampholine (A) PAG plate - LKB 1804.

Solutions:

Electrodes (a) 1 M Sodium hydroxide for cathode.

(b) 1 M O-phosphoric acid for anode.

Fixing Solution: 17.3 g Sulphosalicylic acid.
57.5 g Tricloroacetic acid in
500 ml distilled water.

Staining Solution: 0.460 g Coomassie brilliant blue (R250)
in 400 ml destaining solution.

Destaining Solution: 500 ml ethyl alcohol, 80 ml acetic acid
in 1 litre distilled water.

Preserving Solution: 50 ml glycerol in 500 ml of destaining
solution.

Buffer Solution: pH 7 ± 0.001 was obtained from Beckman.

Preparation of Skeletal and Eye-Lens Proteins Samples:

Muscle Proteins: Approximately 0.3 to 0.5 g of white skeletal muscle tissue was removed from each specimen into a test tube. To each piece an equal weight of cold distilled water was added and homogenised mechanically. Each sample homogenate was centrifuged for 20 min. at $0^{o}C$. Supernatants pipetted into individually labelled eppendorfs for immediate use or storage at $<-30^{o}C$ until required for focusing runs to be made.

Eye Lens Proteins:

Whole eye lenses were dissected from their surrounding tissue, washed in cold distilled water, blotted and homogenised individually in 10 X the weight of each lens of cold distilled water. Homogenates were centrifuged for 30 min. at 2°C. To each sample supernatant 2.5 x its volume of cold revanol solution (2-ethoxy-6,7-diaminocridine lactate) was added to precipitate suspended material which might smear gels otherwise. The mixture was shaken, then allowed to settle by standing in a refrigerator overnight before use or storage at <-30°C.

Isoelectric focusing procedure:

The specimen extracts were isoelectric focused on thin layer polyacrylamide gels using the LKB 2117 Multiphor (LKB-Productor AB). Detailed instructions for using the Multiphor equipment are provided in LKB instruction manual 1-2117-E01 - a copy of which, together with step by step instructions of the total focusing methodology accompanies every package of gels from LKB. The procedure is reproduced in Appendix 1.

Experimental Conditions:

Conditions operative during the present study were as follows :

Constant power	20 W
Maximum voltage	1500 V
Maximum current	50 MA

Constant temperature at (a) Cooler, $2^{o}C$ (b) at gel plate $5^{o}C$.

Total focusing time - 2 h.

* * * * * * * * *

CHAPTER 3

SPECIES TAXONOMY

3.1 General Introduction:

Species taxonomy and classification of most tropical fishes, including those of the study area, are currently based on the morphological attributes of the fishes concerned. However, it has long been acknowledged that populations of organisms, often in the process of adapting to local habitats, acquire some morphological modifications (Merrell, 1981, pp. 278-356). The recognition of intra-specific variability of taxonomic characters has ensured that in the comparison of populations, several individuals of each population are measured, to assess both the plasticity of a trait within a species as well as the possible identity of separate populations.

In several species however, the apparent discreteness or limited plasticity within populations in relation to others has lead ichthyologists to rank populations as 'sub-species' or even new species in a few cases. Classic examples of such ranking, which has been contended by other workers, can be seen in populations of the European Char, genus Salvelinus (L) and the Shad, Allosa fallox (Lacepede) (Frost, 1965; O'Maoileidgh, et al., 1988). Lowe-McConnell (1972) indicated that the African elephant-nose fish, Marcusenius senegalensis, was so widespread that the limited

plasticity of certain meristic features in some populations have lead to the ranking of some populations as sub-species. Then among the tilapiine fishes (genera Oreochromis, Sarotherodon and Tilapia), the extensive distribution of fish with its attendant local modifications in features essential for the identification of species, has been mentioned by many workers (e.g. Fryer and Iles, 1972; Trewavas, 1983; McAndrew and Majumdar, 1983; Pullin, 1985). For example, Trewavas (1983, pp. 515-520) questioned whether members of a series of brackish water populations of Sarotherodon along the West African coast should be recognised as species or sub-species. She regarded them as sub-species of S. melanotheron but Thys (1971) considered the 'populations' as species.

Irrespective of the basis for the upgrading of a population to the rank of sub-species, Mayr (1969) considered the term 'sub-species' as fallacious. He maintained that a 'sub-species' was merely an aggregate of phenotypically similar populations of a species inhabiting a sub-division of the geographic range of the species, and differing in some morphometric characters from other populations. Mayr (1969) therefore suggested that the term population be replaced by "local population" to refer to a community of inter-breeding individuals at a given locality. Mayr has been supported by McFarland et al. (1985) who stated that a population of animals must accumulate numerous small genetic differences before it can become reproductively isolated and ecologically different from the original population.

It is apparent from the above that morphological taxonomy acknowledges intraspecific variation. However, whether an observed morphological difference between populations is genetically controlled or not needs the application of other techniques of study. Electrophoretic analysis of protein variation provides a reliable means of assessing genetic differences between populations (Merrell, 1981, pp. 278-356; Ferguson, 1980, pp. 47; Thorpe, 1983). The usefulness of the electrophoretic approach to species taxonomy rests on three basic properties of the technique. Firstly, the technique is sufficiently rapid to examine many specimens in a relatively short time. Secondly, it is sensitive enough to detect small intraspecies differences or variation that could be of taxonomic importance. Thirdly, and most importantly, although electrophoretic protein patterns in organisms could be influenced by age, sex, physiological condition and environment, these changes are mostly quantitative in nature. That is, the amount of a protein rather than its mobility alters. Generally, changes in mobility have been assumed to have genetic causes (Nei, 1972) because for some enzyme loci, kinetic or some functional differences between isozymes have been demonstrated (Zouros et al., 1982; Powers et al., 1979). However, exceptions to the general trend of thought have been reported where no functional differences between isozymes could be found (Kimura, 1983; Selander and Whittman, 1983).For taxonomic purposes however, only mobility or qualitative differences are of value.

In the present study, meristic counts on individuals from different populations and the plasticity (range) of certain traits within populations are presented and discussed in relation to populations of the same species from different environments, as reported by previous workers. Also, protein characteristics (patterns) of species, considered as distinguishing elements, have been assessed with the intention of identifying taxonomically important proteins for the detection of cogeneric species or confamilial genera, where applicable.

In setting out distinguishing characters of a species in relation to others, the main objective has been to facilitate the ultimate separation of a species from its close relatives. In addition the comparison of morphological characters within populations of the same species can provide further insight into the plasticity of phenotypic characters in different habitats.

It is hoped therefore, that at the end of this chapter the relative stability of morphological and electrophoretic approaches to the taxonomy of species, can be evaluated.

3.2 Morphological Taxonomy:

3.2.1 Family Mormyridae, species Marcusenius senegalensis:

Introduction:

The mormyridae (elephant-nose fishes) are one of the big fish families endemic to Africa. Eleven widely distributed genera of the family have been described from W. Africa, with body structures ranging from very long to very short, all with very small scales. The most recent thorough review of the taxonomy and systematics of the family was reported by Taverne (1972). Leveque and Paugy (1984) adopted Taverne's work with some simplification of key to species identification for the field biologist.

The genus Marcusenius has six species described from W. Africa and they are distinguished from each other primarily on the basis of morphology and counts of lateral line scales together with counts of the anal and dorsal fin rays. However, the species, all prefering calm waters, are so widely distributed that the apparent influence of different ecologies on the critical meristic characteristics (Lowe-McConnell, 1972) might be the major problem in relation to the current taxonomic keys to species.

Distinguishing M. senegalensis from other members of the genus (see Leveque and Paugy, 1984) has in the past, relied on morphological attributes of the species. These include four meristic characters, viz.: Number of branched dorsal and anal fin rays, scales along the lateral line and number of scales around the caudal peduncle.

Results and Discussion:

Counts of these meristic characters on individuals in population samples from the rivers Oti and Pru (both in the Volta basin of Ghana) are presented in Appendix 2 Table A. The observed range and frequency distribution of the characters are presented in Table 3 below. The table also shows the ranges recorded by previous workers in the West African region.

Generally, the observations made on the diagnostic meristic characters of M. senegalensis were compatible with previous reports (Roman, 1966; Lowe-McConnell, 1972; Leveque and Paugy, 1984). The number of scales around the caudal peduncle in all reports, including this study, was twelve (12), thus showing no variation within or between populations of the species. Each of the other three meristic features showed variation within and between the two sub-populations studied here. However, the variation in range of a character between the 'populations' investigated, considered in the light of available literature did not suggest a morphological distinction between the populations studied.

The results presented here and previously reported counts of the meristic features (Table 3) showed clearly that the species exhibited considerable 'localised' morphological modifications or variations. This observation could be deduced from the fact that for all the meristic features considered, Leveque and Paugy (1984), whose analysis

Table 3: Frequency Distribution of Meristic Characters - Marcusenius senegalensis (Steindachner, 1870).

		Number of Dorsal Fin rays.						Previous Studies (Ref. No.) Range in Species		
		22	23	24	25	26	27	1	2	3
M. senegalensis	(Pru-19)			1	2	11	5			
M. senegalensis	(OTI-8)	1	1	0	2	3	1	24-28	26-30	22-31

		Number of branched Anal Fin rays.										
		26	27	28	29	30	31	32	33	34		
M. senegalensis	(Pru-19)						12	4	3			
M. senegalensis	(OTI-8)	1					2	4	0	1	30-34	32-38	28-38

		Number of Lateral-Line Scales.														
		59	65	66	67	68	69	70	71	72	73	74	75	76		
M. senegalensis	(Pru-20)	1	1	3	5	-	4	1	2	2	1					
M. senegalensis	(OTI-8)			1			2		1	1	2			1	63-77	68-72	57-78

		Number of Scales around caudal peduncle			
		12			
M. senegalensis	(Pru-20)	12			
M. senegalensis	(OTI-8)	12	12	12	12

Note: 1 = Roman (1966) - Material from Upper Volta Basin.
2 = Lowe-McConnell (1972) - Volta and Kainji Lakes.
3 = Leveque and Paugy (1984) - West Africa.

involved material from the whole of West Africa, showed the widest range of character states. Although considerable overlapping of ranges of character states is recorded here and in previous works, the limitation of range in relation to area sampled was evident. Lowe-McConnell (1972) noted the species was so widely distributed that sub-species were recognisable. Her observation could be attributed to localised morphological modifications.

The observation that localised variation in the morphology of the species may be associated with different river basins is applicable here. The ranges of meristic characters in this study were more compatible with results of Roman (1966) than to Lowe-McConnell (1972) and Leveque and Paugy (1984). This could have been anticipated since Roman's study material originated only from the northern parts of the Volta basin from which the present study material was collected. The study material of Lowe-McConnell and Leveque and Paugy both included material from other river basins in the region.

Allozyme study (electrophoretic patterns or electromorphs) of the same individuals which showed the morphological variation recorded in Table 3, showed that the two 'sub-populations' were, genetically, very similar and part of the same gene pool. More significantly, all specimens (total 27) were fixed for the same allele at 27 gene loci screened (Table 3.1 Appendix 3). Such a level of genetic monomorphism of proteins is outstanding on its own. To be

observed in a species which shows so much localised morphological variation was even more outstanding and will be fully discussed in an appropriate part of this work. Tentatively, it could be said that these results suggest that electrophoretic identification is more reliable in the identification of populations of this species.

3.2.2 Family Schilbeidae, species: Schilbe mystus, Eutropius niloticus, E. mandibularis, Siluranodon auritus:

Introduction:

The Schilbeids are one of eight siluriform catfishes of Africa with representatives in Asia. The family currently contains eight recognised genera, including Schilbe, Eutropius and Siluranodon, each of which is represented by a single species (Schilbe mystus, Eutropius niloticus and Siluranodon auritus) in the Volta basin of Ghana (De Vos and Leveque, 1983; Leveque and Paugy, 1984). The taxonomy and systematics of family members have always been morphologically based. A current review of the Schilbeidae which started in 1979 is based on morphology, osteology and zoogeography of the fishes involved.

Schilbe mystus and Eutropius species, which are the most robust and widely distributed members of the family (Bell-Cross, 1976), are also the most similar morphologically. Prior to the publication of De Vos' (1984) interim review report, Schilbe mystus had always been distinguished from species of Eutropius by the absence of an adipose fin on the dorsal tail end which is present in Eutropius.

De Vos (1984) reported the presence of the adipose fin on three specimens of S. mystus he found among specimens in two European musea (M.R.AC; Tervurem in Belgium and MNHN, Paris). Then he referred to the apparent lack of consistency in the size of the adipose fin on some Eutropius species, especially E. depressirostris (Peters, 1852) described from South and Southern African rivers. On the basis of these observations, De Vos (1984) suggested that the adipose fin might be a transitional feature and could therefore not be used to separate Eutropius and Schilbe into two genera. He has therefore suggested the creation of a new broad genus, Schilbe-Eutropius, under which the present genera would have the ranks of sub-genera.

Before De Vos' proposal however, the exceptional resemblances between S. mystus and E. depressirostris, had puzzled other workers. For example, Bell-Cross (1976, p. 164), for a description of E. depressirostris, simply wrote "As for Schilbe mystus, but with a small adipose fin which varies from a recognizable adipose". However, he also indicated that E. depressirostris appeared to be closely related to E. niloticus from the Nile, Chad and river basins in W. Africa. Jubb (1957, pers. comm. to Trewavas) in addition to the general similarities between E. depressirostris and S. mystus indicated that anal fin ray counts of E. depressirostris and S. mystus from different water basins suggest E. depressirostris may be grouped together with S. mystus at species level.

Species involved in the present study were Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus. (E. mandibularis was available as tissue samples thus no morphological measurement on the fish is provided here.)

The morphological features on which the identification of various ranks of the family have been based, include meristic attributes of the fishes. The major meristic features on which previous workers (e.g. Daget and Iltis, 1965; Roman, 1966; Lowe-McConnell, 1972; Leveque and Herbinet, 1979-80; Reizer et al., 1980; Leveque and Paugy, 1984) have relied on are: numbers of spinous (unbranched) and branched rays of the dorsal, pectoral, pelvic and anal fins. Also the number of gill rakers on the upper part of the first gill arch has often been used to identify species.

Results and Discussion:

In this study these meristic characters were studied in three of the four species mentioned above. Counts made on individual fishes together with other morphological measurements are presented in Appendix 2, Table B(i), B(ii), and B(iii), for Schilbe mystus, Eutropius niloticus and Siluranodon auritus respectively.

The range and frequency distribution of meristic characters which showed variation between or within the three representatives of the family are presented in Table 4. The table also shows reported ranges of various character states.

Table 4: Family - Schilbeidae. Species - Schilbe mystus, Eutropius niloticus and Siluranodon auritus. Frequency Distribution of Meristic Characters.

	N	No. unbranched Dorsal rays; 0	1	Branched Dorsal rays. 5	6	Previous Studies (Ref. No.) 1	2	3	4	5
S. mystus	(26)		26	2	24	I-6	I-6	I-5-6	I-6	I-6
E. niloticus	(28)		28		26					
S. auritus	(26)	26		26						

		No. of Pectoral Branched rays. 9	10	11						
S. mystus	(26)		20	6				9-11		
E. niloticus	(28)	28						8-11		
S. auritus	(27)		27					9-10		10

		No. of Branched Anal fin rays. 51	52	53	54	55	56	57	58	59	60	61	66	67	68	69	70	71	72	73	74	75	76	77	78	79					
S. mystus	(26)		1	3	3	4	1	4	5	2	2	1															50-62	44-64	44-65		48-62
E. niloticus	(28)	1	2	3	4	7	5	1	2	1	2																48-61		47-62	54-69	53-57
S. auritus	(26)												1	2	3	10	5	2		2						1			67-87		69-75

		No. of Gill Rakers on Upper Part of First Gill Arch. 10	11	12	13	14	15	16	17	69	70	71	72	73	74	75	76	77	78	79					
S. mystus	(26)	1	1	6	15	3																				
E. niloticus	(28)				2	8	12	5	1												Not Available.					
S. auritus	(11)									1	2		1		1		3	1	1	1						

Refs.: 1 = Reizer et al. (1980); 2 = Leveque and Herbinet (1979-1980); 3 = Leveque and Paugy (1984); 4 = Lowe-McConnell (1972); 5 = Roman (1966).

The counts and range of characters' states presently recorded were comparable with previous reports (see Table 4). It was again evident from all available information that individual character ranges' reported by different workers were not the same. This was more evident where within-species variation in character state occurred (Table 4). The discrepancies and overlapping of ranges stated by various workers, coupled with the widest range for all characters being recorded by Leveque and Paugy (1984), was a reflection of the situation discussed earlier in relation to Marcusenius senepalensis.

Taxonomic value assessment of the meristic features studied showed that the meristic characters could be grouped into three. First, there were the characters which showed neither within nor between species (and genera) variation, thus having no taxonomic value with respect to the material studied. These were the unbranched pectoral fin rays - one in all material studied; and the unbranched and branched pelvic fin rays, which were one and five respectively in all fishes.

Second, there was for example, the dorsal branched, unbranched, and the branched pectoral fin rays which showed no or very limited variability within species, but varied distinctly between the species studied. These, considered in the light of published literature (Table 4) were considered taxonomically important in relation to congeneric species.

3.2.3 Family Bagridae, genus Chrysichthys, C. nigrodigitatus, C. auratus:

The genus Chrysichthys, two species of which have been studied here, belong to the family bagridae which, like the schilbeids, are siluriform cat fishes represented in Africa and Asia. Seven species of Chrysichthys have been described from W. Africa, four from Ghana, the present study area (Irvine, 1947; Daget, 1954; Roman, 1966; Lowe-McConnell, 1972; Leveque and Paugy, 1984).

Keys for the identification of species provided by the above works are based on morphology and meristics and body attributes of fishes. The keys broadly agree with one another. However, in the latest of these works (i.e. Leveque and Paugy, 1984), which took into account all previous works, it was indicated that their key was provisional. They also mentioned that a review of the taxonomy of Chrysichthys had been initiated. I have taken these comments to imply that at the time they went to press, Leveque and Paugy may have had some reservations on available keys but preferred to await the outcome of the current review.

Results and Discussion:

Measurements of various body parts and counts of meristic characters of individual fish of the two species studied here are shown in Appendix 2, Table C(i) and C(ii). The frequency distribution of meristic characters, which

showed within and between species variability (branched anal fin rays and gill rakers' number) are shown in Table 5 below. The table also shows counts recorded by previous workers on West African populations of the species.

The rayed dorsal and pelvic fins in both C. auratus and C. nigrodigitatus were consistently invariable within or between species (D.II-6 P.I-5.).

The invariable states of the dorsal and pelvic fin rays were consistent with previous reports (Roman, 1966; Lowe-McConnell, 1972). The recorded range of variability in the anal fin rays for both species were compatible with previous records. However, it was noted that Roman (1966, Table 5) recorded 'fixed' counts for anal fin rays in both species. It was also noted that the lower and upper limits of ranges reported by Lowe-McConnell (1972) for the anal fin rays compared favourably to counts made here. Both apparent discrepancies have been attributed to the limited area of the Volta system sampled by Roman. Inclusion of material from the Kainji basin with Volta basin fish, by Lowe-McConnell (1972) and the fact that C. nigrodigitatus studied here originated from a different river basin (River Densu - Table 1), not sampled by the two workers referred to above, are factors to be considered. Lower gill-raker counts made here on C. auratus were comparable with previous reports (Table 5). However, gill-raker counts made by Roman on C. nigrodigitatus showed a very great departure from the records of Lowe-McConnell, which was comparable to the present results.

Table 5: Frequency Distribution of Meristic Characters in Chrysichthys auratus and C. nigrodigitatus (Fm. Bagridae).

		Number of branched Anal fin rays.				Previous Studies (Ref.No	
		7	8	9	10	1	2
	N						
C. nigrodigitatus	(28)			26	2	9	7-9
C. auratus	(21)	10	7	14		7	6-8

		Number of Gill Rakers on Lower Part of 1st Gill Arch										
		9	10	11	12	13	14	15	16	17		
C. nigrodigitatus	(28)					1	3	21	2	1	11	12-20
C. auratus	(21)	1	15	5							10-11	10-12

		Number of Gill Rakers on Upper Part of 1st Gill Arch															
		13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
C. nigrodigitatus	(28)										2	3	10	5	6	2	None available.
C. auratus	(21)	1			13	6	1										

Note: 1 = Roman (1966); 2 = Lowe-McConnell (1972).

3.2.4 Family Cichlidae - Oreochromis, Sarothorodon, Tilapia: Introduction:

The cichlids studied here were species and populations of Tilapia, Sarotherodon and Oreochromis, all of which were until recently recognised under a broad genus, Tilapia (Trewavas, 1966a, b; Thys, 1966). As a group, they have received more taxonomic attention than any other tropical group of fishes, and are still conveniently referred to as tilapias. In the broad Tilapia genus, Thys recognised seven sub-genera on the basis of reproductive habits. For similar reasons, Trewavas (1966c) grouped all mouth brooding tilapias under Sarotherodon as a sub-genus of Tilapia. She later gave the rank of genus to the Sarotherodon (Trewavas, 1973a, 1980, 1982b). Still later, Trewavas on the main basis of spawning and brooding behaviour of the Sarotherodon, including roles played by parents during brood development separated the Oreochromis from amongst the original Sarotherodon genus and thus the present classification (Trewavas, 1981b, 1982a,b).

Trewavas (1983) has gone further to suggest that the tilapias be recognised as a tribe, tilapiine, until there is a basis to recognise them as constituting a sub-family under cichlidae.

However, the striking resemblances among the tilapias with regard to their distribution and ecology, body shape, skeletal structure, conventional morphometric characteristics,

pigmentation and diet provide basis for other workers to disagree with their classification according to Trewavas (e.g. Thys, 1968b; Peters and Berns, 1982). This is not surprising. With over 700 species known and more to be described (Lowe-McConnell, 1987, pp.27-62), such a species rich complex of very similarly structured fishes can be expected to present controversy if their taxonomy is to be based on morphometrics.

Electrophoresis application has resolved a number of taxonomic issues among temperate fishes as indicated earlier. Among tropical fish groups only the tilapias have been subjected to electrophoresis to obtain interspecies and intergeneric markers. Results obtained prior to 1980 have been summarised by Avtalion (1982). Kornfield et al. (1979) found no karyotype differences among the tilapias. However, they obtained allozyme data that indicated a close proximity between O. aureus and S. galilaeus, with T. zillii more distant from both. Perhaps the most extensive electrophoretic study of tilapias to provide inter-species and generic markers prior to the present work was that of McAndrew and Majumdar (1983). They screened T. zillii, S. galilaeus and nine species of Oreochromis at 23 enzyme loci from muscle, liver and eye extracts. Their results provided significant allelic differences between the genera, according to Trewavas classification.

Results of meristic, starch gel, electrophoresis and isoelectric focusing of proteins are presented below to contribute to the taxonomy of the species studied here.

Results and Discussion:

Species: Oreochromis niloticus; Sarotherodon galilaeus; S. melanotheron; Tilapia busumana and T. zillii.

Morphometric and meristic measurements made on individuals of species and populations of the tilapia group of fishes studied here are in Appendix 2, Table D (i.e. D(i), D(ii), D(iii), and D(iv)). The frequency distribution of meristic characters are given in Tables 6a, 6b, 6c, and 6d below. Table 6, also indicates previous observations by other workers.

Generally results obtained here were comparable with previous estimates. Again available data indicate that local populations may have some amount of specific modifications. However, the excessive movement of tilapia fishes around the world and their potential to hybridize in the wild (Trewavas, 1983; McAndrew and Majumdar, 1983; Pullin, 1985) makes it difficult to discuss variation between populations as 'localised variation'. Further discussion of the morphological differentiation among the tilapias can be seen in the general discussion of this chapter, which follows.

Table 6a: Distribution of Meristic Characters in Oreochromis, Sarotherodon and Tilapia species.

Species	Locality	N	Number of dorsal fin spines (unbranched rays) XIV 14	XV 15	XVI 16	XVII 17	Previous Studies (1); (2); (3) (Range in Species) 1	2	3
O. niloticus	(IAB)	12			1	11	17-18	15-17	16-18
O. niloticus	(MP)	8			2	6			
S. galilaeus	(IAB)	25		1	20	4	15-16	14-17	15-16
S. melanotheron	(Den)	19	1	9	9		-	15-16	15-16
T. zillii	(Den)	21		15	6				
T. zillii	(IAB)	6	1	4	1				
T. zillii	(MP)	18	1	8	9				
T. busumana	(Bos.)	25	3	16	6		-	-	14-16

Note: 1 = Lowe-McConnell (1972); 2 = Trewavas (1983); 3 = Leveque and Paugy (1984).

Table 6b:

Species	Locality	N	Number of branched dorsal fin rays. 10	11	12	13	14	15	Previous Studies (1); (2); (3) (Range in Species) 1	2	3
O. niloticus	(IAB)	12			4	8			12-14		
O. niloticus	(MP)	8			4	4					
S. galilaeus	(IAB)	29				16	13		12-14		
S. melanotheron	(Den)	19	4	14	1					10-12	
T. zillii	(Den)	21			3	12	6		11-13		10-13(12)
T. zillii	(IAB)	6			1	2	2	1			
T. zillii	(MP)	18			6	12					
T. busumana	(Bos.)	25		1	5	18	1		-		10-13

Note: 1 = Lowe-McConnell (1972); 2 = Trewavas (1983); 3 = Leveque and Paugy (1984).

Table 6c:

Number of branched Anal fin rays.

Species	Locality	N	8	9	10	11	12	Previous Studies (1); (2); (3); (4) (Range in Species) 1	2	3	4
O. niloticus	(IAB)	12		2	10			8-10	8-10		
O. niloticus	(MP)	8		2	6						
S. galilaeus	(IAB)	29			3	12	14	10-11	7-10	9-12	
S. melanotheron	(Den)	19		17	2			9	9-10	8-10	
T. zillii	(Den)	21		3	18			7-11	7-10		7-10
T. zillii	(IAB)	6			5	1					
T. zillii	(MP)	18		4	14						
T. busumana	(Bos.)	25	1	1	16	7		-	-	-	10-13

Note: 1 = Roman (1966); 2 = Lowe-McConnell (1972); 3 = Trewavas (1983); 4 = Leveque & Paugy (1984).

Table 6d:

Number of branched Pectoral fin rays.

Species	Locality	N	XIV 11	XV 12	XVI 13	XVII 14	Previous Studies (1); (2); (3) (Range in Species)		
							1	2	3
O. niloticus	(IAB)	12			10	2	15		
O. niloticus	(MP)	8		1	7				
S. galilaeus	(IAB)	29		15	14		14		
S. melanotheron	(Den)	19	2	13	4		14		
T. zillii	(Den)	21			18	3	14-15		
T. zillii	(IAB)	6		2	2	2			
T. zillii	(MP)	18			13	5			
T. busumana	(Bos.)	25	9	14	2				

Note: 1 = Roman (1966); 2 = Lowe-McConnell (1972); 3 = Trewavas (1983).

Table 6e:

Species	Locality	N	Number of Lateral-Line Scales (Total) 28	29	30	31	32	33	34	35	Previous Studies (1); (2); (3); (4) (Range in Species) 1	2	3	4
O. niloticus	(IAB)	12							9	3				
O. niloticus	(MP)	8						4	4					32-33
S. galilaeus	(IAB)	29				3	14	12					29-32	29-30
S. melanotheron	(Den)	19	2	3	12	2							27-30	28-29
T. zillii	(Den)	21		1	9	6	5							27-30 (28-29)
T. zillii	(IAB)	6				1	3	2						
T. zillii	(MP)	18			8	4	5		1					
T. busumana	(Bos.)	25			10	11	4							

Note: 1 = Roman (1966); 2 = Lowe-McConnell (1972); 3 = Trewavas (1983); 4 = Leveque AND Paugy (1984).

Table 6f:

Species	Locality	N	Number of gill rakers on first gill-arch. 12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	Previous Studies (1); (2) (Range in Species)
O. nil.	(IAB)	12																3	2	3	2	1	1		
O. nil.	(MP)	8															1	2		1		1	1	2	
S. gal.	(IAB)	29															1	9	11	6	2				
S. mel.	(Den)	19								2	7	7	3												
T. zil.	(Den)	21		14	7																				
T. zil.	(IAB)	5		1	2	1	1																		
T. zil.	(MP)	18		1	1	1	12	3																	
T. bus.	(Bos)	23						1							2	3	4	9	3	1					

3.3 Discussion:

The results of body measurements and meristic counts made for the purposes of identifying the species investigated were generally comparable with previous studies (see Tables 3, 4, 5 and 6). An assessment of the plasticity of the meristic characteristics in all of the species investigated, together with similar published data on the species from the West African region (Daget, 1956; Roman, 1966; Lowe-McConnell, 1972; Trewavas, 1983; Leveque and Paugy, 1984), brings into focus a number of issues.

Firstly, it was apparent from all available information that the limits of plasticity of a character were not the same in different river basins. For example, the range of dorsal fin rays observed here for Marcusenius senegalensis (from the Volta basin in Ghana) was 22 to 27. Roman (1966), recorded a range of 24-28 based on material from the Volta and other river systems in Upper Volta. Lowe-McConnell (1972), on the basis of fish from the Volta and Kainji lakes, recorded a range of 22-31. Leveque and Paugy considered fish and records from the Volta, Niger, Chad and other smaller river basins in the Ivory Coast.

It is possible that the upper limit of Lowe-McConnell's range, compared to present results, can be accounted for by the inclusion of fish from the Kainji lake (in the Niger basin). The apparent inter-population variability may be attributed to 'localised' adaptation to particular habitats.

However, that would not help to demarcate habitats unless the definition of 'local population' as defined by Mayr (1981) is applied. Biologists interested in the fish could however, be consoled by the fact that the extent of overlapping is extensive and the modes of distribution of character states are similar and stable in all the populations referred to here.

The results also suggest that the taxonomic value of investigations based on a particular population may diminish with increasing physical distance. However, between populations, geographic distance cannot always be related directly to changes in ecology. As can be seen from Tables 3, 4, 5 and 6, the widest range in the expression of meristic characters was, in all cases, recorded by Leveque and Paugy (1984), whose data was based on fish from all worked basins in the West African region. The discrepancies between their work and the present study suggest that an investigation of the influence of particular environments on meristic characters may be useful.

This suggestion is supported by some classical examples of the influence of environmental factors (which might be ill-defined) on meristic characters. For example, Frost (1965) in his report on the Windermere Char (Salvelinus willughbii (Gunther)) populations, indicated that riverine and lake populations of the fish, had statistically different values in gill-raker counts. In a recent study

of the Arctic Char, Salvelinus alpinus (L), Parttington and Mills (1988 - in press) have indicated differences in structure and counts of gill rakers between autumn and spring spawned Char. O'Maoileidgh et al. (1988) have drawn attention to the fact that two races of freshwater shad (Alosa fallux) all derived from a marine ancestor have an increased number of gill rakers compared with the marine species.

Among tropical species, such noted differences between populations have at best been referred to in relation to the geographic position of populations rather than any environmental differences. For example, Leveque and Herbinet (1979-1980) described two populations of Schilbe mystus in the Ivory Coast. The two populations, distinguished by the number (range) of branched anal fin rays and the number of unjoined vertebrae occurred in the northern and southern (coastal) portions of rivers. Considering that tropical rivers cannot effectively be zoned, with respect to river characteristics or fauna composition (Lowe-McConnell, 1987, pp. 3-23), it is here suggested, that perhaps the influence of sea water on the lower reaches of the rivers (sustained salinity differences) could partially account for the differences observed by Leveque and Herbinet.

Lowe-McConnell (1987) indicated that one of the basic problems confronting the tropical freshwater fish biologist, is often the morphological distinction of some sympatric

species. This situation has often been attributed in part to species richness of tropical communities. However, under such conditions, the observation here that the wide application of a taxonomic study, comparing one population to other populations could diminish with increasing distance could be very unwelcome. Yet the complexities of fauna in the tropics may itself provide a consolation. The impracticability of effective zonation of river courses in the tropics effectively implies the duplication of ecological conditions along a river course and in many neighbouring rivers. The situation would then be expected to result in 'homogenisation' of wide areas and thus reduction of inter population differences attributable to ecological differences in habitat. Many of the differences seen would therefore have to be attributed to other causes.

3.4 Protein Taxonomy:

3.4.1 Specific Protein (Allozyme) Patterns:

Introduction to Results:

Observed specific protein (enzymes) and general protein patterns of tissues, considered as expression of taxonomic character states in species are presented below. Specific protein patterns in groups of congeneric species or confamilial genera (where appropriate) have been compared at 27 or 28 skeletal muscle enzyme loci.

Figures 7a, 7b, and 7c show the specific protein patterns observed after starch gel electrophoresis in the schilbeidae, bagridae and cichlidae respectively. For

species comparison, each gene locus was considered as a taxonomic character and genotypes observed in individuals of a species, as the 'range' of character expression within the species.

In each of Figures 7a, 7b and 7c, two alleles and one heterozygote type were assumed at any variable locus as basic possible genotypes. A minumum of three genotypes are therefore shown at each locus. For uniformity in presentation, even where a locus was monomorphic, three representatives have been shown (e.g. at Adh-2 in Fig. 7a). Also, in each of the figures, loci at which no differences in pattern were observed within or between species, have been grouped together. For example, in Fig. 7a, the grouping of Ap-1, Ap-2, Est-1 and αGpdh-1 indicates that the schilbeidae studied were monomorphic for the same allele at each of the loci. However, where more than the basic anticipated combination of alleles were observed at a variable locus, all combinations of alleles (genotypes) have been represented, as for example, at Me-1 in Fig. 7a.

For a brief interpretation of the results therefore, Fig. 7a for example, shows that all individuals of Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus expressed the 'characters' Ap-1, Ap-2, Est-1 and αGpdh in non-variable forms at each locus. At Adh-2, the figure shows that S. mystus and E. mandibularis, while showing no intraspecies variability in 'character' expression were both distinct in different ways from the

Figure 7a: Specific Protein Patterns as Taxonomic Character States.

Family Schilbeidae. Species - Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus.

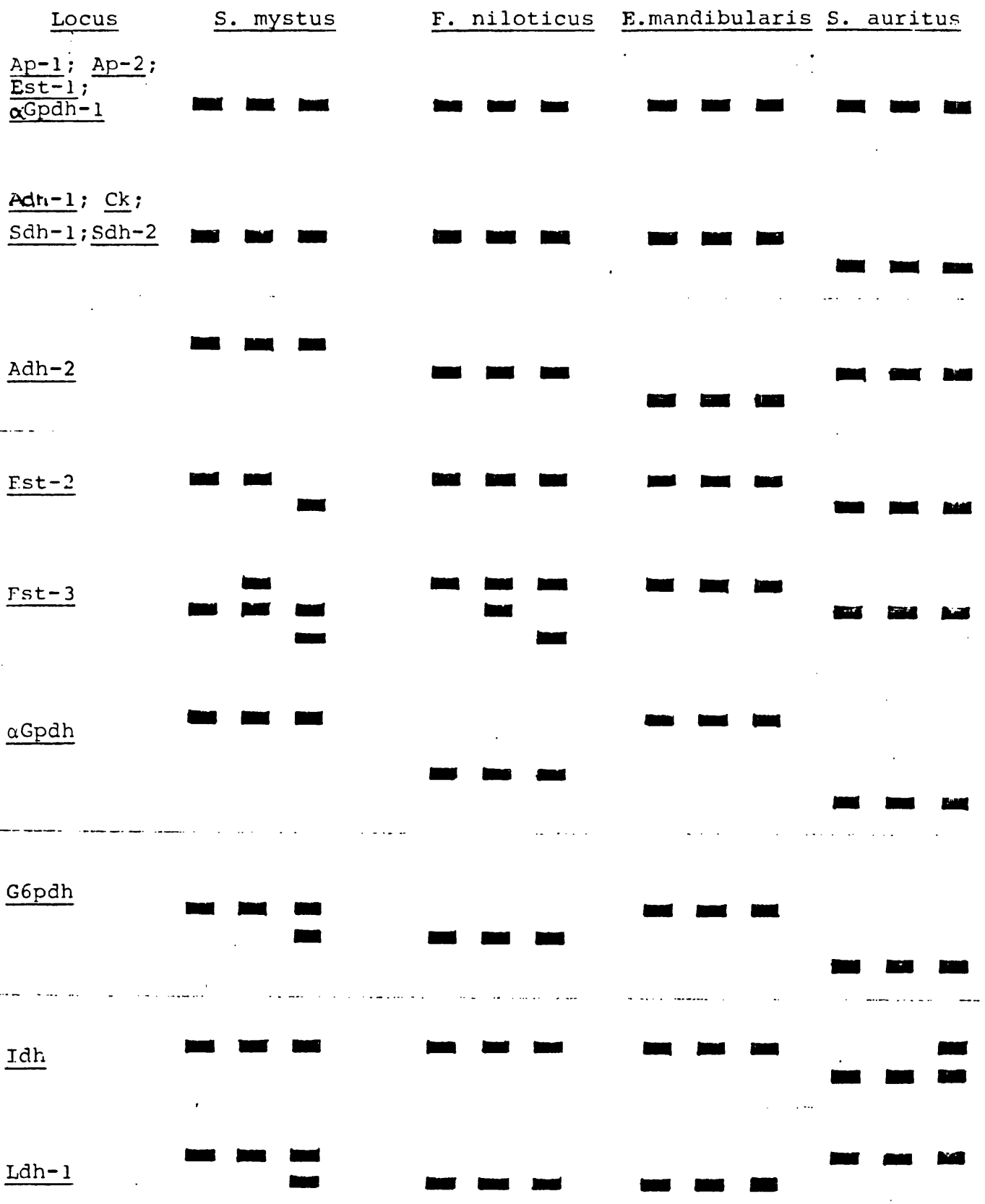


Fig. 7a Contd.

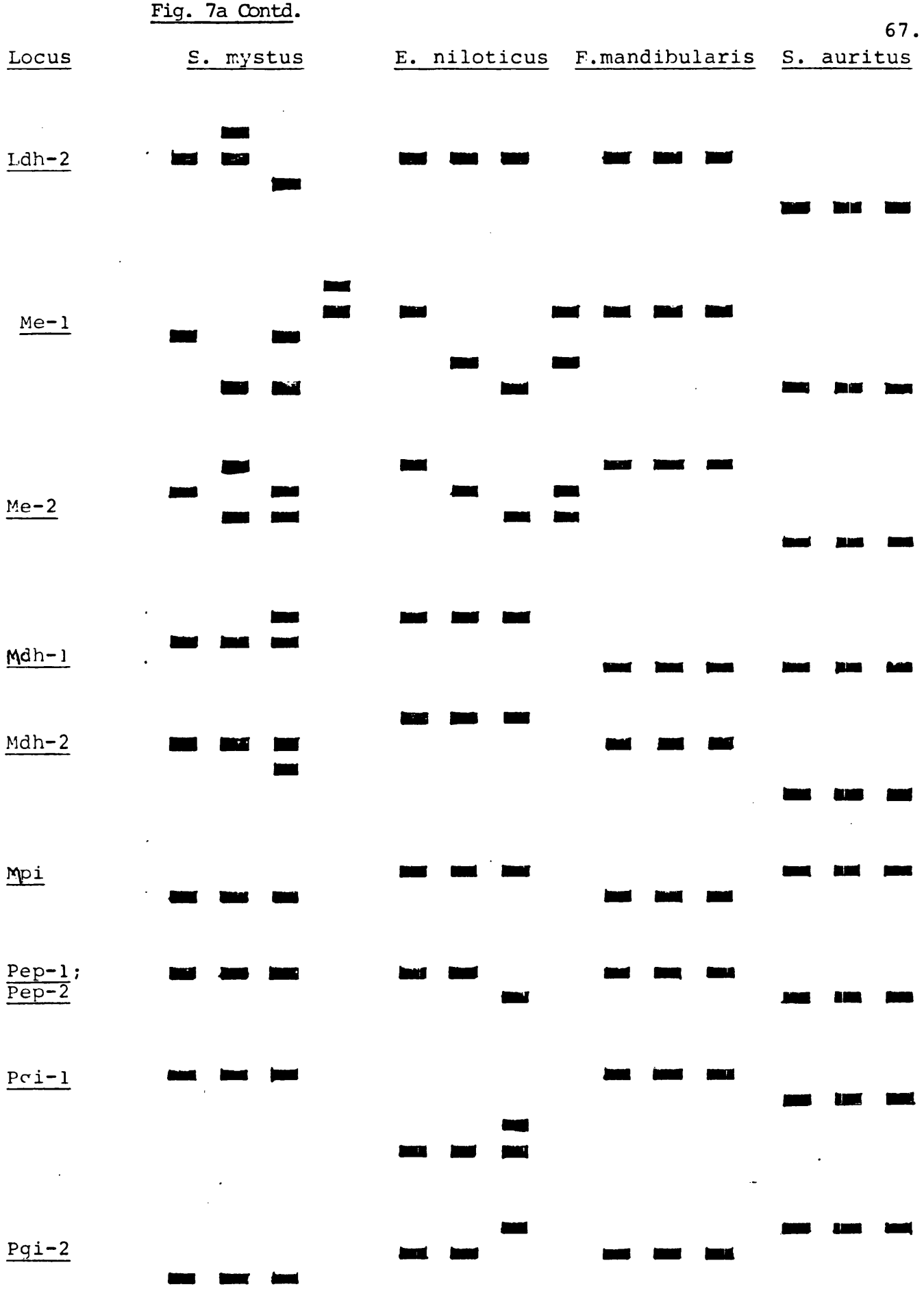

Locus
S. mystus
E. niloticus
F.mandibularis
S. auritus
Ldh-2
Me-1
Me-2
Mdh-1
Mdh-2
Mpi
Pep-1;
Pep-2
Pgi-1
Pgi-2

Fig. 7a Contd.

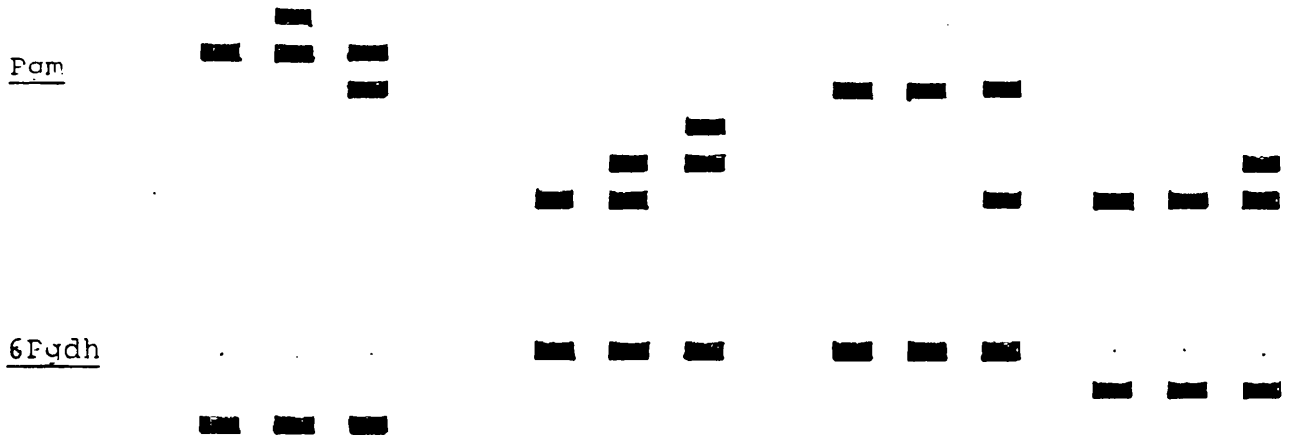

Locus
S. mystus
E. niloticus
E.mandibularis
S. auritus
Pam
6Pgdh

Figure 7b: Specific Protein Patterns as Taxonomic Character States.

Family Bagridae. Species - Chrysichthys nigrodigitatus and C. auratus

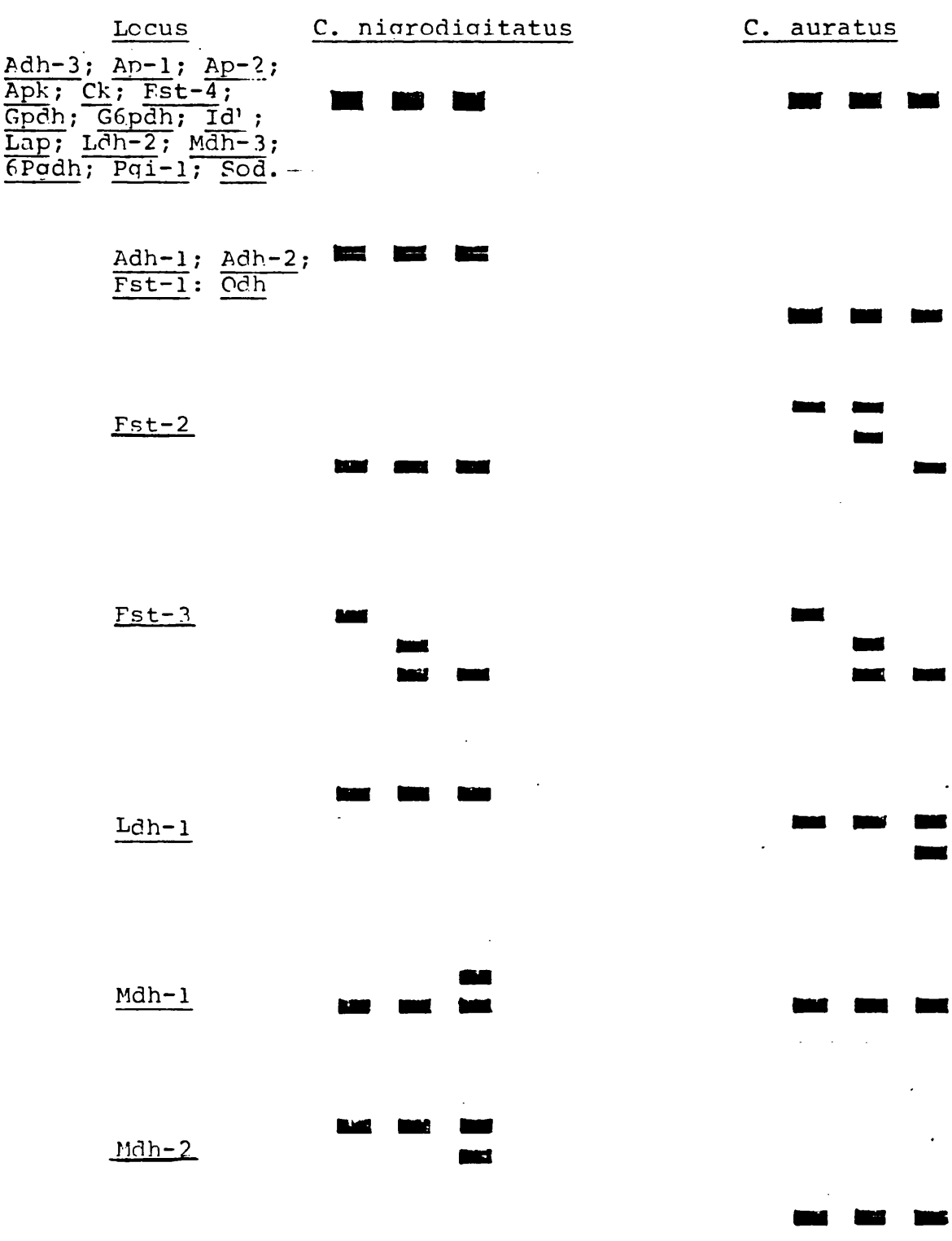


Fig. 7b Contd.

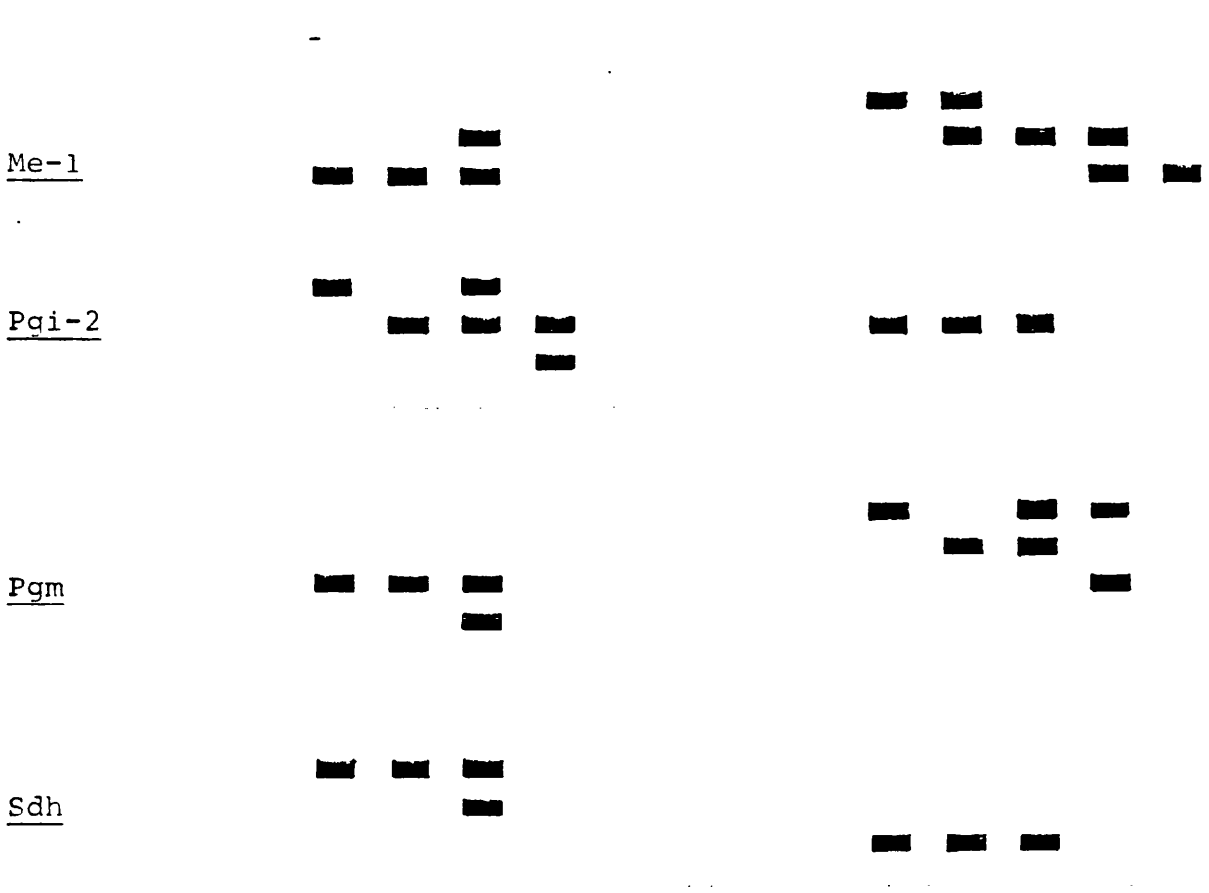


Figure 7c.

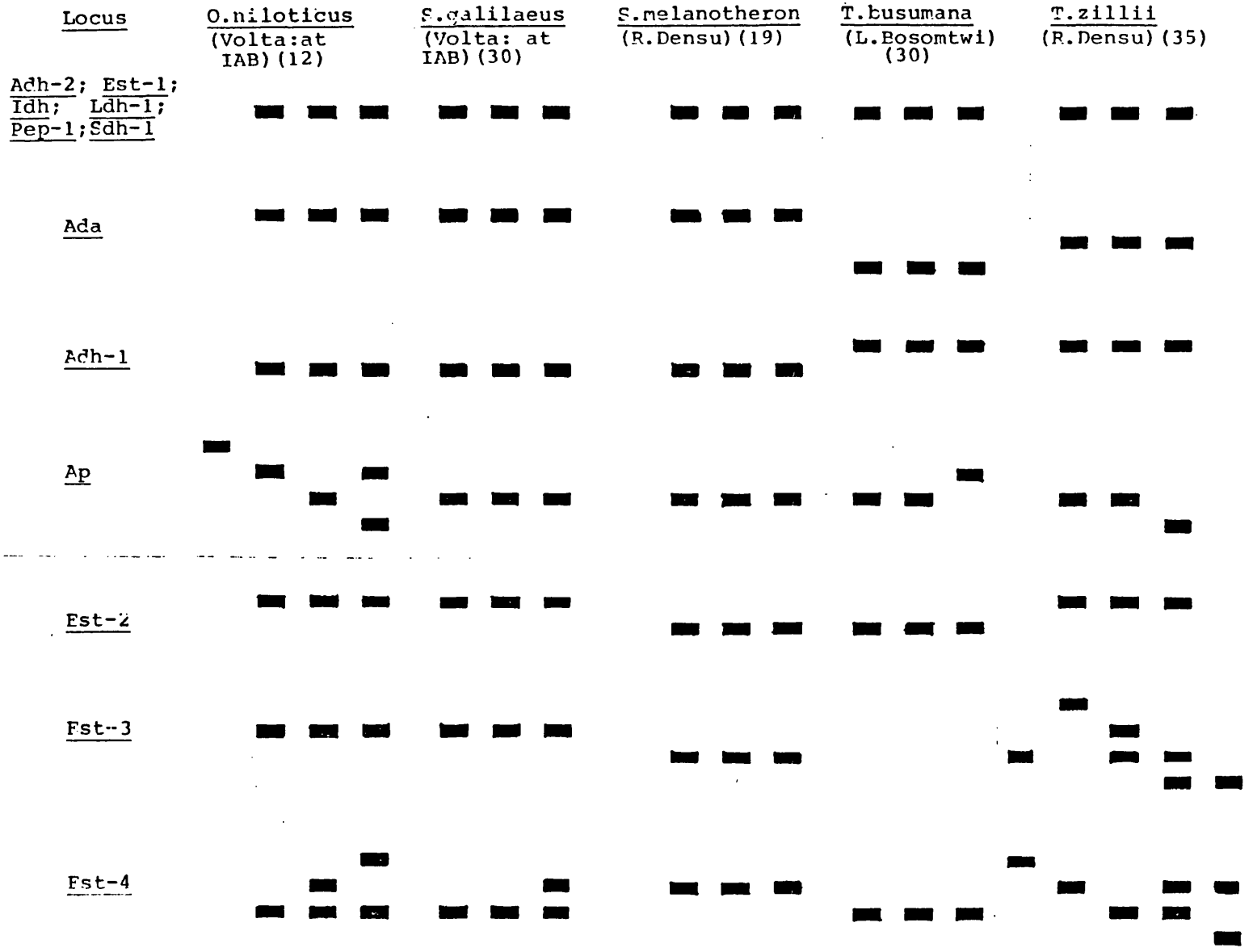

Locus
O.niloticus (Volta:at IAB) (12)
S.galilaeus (Volta: at IAB) (30)
S.melanotheron (R.Densu) (19)
T.busumana (L.Bosomtwi) (30)
T.zillii (R.Densu) (35)
Adh-2; Est-1; Idh; Ldh-1; Pep-1; Sdh-1
Ada
Adh-1
Ap
Est-2
Fst-3
Fst-4

Fig. 7c Contd.

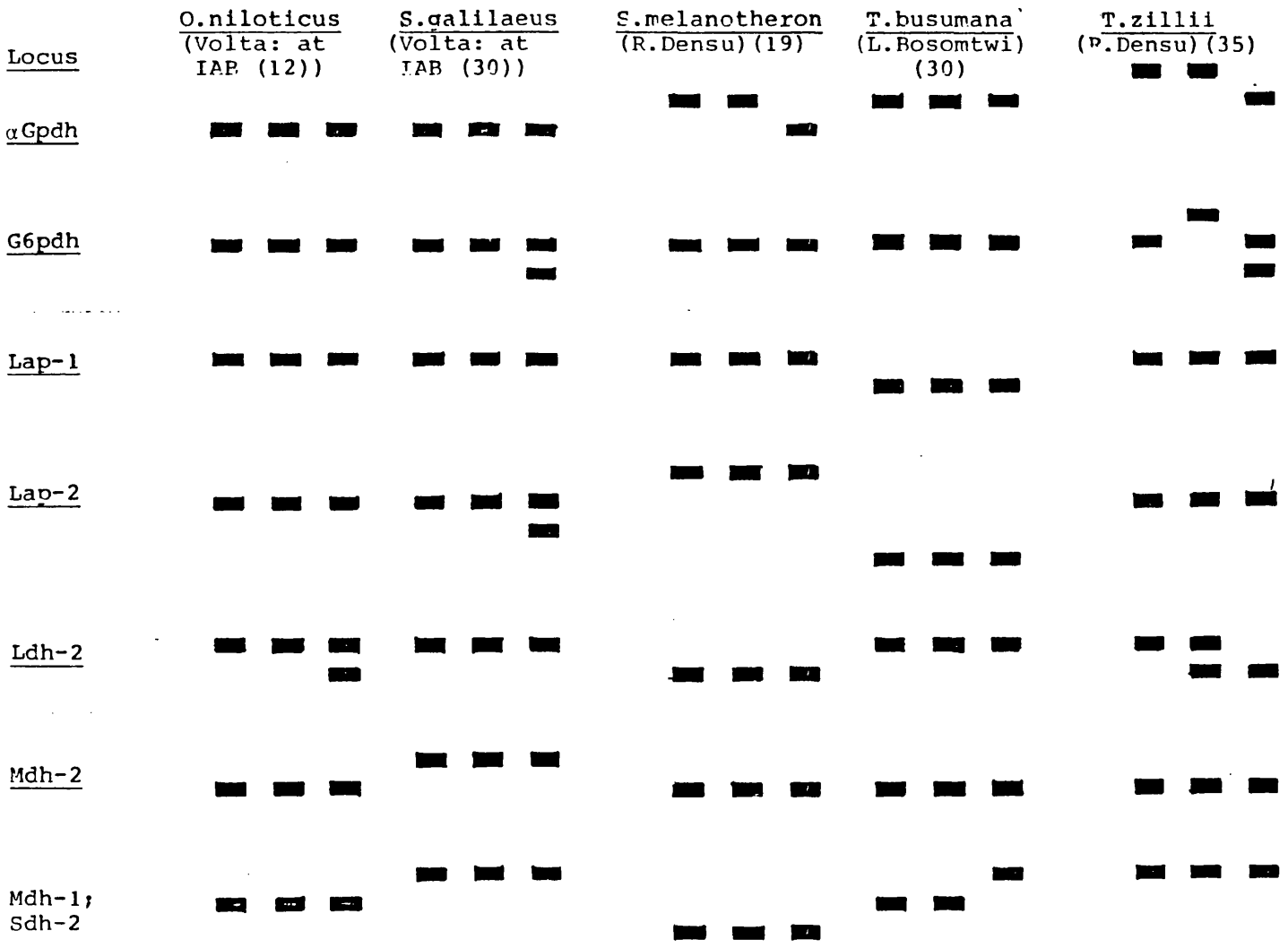

Locus
O.niloticus (Volta: at IAB (12))
S.galilaeus (Volta: at IAB (30))
S.melanotheron (R.Densu) (19)
T.busumana (L.Bosomtwi) (30)
T.zillii (R.Densu) (35)
αGpdh
G6pdh
Lap-1
Lap-2
Ldh-2
Mdh-2
Mdh-1; Sdh-2

Fig. 7c Contd.

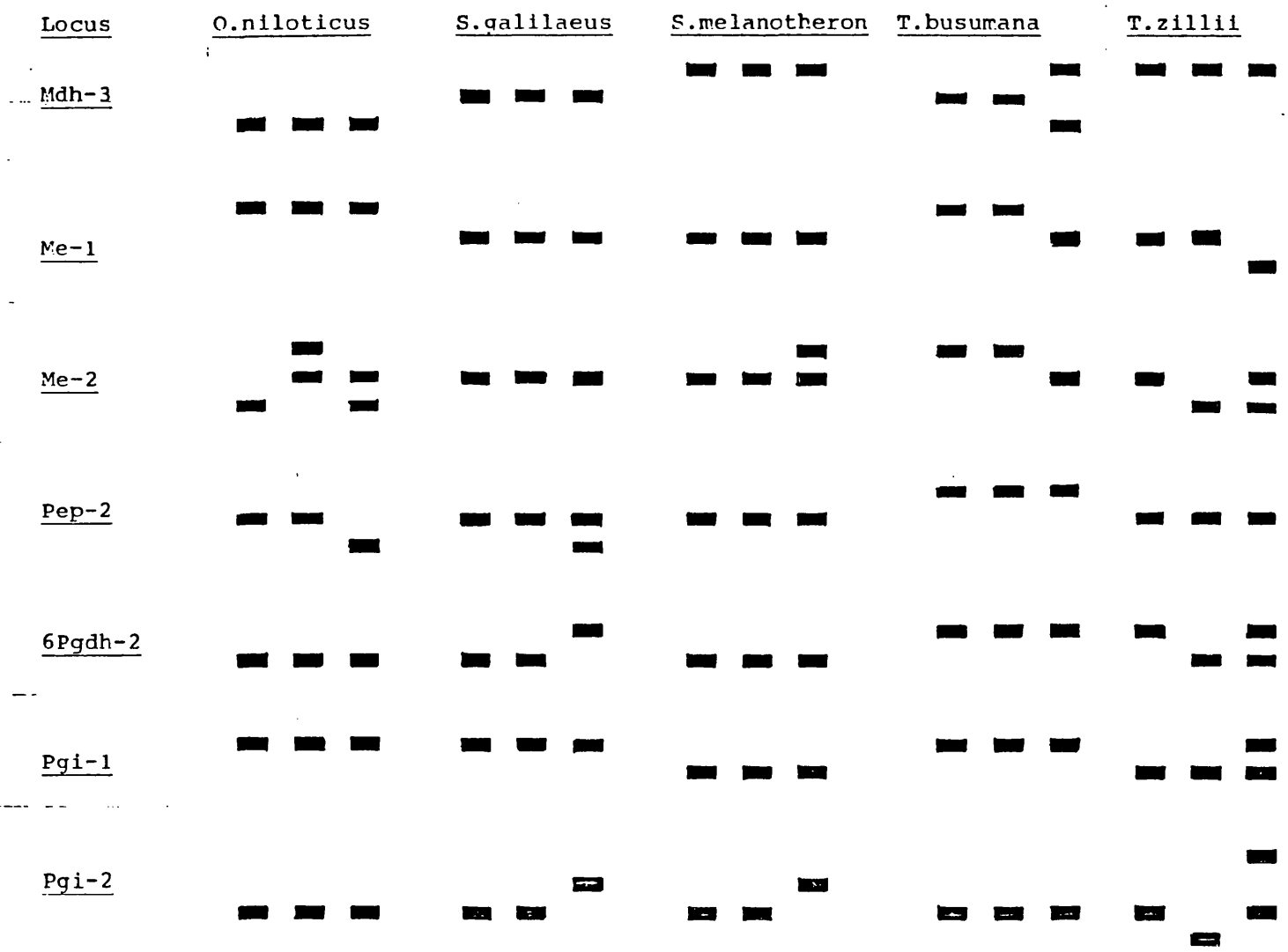
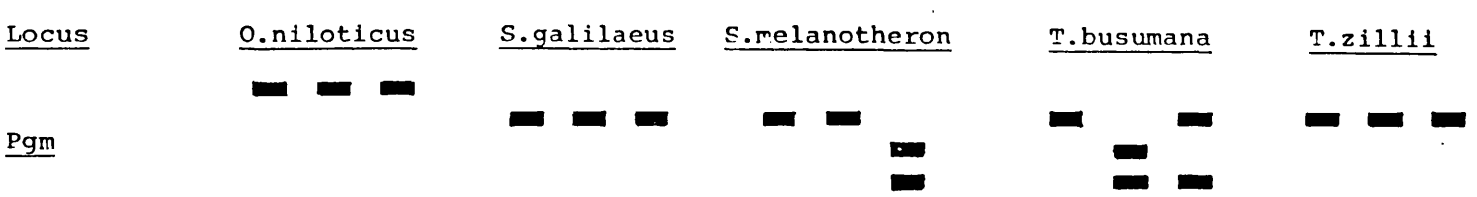

Locus
O.niloticus
S.galilaeus
S.melanotheron
T.busumana
T.zillii
Mdh-3
Me-1
Me-2
Pep-2
6Pgdh-2
Pgi-1
Pgi-2

Fig. 7c Contd.


Locus
O.niloticus
S.galilaeus
S.melanotheron
T.busumana
T.zillii
Pgm

other species in the group. Considering the Est-3 locus as a character, Fig. 7a shows that there is considerable 'overlapping' in expression among the group of related species, as was seen in some of the conventional morphometric taxonomic characters (e.g. Table 4).

The results provide a molecular basis on which unequivocal pair-wise separation of species may be carried out. In the present study, loci at which each of a pair of species was 'fixed' for a different allele were considered as 'discriminating loci' between a pair. Among the four schilbeids an average of 45 individuals were screened at each of 28 enzyme loci to provide the protein patterns. It was thus possible to identify loci which, while not being discriminating, showed significant differential allele frequencies on which species identification could be based. Such loci have, here, been defined as - those at which one of a pair of species had a frequency >0.9 for one allele while the other fish had a frequency <0.1 for the same allele. Estimates of allele frequency were based on observed genotype frequencies - Appendix 3.

Tables 8(i), 8(ii) and 8(iii) show taxonomically important loci between pairs of species within families Schilbeidae, Bagridae and Cichlidae respectively. Two 'sub-populations' of Marcusenieus senegalensis (fm. Mormyridae) were studied. Results of starch gel electrophoresis of 26 individuals at 28 enzyme loci showed that

Table 8(i): Discriminating Loci between pairs of Schilbeidae (No. of Loci screened - 28).

Species Pair	Discriminating Loci					Loci with significant differential allele frequency		
Schilbe mystus/ Eutropius niloticus	Adh-2; Pgi-1;	αGpdh-2; Pgi-2;	Mdh-2; Pgm;	Mpi; 6Pgdh.		Est-3; Me-1;	G6pdh; Mdh-1;	Ldh-2; Sod.
S. mystus/ E. mandibularis.	Adh-2;	Mdh-1;	Pgi-2;	6Pgdh.		Est-2; Sdh-2;	αGpdh-2; Sod.	Idh;
S. mystus/ Siluranodon auritus	Adh-1; Me-2; Pep-1; 6Pgdh	Ck; Mdh-1; Pep-2;	G6pdh; Mdh-2; Pgi-1;	Ldh-2; Mpi; Pgm;		Est-3; Pgi-2.	Idh;	Me-1;
E. niloticus/ E. mandibularis	Adh-2; Mdh-2;	αGpdh-2; Mpi;	G6pdh; Pgi-1;	Mdh-1; Sod.		Me-2;	Pgm.	
E. niloticus/ S. auritus	Adh-1; G6pdh; Mdh-2;	Adh-2; Ldh-1; Pgi-1;	Ck; Ldh-2; 6Pgdh;	Est-2; Me-2; Sdh-1;	αGpdh; Mdh-1; Sod.	Est-3; Pgi-2.	Idh;	Me-1;
E. mandibularis/ S. auritus.	Adh-1; αGpdh-2; Me-2; Pgi-1; Sod.	Adh-2; G6pdh; Mdh-2; Pgi-2;	Ck; Ldh-1; Mpi; 6Pghd;	Est-2; Ldh-2; Pep-1; Sdh-1;	Est-3; Me-1; Pep-2; Sdh-2;	Idh;	Pgm.	

Table 8(ii): Discriminating Loci between two Chrysichthys Species.

(Total number of loci screened - 28).

Species	Discriminating Loci
C. auratus/ C. nigrodigitatus	Adh-1; Adh-2; Est-1; Est-2; Est-3; Ldh-1; Mdh-2; Odh; Sdh.

Table 8(iii): Discriminating Loci between pairs of Cichlid Species.

(Total number of loci screened - 28).

Species	Discriminating Loci
Oreochromis aureus/ O. niloticus	Adh-1; Me-1; Pgm.
Sarotherodon galilaeus/ S. melanotheron	Est-2; Est-3; Est-4; Lap-2; Ldh-2; Mdh-1; Mdh-2; Mdh-3.
Tilapia busumana/ T. rendeli.	Est-1; Adh-1; G5pdh; Lap-1; Mdh-2; Mdh-3; Pep-2; 6Pgdh-2; Pgi-2.
T. busumana/ T. zillii	Est-2; Est-3; Est-3; Lap-1; Lap-2; Mdh-3; Me-2; Sdh-2
T. rendali/ T. zillii	Adh-1; Est-3; Est-4; G6pdh; Lap-1; Mdh-2; Me-2; Pep-2; Sdh-2.

all specimens from both 'sub-populations' were monomorphic for the same allele at 27 loci. At a Pgi locus, one individual was a heterozygote.

3.4.2 General Tissue Protein Patterns: Introduction to Results:

Isoelectric focusing of tissue extracts in thin-layer polyacrylamide gels was also used as a taxonomic procedure in this study. Figures 8(i), 8(ii) and 8(iii) show the proteins' profiles of skeletal muscle extracts of fishes studied. In Figure 8(i) specimens 1 and 2 are profiles of muscle proteins of M. senegalensis from rivers Pru and Oti respectively. Grossly, the two specimens are identical or nearly so, thus each represents the profile of a typical member of the species. The near-identity of the two specimens reflects the uniformity of the two sub-populations as observed during starch gel electrophoresis of specific proteins.

Specimens 3 and 4 are muscle protein profiles of Chrysichthys nigrodigitatus and specimens 5-8, profiles of C. auratus. Each set of the profiles confirms the species specificity of a profile. Comparing the two shows the similarities and differences between the two Chrysichthys species. For example, the two may be distinguished by the mobility of the third anodal protein bands. Fig. 8(ii) shows two specimens each of muscle protein profiles of Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus. The gross similarities among the species is immediately apparent.

However, by the application of protein-band counting and relative mobility of orthologous bands (Ferguson, 1980, pp. 25-27), the individuality of each species profile can

General Protein Profiles in Fishes.

Fig. 8(i) Muscle proteins:

Specimens 1 & 2 = *Marcusenius senegalensis* (Mormyridae)

3 & 4 = *Chrysichthys nigrodigitatus* (Bagridae)

5 - 8 = *C. auratus* (Bagridae)

Figure 8(i)

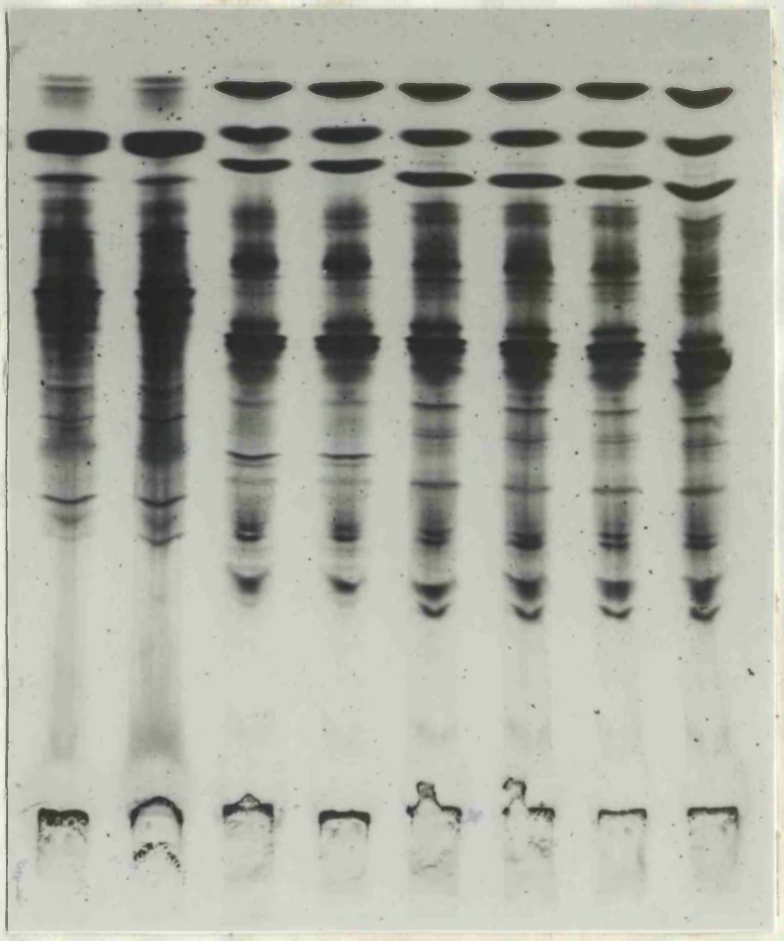


Figure 8(ii): Muscle proteins of Schilbeidae:

Specimens 1 & 2 = Schilbe mystus

3 & 4 = Eutropius niloticus

5 & 6 = E. mandibularis

7 & 8 = Siluranodon auritus

Figure 8(iii): Eye lens proteins of Schilbeidae:

Specimen 1 = Schilbe mystus

2 = Eutropius niloticus

3 = Siluranodon auritus

Figure 8(ii)

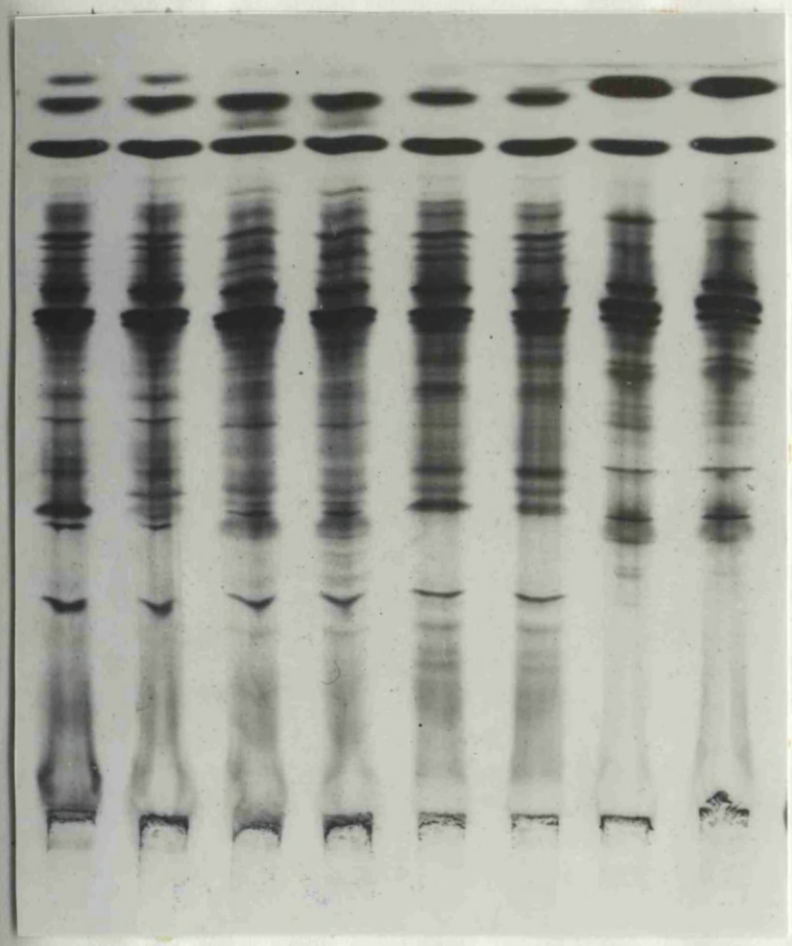


Figure 8(iii)

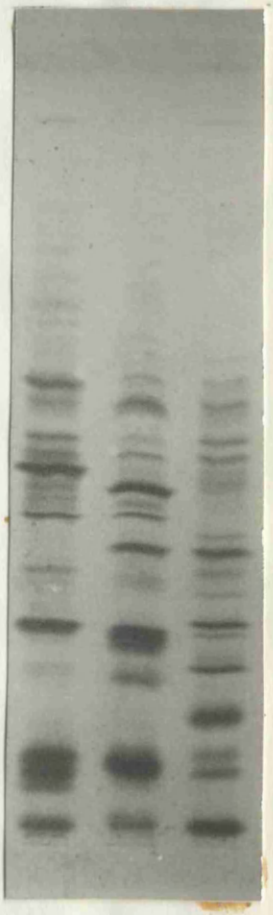


Figure 8(iv): Muscle Protein Profiles of Tilapias (species and stocks):

Specimens 1 & 2	=	Tilapia busumana (wild population, WP)
3,4 & 5	=	T. zillii (Densu WP)
6,7,8 & 9	=	T. zillii (Mixed Pond population)
10 & 11	=	Oreochromis niloticus (IAB)
12	=	O. mossambicus (Swansea)
13 & 14	=	Sarotherodon galilaeus (Stirling)
15,16 & 17	=	S. galilaeus (IAB)

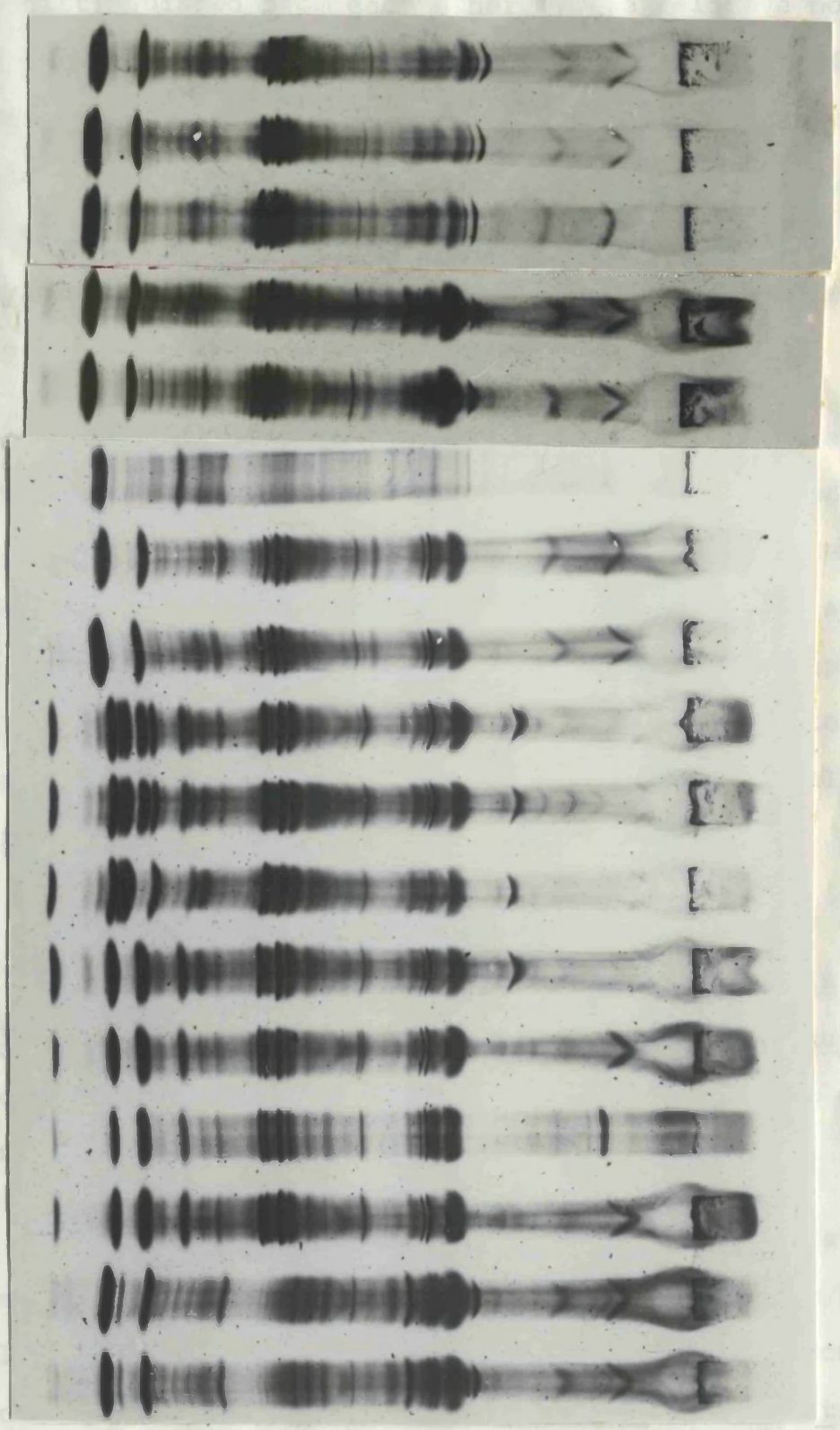


Figure 8(iv)

be distinguished from each other by the relative mobility of the most anodal protein-bands. The relative differences, according to the theory of the technique, indicates the relative differences in the charge of the proteins involved in the bands referred to. Other orthologous bands between any pair of species may be used to identify species (see Figs. 9a and 9b).

Fig. 8(iii) shows the specific profiles of eye lens proteins of Schilbe mystus, Eutropius niloticus and Siluranodon auritus. Eye lens proteins are considered highly species specific (Smith, 1965, 1968), and as can be seen here, the similarities between the three species is less complex, comparing the eye lens profiles to the muscle proteins' profiles; but using eye lens would almost certainly mean sacrificing the specimens involved.

Fig. 8(iv) shows the muscle proteins profiles of five tilapias: Tilapia busumana, T. zillii, Oreochromis niloticus, O. mossambicus and Sarotherodon galilaeus. Grossly specimens of wild populations of a species were consistent in profile.

In Fig. 8(iv) specimens of the T. zillii population of the 'mixed species pond' (MP-specimens 6, 7, 8 and 9) were all different from each other. This observation confirms the suspected introgression of T. zillii by other tilapia species stocks obtained from the mixed pond. Based on the gross profiles (as can be seen with the eye) the stocks of

S. galilaeus (Stirling and IAB) also differed slightly. However, each stock showed a consistent profile. The possibility of observing such protein differences between stocks of a species in one experiment is one of the major advantages of isoelectric focusing over starch gel electrophoresis.

The resemblance, as seen between species in Fig. 8(ii) of such complex electromorphs can only be observed among organisms which are genetically related (Ferguson, 1981). The greater similarity between the profiles of Eutropius niloticus and E. mandibularis, compared to that between any other pair of the four schilbeids (Fig. 8(ii)) is an indication of the closer genetic relationship between species of a genus. The ability of the technique to distinguish such closely related species provided the basis for the application of the isoelectric focusing technique to taxonomic problems in many organisms, including some fish (Chua et al., 1978; Lundstrom and Roderick, 1979; Ferguson, 1981, pp. 92-97; Smith and Jamieson, 1979, 1980 O'Maoleidgh et al., 1988). It could then follow that the ability of electrophoretic techniques, in general, to distinguish genetic differences between populations is perhaps its greatest advantage over morphological taxonomy.

In each of Figs. 9a and 9b, the advantage of determining the range of pH range occupied by bands on a gel during isoelectric focusing was used to identify taxonomically important protein bands. The majority of such bands from

the plates (Fig. 8) are presented in the figures 9a and 9b after calibrating their pH or Isofocusing point (PI) values. Using the eye, very distinct bands were selected for calibration of pH or PI values. Usually, bands common to all species in a group (having no taxonomic value) were ignored. Then, except where differences in intensity of bands was very obvious and important (e.g. Fig. 9a - band with PI value 3.42), such differences have been overlooked. As the legends to Figs. 9a and 9b indicate, it would be possible to rely on one or a few specific protein bands to identify a species among its close relatives.

However, small differences between the migration of orthologous proteins and their relative intensities may be important to distinguish stocks or populations of a species complex. Such fine differences could be obtained, if necessary, by the use of densitometers to scan gels. A densitometer basically provides the following information on the proteins of a profile:

a) the total 'area' occupied by all the proteins in a profile.
b) Picks the peak-bands, and gives their position and intensity, expressed as the proportion of the total area each band occupies as a percentage of the total area.
c) Finally, two profiles could be compared (overlayed) to observe differences in one presentation. Examples of such exercises are presented in Figs. 10 and 11.

Figure 9a: Species Protein Bands with PI Values (from general Protein Profiles) Among Schilbeidae (Skeletal muscle proteins).

Legend: PI = Isoelectric point (= pH value at which protein has zero charge).

S.my = Schilbe mystus

E.ni = Eutropius niloticus

E.ma = Eutropius mandibularis

S.au = Siluranodon auritus

* Note: One common protein band (P1 = 3.70 included). Open bands at P1 3.42 indicate distinct difference in band intensity.

Species	PI Value of Unique Protein
Schilbe mystus	6.90
Eutropius niloticus	3.37
E. mandibularis	6.15; 7.05
Siluranodon auritus	3.45; 3.96; 4.69; 5.17

FIG. 9a.

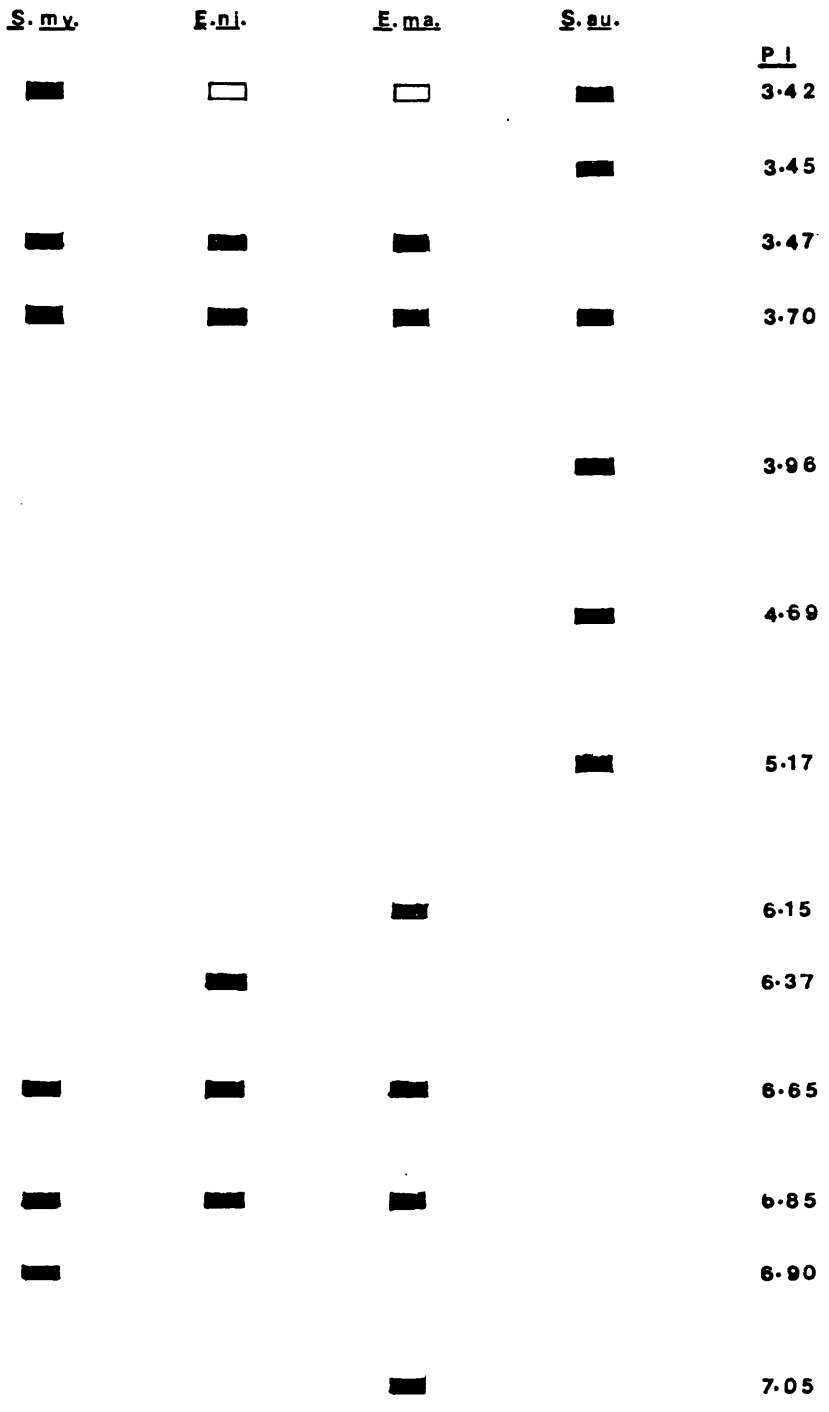

S. my.
E. ni.
E. ma.
S. au.
PI
3·42
3·45
3·47
3·70
3·96
4·69
5·17
6·15
6·37
6·65
6·85
6·90
7·05

Figure 9b: Species/Stock Protein Bands with PI Values Among Tilapias (skeletal muscle proteins).

Legend

Spp = Fish species

PI = Isoelectric point (= the pH value at which protein has zero charge).

A = *Tilapia busumana* (Lake Bosomtwe, typical wild population)

B = *Tilapia busumana* (an odd specimen among a sample of 20. Specimen did not show unusual alleles during starch gel electrophoresis).

C = *Sarotherodon melanotheron* (R. Densu, wild population)

D = *Tilapia zillii* (IAB population)

E = *Tilapia zillii* (River Densu, wild population)

F & G = *Tilapia zillii* (two specimens from Mixed Pond)

H = *Oreochromis niloticus* (Typical of IAB population and a few from Mixed Pond)

I = *Sarotherodon galilaeus* (IAB population).

Note: No attempt has been made to show differences in intensity of bands as seen in original results. The major objective of Fig. 9a and 9b was to show the PI values of peak bands which may be useful in identifying species or populations.

A very conservative interpretation of Fig. 9a shows that each species had a protein specific to it as follows :

Species/Stock	PI Value of Unique Protein
Tilapia busumana (L. Bosomtwi. Wild population)	6.07; 7.32
T. zillii (R. Densu. Wild population)	6.42
T. zillii (IAB)	4.15; 6.70; 6.90; 8.50
Sarotherodon galilaeus (IAB)	3.85; 4.20
S. melanotheron (R. Densu. Wild population)	3.70; 3.80; 4.56; 8.00
Oreochromis niloticus (IAB)	3.87; 6.03; 7.90

Between every pair of species or stocks other unique proteins may be found.

FIG. 9b

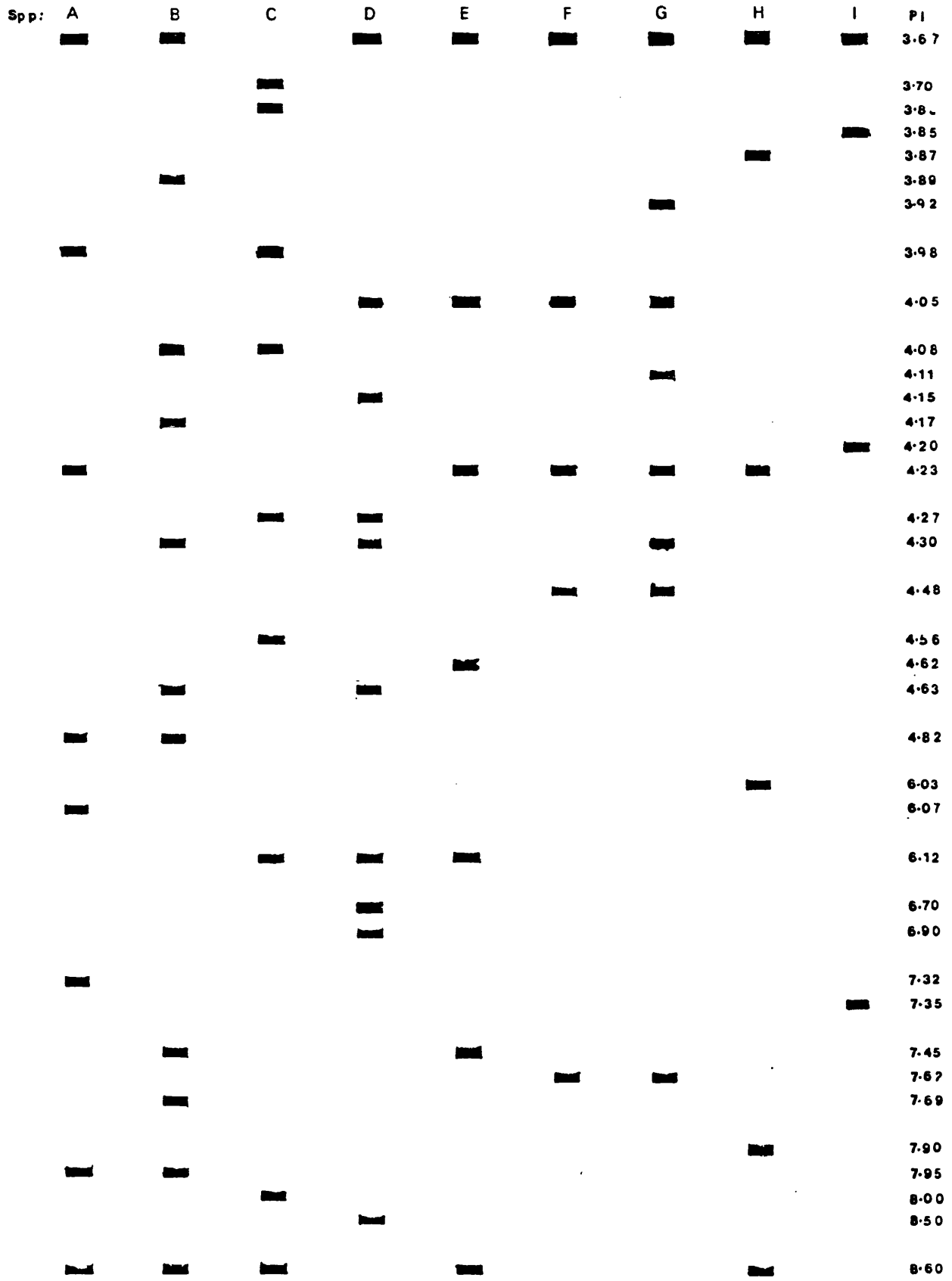

Spp: A B C D E F G H I PI
3·67
3·70
3·8.
3·85
3·87
3·89
3·92
3·98
4·05
4·08
4·11
4·15
4·17
4·20
4·23
4·27
4·30
4·48
4·56
4·62
4·63
4·82
6·03
6·07
6·12
6·70
6·90
7·32
7·35
7·45
7·67
7·69
7·90
7·95
8·00
8·50
8·60

Figure 10a, 10b, and 10c: Densitometer Description of Intensity Profiles of (a) Schilbe mystus, (b) Eutropius niloticus, and (c) Tilapia busumana.

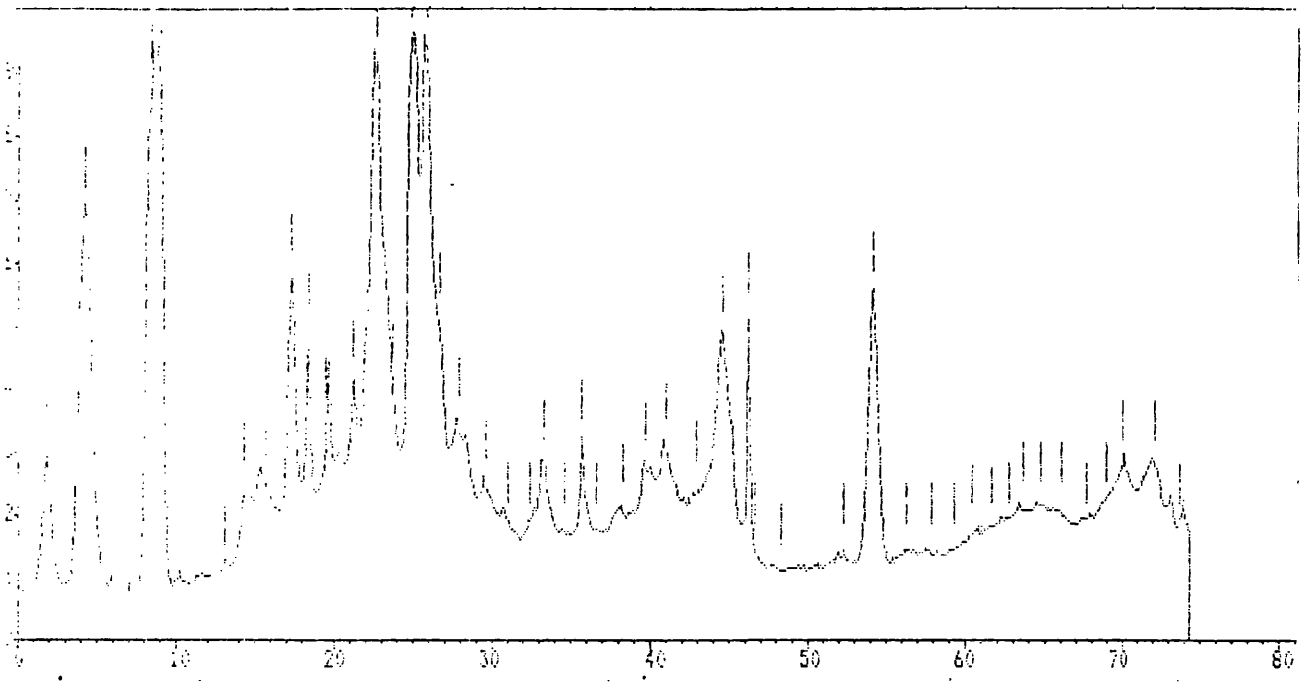
In each of a, b and c, the densitometer indicates that the total area occupied by proteins of the species were 11781.0, 12641.7 and 19797.7 for S. mystus, E. niloticus and T. busumana respectively.

Peak - A number of peak bands detected by densitometer (e.g. 49 in S. mystus, 51 in E. niloticus and 51 in T. busumana).

Position - An indication of the migration of the peaks (bands).

Area - Proportional area occupied by a band in the total area of the profile.

Figure 10a: Intensity Profile of Schilbe mystus.



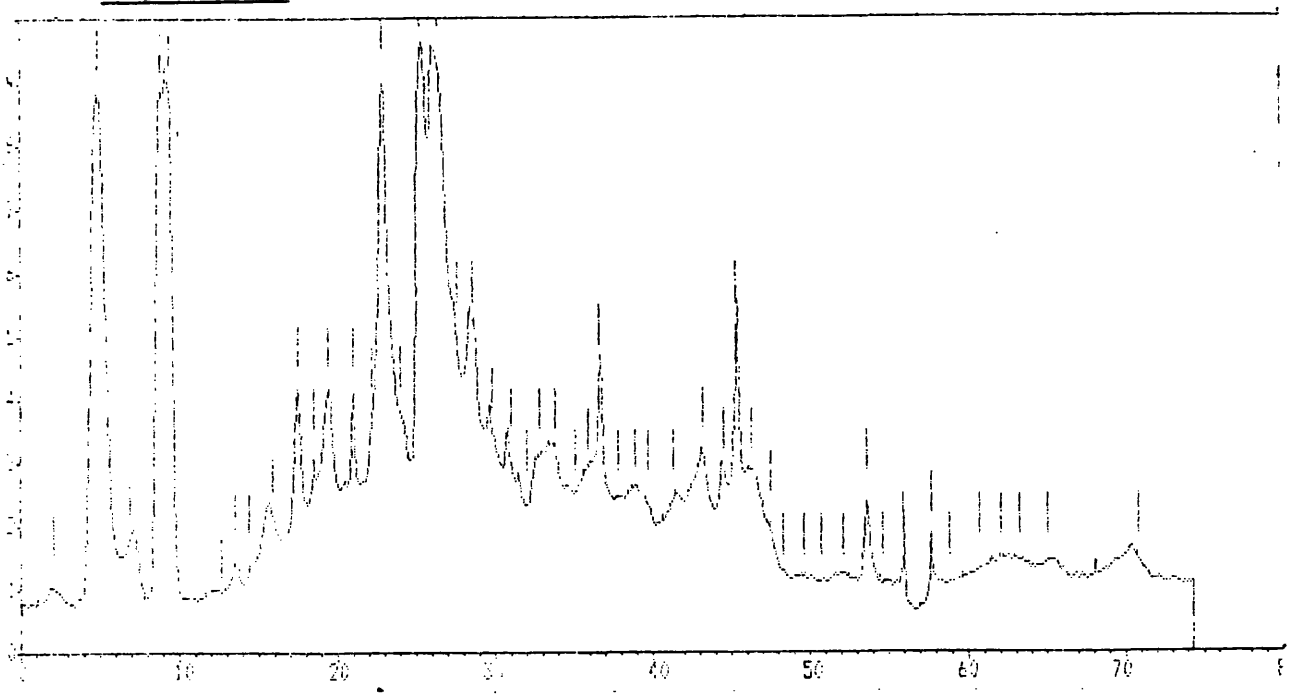
Peak Areas. (Scale = 10, AbsR. = 3.0)

Scaling factor : 1.000; Background : fitted; Peak areas : fitted.

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	1.8	168.0	1.4	26	39.7	135.5	1.1
2	4.2	587.9	5.0	27	41.0	301.9	2.6
3	8.4	535.0	4.5	28	42.9	171.9	1.5
4	9.0	450.7	3.8	29	42.9	88.0	0.7
5	13.1	22.8	0.2	30	44.6	499.1	4.2
6	14.3	90.8	0.8	31	46.2	127.0	1.1
7	15.7	317.0	2.7	32	46.6	15.4	0.1
8	17.3	344.8	2.9	33	48.3	95.2	0.8
9	18.4	196.1	1.7	34	52.3	68.7	0.7
10	19.5	239.1	2.0	35	54.2	372.4	3.2
11	21.2	509.7	4.3	36	56.3	87.2	0.7
12	22.7	854.5	7.3	37	57.9	46.7	0.4
13	23.7	240.1	2.0	38	59.3	58.0	0.5
14	24.9	721.5	6.1	39	60.4	43.8	0.4
15	25.9	656.7	5.6	40	61.6	157.3	1.3
16	26.7	233.0	2.0	41	62.7	69.7	0.6
17	27.9	427.9	3.6	42	63.6	95.1	0.8
18	29.6	154.2	1.3	43	64.7	172.0	1.5
19	31.0	174.1	1.5	44	66.0	128.0	1.1
20	32.4	80.5	0.7	45	67.6	216.6	1.8
21	33.3	142.6	1.2	46	68.9	122.6	1.0
22	34.6	187.7	1.6	47	70.0	260.7	2.2
23	35.7	87.1	0.7	48	72.1	605.6	5.1
24	36.6	76.0	0.6	49	73.7	106.4	0.9
25	38.3	238.2	2.0				

Total Area 11781.0 = 100.0%

Figure 10b: Intensity Profile of Eutropius niloticus.



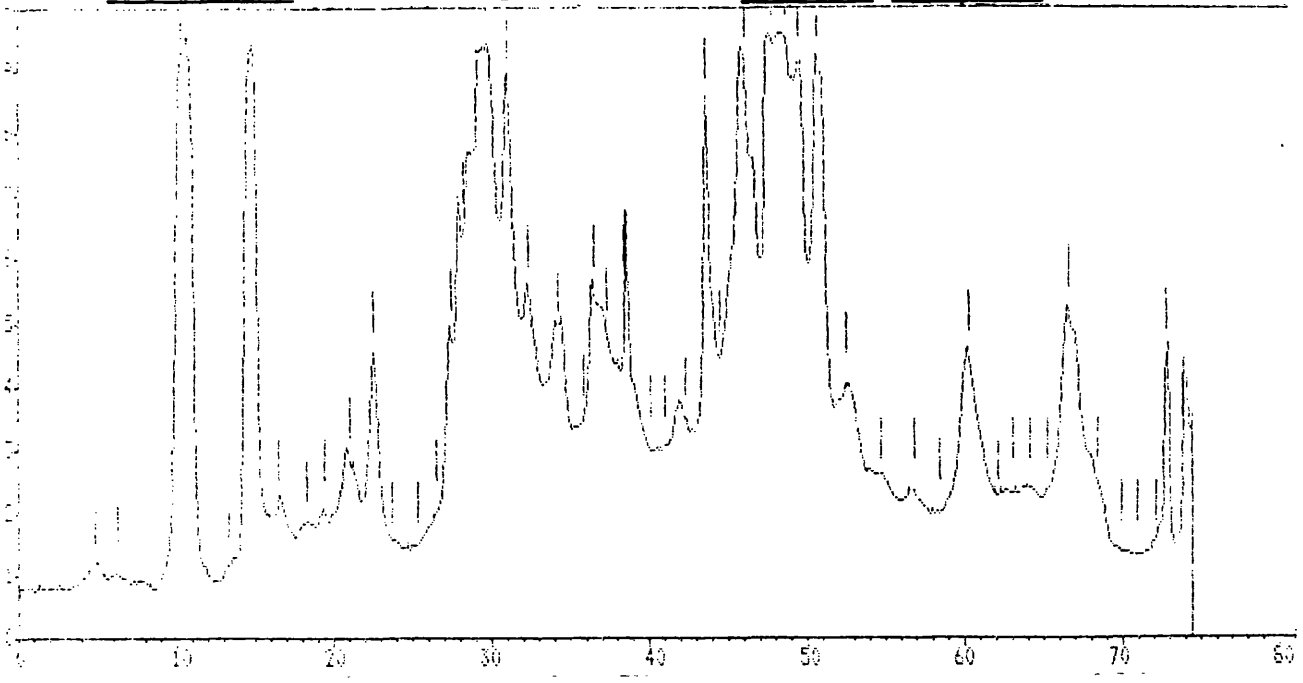
Peak Areas. (Scale = 10, AbsR. = 3.0)

Scaling factor : 1.000; Background : fitted; Peak areas : fitted.

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	2.1	24.8	0.2	27	35.8	134.6	1.1
2	4.9	893.7	7.1	28	36.6	214.8	1.7
3	6.9	164.8	1.3	29	37.8	292.7	2.3
4	8.8	596.3	4.7	30	38.9	108.5	0.9
5	9.4	395.2	3.0	31	39.7	85.7	0.7
6	12.6	42.4	0.3	32	41.3	393.0	3.1
7	13.5	31.5	0.2	33	43.2	327.4	2.6
8	14.4	25.2	0.2	34	44.5	152.6	1.2
9	15.9	278.7	2.2	34	45.3	201.6	1.6
10	17.5	204.3	1.6	36	46.3	313.6	2.5
11	18.5	185.2	1.5	37	47.5	77.9	0.6
12	19.4	275.2	2.2	38	48.3	30.9	0.2
13	21.0	407.5	3.2	39	49.6	92.1	0.7
14	22.2	136.2	1.1	40	50.7	3.2	0.0
15	22.9	571.1	4.5	41	52.1	123.5	1.0
16	24.0	421.0	3.3	42	53.6	67.0	0.5
17	25.3	654.0	5.2	43	54.6	75.9	0.6
18	26.4	1095.4	8.7	44	55.9	30.5	0.2
19	27.6	374.9	3.0	45	57.7	23.0	0.2
20	28.6	415.8	3.3	46	58.8	115.7	0.9
21	29.8	335.3	2.7	47	60.7	71.3	0.6
22	31.0	272.3	2.2	48	62.0	120.0	0.9
23	32.0	103.3	0.8	49	63.2	58.5	0.5
24	32.8	176.1	1.4	50	65.0	180.7	1.4
25	33.8	304.6	2.4	51	70.8	792.9	6.3
26	35.1	179.2	1.4				

Total Area 12641.7 = 100.0%

Figure 10c: Intensity Profile of Tilapia busumana.



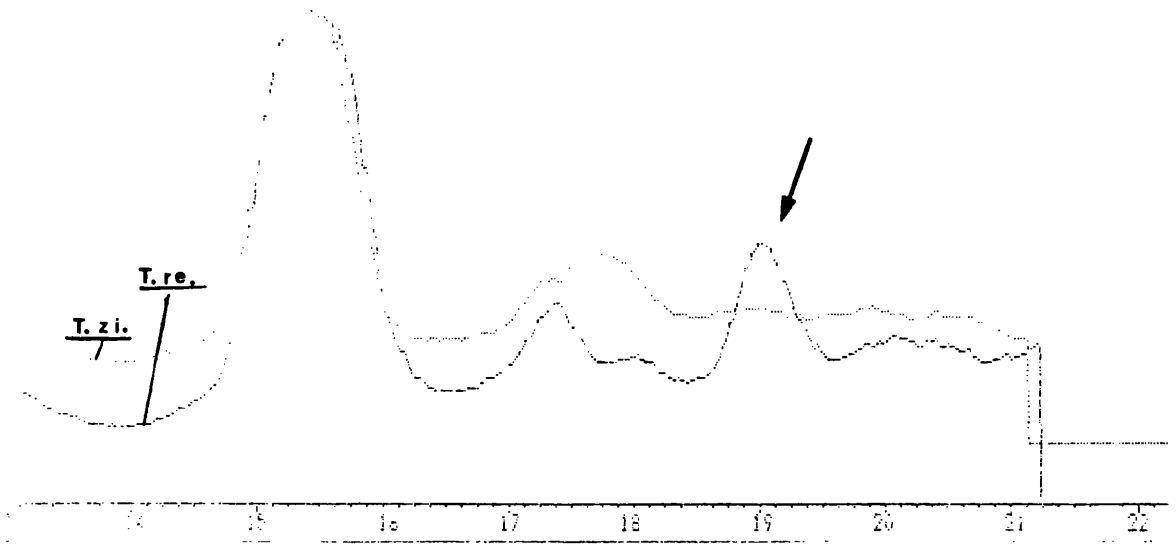
Peak Areas. (Scale = 10, AbsP. = 3.0)

Scaling factor : 1.000; Background : fitted; Peak areas : fitted.

Peak	Position	Area	Area %	Peak	Position	Area	Area %
1	4.8	40.7	0.2	27	41.0	52.2	0.3
2	6.2	20.3	0.1	28	42.3	629.2	3.2
3	10.2	706.4	3.6	29	43.6	309.3	1.6
4	10.9	369.8	1.9	30	44.5	368.0	1.9
5	13.3	27.8	0.1	31	46.1	1457.1	7.4
6	14.7	861.9	4.4	32	47.8	983.5	5.0
7	16.5	227.8	1.2	33	48.7	586.9	3.0
8	18.3	153.1	0.8	34	49.5	633.5	3.2
9	19.4	71.2	0.4	35	50.7	860.6	4.3
10	21.0	384.7	1.9	36	52.5	640.6	3.2
11	22.5	247.1	1.2	37	54.7	317.2	1.6
12	23.7	155.6	0.8	38	56.8	278.9	1.4
13	25.3	92.9	0.5	39	58.4	109.2	0.6
14	26.5	148.3	0.7	40	60.3	660.8	3.3
15	27.4	235.4	1.2	41	62.1	141.2	0.7
16	28.2	379.2	1.9	42	63.0	114.1	0.6
17	29.4	1162.4	5.9	43	64.1	224.0	1.1
18	29.7	524.8	2.7	44	65.2	84.2	0.4
19	31.0	466.5	2.4	45	66.6	733.2	3.7
20	32.3	732.8	3.7	46	68.4	276.2	1.4
21	34.2	731.7	3.7	47	69.9	77.7	0.4
22	35.8	226.4	1.1	48	70.9	91.5	0.5
23	36.5	315.9	1.6	49	72.1	134.2	0.7
24	37.3	262.1	1.3	50	72.8	191.8	1.0
25	38.4	631.1	3.2	51	73.8	300.0	1.5
26	40.1	366.1	1.8				

Total Area 19797.7 = 100.0%

Figure 11a: Comparison of Intensity Profiles of Tilapia zillii and T. rendali.



Peak Areas

Scaling factor : 1.000
Peak areas : fitted

The figure shows very close general protein similarity between T. zillii and T. rendali. Portion of scan shows area of distinct profile difference (arrowed) between the two species.

Abbreviations: T.re = T. rendali
T.zi = T. zillii

Figure 11b: Comparison of Profiles of Tilapia zillii populations.

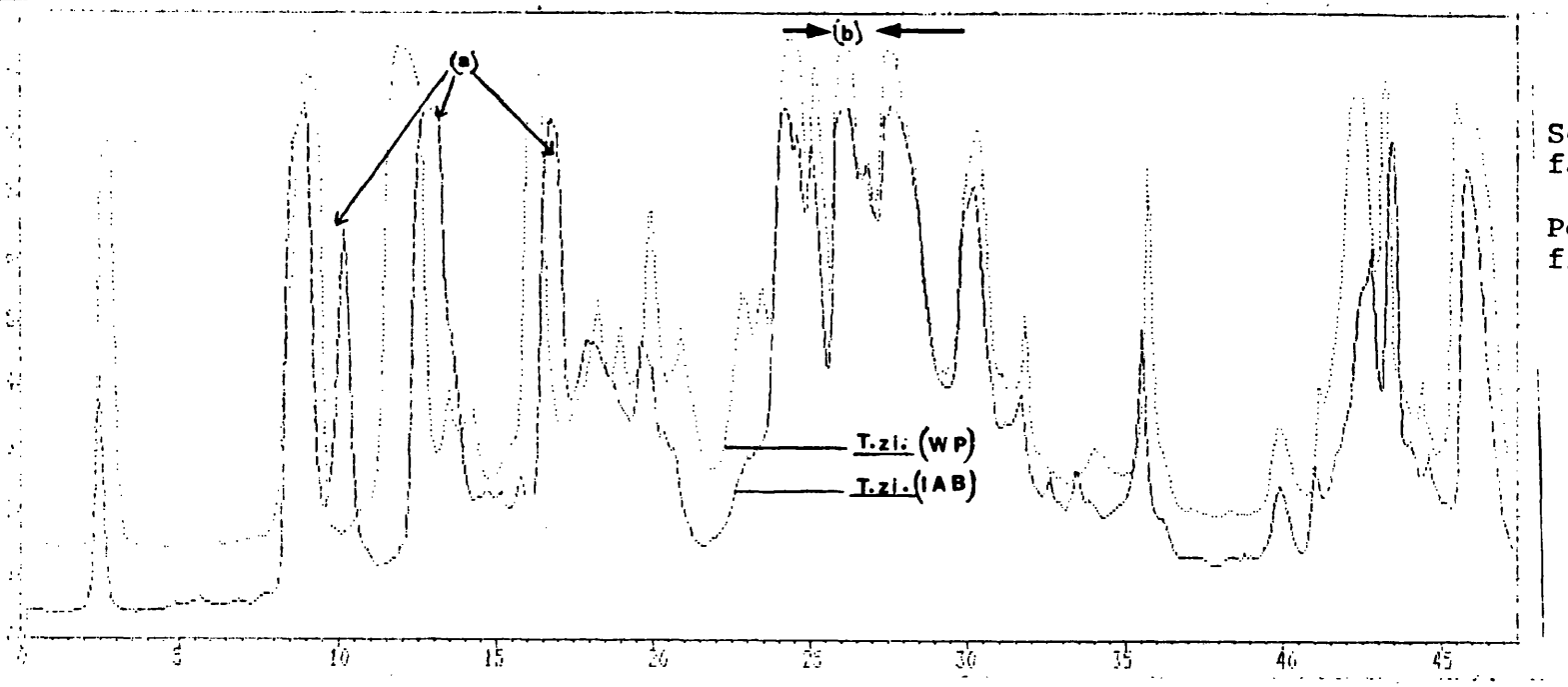


Figure examplifies application of isoelectric focusing of tissue proteins to identification of 'suspected' introgressed or hybrid stocks.

The T. zillii (IAB culture stock) originated from the wild population of river Densu. Points arrowed (a) suggest influences of introgression. Areas within (b) suggest proteins (genes) which show less intense expression in culture stock compared to the parent wild population, which may be attributed to hybridization.

Legend

T.zi = Tilapia zillii
WP = Wild population (river Densu)
IAB = Culture stock (IAB Ponds)

3.5 Discussion and Evaluation of Taxonomic Methods:

In this chapter, three methods (Morphometrics, Starch gel electrophoresis, and Isoelectric focusing of general proteins) have been used to study the taxonomy of species and populations. It was evident from the results that, generally, one or the other method was more reliable as a taxonomic tool than the others. Secondly, perhaps a technique was more productive with reference to particular fish groups. Previous to this work, the taxonomy and systematics of the fishes studied have been based on the morphology and meristic characteristics of the fishes. Thus initial identification of material studied was based on keys to species provided by Roman (1966), Lowe-McConnell (1972), Trewavas (1983) and Leveque and Paugy (1984).

Allowing for intraspecific variation generally, all morphological and meristic data obtained here were comparable to previous reports (see Tables 3 to 6). Differences between populations have usually been attributed to populations having different habitats, without often being able to identify specific ecological conditions which cause increases or limitations in the variability of a character in different populations. It is therefore obvious that small differences between populations which may originate from genetic changes could easily be attributed to ecological differences in habitats.

For example, Lowe-McConnell (1972) pointed out the naming of subspecies of Marcusenius senegalensis, apparently based on modifications of meristic character plasticity in different populations. In two 'sub-populations' of the species studied, some meristic characters showed different extents of plasticity in the sub-populations. It is therefore possible to envisage that an accidental sample containing many 'odd' fishes could have given rise to considering the two sub-populations as constituting separate populations based on meristics. However, results of both electrophoresis of specific proteins and general tissue proteins (isofocusing Fig. 7a) individually showed that the populations of M. senegalensis in the Pru and Oti rivers (Fig. 1) were part of a single gene pool. It is evident therefore that, for a species which is so widely distributed, and apparently exhibits considerable morphological adaptations to local habitats, a more reliable identification of stocks should be based on electrophoresis rather than morphology.

The meristic counts recorded for the identification of the schilbeid fishes showed that often Schilbe mystus and Eutropius niloticus expressed the same characters within very similar ranges. Thus it was necessary to use the expression of combinations of characters to identify fishes. Results of starch gel electrophoresis of individuals including those on which meristic counts were made, revealed a number of discriminating loci between pairs of

species (8 between S. mystus/E. niloticus; 4 for S. mystus/E. mandibularis; 13 for Schilbe mystus/Siluranodon auritus; 8 for E. niloticus/E. mandibularis; 15 for E. niloticus/S. auritus and 21 for E. mandibularis/S. auritus, all out of the same 28 loci screened. See Table 7(i) for specific loci.).

With information on discriminating loci between species available, gel electrophoresis holds many advantages over morphological taxonomy. First many individuals could be screened within a shorter time than they could ever be studied morphologically. The other great advantage in this respect would be that not many individuals would be needed to establish the identity of any specimen. In fact a single specimen each of any two species would be enough, an advantage which is still operative even if specific discriminating loci for species was not available. Many loci in the single individuals could be quickly tested to establish whether any two specimens were the same or different species. Gorman and Renzi (1979) have provided empirical evidence for such work. These advantages of electrophoresis over morphological taxonomy, exemplified by results from the Schilbeids would generally apply for any group of related species as the bagridae studied here.

As can be expected the closer genetically, any two species were, the lower the number of discriminating loci would be between them compared to other pairs of species. Among the schilbidae therefore the results have indicated

that E. mandibularis is more closely related to S. mystus (4 discriminating loci between them) than it is to E. niloticus (with 8 discriminating loci between them). This is interesting, but not a taxonomic problem, it will therefore be discussed under relationships between members of schilbeidae studied (Chapter 4).

Profiles of general muscle and eye lens proteins (Figs. 8ii and 8iii respectively) showed each species to have an individual profile which could not be confused with any other. For purely taxonomic purposes the individualistic profiles, in relation to protein bands migration and intensity, would not require any quantitative assessments to separate one species from another. The taxonomic advantage of isofocusing of tissue proteins over gel electrophoresis here would be as follows : with the starch gel results showing that E. mandibularis was closer to S. mystus than it was to E. niloticus, the isofocusing results shows that when 'all' the proteins with similar properties in the species were compared, the two Eutropius species were more similar to each other than either was to Schilbe mystus (Fig. 9a). The profiles thus remove human or technical bias which might determine which morphological or specific protein characters might be studied by different workers or laboratories in determining identity. Protein profiles can also provide more detail comparison of species by the use of densitometers which estimate both relative positions and intensity of peak (protein) bands (see Figs. 10 and 11).

For those interested in the systematics of the schilbeidae, the observations made here hold a special hope. The hope is, the potential of electrophoresis suggests that the identity of what is nominally known as Eutropius depressirostris (Peters, 1852) but described by many as "just as schilbe except for the presence of an adipose fin, which may be rudimentary in structure" (e.g. Bell-Cross, 1976; Jubb, 1957, pers. comm. to Trewavas), can be determined.

Results obtained here in relation to the taxonomy of the tilapias, by the use of the three methods, also demonstrates other advantages of protein taxonomy over morphological methods. Many authors have enumerated problems associated with the almost futile attempts at resolving the taxonomic and systematic issues of the tilapias based on morphology (e.g. Pullin, 1985; McAndrew and Majumdar, 1983, 1984; Trewavas, 1983). Among the problems, a combination of the following pose a progressive dilemma to the validity of morphological taxonomy of tilapias. Firstly, the morphological and meristic characters used for species identification overlap (see Table 4). Secondly, interspecies hybridization among tilapias in the wild and culture environments do occur (Trewavas, 1983; Taniguchi et al., 1985). These 'natural' characteristics of tilapias, are further complicated by the world wide movement of tilapias. Of course the possibility of 'species' or stocks being moved around having initially been misidentified material cannot be forgotten.

Due to the overlapping of taxonomic characteristics, hybrids of tilapias are almost impossible to identify morphologically, because the hybrids, as in many other organisms, are usually morphological intermediates (Ferguson, 1980; Trewavas, 1983). For example, the range of meristics studied here for T. zillii and O.niloticus, from the mixed-species pond were all within the range observed in the parent stocks (T. zillii (Den) and O. niloticus (IAB) - see Tables 6A, 6B, 6C, 6D and 6E). Similar problems have been resolved in relation to many temperate fish species and stocks (Frost, 1965; Jamieson and Turner, 1978, 1980; Kornfield et al., 1982; Ferguson, 1980, 1981; O'Maoileidgh et al., 1988). Among tropical fishes only the tilapias, as a group of fishes have received some limited attention at resolving the problem of identifying species, stocks and hybrids, with no definite conclusions drawn yet (McAndrew and Majumdar, 1983; Cruz et al., 1982; Taniguchi et al., 1985).

The property of protein electrophoresis which allows for the identification of hybrids is that even sibling species differ considerably in their structural genes and proteins are normally co-dominantly expressed. Thus in many cases, hybrids show a summation of the parental electrophoretic patterns. Where different alleles are present at a locus in the parents, the hybrid will exhibit a "heterozygote" type of pattern. Thus if two species show bands of different electrophoretic mobilities for a

particular protein, then the hybrid will show both bands ("heterozygote") each at approximately half the concentration found in the parent. In the case of multimeric proteins, unique heteromers may be shown in the hybrid pattern, to further help in identification (Ferguson, 1980, pp. 114). In the present study, T. zillii from the mixed pond population especially, shows unique alleles compared to its parent wild stock (T. zillii - Densu), after both starch gel electrophoresis and isoelectric focusing (see allele frequency Table and Fig. 7E). This suggests an introgression of the species with other tilapia species in the mixed pond as suspected prior to experimentation.

In conclusion it is clear that although electrophoresis has its limitations (see reviews of Avise, 1974; Ferguson, 1980; Thorpe, 1982) the technique still holds important advantages as a taxonomic tool over morphological taxonomy. Then between starch gel electrophoresis and isoelectric focusing of proteins, the latter is preferred over the former for the following reasons among several reviewed by Lundstrom and Roderick (1979).

a) Isoelectric focusing of protein patterns are highly reproducible simply by following the explicit instructions (see Appendix 1).

b) Any variation in sample application in electrophoresis can seriously affect results, thus starch gel electro-

phoresis requires great experience to obtain reliable repeats of results. Isofocusing only requires the following of instructions.

c) Isoelectric focusing is an equilibrium technique in which the proteins are limited in how far they can migrate by the pH gradient of gel. In contrast starch gel electrophoresis is time-dependent and may suffer loss of resolution due to buffer used or diffusion.

d) Resolution of general proteins by isoelectric focusing is higher than what may be attained by starch gel electrophoresis (as results here showed).

e) Lastly, separating a great many protein bands (alleles) in the wide range pH may be advantageous to biologists seeking intraspecific variation. However, starch gel electrophoresis provides variable patterns which provide a basis for genetic interpretation only. With isoelectric focusing some very slight variability between population may be due to difference in ecologies, sex or physiological state of individuals.

* * * * * * * * *

CHAPTER 4

TAXONOMIC RELATIONSHIPS AMONGST TILAPIAS AND SCHILBEIDS

4.1 Introduction:

Relationships within and between taxonomic units of major taxa which might suggest associations between, or derivations of units, have been investigated since evolution became a central concept in biology. Studies which have presented relationships based on overall similarity among units with reference to comparable features, are referred to as PHENETIC. Those that suggest derivation of units from a common ancestor imply PHYLOGENY. Both phenetic and phylogenetic relationships, can often be considered as results of systematic investigation in attempts to distinguish them from studies related to evolution as a process and its mechanisms. The school of thought which supports the complete separation of systematics from evolution (transformed Cladism) argue that systematics shows patterns while evolution attempts to explain a process. However, the taxonomic characters on which systematic schemes are based are not attributes of individuals but of populations, which are also the basic units of the evolutionary process (Ayala, 1983). The transformed Cladists, e.g. Cracraft (1983, p. 164), argue that "It is becoming increasingly apparent that systematics

is the area of biology that defines the pattern of organic change through space and time and, consequently, specifies that body of knowledge that theories of evolutionary process must be capable of explaining". Many biologists however, maintain that it is impossible to isolate systematics from evolution (Mayr, 1969b; Simpson, 1961; Dobzhansky, 1973; Thorpe, 1982; Ayala, 1983) and therefore critisize the Cladists. To these critics, Humphries (1983, p. 305) for example, responds as follows: "they confuse the Cladistic activity of ordering characters to their level of universality with causal explanation of hierarchy..." The stand taken here is that, systematics and evolution are complementary components of the study of relationships within and between taxonomic groups. It should probably be said that, when the Cladists have provided arguments to show, that the characters 'ordered' are not products of evolution, then those who oppose them will have a problem. Dobzhansky (1973) stated that "Nothing in biology makes sense except in the light of evolution". Perhaps systematics is the subject which would make least sense if completely separated from evolution.

Until recently, relationships between related organisms had principally been based on morphological and anatomical character states, although attributes such as physiology, embryology, behaviour and ecology of organisms had been used. In the last two decades, the application of various molecular approaches to the study of genetic structure and discreteness of populations, have yielded sources of

information on which relationships have been suggested. The new approaches include quantitative analysis of gel electrophoretic data (allozyme data) isoelectric focusing of tissue proteins and restriction enzyme analysis of DNA, especially that of mitochondrial DNA (mt. DNA). All the new approaches measure characteristics of populations, which are components or 'first-step' products of population genomes. Thus relationships drawn from such studies are considered genetic, and often imply phylogeny.

In this study, some of the morphological and allozyme approaches to taxonomy and systematics have been used. The techniques have therefore been elaborated on and evaluated in the appropriate section. I would therefore, briefly outline the rational behind the use of restriction enzyme analysis of mt. DNA as a tool in systematics here.

Although much valuable information on population structure may be gained through investigation of allozymes, studies in restriction enzyme patterns of mt. DNA have provided additional insight into the mechanisms influencing population genetic structure. This is mainly due to some unique properties of mt. DNA compared to nuclear DNA. For example, there is evidence that mt. DNA evolves much faster than nuclear DNA. Consequently most mt. DNA changes may be neutral (Brown et al., 1982; Brown, 1983). It is therefore usually possible to link together mt. DNA genotypes in convincing phylogenetic trees, in which the mutational changes separating genotypes may easily be

counted (Avise and Lansman, 1983, also referred to in Edwards and Skibinski, 1987). This cannot be done with data on allozyme variation, where genotypes are continually recreated by recombination and segregation of the nuclear DNA.

Perhaps the greatest advantage of mt. DNA restriction enzyme analysis, is that, mt. DNA genotypes are preserved by asexual reproduction, because they are only maternally inherited. Thus, mt. DNA has unique origins and therefore serves as a good indicator of introgression in relation to genotypes evolved through convergent evolution or descent from a common ancestor (Saunders et al., 1986).

It can be said, therefore, that for systematic investigations currently, broad sources of information include morphology and anatomy, allozyme data, which indirectly originate from nuclear DNA and information from mt. DNA. Of these sources of information, it is perhaps only in morphological investigations, that human influences regarding choice of characters may present acceptability problems. It has often been said that the differences between taxonomic schools results from their principles of character choice. Generally, the problem of character choice has been minimised by "objective classification" (Ridley, 1986, p. 3). Ridley defines choice of character in objective classification as the process in which character choice is dictated by theoretical principle. The principle, amongst other things, must specify some

discoverable hierarchical property of nature, which it is desirable and technically possible for classification to represent.

In both allozyme and mt. DNA analysis problems are often technical rather than human judgment

Having identified and measured the morphological or genetic character states to provide the base-line data, two main approaches are available for the construction of relationships among taxa. These are the phenetic and phylogenetic methods. Of course, each of them has versions and modifications. Exponents of the phenetic approach contend that classification should be based on overall (mean) similarities among taxonomic units. Thus, the data base should include all possible characters, each having equal importance. Since the technique finally uses averages of similarities, it has often been equated to numerical taxonomy (e.g. Sneath and Sokal, 1973).

Advocates of phylogenetic systematics (e.g. Henning, 1966), emphasise the sequence of Cladistic splitting of lineages in the origin of taxa. Closely aligned to the phylogenetic approach is the evolutionary method, supported by e.g. Simpson (1961) and Mayr (1969b). Theoretically the major difference between the two has been that, the phylogenetic system does not concern itself with amount and type of change subsequent to the splitting of phyletic lines. The evolutionary systematist, on the other hand,

takes into account the amount and nature of evolutionary change occurring after Cladogenesis.

4.2 Methods:

The fishes studied in this work, as has been indicated earlier, belong to four families :- Mormyridae, Schilbeidae, Bagridae and Cichlidae. The Schilbeidae and Cichlidae included more than two taxonomic units each to merit consideration of their relationships within each family. Among the Cichlidae, populations of species were maintained as individual operational taxonomic units (OTU) in the schematic presentation of relationships. However, among the Schilbeidae material obtained from tributaries of the Volta lake (Fig. 1) were pooled together per species. This was based on an earlier study (Abban and Skibinski, 1988), which showed that within the Volta basin, sub-populations of the species involved here in tributary rivers, were not genetically isolated and thus constituted single biological populations.

Morphological characters (Body proportions and meristic counts) and allozymes, have been used to assess relationships in this work. Adopting the Unweighted Pair-Group Arithmetic Average (UPGMA) clustering method (Sneath and Sokal, 1973, as described by Ferguson, 1980, pp. 166-168), pair-wise comparisons of genetic identities (Nei, 1972) were used to generate the phenetic relationships as briefly exemplified below. Steps taken to adopt the UPGMA method

to meristic data are also outlined. Allele frequency data, based on which, genetic identities were estimated, formed the data base for the generation of phylogenetic relationships.

A computer package, Phylogeny Inference Package (PHYLIP, version 2.8) was employed to generate phylogenetic trees. Within the package a programme, CONTML (Felsenstein, 1982), which operates on the basics listed below, was used to generate "unrooted tree" relationships. The programme uses gene frequencies to construct estimates of the maximum likelihood phylogenetic tree under the following conditions or assumptions:

a) Different lineages split and evolve independently.
b) After two lineages split, their genetic drift proceeds independently.
c) Each gene frequency changes by genetic drift, and
d) Different loci drift independently.

Although the final output by the programme is an unrooted tree, estimates of 'evolutionary lengths' between segments of the tree accompany the tree. These estimates have been used to construct the unrooted trees presented in the results here as the "best trees" (Figs. 12c and 13c).

Procedure of Adapting the UPGMA Approach to Generating Phenetic Relationships based on Meristic Character Counts:

Counts of meristic characters were made on individuals of each population sample to estimate means for character states per population (Tables 9a and 10a). Pair-wise differences per character between populations were then tabulated.

From the Tables of mean pair-differences per character, a cumulative (overall mean differences between pairs of OTUs) mean differences between OTUs were estimated and tabulated in a matrix. (The cumulative mean differences (distances) between pairs of OTU was estimated simply by dividing the sum of differences by number of characters involved.) From the matrix of overall mean differences (Tables 9c and 10c), the step-by-step procedure to identify most similar pairs, described above, was used to generate dendrograms. However, since the data base suggested differences (or distances) between pairs of OTUs, the pair with least difference was clustered at each stage.

4.3 Results:

Generation of Phenetic Relationships (UPGMA method - based on Genetic identities) (Exemplified with Genetic Identity Data among the Schilbeidae):

The initial step towards clustering taxonomic units (OTU) based on overall genetic 'similarities' was to set out pair-wise genetic identities (or distances) in a matrix table (as in Table 11). Then a scale, covering possible range of identities (0.0 to 1.0) was drawn (e.g. Fig. 12a).

Then from the Table, the two OTUs with the highest identity value (e.g. OTUs 2 and 3 in Table 8) were first clustered. The point at which the two OTUs are joined is with reference to the scale, while the distance apart is arbitrary.

The matrix was then reworked with OTUs 2 and 3 combined as a unit (OTU - 2/3). In the new matrix, the identity between any OTU and OTU 2/3 was the mean of the OTU's identity with OTU 2 and 3. For example, the identity between OTU 1 and 2/3 would be (0.488 + 0.615)/2, as in Table 8 below.

Table 8:

OTU	1	2/3	4
1	X	0.552	0.279
2/3		X	0.231

The most similar pair at this stage was OTU 1 and 2/3. They were therefore joined with respect to scale.

Again the matrix was reworked combining OTUs 1 and 2/4 as a unit to estimate its similarity with remaining unclustered OTUs (in the present example, OTU 4), as shown below.

OTU	1/2•3	4
1/2•3	X	0.255

The relationship is then completed by joining OTU 4 to the cluster in relation to the scale.

The stepwise process of constructing the relationships is described by Ferguson (1980, p. 166-168).

The process described above was applied to genetic identity estimates between populations and species of Cichlids presented in Table 11b to generate the phenetic relationships shown in Figure 12a.

Figures 12a and 13a, therefore represent the overall similarity relationships within the Cichlids and Schilbeids respectively. Since the data base for the dendrograms relate to the genetic character states of taxa, the results have been considered as estimates of genetic phenetic relationships, to distinguish them from relationships based on morphometric data.

As indicated earlier in this chapter, meristic data used by previous workers for the identification of species in the two families (see Chapter 3), were subjected to the UPGMA process. Table 9a shows means of counts of meristic characters considered among the Cichlids. From the table mean (per character and overall) differences between pairs of population were estimated, as exemplified in Table 9b. The typical UPGMA matrix set out in Table 9c was derived from Table 9b, and provided the base data for the generation of overall morphometric relationships between the cichlids shown in Fig. 12b.

Table 9a: Means of Meristic Character Counts Among Tilapias.

Populations	CHARACTERS					
	Gill Rakers	Lat. line Scales	Dorsal Spines	D.fin Rays	A.fin Rays	P.fin Rays
O.ni (IAB)	28.9	34.3	16.9	12.7	9.8	13.2
O.ni (MP)	29.8	33.5	16.8	12.5	9.8	12.9
S.ga (IAB)	27.9	32.3	16.1	13.5	11.4	12.3
S.me (Den)	20.6	27.7	15.4	10.8	9.1	12.1
T.zi (Den)	13.3	30.7	15.3	13.1	9.9	13.1
T.zi (IAB)	13.4	32.2	15.0	13.5	10.2	13.0
T.zi (MP)	14.8	31.0	15.4	12.7	9.8	13.3
T.bu (Bos)	26.0	30.8	15.1	12.8	10.2	12.3

Table 9b: Pair-wise Meristic Differences Among Tilapias.

Populations	Gill Rakers	Lat. line Scales	D.fin Spines	D.fin Rays	A.fin Rays	P.fin Rays	MEAN
O.ni(IAB)/O.ni(MP)	0.9	0.8	0.1	0.2	0	0.3	0.383
O.ni(IAB)/S.ga(IAB)	1.0	2.0	0.8	0.8	1.6	0.9	1.183
O.ni(IAB)/S.me(Den)	8.3	4.6	1.5	1.9	0.7	1.1	3.017
O.ni(IAB)/T.zi(Den)	15.6	3.6	1.6	0.4	0.1	0.1	3.567
O.ni(IAB)/T.zi(IAB)	15.5	2.1	1.9	0.8	0.4	0.2	3.483
O.ni(IAB)/T.zi(MP)	14.1	3.3	1.5	0	0	0.1	3.167
O.ni(IAB)/T.bu(BOS)	2.9	3.5	1.8	0.1	0.4	0.9	1.600

Table 9c: Meristic Character Differences Matrix (Tilapias).

Populations	1 O.ni. (IAB)	2 O.ni. (MP)	3 S.ga. (IAB)	4 S.me. (Den)	5 T.zi. (Den)	6 T.zi (IAB)	7 T.zi (MP)	8 T.bu (Bos)
1	X	0.383	1.183	3.017	3.567	3.483	3.167	1.600
2		X	1.167	2.933	3.617	3.500	3.250	1.583
3			X	2.633	3.283	2.933	3.083	1.050
4				X	2.083	2.133	1.817	1.683
5					X	0.450	0.433	2.400
6						X	0.750	2.583
7							X	2.200
8								X

Meristic data on the schilbeids were similarly treated (Tables 10a, 10b, 10c) and from Table 10c the morphometric relationship presented in Fig. 12b was obtained.

The absence of some taxonomic units in the meristic character based relationships compared to the genetic relationships was due to the fact that only tissue samples for the omitted taxa were available. Also meristic characters which did not vary within or between taxa of a family were omitted.

Table 10a: Means of Meristic Character Counts Among Schilbeids.

	CHARACTERS				
Populations	Gill Rakers	D.fin Spines	D.fin rays	P.fin rays	A.fin rays
S.my	12.69	1	6	10.2	56.4
E.ni.	14.82	1	6	9.0	55.3
S.au.	74.27	0	5	10.0	70.6

Table 10b: Pair-wise Differences Among Schilbeids.

Populations	Gill Rakers	D.fin. Spines	D.fin. rays	D.fin. rays	A.fin rays	MEAN
S.my./E.ni.	2.13	0	0	1.23	1.14	0.89
S.my./S.au.	61.58	1	1	0	14.23	15.56
E.ni./S.au.	59.45	1	1	1	15.37	15.56

Table 10c: Meristic Character Differences Matrix (Schilbeids).

Populations	1	2	3
	S.my.	E.ni.	S.au.
1	X	0.89	15.56
2		X	15.56
3			X

Table 11a: Genetic Identity Matrix Among Schilbeids.

Legend:

OTU	=	Operational Taxonomic Unit
S.my	=	Schilbe mystus
E.ni	=	Eutropius niloticus
E.ma	=	E. mandibularis
S.au	=	Siluranodon auritus

Table 11a: Genetic Identity Matrix Among Schilbeids.

OTU	1	2	3	4
	S.my.	E.ni.	E.ma.	S.au.
1	X	0.488	0.615	0.279
2		X	0.642	0.240
3			X	0.183
4				X

Table **11 b.**

Summary of Estimates of Genetic Identity (I) and Genetic Distance (D) among species and stocks of *Oreochromis*, *Sarotherodon* and *Tilapia*. (I - Above diagonal. D - Below diagonal). Based on analysis of 27 enzyme loci .

Species:- (Source)		*O.aur.* (Ster.)	*O.mos.* (Ster.)	*O.nil.* (Ster.)	*O.nil.* (IAB)	*O.nil.* (MP)	*S.gal.* (Ster.)	*S.gal.* (IAB)	*S.gal.* (IAB)	*S.mac.* (Ster.)	*S.mel.* (R.den.)	*T.bus.* (L.Bos.)	*T.ren.* (Ster.)	*T.zil.* (Ster.)	*T.zil.* (R.Den.)	*T.zil* (IAB)	*T.zil.* (MP)
	OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	1	X	0.593	0.800	0.834	0.821	0.726	0.736	0.617	0.654	0.634	0.564	0.407	0.630	0.724	0.636	0.706
	2	0.523±0.159	X	0.610	0.648	0.656	0.518	0.520	0.411	0.636	0.492	0.340	0.482	0.444	0.497	0.466	0.458
	3	0.223±0.096	0.494±0.154	X	0.952	0.953	0.705	0.717	0.598	0.671	0.610	0.553	0.469	0.683	0.621	0.594	0.605
	4	0.182±0.086	0.434±0.142	0.049±0.043	X	0.976	0.724	0.733	0.577	0.687	0.658	0.562	0.415	0.633	0.580	0.568	0.561
	5	0.197±0.090	0.422±0.139	0.048±0.043	0.024±0.030	X	0.747	0.757	0.587	0.722	0.597	0.588	0.441	0.659	0.604	0.556	0.576
	6	0.320±0.018	0.658±0.186	0.350±0.125	0.323±0.119	0.292±0.112	X	0.919	0.647	0.733	0.582	0.514	0.493	0.613	0.651	0.557	0.607
	7	0.307±0.116	0.654±0.185	0.333±0.121	0.311±0.116	0.278±0.109	0.085±0.057	X	0.696	0.699	0.596	0.524	0.492	0.635	0.656	0.573	0.620
	8	0.483±0.152	0.889±0.230	0.514±0.158	0.550±0.165	0.533±0.161	0.435±0.142	0.362±0.127	X	0.604	0.626	0.391	0.561	0.617	0.655	0.584	0.634
	9	0.425±0.140	0.453±0.146	0.399±0.135	0.375±0.130	0.326±0.199	0.311±0.116	0.385±0.126	0.504±0.116	X	0.602	0.504	0.580	0.617	0.641	0.570	0.596
	10	0.456±0.146	0.709±0.196	0.494±0.154	0.566±0.168	0.516±0.158	0.541±0.163	0.518±0.159	0.468±0.149	0.508±0.157	X	0.516	0.529	0.623	0.727	0.598	0.688
	11	0.573±0.169	1.089±0.268	0.592±0.173	0.576±0.170	0.531±0.161	0.666±0.187	0.646±0.183	0.939±0.240	0.685±0.191	0.662±0.186	X	0.443	0.594	0.532	0.489	0.508
	12	0.899±0.232	0.730±0.200	0.757±0.205	0.880±0.229	0.819±0.217	0.707±0.195	0.709±0.196	0.578±0.170	0.545±0.164	0.637±0.182	0.814±0.216	X	0.591	0.600	0.481	0.488
	13	0.462±0.148	0.812±0.215	0.381±0.131	0.457±0.147	0.417±0.138	0.489±0.153	0.454±0.146	0.483±0.152	0.482±0.152	0.465±0.148	0.521±0.159	0.656±0.185	X	0.852	0.771	0.849
	14	0.323±0.119	0.677±0.194	0.476±0.150	0.545±0.164	0.504±0.156	0.429±0.141	0.422±0.140	0.423±0.140	0.445±0.144	0.319±0.118	0.630±0.181	0.511±0.157	0.160±0.080	X	0.823	0.899
	15	0.453±0.146	0.764±0.206	0.521±0.159	0.566±0.168	0.587±0.172	0.585±0.172	0.557±0.166	0.538±0.162	0.562±0.167	0.514±0.158	0.715±0.197	0.732±0.200	0.260±0.105	0.195±0.089	X	0.848
	16	0.348 0.124	0.781±0.209	0.503±0.156	0.578±0.170	0.552±0.165	0.499±0.155	0.478±0.151	0.456±0.146	0.518±0.159	0.374±0.130	0.677±0.189	0.717±0.197	0.162±0.081	0.107±0.065	0.165±0.082	X

Figures 12a and 12b: Phenetic relationships amongst tilapias.

12a: Relationships based on genetic identity.

12b: Relationships based on meristic differences.

Meristic characters used for 12b: Dorsal spines; Dorsal branched rays; Anal branched rays; Pectoral branched rays; Lateral line scale counts and Gill raker counts.

Legend:

O.ni. = Oreochromis niloticus

O.au. = Oreochromis aureus

O.mo. = Oreochromis mossambicus

S.ga. = Sarotherodon galilaeus

S.ga^{+}. = Sarotherodon galilaeus (odd specimen)

S.ma. = Sarotherodon macrochir

S.mr. = Sarotherodon melanotheron

T.bu. = Tilapia busumana

T.re. = Tilapia rendalli

T.zi. = Tilapia zillii

IAB = Institute of Aquatic Biology ponds.

St. = Stirling University.

Den. = River Densu.

MP. = Mixed species Pond

Bos. = Bosomtwi lake.

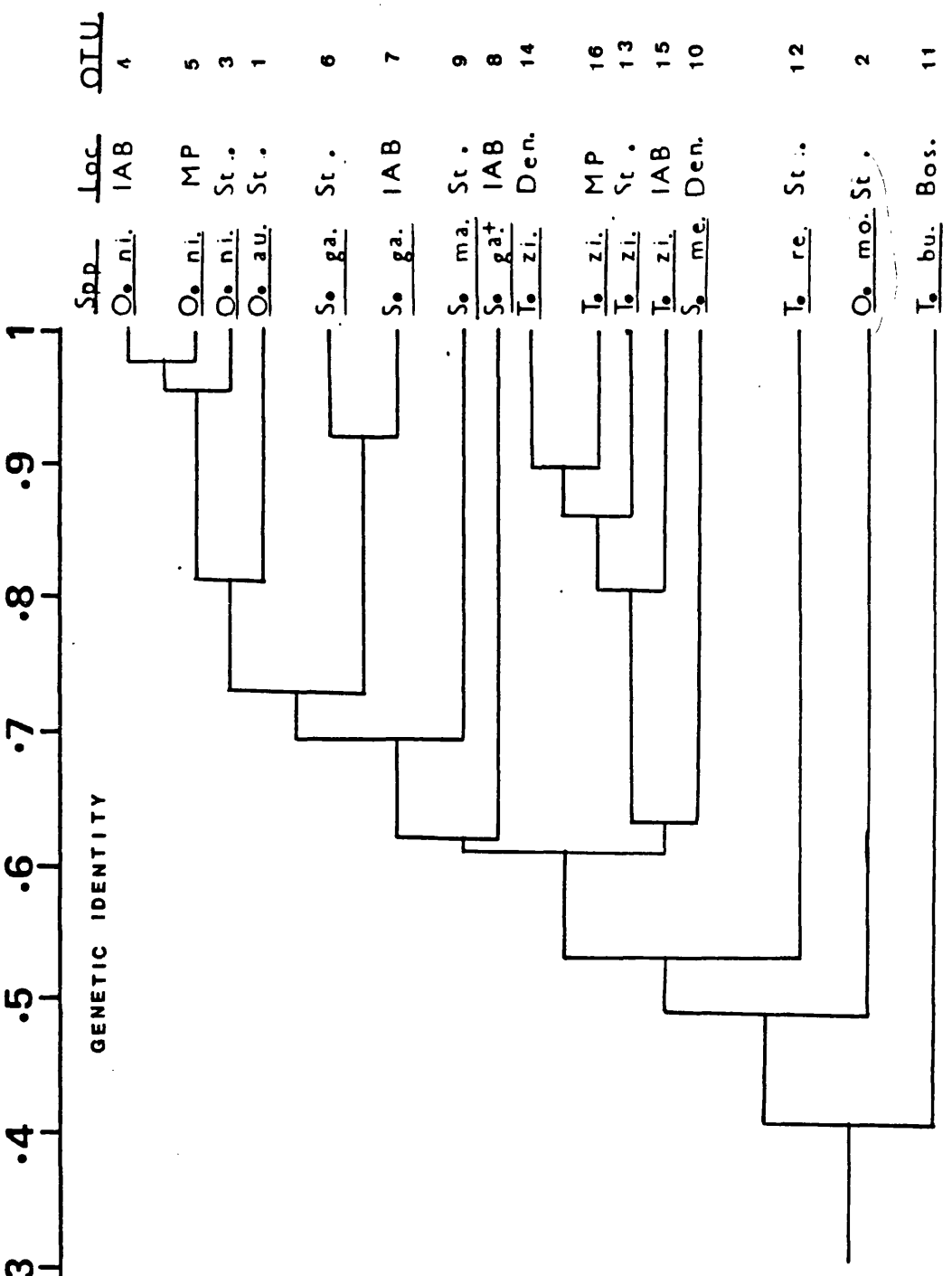


Figure 12a: Phenetic relationships amongst tilapias.

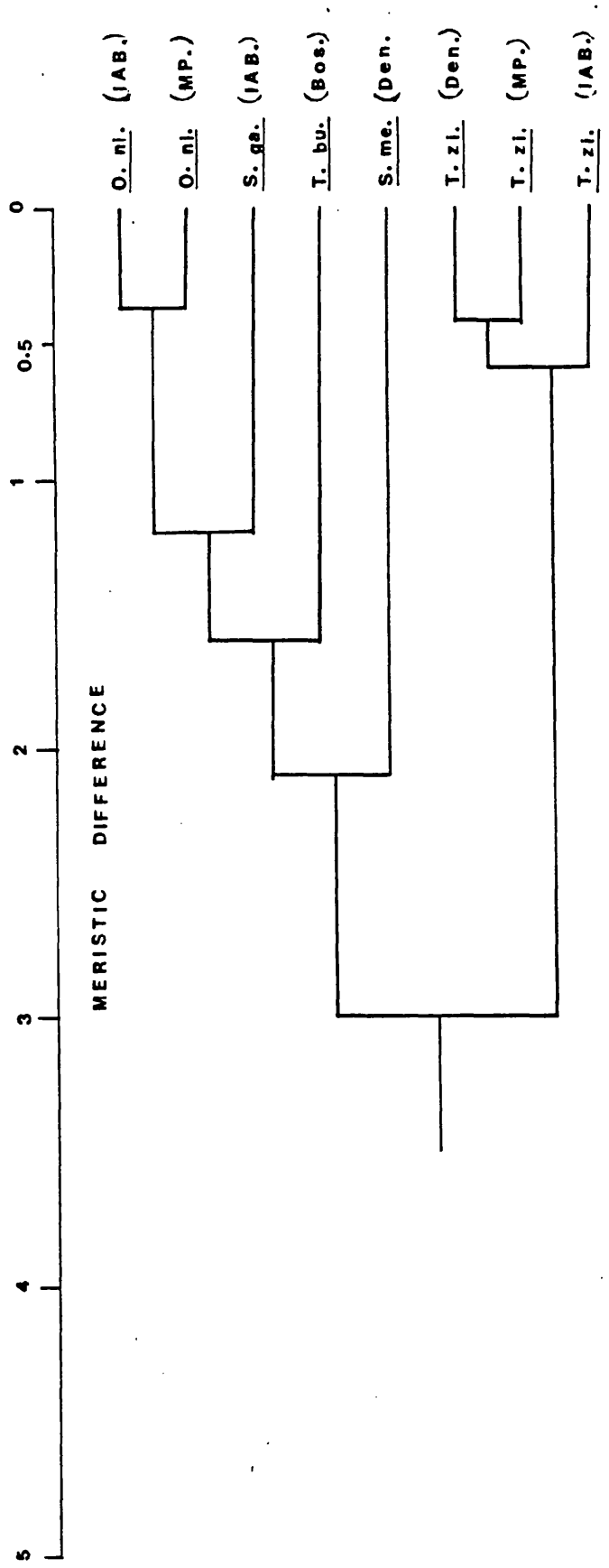


Figure 12b: Phenetic relationships amongst tilapias.

Figure 12c: Unrooted Phylogenetic tree of Tilapias.
(Generated by Computer Programme CONTMYL X)

Legend

Species:

O.au. = Oreochromis aureus.

O.ni. = Oreochromis niloticus.

O.mo. = Oreochromis mossambicus.

S.ga. = Sarotherodon galilaeus.

S.ma. = Sarotherodon macrochir.

S.me. = Sarotherodon melanotheron.

T.zi. = Tilapia zillii.

T.bu. = Tilapia busumana.

( ) = Source of fish

IAB = Institute of Aquatic Biology (Ghana)

Sti. = Stirling University (Scotland)

MP = Mixed Species Pond

L.Bos. = Lake Bosomtwi

R.Den. = River Densu.

* Number Taconomic units in tree were generated by the computer programme.

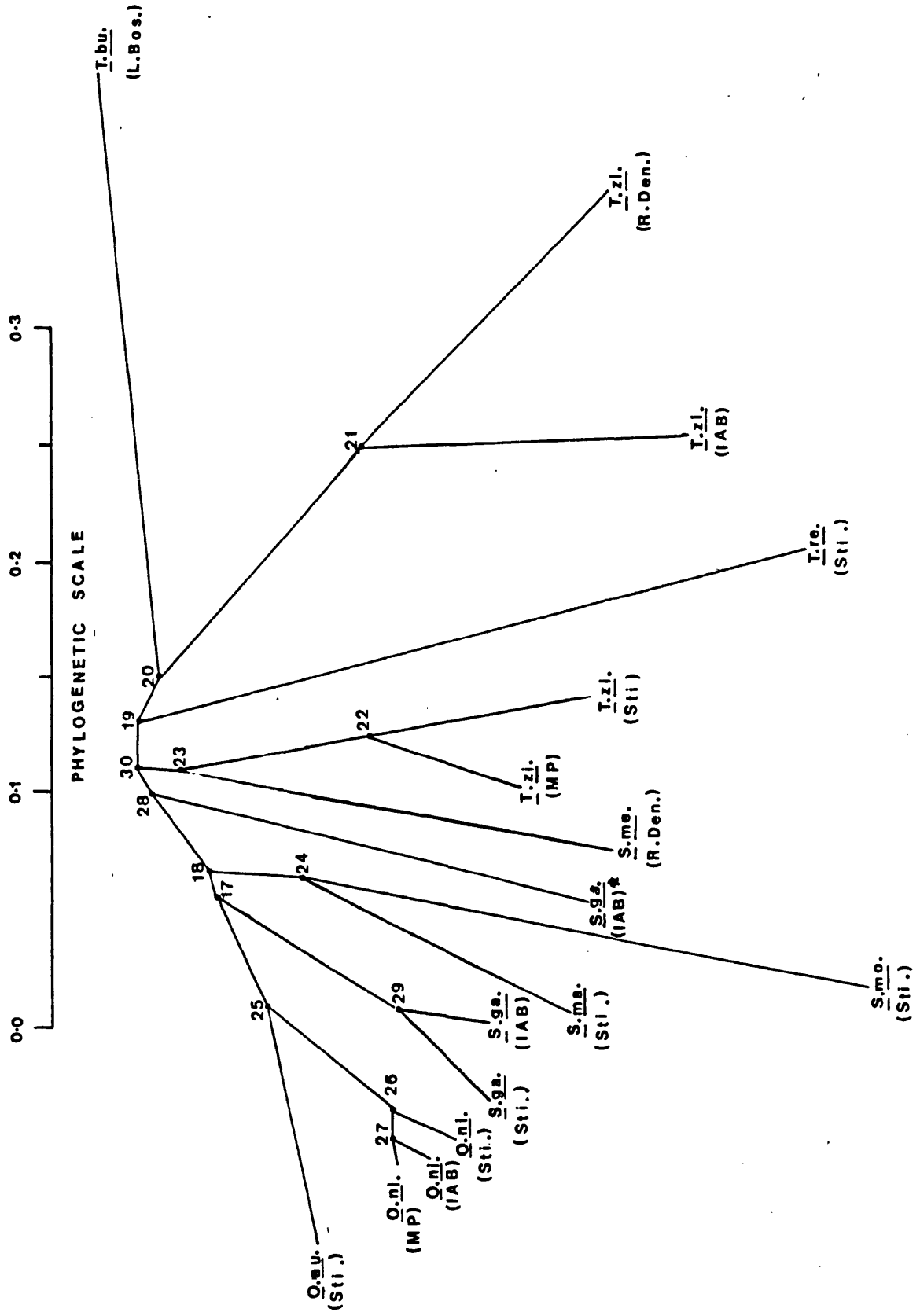


Figure 12c: Unrooted Phylogenetic tree of tilapias.

Estimated Lengths between Taxonomic Units

Including Hypothetical Units Generated by Programme

Between	And	Length
O.au.(Sti.)	20	0.10216
20	25	0.04359
25	17	0.00375
17	22	0.04408
22	24	0.01248
24	27	0.00730
27	S.me.(R.Den.)	0.18852
27	30	0.07574
30	T.zi.(MP)	0.07620
30	T.zi.(Sti.)	0.09368
24	T.bu.(L.Bos.)	0.27982
22	26	0.01041
26	28	0.02030
28	T.re. (Sti.)	0.27983
28	S.ga*.(IAB)	0.18171
26	29	0.16099
29	T.zi.(IAB)	0.14823
29	T.zi.(R.Den.)	0.15174
17	23	0.03902
23	O.mo.(Sti.)	0.25164
23	S.ma.(Sti.)	0.13298
25	21	0.09307
21	S.ga.(IAB)	0.04022
21	S.ga.(Sti.)	0.05598
20	18	0.06756
18	19	0.00721
19	O.ni.(MP)	0.00657
19	O.ni.(IAB)	0.01823
18	O.ni.(Sti.)	0.03406

Figures 13a and 13b: Phenetic relationships among Schilbeids.

13a: Relationships based on genetic identity.

13b. Relationships based on meristic differences.

Legend:

E.ni. = Eutropius niloticus.

E.ma. = E. mandibularis.

S.my. = Schilbe mystus.

S.au. = Siluranodon auritus

Figure 13a.

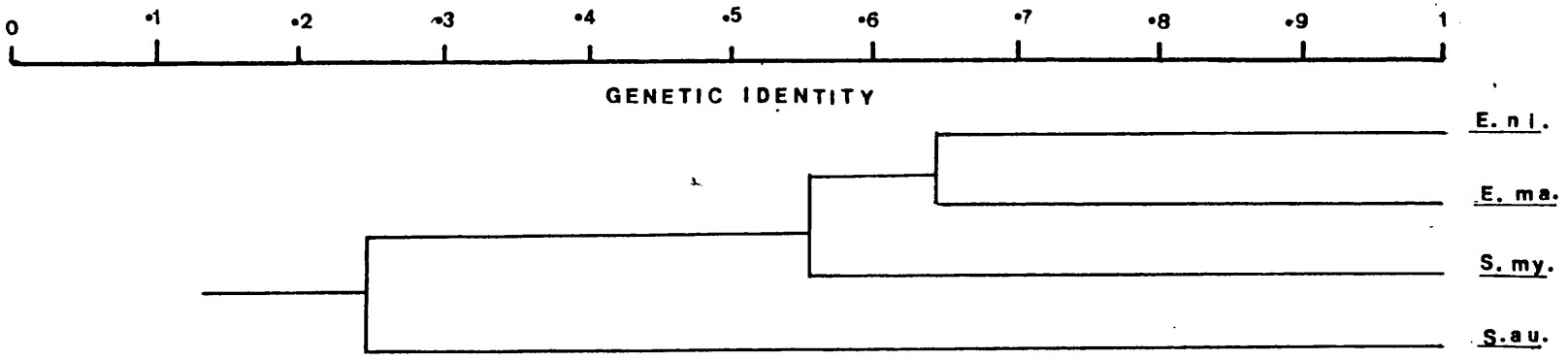
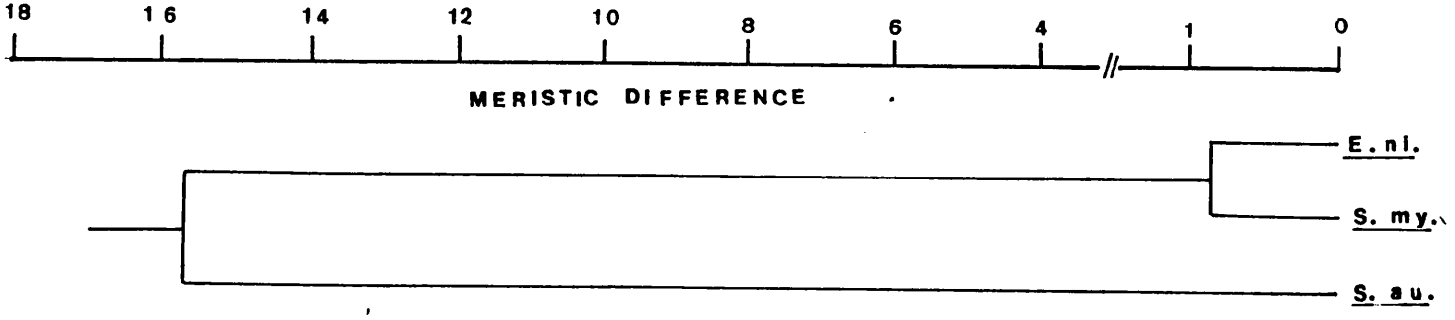


Figure 13b.



Phenetic Relationships among Schilbeids based on Meristic Differences.

Figure 13c: Unrooted Phylogenetic Tree of Schilbeids.

Legend:

S.au. = Siluranodon auritus

E.ma. = Eutropius mandibularis

E.ni. = Eutropius niloticus

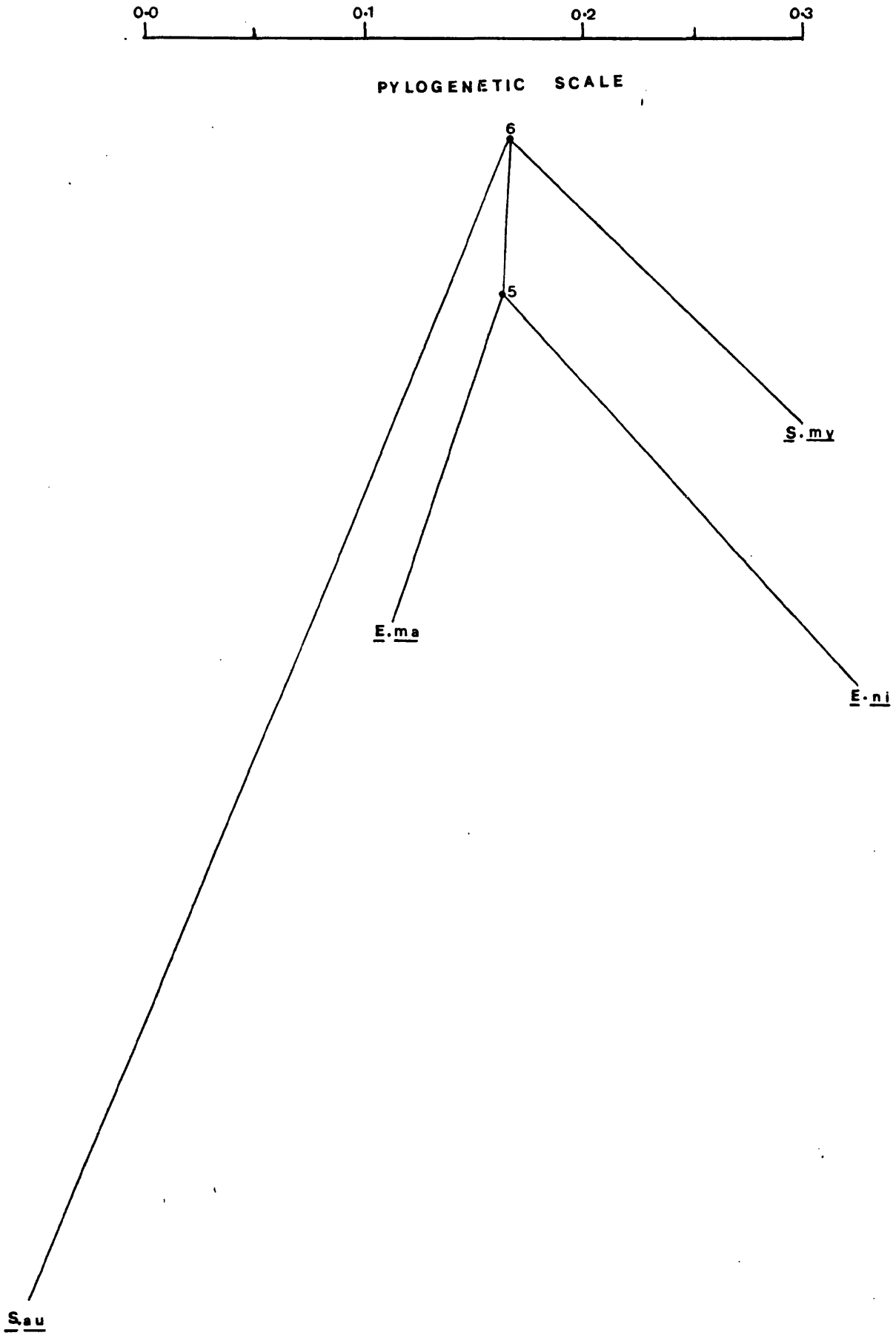
S.my. = Schilbe mystus

5 & 6 = Units generated by computer.

Estimated lengths between taxonomic units.

Between	And	Length
S. mystus	6	0.18295
6	S.auritus	0.57007
6	5	0.07198
5	E. niloticus	0.23827
5	E. mandibularis	0.15906

Figure 13c: Unrooted Phylogenetic Tree of Schilbeids.



4.4 Discussion:

Relationships within populations of Tilapias:

Estimates of relative degrees of genetic divergence between populations, species and genera obtained here were comparable, in relative terms, with previous expectations estimated for fish by Kirpichnikov (1981, pp. 237), and McAndrew and Majumdar (1984) for the Tilapias in particular. The results here therefore support the hypothesis of Trewavas (1980) that Oreochromis, Sarotherodons and Tilapia be recognised as substantive genera. Table 17 (Chapter 5) also shows that similarity between population and species decreases from within Oreochromis > within Sarotherodon > within Tilapia. This observation possibly indicates that the "branching off" of mouth brooders from the ancestral 'tilapia-like' substrate spawners had not occurred "from time to time" as suggested by Peters and Berns (1982). They therefore maintained that the Oreochromis, Sarotheron and Tilapia be retained under the single genus of Tilapia. On the taxonomic status of the fishes, Peters and Berns' consideration had been suggested by Thys (1968b).

The only recorded previous studies on the genetic relationships between tilapias involving some of the taxa investigated here were those of Kornfield et al., (1979) and McAndrew and Majumdar (1984). A direct comparison of the two previous and present study results (Table 12) obtained independently from analysis of geographically separated populations offers both encouragement and caution to all who seek systematic interpretation of electrophoretic

Table 12: Genetic identity values obtained between pairs of species of Tilapiine.

	Ref.	Kornfield et al. (1979)	McAndrew & Majumdar (1984)	Present Study
	Source of Fish	Sea of Galilee	Egypt	Ghana, W. Africa
	No. of loci.	15	25	27
O. aureus/S. galilaeus		0.92	0.738	0.731
T. zillii/S. galilaeus		0.48	0.52	0.646
T. zillii/O. aureus		0.49	0.58	0.663
O. nil/ A. galilaeus		-	0.806	0.733
O. niloticus/T. zillii		-	0.532	0.580

data. The three sets of data are quite comparable except that the I value between O. aureus and S. galilaeus obtained by Kornfield et al. (1979) would usually be considered as an identity value between congeneric species (Kirpichnikov, 1981) rather than confamilial genera. McAndrew and Majumdar (1984) discussed the possible influences of choice and number of loci used in such independent studies on the absolute values obtained. I am of the opinion that the choice of loci should make very little impact and differences in numbers of loci studies would only significantly influence values where the disparity is great. It is more likely that differences in habitat could influence the compatibility of such independent studies. The very high similarity values obtained between taxa in this study compared to previous studies could possibly be attributed to the fact that the environments from which fish were obtained in Ghana were broadly similar, being within a limited geographic area.

The genetic phenetic relationships among the tilapias shown in Figure 12a basically clusters species of Oreochromis, Sarotherodon and Tilapia together. The dendrogram then links the Oreochromis, considered more evolved of the mouth brooding tilapias to the substrate spawners (Tilapia) through the more primitive mouth brooders - Sarotherodon. The situation presented therefore suggests the existence of the three groups as separate evolving entities after their subsequent branching off from their common tilapia-like ancestor. Again estimates

of genetic divergence of species within the nominal genera obtained in this work (Table 17, Chapter 5), showed that divergence values decreased in the following order Tilapia > Sarotherodon > Oreochromis. This observation has also been considered here as an indication of the Tilapia being ancestral to the other genera. The analysis here therefore, generally supports the recognition of the Tilapia, Sarotherodon and Oreochromis as substantive genera as put forward by Trewavas (1980, 1983).

However, advocates of the present alternative hypothesis to that of Trewavas, which considers all the tilapia under one genus, would ask for an explanation for the relative positions of O. mossambicus and S. melanotheron in Figure 12a. The view expressed by exponents of the second hypothesis (e.g. Thys, 1968b; Peters and Berns, 1982), is that, species within the Oreochromis, Sarotherodon and Tilapia groups have branched from the common tilapia-like ancestor from time to time. Under this hypothesis the positions of O. mossambicus and S. melanotheron could easily be explained. The relative position of S. melanotheron, between two Tilapia species, in both phenetic representations of relationships (Figs. 12a and 12b) apparently lends support to Peters and Berns' (1982) considerations on the evolution of the tilapias. However, the phylogenetic relationships among the tilapias (Fig. 12c) shows that S. melanotheron has a "sister-species" relationships with only one of the Tilapia species, as defined by Ridley (1986, pp. 138-149). Considering that

the phenetic technique accepts the short-coming of assuming equal evolutionary rate at each branch, the position of a S. melanotheron as seen in the phenetic relationships may be attributed to the assumption of the technique.

It is interesting that the relative relationships resulting from the present analysis are similar to a study reported by McAndrew and Majumdar (1984). Briefly, their dendrogram involving nine species of the tilapia (from Egypt and East Africa) showed that although Oreochromis niloticus, O. aureus and O. sperulus were closely clustered, their link with O. jipe involved Tilapia zillii. Also O. mossambicus was linked to the O. niloticus group through Sarotherodon galilaeus. What these results probably suggest is that the recognition of Oreochromis, Sarotherodon and Tilapia as substantive genera should allow for the existence of intra-genus complexes of species. Trewavas (1983, Pers. comm. in McAndrew and Majumdar, 1984) indicated that based on anatomical and morphological analysis, O. mossambicus, O. jipe, O. girigan, O. hutei and O. pangani may be placed in a complex a little removed from the main Oreochromis as a genus.

The 'unexpected' relative positions of some species in a dendrogram such as Fig. 12a, or that of other studies, could perhaps be, in part, attributed to the non - inclusion of adequate representatives of the tilapias. With over

70 known species and acknowledged resemblances between genera of tilapias (Trewavas, 1983) the situation observed in Figs. 12a and 12b could be anticipated.

The relationships among the tilapias based on their overall meristic similarities (Fig. 12b) appear rather simpler than Fig. 12a. This was expected since fewer taxa were involved in Fig. 12b. However, the relationships shown present perhaps a more deep rooted problem than that observed in Fig. 12a. In Fig. 12a, T.busumana was at one end of the Tilapia group. Since it is generally considered that T. busumana is a very primitive Tilapia, its position in Fig. 12a presented no problem. In Fig. 12b. T. busumana apparently occupies an almost central position with a species of Sarotherodon immediately on either side. The picture presented by Fig. 12b, if it could be obtained with the inclusion of many more species, could give rise to a third hypothesis regarding the evolution of the tilapiine. What Fig. 12b presently suggests is that considering T. busumana to be very primitive, present day tilapiine could be said primarily to have radiated from their tilapia-like ancestor. This consideration could also have support from the presently known distribution of the tilapiine groups (Thys van den Audenaerde, 1988). A general observation of various presentations of tilapiine distribution in Africa indicates that most Oreochromis, Sarotherodon and Tilapia species have distributions apparently limited by geo-ecological barriers. Thus the evolution of the tilapiine by adaptive radiation could be argued if Fig. 12b involved enough representatives of the fishes.

The major features of the phylogenetic relationships among the tilapias, Fig. 12c, were comparable to the phenetic relationships (Fig. 12a). Oreochromis aureus and stocks of O. niloticus are clustered as species and stocks of Sarotherodon and Tilapia. However, in Fig. 12c, O. mossambicus is more within the 'natural group' of Oreochromis compared with its phenetic position in Figures 12a and 12b. Its position in relation to S.galilaeus and S. macrochir (all culture and experimental stocks) could be attributed to two possible factors. First, Trewavas (1983, pers. comm. in McAndrew and Majumdar, 1984) indicated that O. mossambicus belongs to a complex (including O. jipe, O. girigan, O. huteri and O. pangani) which is a little apart from other Oreochromis. Although Trewavas' observation may be based on non-genetic characteristics, the evolution of the species could have been influenced by ecological factors. Secondly, the Sarotherodon stocks separating O. mossambicus from other Oreochromis in Fig. 12c, are not natural population samples and consisted of very small samples. Thus possible contamination of them cannot be completely ruled out.

The total picture presented by Fig. 12c is perhaps what would be expected by the hypothesis, that mouth-brooders evolved from substrate-spawning ancestors (Peters and Berns, 1978, 1982; Trewavas, 1980; McAndrew and Majumdar, 1984). The results here indicate that T. busumana, the most primitive Tilapia, is phylogenetically further removed from the rest of the fishes involved than any

other species. Thus its primitive link with the ancestral-like tilapia is confirmed.

Relative positions of T. zillii stocks:

Four Tilapia zillii stocks are involved in Fig. 12c. Of these T. zillii (Den) was a sample of a wild population from which T. zillii (IAB) and T. zillii (MP) were derived. Tilapia zillii (Sti) originated from Egypt. However, Figure 12c shows that the 'mixed pond' stock and Stirling stock were genetically more similar than T. zillii (MP) was to its parent wild stock, and the IAB stock. The likely introgression of the T. zillii (MP) stock by other species in the mixed-species pond, earlier discussed, suggests that the T. zillii (Sti) is possibly contaminated. This observation serves as a caution to future attempts to studies which might seek to contribute to tilapia systematics based on cultured and experimental stocks.

Relationships among the Schilbeids:

The phenetic (allozyme and meristic based) and phylogenetic relationships among the schilbeids were comparable (Figs. 13a, 13b and 13c). In the absence of a similar previous study of the family except that implied in their theoretical classification, the uniformity of the relationships shown in the figures by three different approaches strongly suggests the validity of their morphological classification. The results also suggest that the extreme morphological similarities between Schilbe mystus and some

species of Eutropius could only be superficial - possible influences of different ecological conditions.

* * * * * * * * *

CHAPTER 5

GENETIC DIFFERENTIATION AND VARIABILITY IN FISH POPULATIONS

5.1 Introduction:- Variability Parameters:

Five parameters were considered in estimating genetic variability patterns and levels of population variability within and between species studied. These were:

a) Number of loci coding for enzymes in family groups of fish.
b) Distribution of polymorphic enzymes among species and families of fish.
c) Multilocus enzymes and locus variability.
d) Genetic identity and divergence between corresponding ranks of different fish groups.
e) Population variability (heterozygosity).

5.1.1 Results: Number of Loci per Enzyme (Lo):

Seventeen enzymes investigated in thirteen species belonging to eight genera and four families are listed in Table 2 (Chapter 2). Abbreviations of enzymes used in the text and other tables of this work refer to the products of the enzymes at individual gene loci. Table 13 summarises major variability aspects of the enzymes in the fishes. For each enzyme, the table indicates the number of loci coding for it, the maximum number of

Table 13: Summary Notes on Proteins (Enzymes) Investigated.

Legend:

No. of Loci	=	Number of Loci coding for enzyme.
Max. alleles/Locus	=	Maximum number of alleles at any locus.
Het; Ab/Pr.	=	Heterozygotes, Absence or Presence.
Structure, Mon; Dim; Tet.	=	Subunit structure of enzyme. Mon = Monomeric; Dim = Dimeric; Tet = Tetrameric

Table 13: Summary Notes on Proteins (Enzymes) Investigated.

FISH FAMILY:		MORMYRIDAE	SCHILBEIDAE			BAGRIDAE			CICHLIDAE				
SPECIES:		M.se.	S.my.	E.ni.	E.ma.	S.au.	C.ni.	C.au.	O.au.	O.ni.	S.ga.	S.me.	T.bu.	T.zi.
PROTEIN														
ADA	No. of Loci	2	-	-	-	-	1	1	1	1	1	1	1	1
	Max. alleles/Locus	1	-	-	-	-	1	1	1	1	2	1	1	3
	Het. Ab/Pr.	Ab.	-	-	-	-	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.
	Obs. Structure	-	-	-	-	-	-	-	-	-	-	-	-	Mon.
ADH	No. of Loci	2	2	2	2	2	3	3	2	2	2	2	2	2
	Max. alleles/Locus	1	1	1	1	1	1	1	1	1	1	1	1	1
	Het. Ab/Pr.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.
	Structure	-	-	-	-	-	-	-	-	-	-	-	-	-
AP	No. of Loci	2	2	2	2	2	2	2	1	1	1	1	1	1
	Max. alleles/Locus	1	1	1	1	1	1	1	1	4	1	1	1	2
	Het. Ab/Pr	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Pr.	Ab.	Ab.	Ab.	Ab.
	Structure	-	-	-	-	-	-	-	-	Mon.	-	-	-	Mon.
EST	No. of Loci	-	3	3	3	3	4	4	4	4	4	4	3	4
	Max. alleles/Locus	-	3	3	1	1	2	2	1	3	2	1	-	3
	Het. Ab/Pr.	-	Pr.	Pr.	Ab.	Ab.	Pr.	Pr.	Ab.	Pr.	Pr.	Ab.	Ab.	Ab.
	Structure	-	Mon.	Mon.	-	-	Mon.	Mon.	-	Mon.	Mon.	-	-	Mon.
αGPDH	No. of Loci.	1	2	2	2	2	1	1	1	1	1	1	1	1
	Max. alleles/Locus	1	1	1	1	1	1	1	1	2	1	2	1	3
	Het. Ab./Pr.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.
	Structure	-	-	-	-	-	-	-	-	-	-	-	-	-
G6PDH	No. of Loci	1	1	1	1	1	1	1	1	1	1	1	1	1
	Max. alleles/Locus	1	2	1	1	1	1	1	1	1	2	1	1	2
	Het. Ab/Pr.	Ab.	Pr.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Pr.	Ab.	Ab.	Pr.
	Structure	-	Dim.	-	-	-	-	-	-	-	Dim.	-	-	Dim.
IDH	No. of Loci	-	1	1	1	1	1	1	1	1	1	1	1	1
	Max. alleles/Locus	-	1	1	1	2	1	1	1	1	1	1	1	1
	Het. Ab/Pr.	-	Ab.	Ab.	Ab.	Pr.	Ab.	Ab.		Ab.	Ab.	Ab.	Ab.	Ab.
	Structure	-	-	-	-	Mon.	-	-		-	-	-	-	-

Table 13 Contd.

FISH FAMILY		MORMYRIDAE	SCHILBEIDAE			BAGRIDAE		CICHLIDAE					
SPECIES		M.se.	S.my.	E.ni.	E.ma.	S.au.	C.ni.	C.au.	O.au.	O.ni.	S.ga.	S.me.	T.bu.	T.zi.
PROTEIN														
LAP	No. of Loci	-	-	-	-	-	1	1	2	2	2	2	2	2
	Max. alleles/Locus	-	-	-	-	-	1	1	1	2	2	1	1	1
	Het. Ab/Pr.	-	-	-	-	-	Ab.	Ab.	Ab.	Ab.	Pr.	Ab.	Ab.	Ab.
	Structure	-	-	-	-	-	-	-	-	-	Mon.	-	-	-
LDH	No. of Loci	3	2	2	2	2	2	4	2	2	2	3	2	2
	Max. alleles/Locus	1	3	1	1	1	1	2	1	2	1	1	1	2
	Het. Ab/Pr.	Ab.	Pr.	Ab.	Ab.	Ab.	Ab.	Pr.	Ab.	Pr.	Ab.	1	1	Pr.
	Structure	-	Tet.	-	-	-	-	Tet.	-	Tet.	-	-	-	Tet.
MDH	No. of Loci	2	2	2	2	2	3	3	3	3	3	3	3	3
	Max. Alleles/Locus	1	3	1	1	1	2	1	1	1	1	1	2	2
	Het. Ab/Pr.	Ab.	Pr.	Ab.	Ab.	Ab.	Pre.	Ab.	Ab.	Ab.	Ab.	Ab.	Pr.	Pr.
	Structure	-	Dim.	-	-	-	Dim.	-	-	-	-	-	Dim.	Dim.
ME	No. of Loci	2	2	2	2	2	2	2	2	2	2	2	2	2
	Max. alleles/Locus	1	4	3	1	1	2	3	1	3	2	2	2	3
	Het. Ab./Pr.	Ab.	Pr.	Pr.	Ab.	Ab.	Pre.	Pre.	Ab.	Pre.	Ab.	Pre.	Ab.	Pre.
	Structure	-	Tet.	Tet.	-	-	Tet.	Tet.	-	Tet.	-	Tet.	-	Tet.
PEP	No. of Loci	-	2	2	2	2	-	-	2	2	2	2	2	2
	Max. Alleles/Locus	-	1	2	1	1	-	-	1	2	2	1	1	2
	Het. Ab/Pr.	-	Ab.	Ab.	Ab.	Ab.	-	-	Ab.	Pre.	Pre.	Ab.	Ab.	Pre.
	Structure	-	-	-	-	-	-	-	-	Mon.	Mon.	-	-	Mon.
PGI	No. of Loci	2	2	2	2	2	2	2	2	2	2	2	2	2
	Max. Alleles/Locus	2	1	2	1	1	3	1	1	1	2	2	1	3
	Het. Ab/Pr.	Pre.	Ab.	Pre.	Ab.	Ab.	Pre.	Ab.	Ab.	Ab.	Pre.	Ab.	Ab.	Pre.
	Structure	Dim.	-	Dim.	-	-	Dim.	-	-	-	Dim.	-	-	Dim
6PGDH	No. of Loci	1	1	1	1	1	1	1	2	2	2	2	2	2
	Max. Alleles/Locus	1	1	2	1	1	1	1	1	1	2	1	1	2
	Het. Ab/Pr.	Ab.	Ab.	Pre.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Pre.
	Structure	-	-	Dim.	-	-	-	-	-	-	-	-	-	Dim.

Table 13 Contd.

FISH FAMILY:		MORMYRIDAE	SCHILBEIDAE				BAGRIDAE		CICHLIDAE					
SPECIES:		M.se.	S.my.	E.ni.	E.ma.	S.au.	C.ni.	C.au.	O.au.	O.ni.	S.ga.	S.me	T.bu.	T.zi.
PROTEIN														
PGM	No. of Loci	1	1	1	1	1	1	1	1	1	1	1	1	1
	Max. Alleles/Locus	1	3	2	1	2	2	3	1	1	1	2	3	1
	Het. Ab/Pr.	Ab.	Pr.	Pr.	Ab.	Pre.	Pre.	Pre.	Ab.	Ab.	Ab.	Pre.	Pre.	Ab.
	Structure	-	Mon.	Mon.	-	Mon.	Mon.	Mon.	-	-	-	Mon.	Mon.	-
SDH	No. of Loci	1	3	3	3	3	1	1	2	2	2	2	2	2
	Max. Alleles/Locus	1	2	2	2	2	2	1	1	1	2	1	1	2
	Het. Ab/Pr.	Ab.	Pre.	Pre.	Pre.	Pre.	Pre.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Pre.
	Structure	-	Tet.	Tet.	Tet.	Tet.	Tet.	-	-	-	-	-	-	Tet.
SOD	No. of Loci	1	1	1	1	1	1	1	1	1			1	
	Max. Alleles/Locus	1	5	1	1	1	1	1	1	2			1	
	Het. Ab/Pr.	Ab.	Pre.	Ab.	Ab.	Ab.	Ab.	Ab.	Ab.	Pre.			Ab.	
	Structure	-	Mon.	-	-	-	-	-	-	Mon.			-	

MEANS

1) No. of Loci per Enzyme

L

A

alleles per locus, the presence or absence of heterozygotes and observed subunit structure of the enzyme, where heterozygotes had been observed.

The number of loci coding for seven of the 17 enzymes were the same in all fish species and families. For the remaining ten enzymes Lo (number of loci coding for an enzyme), was only uniform within families. Distinct examples of such apparently 'family-associated' Lo were observed for the following enzymes :- AP, ADH, EST, LAP, MDH, and SDH (see Table 13). For example the cichlids (6 species) showed a single 'active' locus, per species for AP. All other family members were active at two loci. For EST, the mormyrid, Marcusenius senegalensis showed no activity (i.e. Lo = 0). For the same enzyme, the schilbeidae (4 species) Lo = 3; in the bagridae and cichlidae (2 and 6 spp. respectively) Lo = 4.

5.1.2 Distribution of Polymorphic Enzymes:

The polymorphic state of an enzyme locus has traditionally been assessed on the basis of the frequency (f) of the most common allele. Thus, as usually stated, it is the polymorphism at a locus that is discussed although very often polymorphism of the enzyme has been implied.

Two common criteria used to describe the polymorphic state of a protein locus (P) have been where f is <0.95

and <0.99 to indicate P at 5% and 1% levels respectively.

Table 14.a shows the distribution of polymorphism for enzymes investigated in all the fish species. The table briefly shows that:

a) The mormyrid was monomorphic for 14 of the 15 enzymes investigated (involving 28 loci).

b) Some enzymes, e.g. EST and ME were widely polymorphic in all fish groups.

c) Some enzymes were distinctly polymorphic or more so in identifiable family-groups of fish. For example, Table 14(a) shows that AP and αGPDH were polymorphic (P .5%) among the cichlids only. On the other hand, SDH was distinctly monomorphic within the cichlidae but highly polymorphic in the schilbeidae. However, PGI, the only enzyme with a polymorphic locus among 15 enzymes investigated in the mormyrid, was monomorphic in all schilbeids. The polymorphism of 6PGDH and LDH were also differentially distributed as can be seen in the table.

d) Tentatively, the table also suggests that, for some enzymes (e.g. MDH) genera within a family may have distinct levels of variability.

Table 14a: Distribution of Polymorphic (P) Enzymes in Fish.

Fm.	MORMYRIDAE	SCHILBEIDAE				BAGRIDAE		CICHLIDAE								
Spp. (Origin)	M.Se.	S.my.	E.ni.	E.ma.	S.au.	C.ni.	C.au.	O.au.	O.ni. (Sti.)	O.nil. (IAB)	O.nil. (MP)	S.ga. (IAB)	S.mel. (Densu)	T.bu. (L.Bo)	T.zi. (Den)	T.zi. IAB	T.zi (MP)
Enzyme																	
AP									++	++	++			++	++		++
EST		+	+			++	++		+	++	++	+			++		+
G6PDH												+			++		
αGPDH									+				++		++	++	++
LDH		++					+			++	++				+		++
ME		++	++			++	++		++	++	++		++	+	++	++	++
MDH		+				++								+			++
PEP			++						++	++		++				++	++
PGI	+					++						++	++		++	++	++
PGM		+	+	++		++	++							+			++
6PGDH			+									++			++	++	++
SDH		++	++	++	++	++											++
SOD		++															
IDH				+													
LAP												+					+

Criteria for Polymorphism (P) P = 0.01 - +; P = 0.05 - ++

5.1.3 Multilocus Enzymes and Locus Variability (P):

Among the seventeen enzymes screened in all species, varying proportions were multi-locus coded in different groups of fishes. Among the multilocus enzymes, 50 to 67% in different fish groups showed polymorphism at a single locus per enzyme. Table 14b shows the multi-locus enzymes in fish families. It also indicates the number of loci coding for an enzyme, the maximum number of loci polymorphic, and the criterion for polymorphism in each species.

A general appraisal of Table 14b revealed that in approximately 70% of situations, not all loci involved in coding for an enzyme were variable. The table also suggests that it is only in "di-locus" enzymes that all loci are possibly variable. It was estimated that in the di-locus enzymes where both loci were polymorphic, 33% of the cases had both loci polymorphic at the 5% level. A further 33% had one of the two variable loci polymorphic only at the 1% level, and the remaining 33% cases had both loci polymorphic at the 1% level.

5.1.4 Subunit Structure of Enzymes:

Genotype frequencies for all species at loci studied are provided in Appendix 3. Table 13 indicates the presence or absence of heterozygous individuals in all populations with respect to the enzymes studied. This information was included in the Table to

Table 14b: Multilocus Enzymes and Locus Variability.

Legend:

No. of loci	=	Number of loci coding for enzyme.
Max. Poly./Spp.	=	Maximum number of loci polymorphic for enzyme in a species belonging to family.
Criterion for P (%)	=	Criterion for polymorphism at locus (in percentage).

Table 14b: Multilocus Enzymes and Locus Variability (P)

Enzyme	Family	Mormyridae	Schilbeidae	Bagridae	Cichlidae
Est.	No. of Loci	-	3	4	4
	Max. Poly./Spp.	-	1	2	2
	Criterion for P.(%)	-	1	5	5
LDH.	No. of Loci	3	2	2	2
	Max. Poly./Spp.	0	2	1	1
	Criterion for P.(%)	-	5	1	1
MDH	No. of Loci	2	2	3	3
	Max. Poly./Spp.	0	2	0	2
	Criterion for P.(%)	-	1	0	1
ME	No. of Loci.	2	2	1	2
	Max. Poly./Spp.	0	2	1	2
	Criterion for P.(%)	-	5∝1*	5	5∝1*[1]
PGI	No. of Loci	2	2	2	2
	Max. Poly./Spp.	1	2	1	2
	Criterion for P.(%)	1	1	5	5∝1*

* 5∝1 = 2 polymorphic loci in a species with one polymorphic at 5% and the other consistently at 1% level.

*[1] = Where if 2 loci are polymorphic both are at 1% but in other species of family with only a locus is polymorphic, the level is 5%

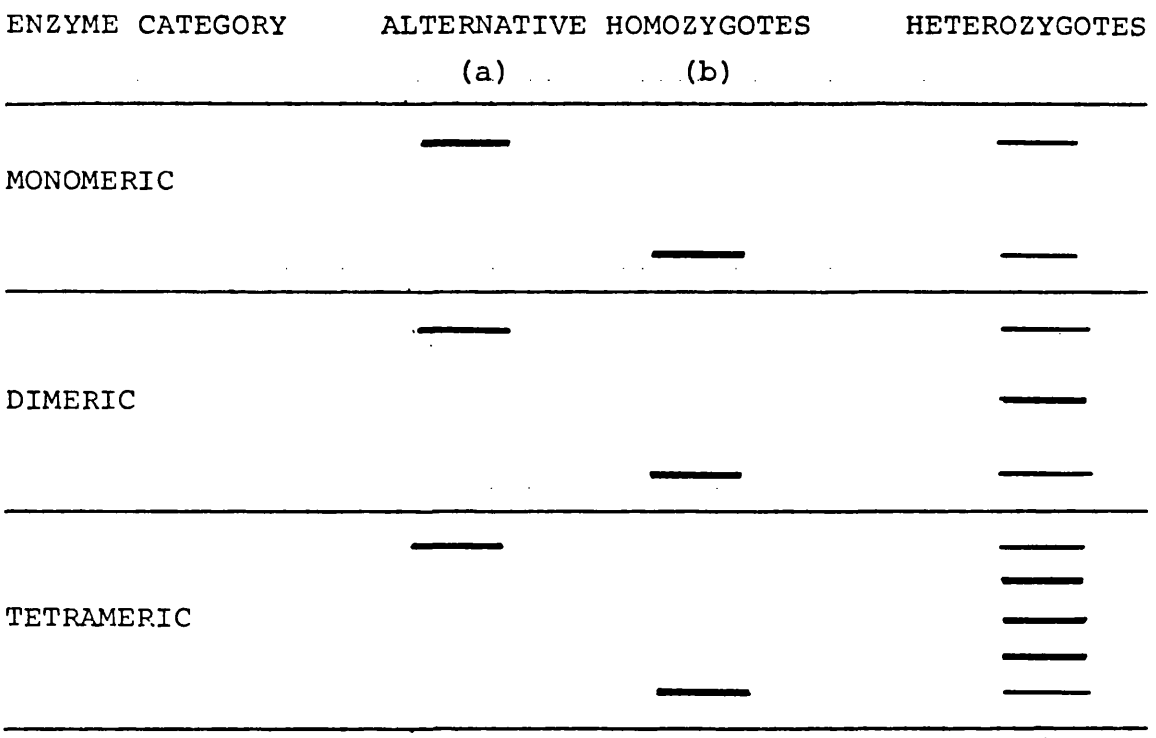
provide a basis for discussion on the subunit structure of the enzymes. Many enzymes are made up of two or more polypeptide chains or subunits (i.e. they are multimeric). After electrophoresis, the pattern of primary isozymes observed, always in heterozygous individuals, depends in general on the number of subunits in the isozyme molecules and hence the number of structurally different polypeptide chains being synthesised.

At any of the loci investigated, two or more alleles could occur (see allele frequency tables). Each allele codes for a distinct version of the particular polypeptide chain. Heterozygous individuals have two alleles, and thus form two structurally different polypeptide chains (see Fig. 15). In the case of monomeric enzymes (e.g. as observed here for ADA, AP, EST, PGM and IDH), the pattern observed in a heterozygote represents a simple mixture of the two possible homozygote patterns (Fig. 15). It must be noted however, that AP, EST and IDH have sometimes been observed showing the structure of dimeric enzymes.

In the case of multimeric enzymes (dimeric or tetrameric here) hybrid heteromeric isozymes are formed in heterozygous individuals with the homomeric isozymes seen separately in corresponding homozygotes. In dimeric enzymes (e.g. G6PDH, MDH, PGI and 6PGDH) heterozygous individuals displayed two homomeric and one heteromeric

form. In tetrameric enzymes (e.g. ME, LDH and SDH) there were two homeric and three heteromeric (hybrid) forms - producing typically, a 5-banded pattern on the gel. The observed patterns which determined the classification of enzymes during this study, are presented in Figure 15 below.

Figure 15: Diagramatic Representation of Subunit Structure Patterns of Enzymes on Gel.



Note: In this representation all enzymes have been allotted two alleles at a locus. There could of course, be only one (in which case no heterozygotes would be observed) but there may be three or more alleles at a locus. However, the number of alleles per locus does not influence the banding pattern of any category of enzyme in heterozygous state.

5.2 Genetic Differentiation Within Fish Families:

5.2.1 Introduction:

Classical Mendelian genetics ascertains the presence of allelic forms of a gene by a study of progenies of parents that phenotypically express varying forms of a gene. The approach could thus not be used to observe their allelic forms in different populations. Protein electrophoresis, among other molecular techniques, provides for the quantitative observation of both similarities and differences in the genetic material of different populations.

Results of electrophoresis of specific enzymes or other proteins expressed in allelic frequencies provides an efficient data for the estimation of genetic differences between populations (see reviews by Ferguson, 1980; Thorpe, 1982). A good aspect of this approach is that the sample of the genome used is selected without prior knowledge of whether the genes sampled are more or less variable than the rest of the genome of populations under investigation. However, electrophoresis as a tool for measuring differences between genomes has its limitations. Perhaps the most critical of them being that the technique resolves only products of structural genes, but none of the regulatory component of the genome.

5.2.2 Results.

Electrophoretic results of specific enzymes, in the four families of fish studied, expressed in allelic frequencies are presented in Tables 15a, 15b, 15c and 15d. With the exception of the mormyridae, more than a population, species or genera were involved in each of the other three families. Thus an estimation of genetic differentiation or divergence between populations, species and genera were possible. The summary of differentiations have been expressed in Nei's (1972) index of genetic Identity (I - see Chapter 2 for definition).

The summaries of identities between taxa of a family are presented diagramatically in Figs. 16a, 16b, 16c, 16d and 16e. Each histogram represents comparison of the two taxa specified. To generate a histogram, allelic frequencies at homologous loci of the two fishes were scored to estimate their genetic identity. Individual locus identities were then classified into 22 classes at 0.05 intervals. The first class (extreme left of histogram) represents the frequency of loci at which the two fishes had an identity of zero; next to it, is the frequency of loci having identity between 0.01 to less than 0.05 (0.01 - <0.05). Thus the last two classes (to the extreme right of the histogram) represented frequencies of loci with 0.95 to less than 1.0 and 1.0 identities respectively. In any pair of species compared, the $I = 0$ and $I = 1$ classes (at extreme left and right respectively) classes indicated the percentage of loci diagnostic and identical respectively.

Table 15a: Allele Frequency and Heterozygosity (Observed - Ho; Expected - He) Values in Marcusenius senegalensis (Mormyridae) at 22 loci.

Locus	Allele	Frequency	Locus	Allele	Frequency	Locus	Allele	Frequency
Ada-1	M	1.000	Ldh-1	M	1.000	Pgi-1	M	1.000
Ho		0	Ho		0	Ho		0
He		0	He		0	He		0
Ada-2	M	1.000	Ldh-2	M	1.000	Pgi-2	F	0.039
Ho		0	Ho		0		S	0.962
He		0	He		0	Ho		0.077
						He		0.073
Adh	M	1.000	Ldh-3	M	1.000			
Ho		0	Ho		0	Pgm	M	1.000
He		0	He		0	Ho		0
						He		0
Ap	M	1.000	Mdh-1	M	1.000			
Ho		0	Ho		0	Sdh	M	1.000
He		0	He		0	Ho		0
						He		0
Fum	M	1.000	Mdh-2	M	1.000			
Ho		0	Ho		0	Sod	M	1.000
He		0	He		0	Ho		0
						He		0
aGpdh	M	1.000	Me-1	M	1.000			
Ho		0	Ho		0			
He		0	He		0	Xdh	M	1.000
G6pdh	M	1.000	Me-2	M	1.000			
Ho		0	Ho		0			
He		0	He		0			
Hk	M	1.000	6Pgdh	M	1.000			
Ho		0	Ho		0			
He		0	He		0			

No. of Individuals screened at all loci = 26.
Proportion of loci Polymorphic (P, where freq. of common < 0.99) = 0.046
Range of: Ho = 0.0 to 0.077; mean 0.004±0.003
He = 0.0 to 0.073; mean 0.003±0.003

Table 15b: Allele Frequency and Heterozygosity (Observed - Ho; Expected - He) Values in Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus (fm. Schilbeidae) at 28 Enzyme loci.

Species		Schilbe mystus	E. niloticus	E. mandibularis	Siluranodon auritus
Source Code		1-01;3-01;4-01, 5-01	1-01,2-01 3-01	10-01	1-01,2-02
Locus					
	F	-	-	-	1.000
	S	1.000	1.000	1.000	-
Adh-1					
	Ho	0	0	0	0
	He	0	0	0	0
		(6)	(6)	(5)	(6)
	F	1.000	-	-	1.000
	M	-	1.000	-	-
	S	-	-	1.000	-
Adh-2					
	Ho	0	0	0	0
	He	0	0	0	0
		(6)	(6)	(5)	(6)
	M	1.000	1.000	1.000	1.000
Ap-1					
	Ho	0	0	0	0
	He	0	0	0	0
		(30)	(25)	(5)	(11)
	M	1.000	1.000	1.000	1.000
AP-2					
	Ho	0	0	0	0
	He	0	0	0	0
		(30)	(25)	(5)	(11)
	F	1.000	1.000	1.000	-
	S	-	-	-	1.000
Ck					
	Ho	0	0	0	0
	He	0	0	0	0
		(30)	(23)	(5)	(25)
	M	1.000	1.000	1.000	1.000
Est-1					
	Ho	0	0	0	0
	He	0	0	0	0
		(101)	(54)	(5)	(62)
	F	0.951	1.000	1.000	-
	S	0.049	-	-	1.000
Est-2					
	Ho	0	0	0	0
	He	0.093	0	0	0
		(101)	(54)	(5)	(62)
	F	0.011	0.952	1.000	-
	M	0.979	0.032	-	1.000
	S	0.011	0.016	-	-
Est-3					
	Ho	0.043	0.095	0	0
	He	0.041	0.092	0	0
		(93)	(63)	(5)	(62)

Table 15b (Contd.)

Species		Schilbe mystus	E. niloticus	A. mandibularis	Siluranodon auritus
Source Code		1-01;3-01;4-01 5-01	1-01,2-01 3-01	10-01	1-01,2-02
	M	1.000	1.000	1.000	1.000
aGpdh-1					
	Ho	0	0	0	0
	He	0	0	0	0
		12	(6)	(5)	(6)
	F	1.000	-	1.000	-
	M	-	1.000	-	-
	S	-	-	-	1.000
aGpdh-2					
	Ho	0	0	0	0
	He	0	0	0	0
		(12)	(6)	(5)	(6)
	F	0.993	-	1.000	-
	M	0.007	1.000	-	-
	S	-	-	-	1.000
G6pdh					
	Ho	0.014	0	0	0
	He	0.014	0	0	0
		(74)	(44)	(5)	(38)
	F	1.000	1.000	1.000	0.017
	S	-	-	-	0.983
Idh					
	Ho	0	0	0	0.035
	He	0	0	0	0.033
		(72)	(25)	(5)	(29)
	F	0.424	-	-	1.000
	S	0.576	1.000	1.000	-
Ldh-1					
	Ho	0.063	0	0	0
	He	0.488	0	0	0
		(79)	(70)	(5)	(81)
	F	0.006	-	-	-
	M	0.943	-	-	-
	S	0.051	1.000	1.000	-
	VS	-	-	-	1.000
Ldh-2					
	Ho	0.013	0	0	0
	He	0.108	0	0	0
		(79)	(71)	(5)	(81)
	VF	0.005	-	-	-
	F	0.005	0.826	1.000	-
	M	0.105	-	-	-
	S	-	0.160	-	-
	vs	0.884	0.014	-	1.000
Me-1					
	Ho	0.137	0.208	0	0
	He	0.208	0.292	0	0
		(95)	(72)	(5)	(72)

Table 15b Contd.

Species		Schilbe mystus	E. niloticus	A. mandibularis	Siluranodon auritus
Source Code		1-01;3-01;4-01 5-01	1-01,2-01 3-01	10-01	1-01,2-02
	F	0.005	0.014	1.000	-
	M	0.984	0.951	-	-
	S	0.011	0.035	-	-
	VS	-	-	-	1.000
Me-2					
	Ho	0.021	0.014	0	0
	He	0.032	0.094	0	0
		(95)	(72)	(5)	(72)
	F	0.006	1.000	-	-
	M	0.994	-	-	-
	S	-	-	1.000	1.000
Mdh-1					
	Ho	0.012	0	0	0
	He	0.012	0	0	0
		(81)	(59)	(5)	(52)
	F	-	1.000	-	-
	M	0.963	-	1.000	-
	S	0.037	-	-	-
	VS	-	-	-	1.000
Mdh-2					
	Ho	0.074	0	0	0
	He	0.071	0	0	0
		(81)	(59)	(5)	(52)
	F	-	1.000	-	1.000
	S	1.000	-	1.000	-
Mpi					
	Ho	0	0	0	0
	He	0	0	0	0
		(24)	(23)	(5)	(20)
	F	1.000	0.833	1.000	-
	S	-	0.167	-	1.000
Pep-1					
	Ho	0	0	0	0
	He	0	0.278	0	0
		(6)	(6)	(5)	(6)
	F	1.000	0.667	1.000	-
	S	-	0.333	-	1.000
Pep-2					
	Ho	0	0	0	0
	He	0	0.444	0	0
		(6)	(6)	(5)	(6)
	F	1.000	-	1.000	-
	M	-	-	-	1.000
	S	-	0.006	-	-
	VS	-	0.994	-	-
Pgi-1					
	Ho	0	0.015	0	0
	He	0	0.012	0	0
		(90)	(69)	(5)	(61)

Table 15b Contd.

Species		Schilbe mystus	F. niloticus	A. mandibularis	Siluranodon auritus
Source Code		1-01;3-01;4-01 5-01	1-01,2-01 3-01	10-01	1-01,2-02
	F	-	0.007	-	1.000
	M	-	0.993	1.000	-
	S	1.000	-	-	-
Pgi-2					
	Ho	0	0	0	0
	He	0	0.014	0	0
		(90)	(69)	(5)	(72)
	VF	0.006	-	-	-
	F	0.989	-	-	-
	M	0.006	-	0.9	-
	S	-	0.008	-	-
	VS	-	0.985	0.1	0.992
	VVS	-	0.008	-	0.008
Pgm					
	Ho	0.023	0.031	0.2	0.016
	He	0.022	0.030	0.18	0.016
		(87)	(65)	(5)	(64)
	F	-	0.011	-	-
	M	-	0.989	1.000	-
	S	-	-	-	1.000
	VS	1.000	-	-	-
6Pgdh					
	Ho	0	0.023	0	0
	He	0	0.022	0	0
		(59)	(44)	(5)	(54)
	F	-	-	-	1.000
	S	1.000	1.000	1.000	-
Sdh-1					
	Ho	0	0	0	0
	He	0	0	0	0
		(6)	(6)	(5)	(6)
	F	-	-	0.2	-
	M	0.167	0.5	0.8	-
Sdh-2					
	Ho	0.333	1.0	0.4	0.167
	He	0.278	0.5	0.32	0.152
		(6)	(6)	(5)	(6)
	VF	0.028	-	1.000	-
	F	0.051	-	-	1.000
	M	0.905	-	-	-
	S	0.006	-	-	-
	VS	0.011	1.000	-	-
Sod					
	Ho	0.023	0	0	0
	He	0.177	0	0	0
		(89)	(74)	(5)	(67)
Ho Range		0.0 to 0.033	0.0 to 0.208	0.0 to 0.4	0.0 to 0.167
mean ± SE		0.028±0.013	0.050±0.019	0.022±0.016	0.008±0.006
He Range		0.0 to 0.488	0.0 to 0.5	0.0 to 0.32	0.0 to 0.152
mean ± SE		0.057±0.020	0.066±0.025	0.019±0.013	0.007±0.006
P		0.179	0.143	0.071	0.036

Table 15c: Allele Frequency, Heterozygosity (Observed, Ho and Expected, He) in Chrysichthys nigrodigitatus and C. auratus at 28 Enzyme loci.

Allele		Frequency	
Species		C. nigro (1)	C. auratus (2)
Locus			
Adh-1	F	1.000	-
	S	-	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Adh-2	F	1.000	-
	S	-	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Adh-3	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Ap-1	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Ap-2	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Apk	M	1.000	1.000
	Ho	0	0
	He	0	0
		(15)	(14)
CK	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(31)

Table 15c Contd.

Allele Frequency			
Species:-		C. nigro	C. auratus
Locus			
Est-1	F	1.000	-
	S	-	1.000
	Ho	0	0
	He	0	0
		(22)	(22)
Est-2	F	-	0.750
	M	-	0.250
	S	1.000	-
	Ho	0	0.227
	He	0	0.375
		(22)	(22)
Est-3	F	0.452	0.568
	S	0.548	0.432
	Ho	0.333	0.046
	He	0.495	0.491
Est-4	M	1.000	1.000
	Ho	0	0
	He	0	0
αGpdh	M	1.000	1.000
	Ho	0	0
	He	0	0
		(15)	(14)
G6pdh	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(31)
Idh	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(31)
Lap	M	1.000	1.000
	Ho	0	0
	He	0	0
		(16)	(19)

Table 15c Contd.

Allele		Frequency	
Species:-		C. nigro	C. auratus
Locus			
Ldh-1	F	1.000	-
	M	-	0.984
	S	-	0.016
	Ho	0	0.032
	He	0	0.032
		(29)	(31)
Ldh-2	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(30)
Mdh-1	F	0.017	-
	S	0.983	1.000
	Ho	0.035	0
	He	0.033	0
		(29)	(30)
Mdh-2	F	0.813	-
	M	0.186	-
	S	-	1.000
	Ho	0.375	1.000
	He	0.304	0.000
		(16)	(16)
Mdh-3	M	1.000	1.000
	Ho	0	0
	He	0	0
		(28)	(31)
Me-1	F	-	0.121
	M	0.069	0.345
	S	0.931	0.535
	Ho	0.138	0.276
	He	0.129	0.580
		(29)	
Odh	F	1.000	-
	S	-	1.000
	Ho	0	0
	He	0	0
		(15)	(14)

Table 15c Contd.

Allele		Frequency	
Species:-		C. nigro	C. auratus
Locus			
6Pgdh	M	1.000	1.000
	Ho	0	0
	He	0	0
		(16)	(16)
Pgi-1	M	1.000	1.000
	Ho	0	0
	He	0	0
Pgi-2	F	0.569	-
	M	0.414	1.000
	S	0.017	-
	Ho	0.414	0
	He	0.515	0
		(29)	(31)
Pgm	VF	-	0.828
	F	-	0.104
	M	0.948	0.069
	S	0.052	-
	Ho	0.104	0.276
	He	0.099	0.299
		(29)	(31)
Sdh	F	0.938	-
	M	0.063	-
	S	-	1.000
	Ho	0.125	0
	He	0.116	0
		(16)	(19)
Sod	M	1.000	1.000
	Ho	0	0
	He	0	0
		(29)	(31)
Range Ho		0.000-0.414	0.0-0.276
Mean Ho		0.054	0.031
1 S.E.		0.042	0.026
Range He		0.0-0.505	0.0-0.580
2 S.E.		0.046	0.044

Table 15d: Allele Frequencies and Heterozygocities (Observed - H_o and Expected - H_e) at 27 Enzyme Loci in *Oreochromis*, *Sarotherodon* and *Tilapia* species and stocks.

GENUS		OREOCHROMIS					SAROTHERODON					TILAPIA					
SPECIES		O.aur.	O.moss	O.nil.	O.nil	O.nil	S.gal.	S.gal.	S.gal.	S.macro.	S.mel.	T.bus.	T.ren.	T.zil.	T.zil.	T.zil.	T.zil
Source/loc.		(Ster.)	(Ster.)	(Ster.)	(IAB)	(MP)	(Ster.)	(IAB)	(IAB)	(Ster.)	(R.Den)	(L.Bos.)	(Ster.)	(Ster.)	(R.Den)	(IAB)	(MP)
OTU		1	2	3	4	5	6	7	8*	9	10	11	12	13	14	15	16
Adh-1	F	1.000	-	-	-	-	-	-	-	-	-	1.000	-	1.000	1.000	1.000	1.000
	S	-	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	-	-	-	-
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(4)	(2)	(6)	(6)	(6)	(3)	(4)	(1)	(1)	(19)	(10)	(2)	(3)	(3)	(3)	(3)
Adh-2	F	1.000	-	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	S	-	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(10)	(2)	(6)	(6)	(6)	(3)	(4)	(1)	(1)	(19)	(16)	(1)	(3)	(9)	(5)	(12)
Ap	VF	-	-	-	0.167	-	-	-	-	-	-	-	-	-	-	-	0.053
	F	1.000	1.000	0.389	0.708	0.300	-	-	-	-	-	0.091	-	-	-	-	0.153
	M			0.222	0.083	0.700	1.000	1.000	1.000	1.000	1.000	0.909	1.000	1.000	0.972	-	0.737
	S			0.333	0.042		-	-	-	-	-	-	-	-	0.028	1.000	0.053
	VS			0.056	-	-	-	-	-	-	-	-	-	-	-	-	-
	H_o	0	0	0.667	0.083	0.0	0	0	0	0	0	0.0	0	0	0.0	0	0
	H_e	0	0	0.685	0.462	0.42	0	0	0	0	0	0.165	0	0	0.054	0	0.428
		(21)	(2)	(9)	(12)	(10)	(3)	(16)	(1)	(1)	(19)	(11)	(1)	(2)	(36)	(3)	(19)
Est-1	M	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(12)	(2)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(31)	(3)	(3)	(31)	(5)	(19)

Table 15d Contd.

Est-2	F	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	-	1.000	1.000	1.000	1.000	1.000
	S	-	-	-	-	-	-	-	-	-	1.000	1.000	-	-	-	-	-
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(12)	(2)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(31)	(3)	(3)	(31)	(5)	(19)
Est-3	F	-	-	-	-		-	-	-	-	-	1.000		-	-	-	-
	M	1.000	-	1.000	1.000	0.938	1.000	1.000	1.000	1.000	-			-	-	-	0.026
	S	-	1.000	-	-	0.063	-	-	-	-	1.000			1.000	0.919	1.000	0.974
	VS	-	-	-	-		-	-	-	-	-		1.000	-	0.081	-	-
	H_o	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0.097	0	0.053
	H_e	0	0	0	0	0.116	0	0	0	0	0	0	0	0	0.149	0	0.051
		(12)	(2)	(7)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(31)	(1)	(3)	(31)	(5)	(19)
Est-4	F	-	-	-	0.042	-	-	-	-	-	-	-	-	-	0.028	-	-
	M	-	-	0.958	0.085	0.083	-	0.05	1.000	-	1.000	-	1.000	1.000	0.875	1.000	1.000
	S	1.000	1.000	0.042	0.875	0.917	1.000	0.95	-	1.000	-	1.000	-	-	0.042	-	-
	VS	-	-	-	-	-	-	-	-	-	-	-	-	-	0.056	-	-
	H_o	0	0	0.083	0.250	0.167	0	0.1	0	0	0	0	0	0	0.139	0	0
	H_e	0	0	0.081	0.225	0.152	0	0.095	0	0	0	0	0	0	0.232	0	0
		(12)	(2)	(12)	(12)	(6)	(2)	(30)	(1)	(1)	(19)	(31)	(2)	(3)	(36)	(5)	(19)
aGpdh	F	-	-	-	-	-	-	-	1.000	-	-	-	1.000	1.000	0.912	0.8	0.118
	M	-	-	-	-	-	-	-	-	-	0.833	1.000	-	-	0.088	-	0.294
	S	1.000	1.000	0.833	1.000	1.000	1.000	1.000	-	1.000	0.167	-	-	-	-	0.2	0.418
	VS	-	-	0.167	-	-	-	-	-	-	-	-	-	-	-	-	0.177
	H_o	0	0	0.167	0	0	0	0	0	0	0	0	0	0	0	0	0.177
	H_e	0	0	0.278	0	0	0	0	0	0	0.278	0	0	0	0.161	0.32	0.694
		(12)	(1)	(12)	(11)	(5)	(3)	(30)	(1)	(1)	(18)	(25)	(2)	(2)	(34)	(5)	(17)
G6pdh	F	-	-				-	-	1.000	-	-	-	-	-	0.030	-	-
	M	1.000	-	1.000	1.000	1.000	-	0.960	-	-	1.000	1.000	-	1.000	0.939	1.000	1.000
	S	-	1.000				-	0.040	-	1.000	-	-	1.000		0.030	-	-
	VS	-	-				1.000	-	-	-	-	-	-		-	-	-
	H_o	0	0	0	0	0	0	0.08	0	0	0	0	0	0	0.061	0	0
	H_e	0	0	0	0	0	0	0.077	0	0	0	0	0	0	0.0117	0	0
		(12)	(2)	(12)	(12)	(6)	(1)	(25)	(1)	(1)	(19)	(20)	(2)	(2)	(33)	(5)	(17)

Table 15d Contd.

Idh	M	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(12)	(2)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(18)	(30)	(2)	(3)	(27)	(5)	(19)
Lap-1	F	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	-	1.000	1.000	1.000	1.000
	M	-	-	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-	-
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(6)	(2)	(6)	(6)	(8)	(2)	(30)	(1)	(1)	(19)	(30)	(2)	(3)	(12)	(5)	(19)
Lap-2	F	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-	-	-
	M	1.00	-	1.000	1.000	1.000	1.000	0.980	1.000	-	-	-	-	1.000	1.000	1.000	0.974
	S	-	1.000	-	-	-	-	0.020	-	1.000	-	-	1.000	-	-	-	0.026
	VS	-	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-	-
	H_o	0	0	0	0	0	0	0.033	0	0	0	0	0	0	0	0	0.053
	H_e	0	0	0	0	0	0	0.039	0	0	0	0	0	0	0	0	0.051
		(6)	(2)	(6)	(6)	(8)	(2)	(30)	(1)	(1)	(19)	(30)	(2)	(3)	(12)	(5)	(19)
Ldh-1	F	-	-	-	-	-	-	-	1.000	-	-	-	-	1.000	-	-	0.790
	S	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	-	1.000	1.000	0.211
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.316
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.331
		(6)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(38)	(3)	(3)	(36)	(5)	(19)
Ldh-2	F	1.000	1.000	1.000	0.917	0.875	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	0.957	1.000	1.000
	S	-	-	-	0.083	0.125	-	-	-	-	1.000	-	-	-	0.043	-	-
	H_o	0	0	0	0.167	0.25	0	0	0	0	0	0	0	0	0.029	0	0
	H_e	0	0	0	0.152	0.219	0	0	0	0	0	0	0	0	0.082	0	0
		(6)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(38)	(3)	(3)	(35)	(5)	(19)
Mdh-1	F	-	-	-	-	-	1.000	1.000	-	-	-	-	-	-	-	0.1	-
	S	1.000	1.000	1.000	1.000	1.000	-	-	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.9	1.000
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.20	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.18	0
		(6)	(1)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(19)	(34)	(3)	(3)	(32)	(5)	(19)

Table 15d Contd.

	F	-	-	-	-	-	1.000	1.000	-	1.000	-	0.030	-	1.000	1.000	1.000	1.000
	M	1.000	1.000	1.000	1.000	1.000	-	-	-	-	-	0.970	-	-	-	-	-
	S	-	-	-	-	-	-	-	1.000	-	1.000	-	1.000	-	-	-	-
Mdh-2	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0.058	0	0	0	0	0
		(6)	(1)	(12)	(12)	(19)	(3)	(30)	(1)	(1)	(19)	(33)	(3)	(3)	(32)	(5)	(19)
	F	-	1.000	-	-	-	-	-	-	1.000	1.000	-	1.000	1.000	1.000	1.000	0.943
	M	-	-	-	-	-	1.000	1.000	1.000	-	-	0.985	-	-	-	-	0.053
	S	1.000	-	1.000	1.000	1.000	-	-	-	-	-	0.015	-	-	-	-	-
Mdh-3	H_o	0	0	0	0	0	0	0	0	0	0	0.029	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0.030	0	0	0	0	0.100
		(6)	(1)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(19)	(34)	(3)	(3)	(31)	(5)	(19)
	F			1.000	1.000	1.000	-	-	-	1.000	-	0.971	-	1.000	-	0.167	-
	M	1.000		-	-	-	1.000	1.000	1.000	-	1.000	0.029	1.000	-	0.973	0.833	1.000
	S		1.000	-	-	-	-	-	-	-	-	-	-	-	0.027	-	-
Me-1	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.333	0
	H_e	0	0	0	0	0	0	0	0	0	0	0.056	0	0	0.533	0.278	0
		(12)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(34)	(2)	(1)	(37)	(3)	(5)
	F	-	-	0.125	0.05	-	-	-			0.079	0.970	1.000	-	-	-	-
	M	1.000	1.000	0.875	0.10	0.714	1.000	1.000			0.921	0.030	-	1.000	0.881	-	0.921
	S	-	-	-	0.85	0.286	-	-	1.000		-	-	-	-	0.119	1.000	0.079
	VS	-	-	-	-	-	-	-		1.000	-	-	-	-	-	-	-
Me-2	H_o	0	0	0.083	0.200	0.0	0	0	0	0	0.158	0	0	0	0.048	0.0	0.158
	H_e	0	0	0.219	0.265	0.408	0	0	0	0	0.146	0.058	0	0	0.210	0	0.146
		(12)	(2)	(12)	(10)	(7)	(2)	(30)	(1)	(1)	(19)	(33)	(2)	(3)	(42)	(3)	(19)
Pep-1	M	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(12)	(2)	(12)	(12)	(6)	(3)	(21)	(1)	(1)	(19)	(14)	(2)	(3)	(27)	(3)	(12)
	F	-	-	-	-	-	-	-	-	-	-	1.000	-	-	-	0.167	0.25
	M	1.000	1.000	0.846	0.9	1.000	0.667	0.929	1.000	1.000	1.000	-	-	1.000	1.000	0.833	0.75
Pep-2	S	-	-	0.154	0.1	-	0.333	0.071	-	-	-	-	-	-	-	-	-
	VS	-	-	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-
	H_o	0	0	0.154	0.0	0	0	0.143	0	0	0	0	0	0	0	0.333	0
	H_e	0	0	0.261	0.18	0	0.444	0.132	0	0	0	0	0	0	0	0.278	0.375
		(12)	(2)	(13)	(10)	(6)	(3)	(21)	(1)	(1)	(19)	(12)	(2)	(3)	(27)	(3)	(12)

Figure 15d cont'd.

	F												1.000				0.167
	M							0.1				1.000		1.000	0.833	0.8	0.833
	S	1.000	1.000	1.000	1.000	1.000	1.000	0.9	1.000	1.000	1.000				0.167	0.2	
6Pgdh-2	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0.067	0	0
	H_e	0	0	0	0	0	0	0.18	0	0	0	0	0	0	0.278	0.32	0.278
		(12)	(2)	(6)	(6)	(6)	(3)	(30)	(1)	(1)	(19)	(8)	(2)	(3)	(15)	(5)	(18)
	F		1.000													1.000	
	M			1.000	1.000	1.000	1.000	1.000		0.5		1.000	1.000	1.000	0.014		
	S	1.000							1.000	0.5	1.000				0.986		1.000
Pgi-1	H_o	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0.028	0	0
	H_e	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0.028	0	0
		(12)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(18)	(25)	(3)	(3)	(36)	(5)	(19)
	VF	-	1.000	-	-	-	0.5	-	-	-	-	-	-	-	0.026	-	-
	F	-	-	-	-	-	-	0.155	0.5	1.000	0.211	-	-	-	-	0.4	0.368
	M	1.000	-	1.000	1.000	1.000	0.5	0.885	0.5	-	0.790	1.000	-	1.000	0.053	0.6	0.421
	S	-	-	-	-	-	-	-	-	-	-	-	1.000	-	0.921	-	0.211
Pgi-2	H_o	0	0	0	0	0	1.0	0.0	1.0	0	0	0	0	0	0.053	0.40	0.421
	H_e	0	0	0	0	0	0.5	0.204	0.5	0	0.331	0	0	0	0.148	0.48	0.643
		(12)	(1)	(12)	(12)	(8)	(2)	(26)	(1)	(1)	(19)	(25)	(2)	(3)	(38)	(5)	(19)
	F		1.000	1.000	1.000	1.000											
	M	1.000					1.000	1.000	1.000	1.000	1.000	0.956	1.000	1.000	1.000	1.000	0.737
	S											0.015					0.158
Pgm	VS											0.029					0.105
	H_o	0	0	0	0	0	0	0	0	0	0	0.059	0	0	0	0	0.158
	H_e	0	0	0	0	0	0	0	0	0	0	0.085	0	0	0	0	0.421
		(12)	(1)	(12)	(12)	(8)	(3)	(25)	(1)	(1)	(19)	(34)	(2)	(3)	(36)	(5)	(19)
	F															1.000	0.909
	S	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.091
Sdh-1	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.182
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.165
		(12)	(2)	(12)	(11)	(6)	(3)	(27)	(1)	(1)	(19)	(19)	(2)	(2)	(27)	(5)	(11)
	F	-	-	-	-	-	-	1.000	1.000	-	-	-	-	-	-	-	-
	M	1.000	-	1.000	1.000	1.000	1.000	-	-	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000
	S	-	1.000	-	-	-	-	-	-	-	-	-	1.000	-	-	-	-
Sdh-2	H_o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H_e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		(12)	(2)	(12)	(11)	(6)	(3)	(27)	(1)	(1)	(19)	(19)	(2)	(2)	(27)	(5)	(19)

5.2.3 Among Schilbeidae:

Results obtained here (Figs. 16a, 16b, 16c, 16d and 16e) show that among the Schilbeidae, Eutropius mandibularis and Siluranodon auritus (Fig. 16a.vi) constituted the most diverged pair of species of the family. This is indicated by the pair having the highest frequency of loci in the zero identity class and also the lowest frequency of loci with complete identity (i.e. $\underline{I} = 1$). Thus the mean genetic identity between the pair, $\underline{I} = 0.183$, was the lowest among pairs of Schilbeidae studied.

On the other hand, Eutropius niloticus and E. mandibularis (Fig. 16a.iv) show that they were genetically the closest related pair among the Schilbeids. Although having about the same frequency of loci with identity '1' as the Schilbe mystus/ E. mandibularis pair, the Eutropius species pair had the lowest frequency of the $\underline{I} = 0$ or nearly zero identity classes. The 0.642 mean identity between the two species of Eutropius, being the only congeneric species among the Schilbeids studied, could have been expected to be the highest within the family.

5.2.4 Among Tilapias:

Genetic differentiation within three genera of the family Cichlidae are presented in Fig. 16b, 16c and 16d for Oreochromis, Sarotherodon and Tilapia respectively Within Oreochromis, three species, O. aureus, O. mossambicus and O. niloticus, the latter consisting of three populations, were investigated. The comparative distribution of identities between pairs of Oreochromis (Fig. 16b) shows that where two species are compared, the distribution of loci shows a 'U' shape or nearly so. However, the distribution of populations shows a characteristic 'L' shape (e.g. Fig. 16b (ix) and (x)).

The 'U' shape indicates that at any given locus in most cases, the two species, under consideration, are either identical or distinctly different in allelic composition. The 'L' shape suggests that at many loci, the two populations involved are identical at most loci and very few loci of the populations have an identity of zero or nearly so; thus the relatively higher values of I between populations compared to I's between species. These general patterns are repeatedly observed in comparisons of distributions of loci with respect to genetic identity within the genera Sarotherodon and Tilapia (Figs. 16c and 16d).

5.2.5 Between two Chrysichthys:

The identity distribution of loci between the two species of Chrysichthys (fm. Bagridae) also showed the characteristic 'U' shape observed between species of other genera (Fig. 16e).

Variations in the 'arm' length of the 'U's and 'J's in similar ranks of different fish families in Figure 16 show differences in the extent of genetic divergence in the various groups that are consistent with their classification to the same rank by conventional systematics.

Figure 16a-e: Distribution of Loci with respect to genetic identity in pairs of species of fish groups.

Legend with respect to abbreviations of fish names used in Figures.

Fig. 16a: Family Schilbeidae

S.my. = Schilbe mystus

E.ni. = Eutropius niloticus

E.ma. = Eutropius mandibularis

S.au. = Siluranodon auritus

Fig. 16b: Family Cichlidae, genus Oreochromis

O.au = Oreochromis aureus

O.mo. = Oreochromis mossambicus

O.ni.(ste) = Oreochromis niloticus (Stirling)

O.ni.(IAB) = O. niloticus (Institute of Aquatic Biology, Ghana).

O.ni. (MP) = O. niloticus (Mixed species Pond).

Fig. 16c: Family Cichlidae, genus Sarotherodon

S.ga. = Sarotherodon galilaeus

S.ma. = S. macrochir

S.me. = S. melanotheron

Fig. 16d: Family Cichlidae, genus Tilapia

T.bu. = Tilapia busumana

T.re. = T. rendeli

T.zi. = T. zillii

General

Abbreviations for sources of species in brackets against species names.

Den = Densu river, Ghana.

IAB = Institute of Aquatic Biology, Ghana.

M.P. = Mixed species pond (a private farm, in Ghana).

Ste. = Stirling University, Scotland.

Figure 16a:
Frequency Distribution of Loci with Pespect to Genetic Identity (I) in Pairs of Species in Family Schilbeidae based on 28 Loci.

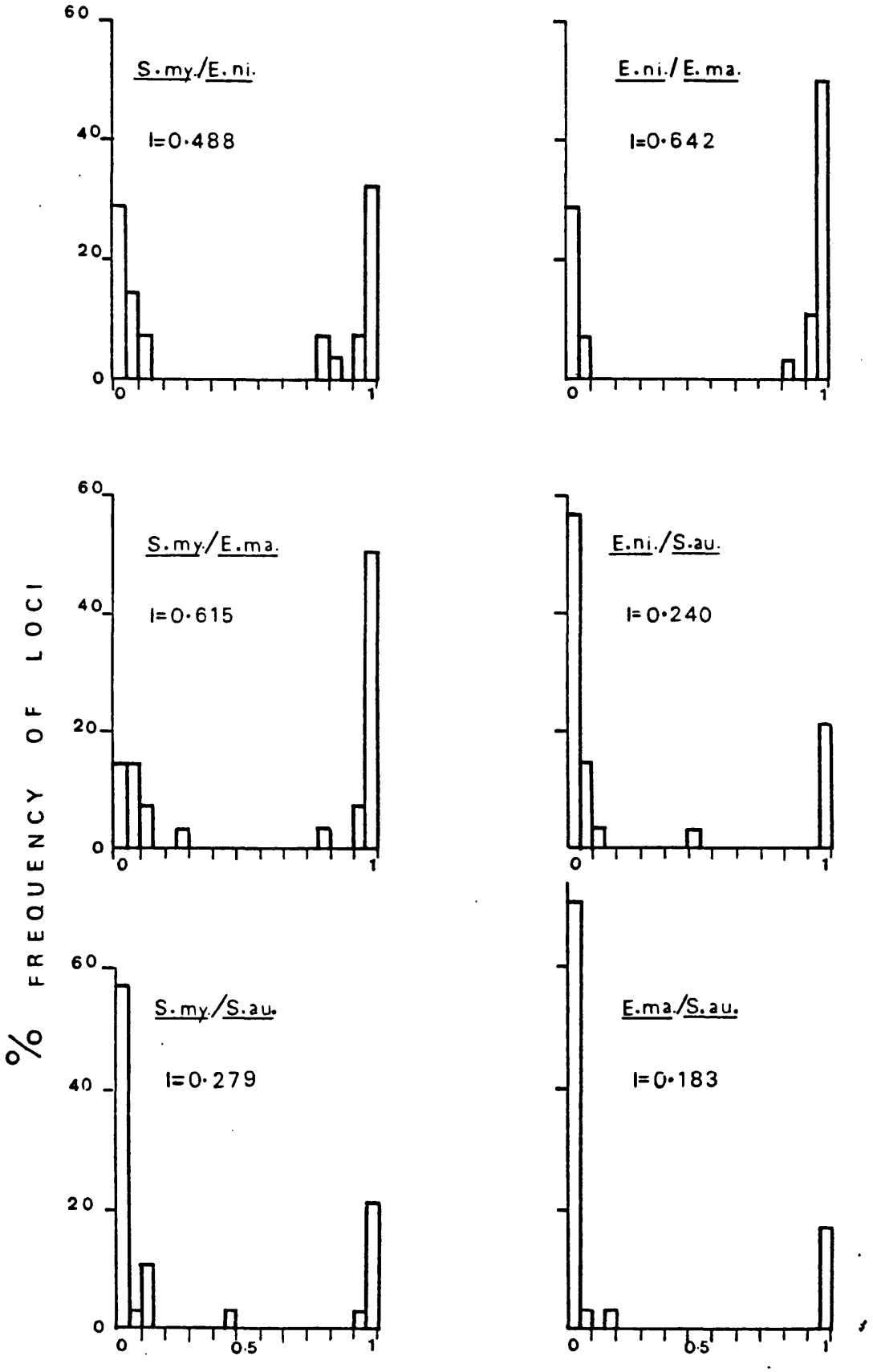


Figure 16b:
Distribution of Loci with Respect to Genetic Identity among Stocks and Species of Oreochromis (fm. Cichlidae). Based on 27 loci.

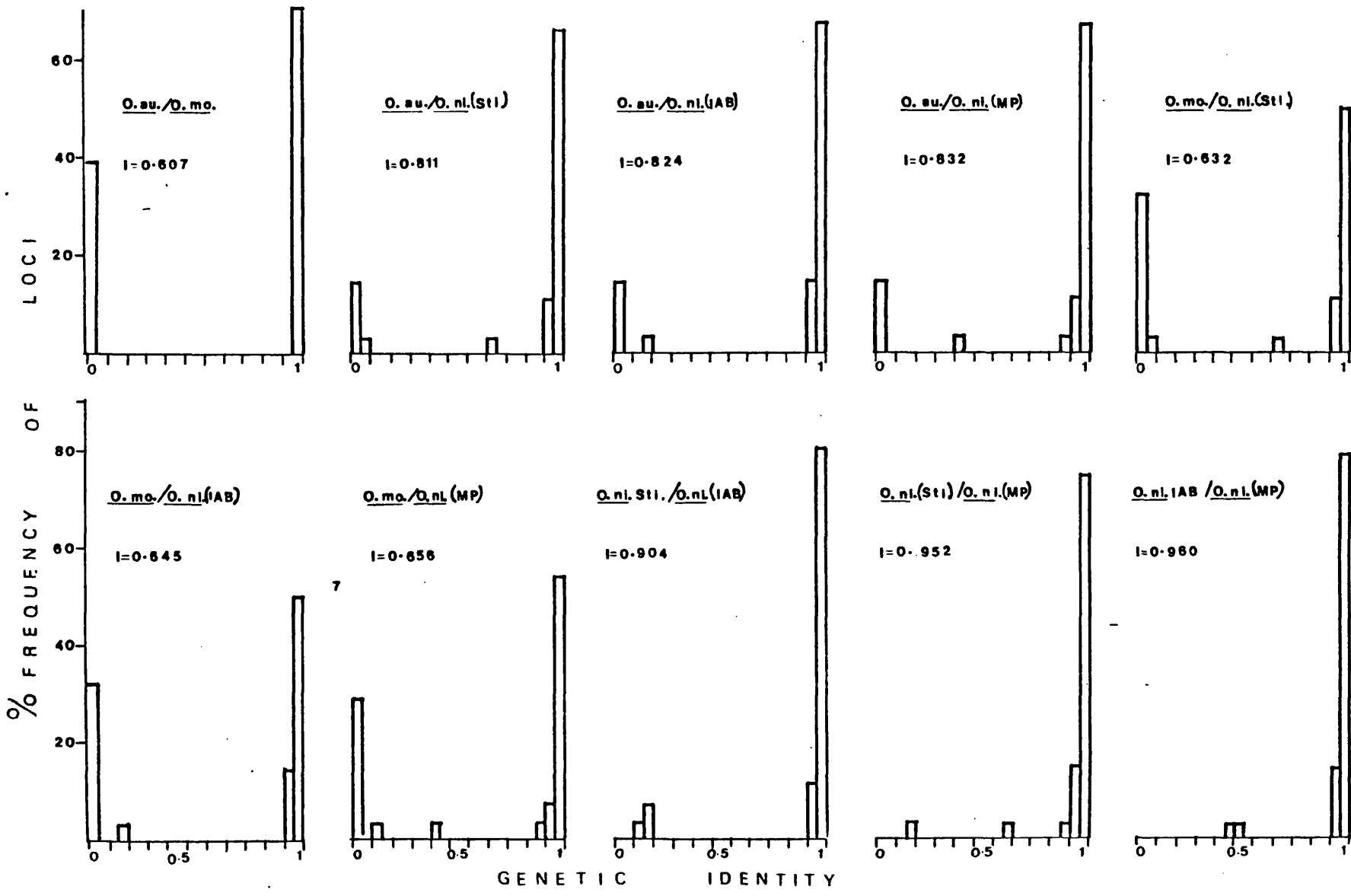


Figure 16c:
Distribution of Loci with Respect to Genetic Ideneity Among Stocks and Species of Sarotherodon (fm. Cichlidae). Based on 27 loci.

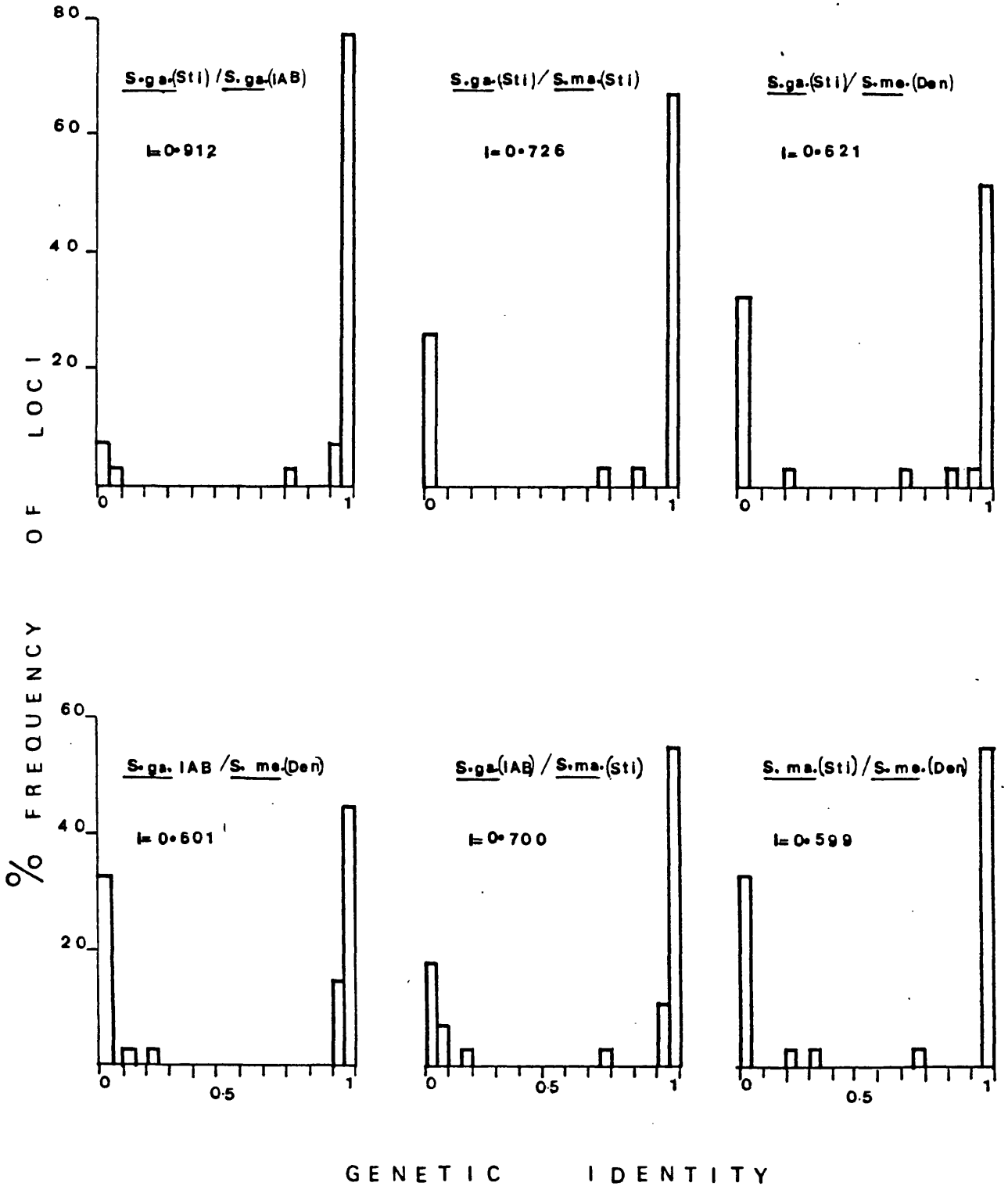


Figure 16d:

Distribution of Loci with Respect to Genetic Identity Among Stocks and Species of Tilapia (fm. Cichlidae). Based on 27 loci.

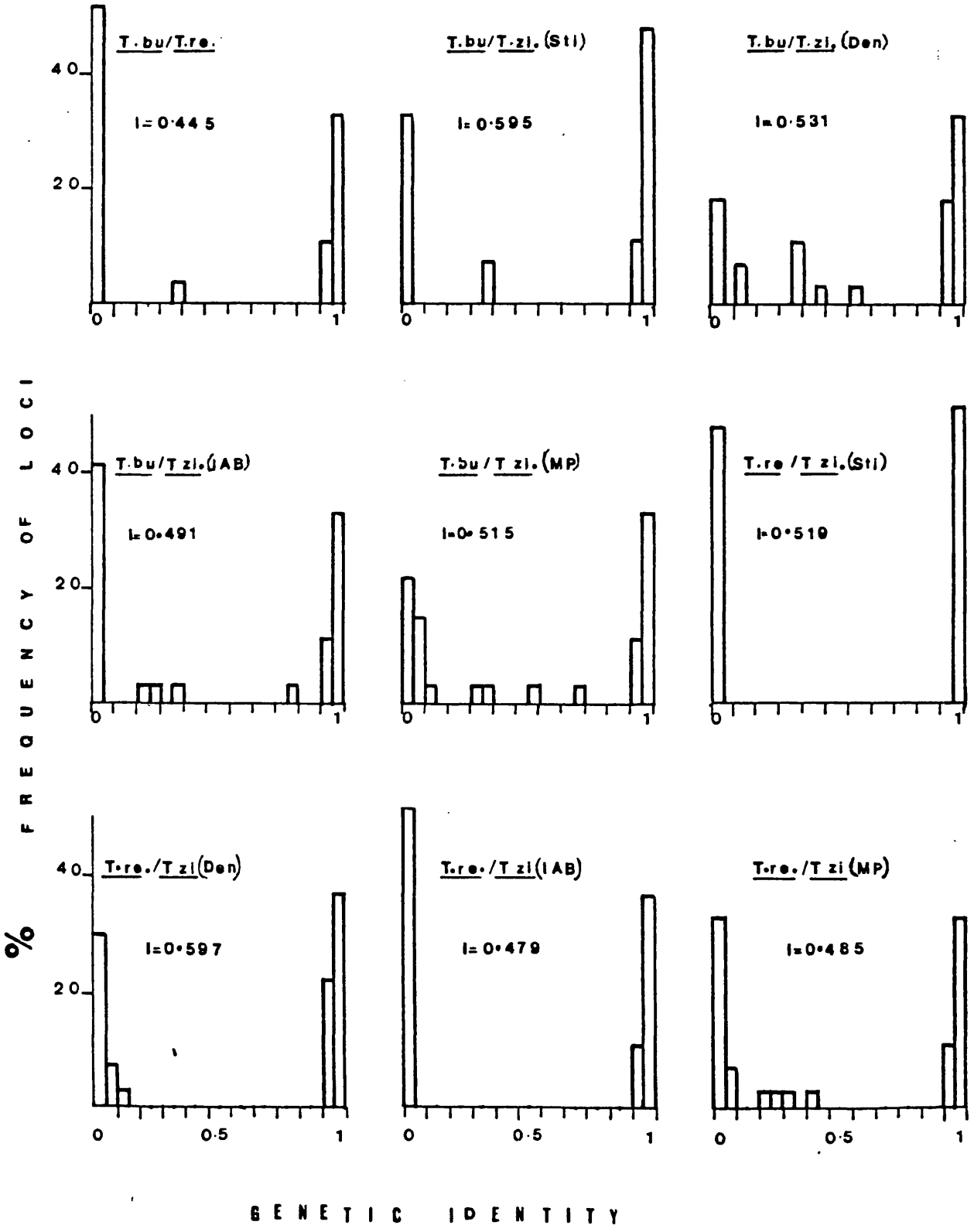
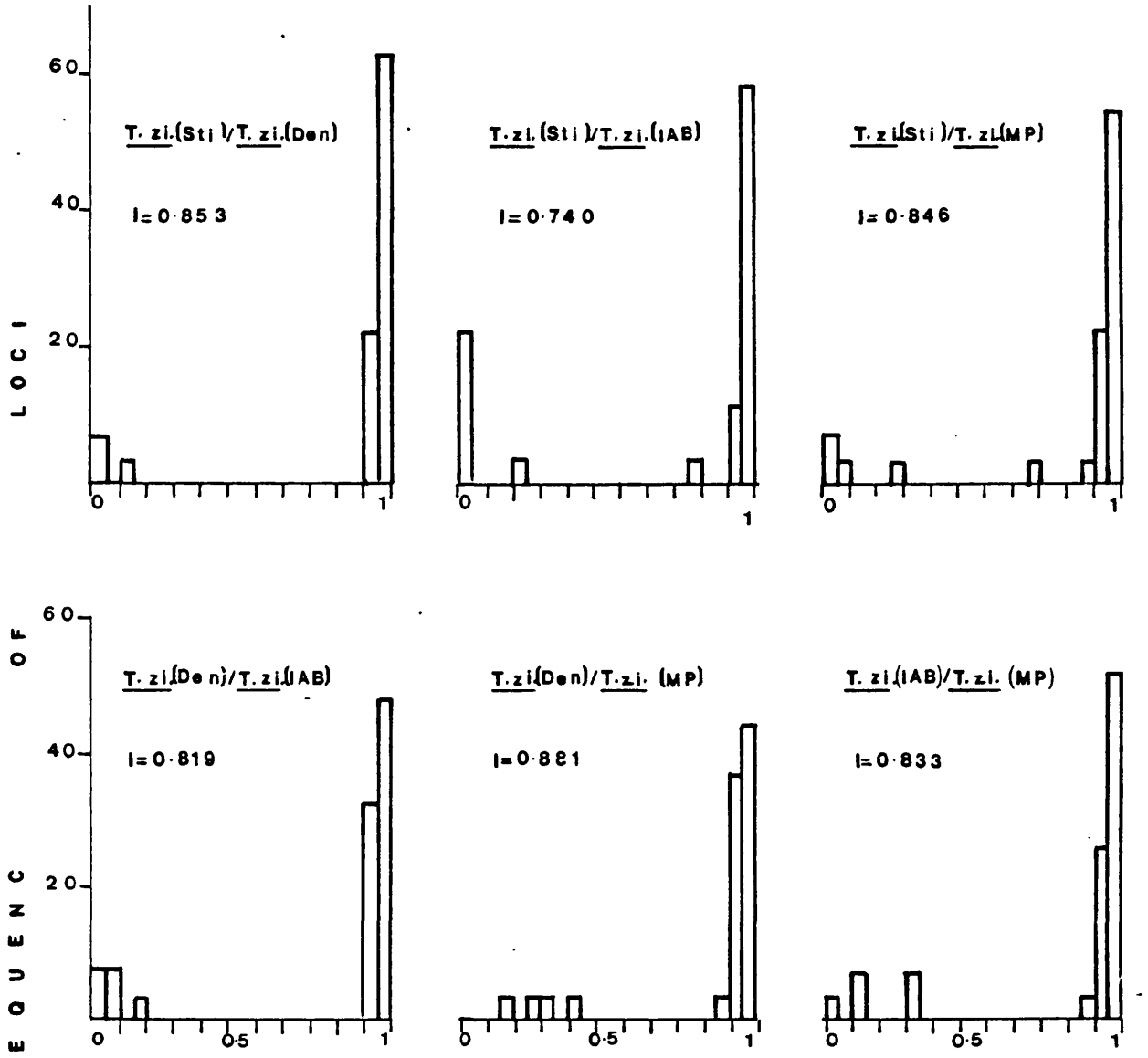
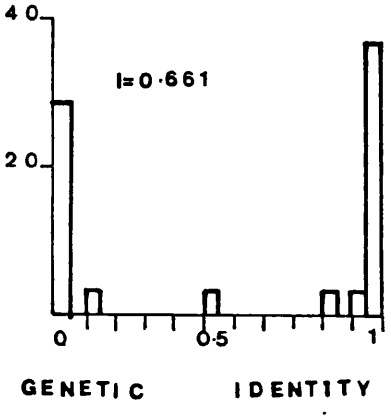


Figure 16d Contd.



Distribution of Loci with Respect to Genetic Identity between Chrysichthys auratus and C. nigrodigitatus (Bagridae). Based on 27 loci.

Figure 16e:



5.3 Discussion:

The distribution of loci with respect to genetic identity between pairs of species in families studied was generally bimodal (see Figs. 16a - 16e). This pattern of distribution was also observed among nine closely related fish genera from California (Avise and Ayala, 1976). Ayala (1975) associated such bimodal allozyme differentiation with sexually reproducing animals in which speciation occurs according to the allopatric or peripatric model. Since Ayala's assessment, the pattern has been noted in other major taxa including plants, insects and rodents (Gottlieb, 1975; Bullini and Sbordoni, 1980; Benado et al., 1979).

From various studies, Ayala (1983) estimated that species showing the typical bimodal distribution have 20 to 30% of loci between species to be diagnostic. In the present study, 29 and 32% of loci between species of Eutropius (Schilbeidae) and Chrysichthys (Bagridae) respectively, were diagnostic. Among the tilapias, percentage diagnostic loci between species of Tilapia, Sarotherodon and Oreochromis, were 32, 29 and 11% respectively. Thus, based on the assessment of Ayala (1983) speciation among fishes studied here involved mechanisms related to allopatric or peripatric speciation. However, between Oreochromis species the atypical differentiation (11% of diagnostic loci) may be likened to what Benado et al. (1979) observed among species of spiny rats, Proehimys guaire. Among the rats, species were said to

be parapatrically distributed and morphologically almost indistinguishable but reproductively isolated. The characteristics of these rat species may be said to reflect those of the tilapias in general. White (1978) suggested that, such low differentiation between species, as observed between the Oreochromis species here, to be what would occur in genera in which species arise rapidly by any of the processes which might come under 'quantum' or 'saltational' speciation events.

Fryer and Iles (1969) among others, have attempted to explain the lack of morphological differentiation among the tilapias of the East African lakes. They suggested conservatism of the alleged generalised condition of tilapias. However, Trewavas (1983) indicated that defining the 'generalised' form of tilapias would be a difficult task. This is because, irrespective of morphological similarities, many important specialized features exist between the major tilapia groups, with respect to especially, trophic and reproductive habits. The mouth brooding habits of Sarotherodon and Oreochromis, for example, could not be described as habits lacking in specialization. In the many explanations offered to explain the lack of morphological differentiation among the tilapias, both sympatric and allopatric models of speciation have been sited among various communities of Tilapia, Sarotherodon and Oreochromis (see Trewavas, 1983, pp. 511-521).

Perhaps the only conclusion which may be drawn from all the estimates of divergence here is that genetic and morphological divergences do not always proceed in parallel. And as Kirpichnikov observed, the rate of mutation relates to both time and specific features of speciation in different evolutionary branches.

The extent and often the model of speciation in related organisms has frequently been deduced from Nei's identity values between various ranks (populations, subspecies, species and genera) of the related groups. Table 12 summarises and puts into better perspective identity values 'spread out' in Figures 16a to 16e, by virtue of presenting the distribution of differentiation. More importantly, Table 12 compliments values in Fig. 16 with the inclusion of identity values between confamilial genera of all the groups of fishes. The Table shows that the extent of divergence between comparable ranks varied in different groups of fish. This variation has been previously observed among other fish groups. For example, among eight species of Pacific Salmonids, Kirpichnikov (1981, pp. 228-239) reported that the mean similarity value between pairs of species was 0.46, although very high values, up to 0.9, were estimated between some species. Here, estimates between species of Eutropius, Oreochromis and Sarotherodon (0.642, p.691±0.094 and 0.649±0.053) may be accepted as being comparable. However, Tilapia species showed a mean identity value of 0.499±0.039.

Table 12 : Summary of Genetic Differentiation (Genetic Identity, I) in Similar Taxa of Different Fish Groups.

Family (genus)	I between Populations	Mean	I between Species	Mean	I between Genera	Mean
					0.488	
Schilbeidae	------	-----		----	0.615	
Schilbe	------	-----			0.279	0.361 ± 0.163
Siluranodon	------	-----			0.240	
Eutropius	------	-----	0.642	—	0.183	
Cichlidae	0.904		0.607			
	0.952	0.939	0.822	0.691	0.731	
Oreochromis	0.960	±0.025	0.644	± 0.094	0.654	
					0.634	
					0.407	
Sarotherodon	0.912	-----	0.726		0.564	0.582 ± 0.089
			0.621	0.649	0.674	
			0.601	± 0.053	0.514	
			0.700		0.493	
			0.599		0.607	
Tilapia	0.853				0.529	
	0.740	0.829	0.445	0.499	0.516	
	0.846	±0.044	0.533	± 0.039	0.659	
	0.819		0.520			
	0.881					
	0.833					
Bagridae	----------	-----	0.661	-----	-----	
Total Mean	0.870 ± 0.063		0.625 ± 0.092		0.517 ± 0.154	

Summarising all previous studies, the range of identity between conspecific populations was estimated to be 0.99 to 0.97. The generally lower values obtained here for the tilapias may be explained by three factors which are not conclusive. Firstly, some of the stocks investigated originated from cultured and experimental stocks, which if they had been inbred could contribute to a general lowering of identity with the wild stocks included here. Secondly, although the tilapias are cichlids among which high identity values have been reported (Kornfield, 1978), the tilapias have not been involved in the explosive morphological divergence of other cichlids, not even in the African Great lakes. Thirdly, available literature clearly indicates variation in different groups of fishes (Kirpichnikov, 1981). Thus it is possible that previous data on tilapias could have involved more Oreochromis species compared to Sarotherodon and Tilapia species. The range of identity between species of cichlids of Lake Malawi was 0.86 to 0.99(Kornfield, 1978).

With respect to confamilial genera, Kirpichnikov's review indicated that identity value between genera did not exceed 0.40 except in Cyprinids. It would therefore seem that value for Schilbeidae (0.361±0.163) agrees with expectations. The values obtained between tilapia genera was higher. Data from the Lake Malawi cichlids provides no reference point at the generic level. However, the range of identity values obtained here (0.493 to 0.731) compares favourably with that of McAndrew and Majumdar (1984), who investigated

seven Oreochromis species and one each of Tilapia and Sarotherodon. Their identity values between genera ranged from 0.391 to 0.738.

However, speciation among tilapias and other cichlids remain incompletely resolved. A fair discussion of the subject is beyond the scope of this work, but good accounts, based mostly on morphology, have been given by several authorities since the 1920's (e.g. Regan, 1920; Greenwood, 1984; Trewavas, 1981b, 1982a; 1983; McAndrew and Majumdar, 1984). It would suffice here to say that the lack of morphological differentiation among tilapias compared to the cichlids of the Great African Lakes, where a 'flock' of cichlids have revealed explosive speciation events still receives attention.

5.4 Polymorphism and Heterozygosity Among Populations:

5.4.1 Introduction:

Variability estimates (Polymorphism (P) and Heterozygosity (H)) of fish and other populations, have often been associated with various environmental and life zone characteristics. Most reviews on the subject have demonstrated the adaptive significance of both polymorphism and heterozygosity (Lewontin, 1974; Ayala, 1976; Nevo, 1978; Nevo et al., 1984, pp. 11-137; Selander and Whittman, 1983). However, some studies question the environmental dependence of population variability (Whitt, 1983; Aspinwall, 1974; Kimura, 1983; Levie et al., 1987).

The review of Nevo *et al.* (1984), based on 183 fish, species related variability to seven ecological, five demographic and nine life history characteristics making the review perhaps the most thorough analysis available. Their conclusions contained many trends on the patterns and levels of protein variability in fish. For example, they estimated the mean heterozygosity of fish to be 0.052. In relation to life-zone, the review estimated that mean H decreased from 0.067 > 0.047 > 0.037 in tropical, temperate and arctic populations respectively. Considering various parameters associated with population variability Nevo *et al.* (1984) estimated that tropical, medium sized, regionally distributed fish populations, such as those studied here were expected to have a mean observed heterozygosity of 0.074.

5.4.2 Results:

Estimates obtained for the mormyrid, schilbeidae, bagridae and cichlidae studied here are presented in Tables 15a, 15b, 15c and 15d respectively. Each table provides H values at individual loci and population P and H. Heterozygosity estimates have been presented as Ho for observed heterozygosity and He, for calculated or expected heterozygosity. For an overall appraisal of variability levels in all populations Table 16 has been provided as a summary. Table 16 excludes five of the tilapia populations in Table 15d. This was because sample sizes of the omitted populations were considered too low

Table 16: Summary of Protein Variability in Fishes Studied.

FAMILY:	MORMYRIDAE	S C H I L B E I D A E				BAGRIDAE		C I C H L I D A E								
Species: (Type of Population	M.sen. (WP)	S.mys. (WP)	E.nil. (WP)	E.man. (WP)	S.aur. (WP)	C.nig. (WP)	C.aur. (WP)	O.aur. (EXP)	O.nil. (EXP)	O.nil. (CP)	O.nil. (MP)	S.gal. (CP)	S.mel. (WP)	T.bus. (WP)	T.zil. (WP)	T.zil. (MP)
No. of Loci	22	28	28	28	28	28	28	27	27	27	27	27	27	27	27	27
Proportion of Loci Polymorphic (P) (Criterion = 0.95)	0.0*	0.179	0.143	0.071	0.036	0.214	0.143	0.0*	0.185	0.185	0.185	0.111	0.111	0.074	0.296	0.333
Obs.Het.(Ho) Range (at Loci)	0-0.077	0-0.333	0-0.208	0-0.4	0-0.147	0-0.414	0-0.276	0-0	0-0.667	0-0.250	0-0.167	0-0.143	0-0.158	0-0.059	0-0.139	0-0.421
Ho Mean ± S.E.	0.004	0.028	0.014	0.022	0.008	0.054	0.031	0.0	0.043	0.026	0.011	0.013	0.006	0.003	0.019	0.057
	±0.003	±0.013	±0.009	±0.016	±0.006	±0.042	±0.026	0.0	±0.025	±0.012	±0.007	±0.007	±0.006	±0.002	±0.006	±0.019
Exp.Het.(He) Range	0-0.073	0-0.488	0-0.5	0-0.32	0-0.152	0-0.505	0-0.58	0	0-0.685	0-462	0-0.42	0-0.204	0-0.331	0-0.165	0-0.278	0-0.694
He Mean ± S.E.	0.003	0.057	0.067	0.019	0.007	0.060	0.064	0	0.056	0.048	0.041	0.027	0.029	0.016	0.056	0.136
	±0.003	±0.020	±0.025	±0.013	±0.006	±0.046	±0.044	0	±0.027	±0.020	±0.021	±0.010	±0.015	±0.007	±0.014	±0.035

Note:
WP = Wild Population
CP = Culture Stock
EXP = Experimental Stock
MP = Sample from a private farm pond having a mixture of tilapia.

* P at 0.99 criteria in M. senegalensis and O. aureus were 0.046 and 0.0 respectively.

to provide dependable comparative information. The populations excluded in Table 16 were those of Oreochromis mossambicus, Sarotherodon galilaeus, S. macrochir, Tilapia rendalli and T. zillii obtained from Stirling. Results from these samples have, however, been used where appropriate in this work.

5.4.3 Discussion of Polymorphism (P) Among Populations:

Polymorphism (P 0.005) among all populations (Table 16) ranged from 0.0, in the mormyrid, Marcusenius senegalensis to 0.333 in the cichlid, Tilapia zillii (MP). Nevo et al. (1984) and Powell (1975) estimated the mean P for fish to be 0.209 and 0.220 respectively. It is possible that the P value for the T. zillii (MP) could have been influenced by introgression of genetic material from other species in the mixed-species pond (MP) as evidenced by Tanuguchi et al. (1985). However, the highest P of 0.296 (excluding the Mixed Pond T. zillii) was still recorded in a wild population of T. zillii. Thus the total range of P (0.05) among 'true' populations here was 0.0 to 0.296. It could be argued that, for fish within the same life-zone and often sympatric, the range was too wide. That is, if life-zone of major taxa, irrespective of evolutionary position or history of minor taxa involved was significant. The above observation is based on the estimation that differences between P values of fish of major life-zones (arctic, temperate and tropical) provided by Nevo et al. (1984) show that between arctic and temperate fishes the

difference in P value was 0.010, 0.020 between temperate and tropical fish and 0.030 between arctic and tropical fish.

Considering the populations studied here in their family groups however, a trend of increasing mean P with standard errors was observed as follows:

Mormyridae	Schilbeidae	Bagridae	Cichlidae
(P SE = 0.0±0	> 0.107±0.064	> 0.179±0.050	> 0.185±0.091)

Figure 14 shows a schematic relationship of P values within and between families. Although P is dependent on sample size, Table 16 together with Figure 14 at least suggests that, for each major taxa, there was an upper limit, to the proportion of genomic polymorphism. The figure also suggests that within each family minor taxa may have trends of P which might grossly reflect their systematic position. In the present study O. niloticus originating from Ghana and Egypt showed the same level of polymorphism (P = 0.185). These results compare favourably with estimates made by McAndrew and Majumdar (1983) of P = 0.180 for the same species. Also the P value of 0.111 obtained for two species of Sarotherodon, suggests some uniformity with respect to polymorphism in the genus. Tilapia species may then be considered as most polymorphic among the tilapiine. Although overlapping of P values at the 'lower ends' could be expected, the 'wide-range' of P values for Tilapia species may be partially attributed to the estimated evolutionary

Figure 14: Species Polymorphism - A schematic diagram.

Figure shows range of fish families investigated. In each family, the sequence of species reflects increasing polymorphism in species within the family.

Abbreviation

M.se = Marcusenius senegalensis

S.au = Siluranodon auritus

E.ma. = Eutropius mandibularis

E.ni. = E. niloticus

S.my. = Schilbe mystus

C.au. = Chrysichthys auratus

C.ni. = C. nigrodigitatus

T.bu. = Tilapia busumana

S.me. = Sarotherodon melanotheron

S.ga. = S. galilaeus

O.ni. = Oreochromis niloticus

T.zi. = Tilapia zillii

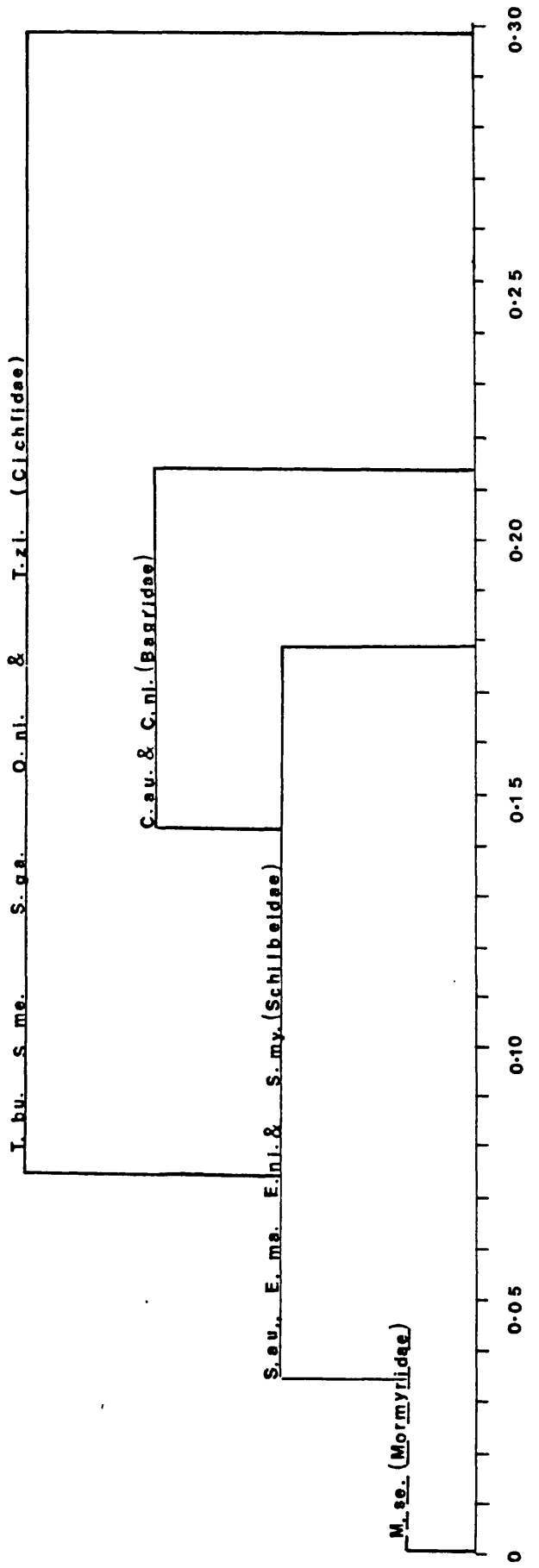


Figure 14: Species Polymorphism - A Schematic Diagram.

distance between T. busumana and the rest of the Tilapia species (section 4.4). The low P value obtained for T. busumana compared to T. zillii may also reflect the restricted drainage of Lake Bosomtwi (see Section 2.1), from where the sample studied here was obtained.

It is interesting to consider that in the evolutionary ordering of African freshwater fish families, the status among the families involved here were as follows : Mormyridae < Schilbeidae < Bagridae < Cichlidae (Lowe-McConnell, 1987, pp. 329-337). Figure 14 here also shows that the level of polymorphism of the families decreased in a similar sequence as their evolutionary status. The results here would therefore generally support the association between protein polymorphism and protein evolution indicated by Kimura (1983, pp. 251). Although, how the relationship supports neutral theory, according to Kumura is not clear to me.

5.4.4 Discussion of Heterozygosity (H) Among Populations:

It has already been indicated that heterozygosity (H) is considered a more reliable measure of population variability than P because H is a better measure of genetic variability and independent of sample size (Gorman and Renzi, 1979; Nevo et al., 1984). The mean observed heterozygosity (Ho) values for the thirteen populations presented in Table 16 ranged from 0.004 ± 0.003 to 0.056 ± 0.019. Considering only the ten wild populations (WP), the highest mean Ho was obtained in the bagridae, Chrysichthys nigrodigitatus with the value of 0.054 ± 0.042.

The Ho values for the ten wild populations were all below what would have been expected (i.e. 0.074) for tropical, medium sized, regionally distributed mainland fishes according to Nevo et al. (1984). The values were also lower than the mean value expected for tropical fishes generally (i.e. 0.067 - according to Nevo et al., 1984). Nine of the populations' mean values were also below what would be expected for temperate and even arctic fish populations - i.e. 0.047 ± 0.029 and 0.037 ± 0.026 respectively according to Nevo et al. (1984) (see Table 17 for differences between Nevo et al. estimates and the present).

Consideration of other ecological and life history characteristics (body size in particular), in an attempt to explain the unexpected low levels of heterozygosity

obtained here revealed that such low variabilities have been associated with isolated populations. Avise and Selander (1972) and Webster et al. (1972) respectively described such low variability in cave populations of the fish Astyanax mexicanus (Hubb and Innes) and in populations of the lizard, Anolis species on the South Bimini Island. Cruz et al. (1982) also reported low variability in a specialised population of T. zillii discovered in a heated effluent stream in England. The populations investigated presently could not be considered isolated. All the fishes have been described from various tropical and subtropical regions (De Vos and Leveque, 1983; De Vos, 1984; Trewavas, 1983; Leveque and Paugy, 1984). However, peculiar environmental conditions of the populations, discussed later, could account for the heterozygosities observed.

Body size of fishes is the only characteristic that opponents of the life-zone and ecological amplitude hypotheses (the neutralists) consider to influence heterozygosity levels (Nei, 1975). According to the neutralists' view, heterozygosity decreases with increase in size. However, the review of Nevo et al. (1984) involving 183 species of fishes detected no significant differences in fish heterozygosity in relation to species size. Even if the neutralist view that larger animals generally have smaller effective population size and thus lower variability levels (Kimura, 1983, p. 255) was to be considered, the estimated mean heterozygosity for medium sized fishes was

0.049 ± 0.029 (Nevo et al., 1984). Only two of the ten medium sized wild fish populations studied here conformed to the estimated value.

Since the main aspects of the existing hypotheses on variability levels do not sufficiently explain the results obtained for ten species originating from a limited area, it is necessary to consider specific ecological factors operative in the area. The river sources of the fishes were all typical tropical savanna types. Being so, they experience a single flood period during the year, from mid-May till September. This is then followed by a relatively longer dry period, from September to May, during which most of the rivers involved here would be reduced to a series of pools (Holden, 1963; Hopson, 1965; Abban, 1979; Abban and Samman, 1982; Lowe-McConnell, 1987). It is thus evident that an annual reduction in habitable aquatic space for the fish populations occurs regularly. Such situations have been known to exert a 'crash' on population sizes, which in turn could result in reduction of variability as sudden stochastic effects, with no selective advantage for any genotypes or individuals (Merrell, 1981). The hydrology of the rivers could also lead to regular bottle-necking resulting in an annual reduction of effective population size. Frankel and Soule (1981) have shown how such regular bottle-necking could directly influence the genetic structure of populations.

There are also documented human influences, such as excessive fishing pressure and poisoning of restricted dry season pools in the Ghanaian rivers which must have influenced the effective population size of fish populations (Irvine, 1947). Examples of reduction in variability attributed to regular bottle-necking, founder effect and human influences have been described by Selander et al. (1971) in relation to the old mouse, Peromyscus polionotus (Osgood).

Other special features of the freshwater fishes of West Africa which could be directly related to the low variability values obtained, could be their relic status and endemism. Lowe-McConnell (1987, pp. 27-45) mentioned that eighteen of the big fish families in Africa, including the mormyridae and Schilbe mystus are representatives of archaic elements with relatives in South America and Australia. Presently, the most ancient of the African freshwater fishes are said to be restricted to West Africa and the Nile. Thus, although the fishes may show regional distribution and are not isolated, their relic and endemic status could account greatly for their levels of heterozygosity. Nevo et al. (1984) estimated the mean heterozygosity for relic and endemic fish species to be 0.016 ± 0.011. Taking into account that their assessment was based on the analysis of six species, the expected heterozygosity value indicates that results obtained here are consistent with their results.

It is however, difficult at present to determine to what extent any of the possible causes (hydrology, human influences and the relic status of the fishes) has contributed to the low variability levels obtained here. Recently Gyllensten (1985) has cautioned that freshwater fishes, would in general, be expected to have lower variability levels than marine species because of land barriers. He also indicated that different fish groups may have different levels of variability. These observations were made against a background of work which had not considered marine and freshwater fishes separately with regards to variability.

Of the fish groups studied here only some of the cichlid species had been previously studied electrophoretically because of their aquaculture research importance (Kornfield et al., 1982; Cruz et al., 1982; FAO, 1980; McAndrew and Majumdar, 1983; Tanuguchi et al., 1985). McAndrew and Majumdar; and Tanuguchi et al., also obtained similarly low variability values for the wild populations of the tilapias they studied. The former authors attributed their results to bottle-necking and inbreeding effects. Results here suggest that different fish groups, very likely, have different 'basic' levels of variability, which would be a reflection of their protein evolution and evolutionary history (Kimura, 1983). However, measured variability should be a product of the 'expected' and the environment. The near-monomorphic state of the mormyrid, M. senegalensis (H_o = 0.004 ± 0.003) calls for special

comment. Considering that the 26 individuals screened were obtained from two rivers and were sympatric with S. mystus, E. niloticus and C. nigrodigitatus (Ho being 0.028 ± 0.013; 0.014 ± 0.009 and 0.054 ± 0.042 respectively), the results obtained might suggest that monomorphism was a characteristic of the mormyrid.

Usually genetic variability at loci and within species is discussed with passion. However, monomorphism has largely been ignored possibly because it does not lend itself to discussion on evolution and its mechanisms. It is however, acknowledged that monomorphism is often a species rather than a population characteristic (Altukhov et al., 1972; Altukhov, 1982; Omelchenko, 1974).

Where monomorphism as observed here is under discussion, it is usual to consider the following :

i) The sufficiency of sample;
ii) The adequacy of the method of resolution;
iii) Whether this monomorphism is characteristic of a particular population, and
iv) The thesis about absolute relations between genetic monomorphism of proteins and the viability of taxa would probably have to be proved.

Twenty-six individuals from the Volta basin population of a fish could probably be regarded a small sample. However, the screening of the same individuals at 28 loci to detect protein variability could not be considered insufficient. Nei and Roychoudhury, (1974) emphasized that one obtains

far more information in an electrophoretic study by increase in number of loci sampled rather than the number of individuals. Furthermore, Gorman and Renzi (1979) presented empirical evidence indicating that estimates of genetic distance were relatively sample-size independent. Thus with complete identity ($\underline{I} = 1$) between the two 'subpopulations' of M. senegalensis, the lack of variability in the population as a whole, should be a characteristic of the species.

The lack of variability at 28 loci in 26 individuals of a tropical, unisolated fish population, could be unusual enough to question the methods used. The methods were however, adequate in resolving the same proteins in twelve other species in 19 populations involved in this study. There is also evidence that loci that are highly variable under one condition may have their variability increased by more refined methods while monomorphic loci remain so when carefully examined (Lewontin, 1978, pp. 467).

It has long been established that the catalytic centre of an enzyme is relatively constant compared to its less significant regions (Kimura, 1983, p. 156). For example, the amino acid composition of LDH in vertebrates varies, but the basic catalytic unit of the enzyme (the lactic centre, consisting of 12 amino acid residues) is identical in all cases studied (Muller & Kaplan, 1966). Also the histidine positions 58, 63, 87 and 92 of both α- and β- chains of haemoglobin molecules are completely invariant and have

been kept as such in many vertebrates for over 500 million years (Jukes, 1971; also see Kimura and Ohta, 1973; Berger and Weber, 1974). It was therefore considered that in this particular environment, in which variability at the protein level of twelve other species has been considered lower than expected (Ref. to Nevo et al., 1984), a species could be encountered in which maintenance of the basic (functionally important units) has become crucial. However, the fact that the individuals screened originated from two sub-populations may provide some indication that monomorphism observed will be a feature of the species rather than the population (Altukhov, 1982).

In seeking a possible explanation for the near monomorphism in the mormyrid in relation to the possible extinction of the species, certain concepts of molecular evolution must be considered. First, polymorphism in natural populations is acknowledged and average heterozygosity in natural populations range from 0.0 to 0.3 (Kimura, 1983). It is also generally accepted that mutations contribute to the evolution of alleles at an approximate rate of $10^{-5} \sim 10^{-6}$ per gamete per generation. However, only about 1/10 of the amino acid altering mutations per generation survive to enter the reproductive population. This, according to Kimura (1983, pp. 321) is because the 1/10 of mutations are essentially neutral while the 9/10 are "selectively" deleterious.

From this common basis selectionists generally suggest the observed heterozygosity of populations is influenced by their environment and maintain that variability can usually be advantageous. However, under certain constraints and environmental heterogeneity, an allele having high plasticity would be favoured, thus encouraging monomorphism. Thus the near-monomorphism observed in the mormyrid here would be considered as an adaptive strategy.

Such ecological hypotheses, assuming adaptive strategies, are considered by exponents of the neutral theory as elusive and difficult to quantify. They submit that the average heterozygosity of a population or species is in many ways related to the effective population size of the organisms, and has little or nothing to do with the environment. Kimura (1983) for example, suggests that low heterozygosity is common in bigger mammals because by virtue of the big body size, the organisms have low effective population sizes. Kimura also suggests that polymorphism is simply a transient phase of molecular evolution. Thus the level of polymorphism would strongly be influenced by structural and functional constraints of molecules, and be independent of the environment.

The problem with the above and the situation in the mormyrid is that, the fish could not be considered big in body size and thus automatically have a low effective population size. Secondly, fishes being the most primitive

among vertebrates, their genomes would be expected to be most flexible. Thus although the relationship between polymorphism and general molecular evolution is acceptable, it does not explain the observations made here. However, if it were acceptable that the observed heterozygosity of a species or population was the product of transient evolution and the influence of the environment, or a characteristic of a species, individual groups of organisms could receive more liberal considerations.

It is therefore sufficient to conclude that :

a) The near-monomorphism observed in M. senegalensis could indicate that the phenomenon was more related to the species than to the population studied.

b) Hereditary variability at the protein level could be very low without putting a population or species on the path of extinction.

c) It also suggests that protein variability, while having its advantages for some taxa, may not be vital for others.

d) If (c) is acceptable, then it would follow that some alleles, perhaps the most primitive, could be multipurpose in function.

The summary of variability estimates in the fish populations studied (Table 16) shows that the calculated heterozygosity (He) in all the natural populations

were lower compared to their corresponding observed heterozygosity (Ho). The observed heterozygosities express the proportion of heterozygous individuals in each population. However, it could have been expected that Ho and He values of individual populations would be quite similar (Ferguson, 1980). The expectation is based on a principle generally referred to as the Hardy-Weinberg equilibrium or law (H-W-equilibrium). According to the H-W-equilibrium, in sexually outbreeding natural populations (as those studied here), the inherent random mating proportions of gametes ensures that observed proportions of heterozygotes in a population would not be distinctly different from the expected. However, many populations under various conditions have shown shifts from this equilibrium and often shown 'excess' of homozygotes.

Attempts to account for deviations from H-W-equilibrium in various populations generally, have considered the following pressures: mating choice; mutations, migrations, genetic drift and natural selection. Saura (1983) indicated that obstructions to movement and mere physical distance in the absence of barriers could also influence heterozygosity values. This is because, as a result of any or a combination of these factors operating, the net effect would be that the total gene pool of a population will not be randomly available to future generations as compared to their ancestors (Saura, 1983), because populations become subdivided.

Among the factors which influence the equilibrium of heterozygosity in populations, only selection was accepted to be operating, and could be directional in the type of homozygotes that eventually dominate. The other factors, including environmental heterogeneity, effectively reduce the proportion of heterozygous individuals by reducing the effective population size of populations (see Wilkins, 1977; Nevo et al., 1984, Badino and Sella, 1980; Lavie et al., 1987; Beardmore and Morris, 1978 - who attribute deviations from H-W-equilibrium to various factors and combinations of them).

Considering the populations studied here, an apparently obvious factor operating to lower heterozygosity in the populations would be genetic drift associated with population crash and bottle-necking. The hydrology of the rivers, elaborated in Chapter 2, shows that the populations studied go through regular 'crashes' during the long dry periods of the rivers (Dec. to May). During these months, the fishes regularly have to live in dry season pools often separated by kilometres of dry land. The ecology therefore puts fishes under physical pressure and increases competition for habitable space and resources. More importantly, a regular occurrence of the condition could lead to population subdivisions. Beardmore and Morris (1978) concluded that when competition, as a biotic factor, is taken into account, reduction in niche-width leads to a general reduction in heterozygosity.

Then at the peak of the dry period (March-April), when most sections of the rivers completely dry up, fishes which could not reach the 'dry season pools' in good time, would naturally be expected to be eliminated. Usually the stranded fish are easily fished by humans or picked on by birds (in protected sections of the Volta system). Extreme bottle-neck effects on population heterozygosity has often been exemplified with isolated or cave populations, but the example of 'crashed' northern elephant seal population is also often sited (Avise and Selander, 1972; Sbordoni et al., 1981).

The populations studied may not be considered isolated in various parts of the Volta basin. However, it can be suggested that, before the man-made Volta lake was formed in 1964, the hydrology of the rivers could have isolated various segments of the population over long periods. Thus possibly confining 'sub-populations' to areas within the basin. Studies on the land snail, Cepaea nemoralis, have been used to show that 'area-effect' could result in extensive changes in allele frequencies and encourage monomorphism (Murray, 1972). However, investigations related to area-effect in other organisms have shown that not all enzyme alleles are associated with area effects which can be observed morphologically (Caugant et al., 1982; Jones et al., 1980).

There is no direct evidence of selection operating to reduce populations' sizes in the Volta basin. However,

Table 17: Differences in Heterozygosity of fish in relation to characteristics (Nevo et al., 1984 and present estimates).

Parameter		N	Mean	Sd	P	
All fish	1	183	0.051	0.035	4.67	***
	2	10	0.023	0.017		
Tropical fish	1	11	0.067	0.025	4.75	***
	2	10	0.023	0.017		
Medium tropical	1	18	0.058	0.040	3.226	**
	2	10	0.023	0.017		

N = Number of species.

P *** at < 0.001

** at < 0.002

1 Nevo et al. (1984) Estimates.

2 Present study estimates.

1 and 2 have been computed statistically using a t test
**, P< 0.01; ***, P<0.001.

Chitty (1960) hypothesized that selection operates with unequal intensity at different stages of life cycles, which changes genetic constitution of populations. Later, Semeonoff and Robertson (1968) and Gaines and Krebs (1971) demonstrated that the frequency of some enzyme alleles change with population decline. Thus, it is possible to attribute a measure of the reduction in observed heterozygosity in the populations studied to the various factors which primarily reduce population sizes.

* * * * * * * * *

CHAPTER 6

GENERAL DISCUSSION

Investigations carried out in this study relate to morphological and biochemical taxonomy, systematic relationships, genetic differentiation and population structure of 13 species of African freshwater fishes. The fishes belong to eight genera and four families of commercially important fishes.

The taxonomic studies showed that, generally, the protein electrophoretic approach to species identification supports existing morphological separation of related taxa. However, the biochemical approach was faster, more consistent and reliable compared to results obtainable from morphological analysis. This was apparently because environmental influences on populations and individuals (during development) did not interfere with protein identification techniques. For example, Marcusenius senegalensis (mormyridae) obtained from two sub-populations in the Volta basin, showed considerable meristic variation, as expected according to Lowe-McConnell (1972). Analysis of 26 individuals at 22 gene loci however, showed that all individuals were identical (genetic identity = 1). The results on the mormyrid suggest that, perhaps like other species in the Volta basin, M. senegalensis within the basin belonged to a single gene pool. Among the schilbeids

(Schilbe mystus, Eutropius niloticus and Siluranodon auritus) samples of each species obtained from several sources within the Volta basin also showed that they constituted single biological populations.

De Vos and Leveque (1983) concluded that there existed only one species of Schilbe, Eutropius and Siluranodon in the Volta basin of Ghana, based on morphological analysis of samples. A recent report (Abban and Skibinski, 1988) however, showed that, based on starch gel electrophoresis of proteins, there could be cryptic species of Schilbe in addition to the main S. mystus in Volta basin. None of the material studied during the present study was however, genetically different from the 'main-type' of Schilbe. Due to close morphological resemblances between species of Eutropius and also between Schilbe mystus and some species of Eutropius, the advantages of electrophoretic identification over morphology became again very clear.

Usually morphological separation of tilapias is a nightmare to those who seriously attempt it, mainly because morphological and meristic characters of species and even genera often overlap. Starch gel electrophoresis of Oreochromis, Sarotherodon and Tilapia species, showed that irrespective of the development of specialised breeding and feeding habits in the three genera, considerable overlapping of protein character states existed between the groups. However, there were enough protein markers to

identify species even if the proportion of diagnostic loci between tilapias was less than observed amongst the schilbeids or bagridae.

In the last 30 years a great deal of time and effort has been put into the development of biochemical techniques for the identification of fish species (see reviews by Utter et al., 1973; Ferguson, 1980; Lundstrom, 1977). The application of these techniques, mainly gel electrophoresis and isoelectric focusing of proteins, have become everyday tools for the biologist in industrial countries. It is interesting that in Africa and other tropical regions, where, because of the richness of fauna, consistent correct species identification of fish still complicates studies, the new approaches are operationally unavailable. In a continent where fish is often the only source of animal protein in diets of indigenous populations, it is economically very important that fish species, stocks and populations (as defined by Ihssen et al., 1981) be properly identified. Many authors have already mentioned the importance of such knowledge as a basis for the proper utilization, protection and development of fisheries on the African continent. It would suffice here to say that the need of knowing what species exist and their distribution is urgent. The urgency has come about by several human modifications of inland drainage systems by mainly the construction of dams on rivers and diversion of rivers. These changes effectively redistribute fish

fauna and bring into single communities species which might be difficult to separate when sympatic.

There is a great hope that the protein deficiency in less developed countries will be reduced by the development of fish culture. However, the very limited publications of application of electrophoretic analysis of tropical fishes (Kornfield, 1978; McAndrew and Majumdar, 1983, 1984; Tanuguchi et al., 1985), show that the development of tilapia culture ('the hope') would not go very far without the application of available molecular approaches to the identification of species.

Determination of biological relationships amongst related taxa (systematics) after their individual identification is a very old science which influences all biological studies (Dobzhansky, 1973). This aspect of biology has benefitted immensely from recent molecular biological studies. This is because molecular evolutionary investigation has given rise to a number of theories concerning the nature of proteins which are currently analysed for the identification of species and populations. One of these theories is that of the 'Molecular Clock', which concerns the rate of protein evolution. Simply stated, the molecular clock theory implies that proteins evolve at an approximately constant rate. This theory has been justified mainly by the argument that protein evolution is selectively neutral (Kimura, 1983). Although selective justifications have also been offered for the

theory, it is mainly the justification of the neutralists that has made the application of the molecular clock theory to systematics active.

Stemming from the theory it follows that differences between proteins of species should be exactly proportional to the recency of their common ancestor. Therefore species with more similar proteins should have more recent common ancestors. This formed the basis for the two approaches used in constructing relationships amongst the schilbeidae and tilapiine studied here.

For each related group overall similarities of meristic and allozyme character states were used to construct relationships. Since the technique does not consider the evolutionary pathway of species or taxonomic units involved, it is usually termed PHENETIC (Figs. 12a, 12b, 13a and 13b in Chapter 4). Then the allozyme data was again used to construct relationships which take into account the most likely evolutionary pathways of the species involved. Thus the resulting relationships were considered as EVOLUTIONARY (Figs. 12c and 13c for the tilapias and schilbeids respectively).

The results for the schilbeids, Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus by both approaches basically agreed. The two Eutropius species showed that they had a close "sister-species" relationship as defined by Ridley (1986). While

the four species share a more distant ancestor. The results also showed that although S. auritus was far more removed from the Eutropius species than Schilbe was, the Schilbe stalk was not involved with the Eutropius. Thus the morphological resemblances between Schilbe mystus and some species of Eutropius could be related to the relatively shorter independent evolutionary histories the two stalks have had after separation from the common ancestor to the four species. The results here cannot in any way support the new proposal put forward by De Vos (1984) suggesting that the genera Schilbe and Eutropius be combined into a single genus based on morphological similarities. It may also suggest that environmental influences on the morphometrics of Schilbeidae could be very prominent. Thus where there are problems comparing material from different ecological circumstances, protein analysis should be used to support morphological analysis.

Among tilapias three species each of Oreochromis, Sarotherodon and Tilapia were involved in the construction of relationships among the family members. However, for the phenetic relationships based on meristic data only one Oreochromis, two Sarotherodon and two Tilapia species were involved. However, some comparisons could be made between the allozyme based and meristics based phenetic relationships. The most outstanding disparity between the two phenetic relationships obtained was in connection with the relative position of T. busumana in the meristic based relationships.

In the allozyme based phenogram (Fig. 12a), T. busumana, which is considered the least advanced fish among the nine species, occupied a position which was as expected. That is as an outgroup. This expected position was somewhat confirmed in the evolutionary relationships. However, based on meristics T. busumana became 'centrally' placed. "Radiating" from this primitive tilapia was Sarotherodon on either side. On one side the Sarotherodon was connected to Oreochromis and the other to Tilapia (Fig. 12b). What makes this distribution more interesting is the fact that S. melanotheron which seemed to come between T. busumana and the more evolved T. zillii, is also the least developed compared to S. galilaeus which falls on the opposite side of T. busumana with the most recent genus, Oreochromis, based, for example, on reproductive habits. This arrangement would very much support the exponents of the hypothesis that the evolutionary relationships between Oreochromis, Sarotherodon and Tilapia species could not have been sequential (Peters and Berns, 1982).

However, the unrooted evolutionary relationships obtained (Fig. 12c), clearly suggest that T. busumana is evolutionarily more distantly removed from all the Sarotherodon species than it is from Tilapia species. In a species complex with over 70 known species (Trewavas, 1983), a significant contribution to the systematics of the tilapias may have to involve a fair representation of

all major known groups. More significant for both theoretical systematists and tilapia culture scientists, the relative positions of especially T. zillii populations in the relationships obtained suggest that, perhaps tilapiine species could be easily introgressed by other species within the complex. The evolutionary relationships show that T. zillii (MP) is genetically clearly removed from its parent population in the Densu river. The mixed species pond (MP) population which was suspected to have been introgressed by other tilapia in the pond, is also more closely related to the T. zillii stock obtained from Stirling than it is from T. zillii (IAB), which also has the Densu stock as parent population. If culture conditions within a limited time span (less than 10 years in the MP) could bring so much divergence between a parent and derived population of T. zillii, as observed, then there is caution here for the use of cultured stocks of tilapia in the determination of relationships within the complex. The results should also make culturalists aware that unless great care is taken to avoid species mixing under culture conditions, expectations of species performance can easily be undermined by genetic introgression.

Another legacy of molecular advances in genetics to knowledge on fish biology is the possibility of quantifying the extent of biological differentiation involved in the separation of related taxonomic units. Analysis of genetic divergence between taxa directly contributes to systematic consideration of related organisms irrespective of morpho-

logical differences. Results obtained here in relation to divergence among related taxa suggest that genetic similarity or Identity of populations within genera, decreases with increasing taxonomic diversity, as previously indicated by other workers (Ayala and Tracy, 1974; Avise, 1977). It would therefore seem that the results also support the proposed probability of a correlation between biochemical diversity and taxonomic separation as suggested by Thorpe (1983). However, the mean identity between pairs of conspecific populations of Oreochromis, Sarotherodon and Tilapia (cichlidae) were 0.939±0.025, 0.912 and 0.829±0.044 respectively. From the estimates of differentiation between congeneric species of the family, it is apparent that the proposed correlation (Thorpe, 1983) could be probable within genera only, but not across wider taxonomic ranges. Thus the hypothesis that the amount of biochemical divergence between conspecific populations, congeneric species and confamilial genera may be roughly similar across a wide range of taxa (Thorpe, 1983), cannot be supported here. As observed in present analysis, the mean genetic divergence among conspecific populations of Tilapia was 0.829±0.044, which is below the expected value of 0.9 (by Thorpe, 1983). However, within Oreochromis and Sarotherodon, mean identity between conspecific populations were above 0.9. It would seem theoretically arguable, that if genetic divergence and taxonomic ranking were both functions of evolutionary time; the almost uniform amount of biochemical divergence between

conspecific populations, congeneric species and confamilial genera across a wide range of taxa could not be expected. For example, in a family such as the cichlidae, the genus Tilapia may be considered as having diverged from the common ancestor prior to other known genera. It would be expected, as in fact results here show, that genetic divergence among species of Tilapia would be greater than that observed among congeneric species of more recent genera of the family such as Oreochromis.

Protein polymorphism in natural populations as revealed by electrophoresis is perhaps the most widely discussed phenomenon of molecular genetics. It has been repeatedly confirmed by many workers (Lewontin and Hubby, 1966; Lewontin, 1974; Nevo, 1978; Kirpichnikov, 1981; Nevo et al., 1984; Gyllensten, 1985; Kimura, 1983). However, the quantitative significance of deterministic and stochastic forces influencing polymorphism remains actively contested by different biological schools of thought. Together with the acceptance of polymorphism as a natural phenomenon, levels of polymorphism or variability have been estimated for almost all major taxonomic groups (Lewontin, 1974; Ayala, 1976; Powell, 1975; Nevo et al., 1984). All reports on polymorphic levels of gene loci and populations have emphasised the proportion of polymorphic loci (P) and heterozygosity (H) as measures of variability. Of the two measures, H has been preferred. It is highly correlated with P but more importantly, H measures variability independent of sample

size and criteria used to estimate P in any single situation (Nevo *et al.*, 1984).

It is perhaps surprising that the number of loci coding for an enzyme in a taxon or the possible relationship between number of loci and the conventional measurement of *P* and *H* have received only passing references (as in Kirpichnikov, 1981; Lavie *et al.*, 1987). Although it has been observed that a progressive increase in number of loci occurs from the most primitive fishes to the most advanced species (Markert *et al.*, 1975; Fisher and Whitt, 1978; Whitt, 1981b; all quoted by Whitt, 1983), it has also been suggested that increase in numbers of isozyme loci appear to be due to both ancient and recent polyploidization events as well as tandem-duplication (Whitt 1983; Harris and Hopkinson, 1978, pp. 1-2). Evidence here suggests that protein polymorphism is not completely independent of protein evolution, as all the above and Kimura (1983) indicate. It can be said, therefore, that the process of genetic differentiation of major taxa (e.g. families) may be deduced from the number of loci coding for enzymes in general.

Results obtained here show a general uniformity in the number of loci coding for an enzyme within a family of fishes. Often, the number of loci for an enzyme within a family differ from that of other families. For example, no esterase activity was observed in the mormyrid, *Marcusenius senegalensis* indicating "zero locus". This

observation, despite trying different extraction, gel and electrode buffers, could however be interpreted as being due to the fact that the product(s) of the esterase gene locus in the species were not reactive to the substrate provided in the staining recipe. However, the "zero locus" interpretation given here holds because all other species reacted to the same procedure. For the same enzyme, esterase, Schilbe mystus, Eutropius niloticus, E. mandibularis and Siluranodon auritus (fm. Schilbeidae), three loci could be scored. The bagridae, Chrysichthys auratus and C. nigrodigitatus together with all the five cichlids were active at four loci. Other examples of this apparently family related "number of loci per enzyme" were observed in connection with ADH, AP, αGPDH, 6GPDH and SDH.

The need to account for the number of loci in the estimation of variability for a large assemblage of organisms such as fishes becomes more obvious when it is appreciated that in multilocus enzymes only one locus is usually polymorphic (Kirpichnikov, 1981, pp. 158-199). In the present study also, only one locus in multilocus enzymes were appreciably polymorphic. If, as revealed by Kirpichnikov's review and shown to some extent by results here, loci in multilocus enzymes are not equally polymorphic in most cases, then the following merit consideration in the estimation of variability:

i) The number of loci coding for individual enzymes in taxa being compared - to compensate for differences

in variability which might be attributed to differences in number of loci coding for an enzyme in two taxa.

ii) Perhaps enzyme and population variability should be estimated on the basis of each enzyme having a single locus and as many alleles as there may be.

iii) The possibility of considering number of loci per enzyme as a gross indicator of the differentiation process within fishes.

Information on individual enzyme polymorphism in fishes has been summarised by Kirpichnikov (1981, pp. 185-199). Nevo et al. (1984), while reviewing variability in 183 species of fish in relation to various ecological, demographic and life history characteristics, considered the polymorphic levels in fish categories in relation to parameters considered. Both accounts in spite of their depth of analysis made no distinction between marine and freshwater fishes. However, Avise and Smith (1974a) had reason to believe that the discontinuous nature of the aquatic habitat may affect the genetics of fresh water fishes in a way which would lower heterozygosities. Later, Gyllensten (1985) indicated that heterozygosity was higher in marine compared to freshwater species. He went further to suggest that more attention was to be paid to differences in the distribution of variability within species in different groups of fishes. Thus, the possible differences between freshwater and marine fishes with respect to population variability and locus polymorphism

of enzyme remains unresolved. Distribution and level of polymorphism (P) of twelve enzymes in thirteen species, representing four freshwater fish families show that some enzymes, here EST and ME, were widely polymorphic. Kirpichnikov (1981) also considered EST as being highly polymorphic in many species and attributed the situation to the varied metabolic activities to which esterases were associated. He however, found information to be scarce on the polymorphism of malic-enzyme (ME). I suspect the situation could be attributed to the majority of species reviewed by Kirpichnikov being marine. There is therefore reason to suggest that, enzymes found to be commonly polymorphic in marine fishes may not necessarily be expected to be so in freshwater species. The same reason could account for his considering IDH as highly polymorphic while it was observed here to be monomorphic in nearly all species.

Lactate dehydrogenase (LDH) also considered by Kirpichnikov (1981) to be commonly polymorphic among fishes, could not be considered as such based on present results. The monomorphism of loci of the enzyme in the two species of Chrysichthys, representing family bagridae was distinctive. Together with that the distribution and polymorphic criteria of six of the thirteen enzymes studied strongly indicated family group association.

The present results may be considered inadequate to support broad generalizations. However, that all the

species involved were sympatric in the study area rivers, made the differences in the distribution patterns and levels of polymorphism in the different groups of fishes of significance biologically. It can therefore be concluded that the total freshwater environment, while influencing enzyme polymorphism, affects different fish groups differentially. It is very possible that the differences between fish groups could be a reflection of their evolutionary status as well as their niches in the water.

Observations made here suggest that the general expected patterns and levels of variability in populations may need a further consideration of freshwater fishes as distinct from marine fishes. Also evolutionary histories of species as well as populations are necessary parameters. Finally, it may be necessary to accumulate more information on tropical fishes for generalisations to be made.

* * * * * * * * *

APPENDIX I

ENZYME STAINING RECIPES

ADA

Adenosine	15 mg.
Sodium Asenate	50 mg
MTT	7 mg
XOD	50 µl
NP	20 µl
PMS	Trace
Tris-HCl	30 mls.

Agar overlay

ADH

NAD	15 mg.
MTT	6 mg.
Tris-HCl (0.2M) pH 9.0	30 mls.
Iso-propanol	0.75 mls.
PMS	Trace

AP

Glycyl-L-leucine	20 mg.
Peroxidase	10 mg.
L-amino acid oxidase	5 mg.
δ-diamisidine	5 mg.
$MnCl_2$	10 mg.
Tris-HCl (pH 8)	25 mls.

Agar overlay

CK

Phosphocreatine	40 mg.
ADP	15 mg.
Glucose	30 mg.
$MgCl_2$	5 mg.
NADP	6 mg.
MTT	7 mg.
HK/G6PDH	30 µl
PMS	Trace
Tris-HCl	25 mls.

Agar overlay

EST

Fast Blue RR (or BB)	20 mg.
αnaphthyle acetate	2 ml.
Tris maleate pH 5.3	30 mls.

Incubate gel in Tris maleate pH 5.3 - 30 minutes.

FUM

NAD	30 mg.
MTT	7 mg.
Fumic Acid	200 mg.
PMS	Trace
MDH	5 l
Tris-HCl pH - 7.0	30 mls.

αGPDH

NAD	15 mg.
MTT	7 mg.
α-glycerophosphate	20 mg.
EDTA	60 mg.
PMS	Trace
Tris-HCl pH 8.0	30 mls.

G6PDH

Glucose-Phosphate	10 mg.
EDTA	10 mg.
NADP	7 mg.
MTT	7 mg.
PMS	Trace
Tris-HCl pH 7.1	3.3 mls.
H_2O	30 mls.

HK

Glucose	60 mg.
$MgCl_2$	100 mg.
ATP	15 mg.
NADP	5 mg.
MTT	7 mg.
6GPDH	20 µℓ
Tris-HCl pH 7.0	30 mls.
PMS	Trace

IDH

Sodium Isocitric Acid	50 mg.
$MgCl_2$	10 mg.
NADP	4 mg.
MTT	7 mg.
PMS	Trace
Tris-Hcl	30 mls.

LDH

NAD	15 mg.
MTT	7 mg.
Na Lactate	1 ml.
PMS	Trace
Tris-HCl	30 mls.

MDH

L-Malic Acid	150 mg.
MTT	6 mg.
NAD	10 mg.
Tris	600 mg.
PMS	Trace
H_2O	30 mls.

ME

DL Malic Acid	60 mg.
NADP	10 mg.
$MgCl_2$	10 mg.
MTT	7 mg.
PMS	
Tris-HCl	30 mls.

MPI

Mannose-6-phosphate	10 mg.
NADP	5 mg.
MTT	7 mg.
$MgCl_2$	10 mg.
G6PDH/PGl	70 µl/30µl
Tris HCl pH 8.0	25 mls.
PMS	Trace
Agar overlay	

ODH (Octonol dehydrogenase)

NAD	15 mg.
MTT	6 mg.
Octanol	0.75 mls.
Tris-HCl 0.2M pH 9	30 mls.
PMS	Trace

6PGDH

6-Phosphogluconic Acid	10 mg.
NADP	5 mg.
MTT	5 mg.
Tris-HCl pH 8.0	40 mls.
PMS	Trace

PEP

Leucyl-L-leucine	20 mg.
Peroxidase	10 mg.
L-amino-acid-oxidase	5 mg.
σ-Diamisidine-HCl	5 mg.
Tris-HCl pH 8.0	20 mls.

Agar overlay.

PGI

Fructose-6-Phosphate	20 mg.
NADP	4 mg.
$MgCl_2$	20 mg.
MTT	7 mg.
6-GPDH	50 µℓ
PMS	Trace
Tris-HCl 0.2 M	30 mls.

PGM

$Na\text{-}G\text{-}PO_4$	50 mg.
$MgCl_2$	70 mg.
NADP	3 mg.
MTT	7 mg.
Tris-HCl pH 8.0	30 mls.
G6PDH	50 µℓ
PMS	Trace

SDH

Sorbital	150 mg.
NAD	15 mg.
$MgCl_2$	10 mg.
MTT	7 mg.
PMS	Trace
Tris-HCl	30 mls.

SOD

MTT	7 mg.	(a) in 15 mls. of $Tris\text{-}HCl_2$ pH 8.0
$MgCl_2$	20 mg.	
PMS	Trace	

b) Leave (a) under UV light for 15 min. till gel goes violet.

c) Pour on the remaining solution and incubate at $30^{o}C$.

XDH

Hypoxanthine	20 mg.
NAD	15 mg.
MTT	7 mg.
PMS	Trace
Tris-HCl pH 8.0	30 mls.

BUFFER SOLUTIONS

Continuous Tri-citrate (CTC):

Electrode: 0.25 M, 0.057 citric acid pH 8.0
i.e. 30.29 g. Tris and 11.98 g. citric acid in 1 ℓ Dis.H_2O.

Gel buffer: 8½ ml. Electrode buffer in 211.5 ml. Dist.H_2O to make 220 mls. (Ward and Beardmore, 1977).

Continuous Tris EDTA-Borate (TEB):

Electrode: 0.50 Tris; 0.016 M EDTA; 0.24 Boric acid pH 8.5, i.e. 60.57 Tris + 5.99 EDTA and 15.0 Boric acid in 1 ℓ Dis.H_2O.

Gel buffer: Dilute electrode buffer with distilled water 1:10 (22 mls. of buffer:198 H_2O) (Shaw and Prasad, 1970).

Discontinuous Tris Citrate (Poulik, DTC):

Electrode: 0.30 Borate, 2.40 Sodium hydroxide per liter of dist. water pH 8.2.
i.e. 18.55 g. Boric acid; 2.40 g. NaOH, per litre of dist. water.

Gel buffer: 0.076 M Tris; 0.005 M citric acid pH 8.7
i.e. 9.21 g. Tris; citric acid 1.05 in 1 litre dist. water. (Ward and Beardmore, 1977).

APPENDIX Ib:

HOW TO ANALYSE PROTEINS WITH LKB AMPHOLINE^{R} PAG PLATES STEP BY STEP

1. Connect the Multiphor to the Multitemp thermostatic circulator and set a temperature given in Practical Information. If another circulator is used, make sure it delivers a flow of 6-10 ℓ/min. Switch on the Multitemp II 20 minutes before starting the experiment. Make up the electrode solutions (see Practical Information) in the bottles provided. Cut off the spout. Store the bottles in a refrigerator.

2. Open the package from the transparent side.

Note: The gel surface is in contact with the aluminium cover. Cut on all four sides about 1 cm from the gel edge. Do not cut away the gel support protruding at the ends. If only part of the plate is to be used, cut through the package with sharp scissors, reseal the package with sticky tape or plastic film and store the rest of the plate in the refrigerator for later use. Remove the transparent plastic. Separate the gel from the aluminium cover and place the gel on the Multiphor.

3. Place the gel on the cooling plate, with some insulating fluid (Kerosene or light paraffin oil) in between, and centre it to the middle of the plate. Use a minimum of fluid to avoid trapping air bubbles. If you use the Multiphor with the glass cooling plate, first lay a template on the cooling plate with insulating fluid in between, then proceed as above.

4. Soak the electrode strips evenly with the appropriate electrode solutions. Remove excess solution with tissue paper or filter paper.

Apply the electrode strips close to the long edges of the gel (Cathode strip to the left, Anode strip to the right). Using sharp scissors, cut off the parts of the strips which protrude beyond the end of the gel.

5. Sample application: Apply dry sample application pieces to the surface of the gel using the template screen printed on the cooling plate (or paper template, if used) as a guide.

The best application area can be determined in a trial run by applying the sample at different positions, so that the proteins do not focus underneath the application piece. Most proteins give best results when applied 1-3 cm. from the cathode. Apply the samples (recommended volume 15-20/µℓ conc. 0.5-2 mg/ml for Coomassie staining) by means of micropipette. Very small volumes (e.g. 2/µℓ

can be applied as droplets directly onto the gel surface.

6. Place the electrode holder on the Multiphor unit and align the electrodes with the centre of the electrode strips. Finally place the safety lid in position.

7. Connect the power supply. Follow practical information for values to set and running times. Do not run the electrofocusing experiment too long, otherwise the pH gradient will start to drift towards the cathode.

8. Remove the sample application pieces after approximately half the focusing time. Switch off the power, take off the cover lid and electrode holder, and remove the pieces with forceps. Replace the covers and continue the experiment.

9. The pH gradient can be determined by placing a surface pH electrode on the gel at several positions (approx. 10) between the anode (+) and cathode (-). The pH electrode should be calibrated and the pH determined at the temperature of the experiment. Plot the pH curve on the Experimental result form. It is advisable to refocus the zones for 10 min. after measuring the pH.

10. Fixing and staining. Remove the electrode strips by using forceps. Immediately place the PAG plate in the Multiphor Staining Kit containing fixing solution (for all solutions see Practical Information) for 0.5-1 hour. This solution precipitates the proteins and allows the Ampholines to diffuse out.

Before staining, wash the PAG plate in destaining solution for 5 minutes. Remove the destaining solution and stain the PAG plate for 10 minutes in staining solution which has been preheated to 60°C. Cover the staining dish. Destain the PAG plate with several changes of the destaining solution until the background is clear.

11. Preserving gel. Place the destained PAG plate in the glycerol preserving solution for 1 hour. Then place it on a glass plate and allow it to dry at room temperature until the gel has a sticky surface.

12. Cover the gel with the plastic preserving sheet, by carefully rolling it into the gel using a rubber roller. Avoid trapping air bubbles. In order to remove excess glycerol on the plastic support film use alcohol or water.

APPENDIX 2 - Table A: Marcusenius senegalensis (Steindachner, 1870). R. Pru (Volta basin). Morphological measurements.

Sp.No.	S.L.	B.D.	H.L.	E.D.	Iob.D.	D	A	Ll.Sc.	S.
1	168.8	43.2	32.3	2.9	11.2	26	31	65	12
2	165.8	47.3	34.0	3.0	11.4	26	32	66	12
3	162.6	52.9	35.5	3.1	12.9	26	31	67	12
4	180.5	47.5	34.5	3.2	11.3	26	32	72	12
5	173.0	42.5	34.0	2.7	10.7	27	31	59	12
6	138.4	40.4	28.5	2.7	9.7	26	31	73	12
7	156.9	38.9	33.0	2.7	9.9	27	31	67	12
8	132.5	37.4	28.9	2.9	8.9	25	31	71	12
9	137.8	39.4	28.4	2.5	9.2	24	30	71	12
10	143.5	42.4	29.0	2.8	10.2	26	31	66	12
11	144.5	40.1	31.7	2.7	10.7	26	31	69	12
12	126.5	33.3	24.5	2.4	7.5	27	31	69	12
13	120.5	34.1	25.0	2.6	8.6	27	33	66	12
14	128.1	30.3	24.8	2.4	7.4	26	30	69	12
16	139.5	36.1	28.5	2.5	9.4	-	-	67	12
17	123.1	30.4	25.0	2.5	8.3	26	32	69	12
18	116.8	24.5	22.7	2.2	6.7	25	32	70	12
19	174.4	52.5	34.0	2.4	10.6	27	33	67	12
21	167.8	42.8	31.8	2.5	10.6	26	31	67	12
22	186.0	50.6	34.5	2.9	11.2	26	33	72	12
					M. senegalensis (Oti R. Volta Basin)				
1	180.0	48.3	37.0	3.2	12.1	26	32	76	12
2	134.4	33.9	28.0	2.8	8.1	25	31	73	12
3	165.0	44.4	32.5	2.8	10.0	26	32	72	12
4	143.6	40.4	28.5	2.5	8.8	25	30	69	12
5	156.1	43.5	31.9	2.4	9.8	26	32	69	12
6	157.4	45.1	32.4	2.5	10.9	23	32	73	12
7	183.8	53.5	49.7	2.8	12.8	27	31	66	12
8	178.7	46.5	34.7	2.5	11.8	22	34	71	12

Fish: Schilbe mystus Coll. Ped.: June-August, 1986. APPENDIX 2 Table B(i)

Loc.: Oti/Sabari (1-16) Sub.: Morphological Measurements.
Pru/Asubenda (17-26)

	St.L.	B.Dth.	H.L.	H.W.	M.W.	E.D. (H)	E.D. (V)	Iob.W.	Sn.Lth.	Pec.	Pel.	An.	Dor.	Sn.D.	Sn-Ad.	Sn.Pel.	D. B.Lth.	Ad. B.Lth.	Pel. B.Lth	An. B.Lth	C.P. Lth.	C.P. Wth.	Gill rakers	D.Sp. Lth.	Pec-Pel.	Distance between Ant. Nos.	Post. Nos.	Adipose (+)/(-)
1	148.3	37.4	32.3	23.8	18.2	5.6	5.6	19.0	6.0	I-11	I-5	III-59	I-6	49.0	-	56.0	9.9	-	8.2	75.7	8.8	10.6	12	25.7	23.4	12.1	11.2	-
2	170.4	40.4	36.7	26.8	18.7	6.8	6.8	18.2	5.6	I-10	I-5	III-56	I-6	53.5	-	62.7	10.4	-	8.8	96.8	8.9	12.4	13	27.2	30.7	13.8	11.6	-
3	205.6	54.8	46.3	35.5	25.5	6.8	6.8	26.4	10.0	I-10	I-5	III-55	I-6	69.1	-	75.8	13.4	-	10.7	102.3	12.5	16.7	12	34.3	28.5	18.3	15.8	-
4	156.9	41.5	35.7	26.1	18.2	6.5	6.5	17.9	7.4	I-10	I-5	III-58	I-6	53.8	-	61.7	9.1	-	7.2	77.6	8.9	10.75	13	30.2	25.5	13.0	10.0	-
5	161.4	39.7	36.4	25.4	19.2	6.3	6.3	19.5	6.7	I-11	I-5	III-55	I-6	53.8	-	58.8	11.3	-	8.0	80.7	8.0	11.1	12	28.7	24.5	11.8	10.6	-
6	208.5	56.4	47.2	33.5	23.3	7.0	7.0	23.2	12.0	I-10	I-5	III-54	I-5	72.6	-	79.1	13.7	-	9.9	108.4	10.5	15.0	13	34.4	32.7	16.4	14.4	-
7	234.3	65.2	51.7	40.5	28.6	7.4	7.4	30.9	12.1	I-10	I-5	III-55	I-6	82.8	-	92.4	12.2	-	11.4	119.5	13.7	18.6	13	37.7	40.4	19.6	17.3	-
8	203.1	57.0	44.5	34.1	24.7	8.4	8.4	23.7	7.9	I-11	I-5	III-58	I-6	67.8	-	72.5	13.4	-	11.5	108.1	12.8	14.0	13	32.6	34.9	17.6	14.7	-
9	193.9	46.8	40.7	30.5	21.7	7.1	6.3	21.8	9.9	I-10	I-5	III-61	I-6	60.6	-	73.1	11.1	-	8.8	101.5	13.0	12.9	14	-	32.0	15.4	12.8	-
10	106.7	22.9	23.15	15.5	12.8	5.6	5.0	11.9	5.1	I-10	No Pelvic	III-60	I-5	33.5	-	-	6.3	-	-	55.8	6.3	6.6	13	16.6	-	9.2	7.2	-
11	233.0	65.2	52.4	38.2	28.5	8.3	8.3	29.4	14.3	I-11	I-5	III-57	I-6	79.7	-	90.6	15.4	-	12.7	114.6	12.8	17.4	13	40.7	38.0	19.4	17.7	-
12	222.0	61.2	49.6	37.6	28.1	7.7	6.9	26.6	12.8	I-10	I-5	III-55	I-6	72.4	-	81.2	15.5	-	11.5	107.1	15.0	16.0	13	37.3	34.0	18.8	17.1	-
13	224.0	60.8	47.7	35.1	26.4	7.4	7.4	26.9	13.0	I-10	I-5	III-58	I-6	72.7	-	82.0	15.1	-	12.2	117.4	13.5	17.1	10	-	36.7	17.8	16.3	-
14	228.8	68.9	50.1	37.0	27.0	7.4	7.3	26.7	11.5	I-11	I-5	III-57	I-6	76.4	-	85.0	14.4	-	12.1	110.3	14.6	15.5	12	32.2	36.1	19.5	16.0	-
15	226.7	61.3	48.9	37.0	26.7	7.7	7.0	27.4	12.1	I-10	I-5	III-53	I-6	76.8	-	82.0	14.5	-	12.0	117.5	13.0	16.7	13	-	36.7	18.6	16.4	-
16	198.0	49.5	41.75	30.9	21.6	7.3	6.8	23.0	9.8	I-11	I-5	III-59	I-6	64.4	-	64.7	13.1	-	10.3	107.9	11.9	14.9	11	33.0	27.0	15.7	14.0	-
17	126.5	27.3	26.6	17.7	12.0	4.8	4.7	10.4	7.2	I-10	I-5	III-58	I-6	41.5	-	44.3	7.7	-	6.0	71.7	7.2	10.3	12	18.5	17.5	8.2	7.0	-
18	146.4	32.9	32.3	22.6	16.0	6.4	5.9	13.5	7.4	I-10	I-5	III-57	I-6	47.8	-	56.9	7.7	-	7.6	70.0	8.8	12.0	13	21.6	22.1	10.3	9.1	-
19	111.1	26.5	27.6	17.8	14.1	4.0	4.5	11.6	7.6	I-10	I-5	III-58	I-6	38.3	-	40.5	6.9	-	4.3	56.6	6.5	6.4	13	19.2	12.7	9.4	8.5	-
20	162.7	40.4	38.0	28.2	20.0	5.5	5.1	18.7	8.2	I-10	I-5	III-53	I-6	55.0	-	63.5	9.8	-	6.9	82.8	9.4	11.4	13	29.6	26.0	13.3	11.4	-
21	115.2	24.6	26.6	16.7	10.4	5.2	4.5	10.6	5.9	I-10	I-5	III-52	I-6	38.9	-	44.1	6.3	-	5.5	58.5	5.6	8.9	14	17.4	18.3	7.5	6.8	-
22	106.3	21.1	24.0	15.2	9.8	4.2	3.6	9.2	6.0	I-10	I-5	III-57	I-6	33.3	-	38.1	6.9	-	4.6	57.3	4.6	8.1	12	17.0	14.9	7.0	6.5	-
23	152.9	32.2	34.0	21.5	14.3	6.4	5.0	14.0	8.2	I-10	I-5	III-60	I-6	49.3	-	56.5	8.2	-	7.1	76.4	10.4	10.6	13	-	22.9	9.5	9.2	-
24	138.6	30.0	32.7	19.6	15.1	5.2	5.0	13.0	6.7	I-10	I-5	III-54	I-6	48.3	-	51.2	7.9	-	6.8	65.4	8.7	10.1	13	23.2	18.8	10.9	9.4	-
25	150.8	36.4	37.8	25.2	16.8	6.6	6.1	15.8	8.9	I-10	I-5	III-54	I-6	53.5	-	64.2	9.8	-	7.0	81.4	9.0	13.1	13	22.4	24.3	12.2	10.4	-
26	115.8	22.6	24.7	15.8	11.1	5.8	4.6	10.1	5.8	I-10	I-5	III-53	I-6	35.9	-	39.5	6.0	-	5.8	62.3	6.3	8.4	14	18.8	15.5	7.9	7.1	-

Fish: Eutropius niloticus Coll. Ped.: (June-July, 1986.) APPENDIX 2 Table B(ii)

Loci: 1-15; Oti/Sabari. 16-28 Wt. Volta/Daboya Sub.: Morphological Measurements.

	St.L.	B.Dth.	H.L.	H.W.	M.W.	E.D. (H)	E.D. (V)	Iob.W.	Sn.Lth.	Pec.	Pel.	An.	Dor.	Sn.D.	Sn-Ad.	Sn-Pel.	D. B.Lth.	Ad. B.Lth.	Pel. B.Lth.	An. B.Lth.	C.P. Lth.	C.P. Wth.	Gill rakers	D.Sp. Lth.	Pec.-Pel.	Distance between Ant. Nos.	Distance between Post Nos.	Adipose (+)/(-)
1	121.9	25.3	22.0	14.6	9.2	6.0	5.2	8.6	4.4	I-9	I-5	III-56	I-6	35.2	103.1	40.3	6.9	1.0	7.8	59.0	13.5	10.3	15	18.3	19.5	5.4	5.7	+
2	107.5	23.7	20.5	13.7	8.3	5.9	4.4	7.5	4.0	I-9	I-5	III-54	I-6	31.2	92.4	34.6	6.4	1.0	6.9	51.9	11.6	9.1	15	13.4	17.1	4.5	5.5	+
3	109.8	22.5	20.9	13.4	8.4	5.5	4.3	7.4	3.5	I-9	I-5	III-55	I-6	32.2	92.2	38.6	6.3	1.0	6.3	56.7	10.5	9.0	15	16.9	17.5	4.7	5.5	+
4	115.5	25.5	21.0	13.7	8.1	9.9	5.0	8.5	4.1	I-9	I-5	III-56	I-6	33.8	96.0	37.5	6.1	1.1	5.8	58.7	13.0	8.7	14	17.3	17.5	5.5	5.9	+
5	113.4	23.9	21.2	14.0	8.3	5.5	4.5	8.3	3.2	I-9	I-5	III-56	I-6	34.3	94.6	40.8	6.5	1.1	6.8	55.5	12.5	8.5	16	16.6	20.6	5.6	5.8	+
6	123.9	28.6	22.9	15.3	9.0	6.5	6.0	9.1	5.0	I-9	I-5	III-51	I-6	35.5	102.2	42.9	6.1	1.2	7.5	60.7	14.9	10.4	14	17.5	20.0	5.4	5.9	+
7	110.8	26.4	21.0	14.5	8.5	6.1	5.2	8.7	3.5	I-9	I-5	III-55	I-6	33.4	95.7	38.5	7.2	1.0	6.6	55.6	11.6	9.5	15	16.2	18.5	5.1	5.4	+
8	117.8	26.4	21.8	13.0	8.8	5.4	4.9	8.6	3.4	I-9	I-5	III-53	I-6	34.6	100.5	39.5	6.6	1.2	8.5	58.5	12.9	8.8	15	17.6	17.7	5.1	6.0	+
9	109.4	22.5	20.2	13.5	8.5	5.1	4.4	7.6	3.7	I-9	I-5	III-55	I-6	30.7	92.6	39.3	5.6	0.9	5.5	52.5	12.0	8.5	15	16.4	16.8	5.0	5.4	+
10	128.9	28.0	23.4	14.9	10.0	5.5	5.0	10.2	3.9	I-9	I-5	III-57	I-6	36.9	110.0	48.1	7.0	1.2	8.2	61.6	13.4	10.3	15	20.8	24.7	5.5	6.7	+
11	111.2	24.3	21.7	14.2	8.9	6.0	5.1	8.5	3.1	I-9	I-5	III-52	I-6	33.2	94.4	40.3	5.8	1.1	7.7	52.1	10.4	9.2	15	17.1	17.7	5.3	6.0	+
12	146.4	31.5	27.3	18.8	11.5	6.0	5.1	11.4	4.9	I-9	I-5	III-59	I-6	43.0	126.7	51.5	8.5	1.5	9.4	72.7	15.0	11.8	15	19.9	24.5	7.5	8.0	+
13	118.2	27.4	22.8	14.8	9.1	5.5	4.8	9.8	3.8	I-9	I-5	III-52	I-6	37.0	102.4	42.8	7.1	1.5	6.8	58.5	11.8	9.5	16	18.0	20.2	6.2	6.7	+
14	103.3	23.4	19.0	13.0	7.9	5.5	4.9	7.7	3.3	I-9	I-5	III-56	I-6	30.8	86.5	35.7	6.2	1.0	6.9	50.4	11.0	8.7	14	13.1	16.3	4.5	5.1	+
15	162.2	35.6	31.0	21.0	13.0	6.5	5.6	12.5	6.0	I-9	I-5	III-54	I-6	49.9	136.9	55.4	9.5	1.8	9.5	78.8	18.4	13.1	15	23.7	24.7	8.0	8.5	+
16	110.0	23.6	20.5	13.8	8.2	5.8	4.9	7.8	4.5	I-9	I-5	III-53	I-6	31.7		38.9	6.3	1.1	6.4	53.8	11.8	9.2	16	14.8	18.5	5.2	5.6	+
17	127.6	23.9	23.8	15.5	9.9	7.9	6.5	9.1	4.5	I-9	I-5	III-55	I-6	38.5		44.0	7.6	1.5	8.2	62.2	12.3	9.2	17	18.9	22.5	6.1	6.4	+
18	107.8	24.5	20.1	13.5	8.3	5.3	4.8	8.1	3.7	I-9	I-5	III-60	I-6	32.2		38.6	5.2	1.3	5.6	51.1	11.2	8.6	13	14.6	17.4	5.1	5.4	+
19	108.1	23.8	20.4	13.3	8.7	5.5	4.6	9.0	6.1	I-9	I-5	III-54	I-6	32.0		39.3	6.0	1.0	6.1	51.4	11.4	8.9	15	15.2	17.4	5.1	5.6	+
20	102.8	23.8	19.0	11.9	7.8	5.3	4.3	7.8	3.3	I-9	I-5	III-55	I-6	28.9		34.5	5.9	1.2	4.8	51.5	10.1	8.5	16	15.1	16.5	4.5	5.2	+
21	110.7	23.9	20.5	14.5	9.0	5.1	4.5	8.5	3.0	I-9	I-5	III-55	I-6	32.1		39.4	6.1	1.1	5.5	52.5	10.9	8.9	14	15.0	19.4	4.5	5.8	+
22	104.1	24.2	20.5	13.3	8.0	5.7	4.5	8.5	3.1	I-9	I-5	III-54	I-6	31.2		36.7	6.4	1.2	6.4	50.7	10.0	7.8	13	16.9	18.1	4.3	4.5	+
23	114.2	25.4	21.5	13.2	8.3	5.7	4.8	9.3	4.7	I-9	I-5	III-56	I-6	34.5		40.0	6.4	1.5	7.3	57.8	11.3	8.8	14	15.4	17.9	5.3	5.5	+
24	113.3	24.8	20.8	14.5	8.2	6.0	5.6	8.6	4.2	I-9	I-5	III-53	I-6	33.3	94.1	39.8	6.0	0.9	7.1	55.0	11.0	9.6	14	17.1	19.5	5.4	5.5	+
25	123.3	24.4	22.2	15.0	9.4	6.4	4.9	9.9	4.5	I-9	I-5	III-58	I-6	34.6	103.4	41.4	7.1	1.2	6.7	62.8	12.8	10.2	16	19.0	18.3	5.3	6.1	+
26	108.5	23.2	21.5	13.9	7.9	6.3	5.0	8.5	3.6	I-9	I-5	III-55	I-6	32.8	92.7	39.7	5.9	1.4	6.5	54.6	10.0	9.1	15	17.5	18.5	5.3	5.4	+
27	105.6	23.8	20.5	13.4	7.8	5.4	4.3	8.5	3.6	I-9	I-5	III-58	I-6	31.7	91.5	37.0	5.6	1.0	6.3	54.4	11.5	8.5	14	17.1	16.9	4.5	4.5	+
28	108.8	23.8	20.5	13.4	8.2	5.9	5.1	8.4	3.4	I-9	I-5	III-60	I-6	33.1	93.3	37.4	5.7	1.2	6.6	51.0	11.2	8.5	14	17.6	18.8	5.2	5.5	+

NOT MEASURED

Fish: Siluranodon uritus Coll. Ped.: June-July 1986. APPENDIX 2 Table B(iii)

Loc.: Black Volta/Bamboi Sub.: Morphological measurements

	St.L.	B.Dth.	H.L.	H.W.	M.W.	E.D. (H)	E.D. (V)	Iob.W.	Sn.Lth.	Pec.	Pel.	An.	Dor.	Sn.D.	Sn-Ad.	Sn.Pel.	D. B.Lth.	Ad. B.Lth.	Pel. B.Lth.	An. B.Lth.	C.P. Lth.	C.P. Lth.	Gill rakers	D.Sp. Lth.	Pec./Pel.	Distance between Ant. Nos.	Distance between Post Nos.	Adipose (+)/(-)
1	106.9	22.6	19.9	10.7	7.7	4.4	3.9	8.8	3.5	I -10	I-5	74	0-5	29.6	-	31.0	1.9	71.4	3.1	-	2.5	8.0	-	-	5.1	5.1	3.1	-
2	107.5	23.7	21.3	13.0	8.1	4.6	3.7	9.4	3.9	I -10	I-5	70	0-5	29.9	-	32.5	1.7	70.1	3.5	-	2.2	8.5	-	-	5.3	5.3	3.7	-
3	104.6	23.2	21.2	12.5	7.1	4.0	3.5	9.6	3.5	I -10	I-5	69	0-5	30.4	-	32.1	2.0	68.8	2.7	-	3.2	9.0	-	-	5.3	5.3	3.3	-
4	104.3	24.8	21.1	12.8	7.0	4.8	4.0	8.7	3.2	I -10	I-5	70	0-5	27.1	-	29.0	1.9	66.1	3.0	-	2.5	7.9	-	-	4.8	4.8	2.9	-
5	107.5	24.8	21.0	11.4	7.5	4.4	3.6	9.1	3.4	I -10	I-5	68	0-5	31.3	-	32.5	1.7	67.8	2.3	-	3.5	7.5	-	-	4.6	4.6	3.2	-
6	101.4	24.0	20.5	9.5	7.3	4.5	4.0	8.0	3.7	I -10	I-5	70	0-5	29.4	-	33.5	1.8	63.7	3.3	-	2.8	7.6	-	-	4.6	4.6	3.2	-
7	104.5	24.8	21.4	10.0	7.7	4.3	4.2	8.7	4.0	I -10	I-5	74	0-5	31.5	-	34.2	1.8	67.5	3.1	-	2.7	8.3	-	-	4.0	4.0	2.7	-
8	103.9	25.0	21.1	9.5	7.6	4.3	4.0	7.6	3.9	I -10	I-5	-	0-5	29.5	-	35.0	2.0	55.1	3.2	-	2.5	8.6	-	-	4.4	4.4	3.0	-
9	96.0	22.4	18.8	9.5	6.9	3.7	3.2	7.5	3.2	I -10	I-5	70	0-5	27.9	-	31.9	2.0	60.6	2.8	-	2.5	7.8	-	-	4.0	4.0	2.4	-
10	108.5	25.0	20.7	11.2	7.3	4.3	3.6	8.6	3.5	I -10	I-5	70	0-5	30.7	-	33.4	2.0	71.7	2.8	-	2.6	8.9	-	-	4.9	4.9	3.6	-
11	107.8	25.2	21.3	11.6	8.1	4.4	3.5	8.0	3.4	I -10	I-5	71	0-5	32.4	-	34.0	2.1	67.6	3.0	-	2.7	8.7	-	-	5.3	5.3	3.2	-
12	103.4	23.3	21.4	11.4	8.0	4.0	3.2	9.1	2.7	I -10	I-5	67	0-5	31.7	-	36.4	1.6	72.1	2.9	-	3.1	8.2	-	-	4.7	4.7	3.1	-
13	113.3	27.9	22.2	11.3	8.0	5.6	4.2	8.6	3.9	I -10	I-5	70	0-5	31.8	-	37.6	2.3	73.1	3.0	-	2.7	8.5	-	-	5.5	5.5	3.5	-
14	106.9	23.4	20.2	9.8	8.0	4.5	3.6	8.4	3.5	I -10	I-5	70	0-5	28.5	-	35.7	1.6	68.9	2.4	-	2.8	7.9	-	-	4.4	4.4	2.2	-
15	105.7	23.3	20.3	10.5	7.0	4.7	3.8	7.6	3.5	I -10	I-5	70	0-5	30.3	-	34.5	2.1	69.8	2.4	-	3.4	8.2	-	-	4.4	4.4	2.5	-
16	109.6	24.0	21.4	11.8	8.0	5.0	4.1	8.1	3.4	I -10	I-5	71	0-5	31.3	-	34.4	2.2	70.2	2.4	-	4.1	8.7	-	-	5.9	5.9	3.0	-
17	101.1	22.5	20.1	11.3	8.1	4.8	3.5	8.6	3.2	I -10	I-5	68	0-5	28.1	-	31.7	1.9	65.7	2.5	-	3.5	8.7	74	-	4.8	4.8	2.5	-
18	109.4	23.1	21.3	11.9	8.4	4.8	3.5	8.6	3.7	I -10	I-5	72	0-5	31.5	-	33.6	2.3	66.9	2.4	-	2.8	8.4	77	-	5.4	5.4	3.1	-
19	112.3	23.4	20.7	12.9	8.7	4.9	3.8	10.5	3.4	I -10	I-5	71	0-5	31.6	-	34.0	1.8	72.4	3.2	-	2.3	8.5	79	-	5.2	5.2	3.5	-
20	105.4	23.6	21.2	10.4	7.6	4.7	3.5	8.7	3.3	I -10	I-5	71	0-5	30.4	-	34.3	2.1	67.2	2.5	-	3.0	8.3	78	-	5.4	5.4	3.6	-
21	108.2	24.8	21.2	10.3	8.0	4.8	3.7	8.5	3.4	I -10	I-5	72	0-5	30.0	-	33.8	2.3	71.5	3.5	-	3.7	8.5	75	-	5.7	5.7	3.4	-
22	112.0	26.0	21.8	10.6	8.8	4.6	3.5	9.5	3.5	I -10	I-5	79	0-5	31.4	-	34.3	2.0	74.5	3.3	-	3.5	8.5	76	-	4.9	4.9	3.0	-
23	110.7	23.3	21.5	12.5	8.1	4.5	3.3	8.6	3.6	I -10	I-5	69	0-5	31.3	-	29.4	2.9	72.7	2.5	-	3.3	7.9	76	-	5.6	5.6	3.1	-
24	111.3	24.2	21.2	11.4	8.4	4.5	3.5	10.4	4.3	I -10	I-5	70	0-5	31.6	-	36.4	2.4	71.5	3.0	-	4.0	9.2	70	-	5.5	5.5	3.5	-
25	109.5	25.5	21.3	11.5	7.3	5.7	4.3	9.5	3.6	I -10	I-5	71	0-5	31.3	-	33.0	2.2	70.0	3.4	-	3.6	8.5	72	-	4.8	4.8	3.2	-
26	102.2	24.0	20.1	-	7.0	4.6	3.9	8.3	2.9	I -10	I-5	69	0-5	29.5	-	33.1	2.0	65.5	2.9	-	2.5	8.1	70	-	4.8	4.8	2.8	-
27	111.3	25.0	20.7	-	8.2	5.1	4.0	10.4	3.5	I -10	I-5	70	0-5	30.7	-	36.5	2.0	73.3	3.7	-	2.7	8.7	69	-	5.4	5.4	3.8	-

APPENDIX 2 - Table C: Taxonomical measurements. Family - Bagridae.
Species: Chrysichthys nigrodigitatus and C. auratus.

Table C(i)

Chrysichthys auratus body measurements.

	S.L.	B.D.*	H.L.	Iob.D	Ad.Bl.	Dr.Bl.	Sn-D	D-Ad	D	Pec.	Pel.	An.	Gill R. No. L.	Gill R. No. U.
1	94.2	15.1	29.4	4.5	10.8	13.1	35.5	27.2	II-6	-	I-5	IV-8	10	16
2	99.4	17.5	28.2	4.6	11.1	12.4	36.4	26.4	II-6	I-9	I-5	IV-7	11	17
3	99.3	-	28.8	5.0	13.4	12.5	35.8	25.8	II-6	I-9	I-5	IV-9	11	17
4	93.4	15.8	28.1	4.5	10.7	12.1	33.5	24.3	II-6	I-8	I-5	IV-7	10	17
5	103.8	18.9	30.9	5.7	9.6	14.1	39.6	27.1	II-6	-	I-5	IV-7	10	16
6	108.2	19.2	30.5	6.2	11.6	13.3	39.4	26.1	II-6	-	I-5	IV-9	10	16
7	79.5	17.8	28.9	4.6	11.8	12.7	35.9	26.0	II-6	-	I-5	IV-7	10	16
8	105.9	20.5	29.7	5.0	11.7	14.3	37.9	31.4	II-6	-	I-5	IV-8	10	16
9	93.3	17.2	27.5	4.5	10.0	12.5	34.9	22.9	II-6	-	I-5	IV-7	10	16
10	97.2	19.3	29.7	4.5	9.8	11.7	36.8	26.5	II-6	-	I-5	IV-7	10	16
11	103.1	19.9	30.7	5.4	13.8	12.8	38.6	26.2	II-6	-	I-5	IV-7	10	17
12	97.5	18.0	29.1	4.6	10.2	12.2	36.5	27.3	II-6	-	I-5	IV-8	11	16
13	102.8	19.8	30.4	4.7	12.2	13.0	38.4	26.0	II-6	I-7	I-5	IV-7	10	13
14	82.9	14.4	25.0	3.7	8.5	11.2	31.2	22.5	II-6	-	I-5	IV-7	10	16
15	88.4	15.2	27.1	4.7	9.4	11.1	33.5	21.4	II-6	-	I-5	IV-9	10	17
16	101.0	17.5	29.4	5.0	11.8	13.0	36.8	27.5	II-6	-	I-5	III-8	9	16
17	102.3	18.8	30.5	5.8	12.4	13.9	38.5	38.5	II-6	-	I-5	IV-8	10	7
18	101.7	18.5	29.8	5.4	12.3	11.8	37.7	26.1	II-6	-	I-5	IV-9	11	16
19	104.9	18.7	32.0	5.9	12.7	13.9	39.8	26.5	II-6	-	I-5	IV-7	10	16
20	102.1	17.7	30.0	5.8	13.4	13.7	37.7	25.2	II-6	-	I-5	IV-8	11	18
21	104.3	18.4	31.2	5.5	12.5	14.0	38.4	25.3	II-6	-	I-5	IV-8	10	16

* Not to be used in analysis.

Table C(ii): Taxonomical Measurements. Chrysichthys nigrodigitatus.

	S.L.	D.B.	H.L.	Iob.D.	Ad.Bl.	Dr.Bl.	Sn-D	D-Ad	D	Pec.	Pel.	An.	Gill R. No. L	Gill R. No. U	CP.W.	CP.L.
1	175.3	33.0	49.1	11.5	16.5	23.3	63.8	46.5	II-6	I-9	I-5	V-9	15	24	16.2	29.5
2	163.1	30.7	47.4	10.9	13.4	23.0	60.6	47.8	II-6	I-9	I-5	V-9	15	24	15.3	26.8
3	163.4	31.3	44.6	10.5	15.8	24.4	58.8	46.0	II-6	I-9	I-5	V-9	14	24	15.2	26.7
4	166.1	32.5	48.4	10.7	15.9	23.1	61.7	44.5	II-6	I-9	I-5	V-9	15	24	15.5	27.9
5	146.9	29.2	40.0	10.3	14.6	21.3	53.6	40.4	II-6	I-9	I-5	V-9	15	23	14.4	25.1
6	174.9	33.0	48.5	11.0	16.8	23.7	63.8	49.5	II-6	I-9	I-5	V-9	13	24	15.5	27.3
7	183.8	32.9	51.2	11.6	15.8	24.2	67.3	50.1	II-6	I-9	I-5	V-9	16	25	16.5	29.9
8	146.3	27.5	39.3	9.6	10.4	20.7	52.8	40.7	II-6	I-9	I-5	V-9	15	25	14.5	23.6
9	172.2	35.8	49.3	11.6	14.4	23.5	64.8	47.2	II-6	I-9	I-5	V-10	15	27	16.6	26.7
10	178.6	34.3	47.5	12.1	17.5	25.6	62.5	51.0	II-6	I-9	I-5	V-9	14	24	16.5	30.4
11	142.6	27.6	38.1	9.0	13.8	20.7	51.4	40.3	II-6	I-9	I-5	V-9	14	23	13.9	22.8
12	164.4	30.5	46.1	10.6	12.9	23.1	60.0	46.4	II-6	I-9	I-v	V-9	15	27	16.0	26.5
13	158.0	32.5	44.8	11.1	14.6	21.4	57.6	45.9	II-6	I-9	I-5	V-9	15	24	15.2	26.5
14	156.6	28.3	40.9	9.5	15.3	22.9	56.0	45.5	II-6	I-9	I-5	V-9	15	26	13.8	26.8
15	187.1	34.6	51.1	12.4	17.3	25.7	66.3	50.4	II-6	I-9	I-5	V-9	15	22	17.5	32.9
16	147.7	30.1	40.9	8.7	13.5	22.0	54.7	52.5	II-6	I-9	I-5	V-9	15	22	16.0	25.9
17	163.4	29.5	46.3	10.8	14.8	22.1	60.9	46.1	II-6	I-9	I-5	V-9	15	23	15.4	27.2
18	180.0	35.7	48.5	12.2	17.5	25.5	62.9	51.3	II-6	I-9	I-5	V-10	15	24	17.5	30.5
19	170.5	32.3	46.9	11.2	14.9	24.3	60.7	48.2	II-6	I-9	I-5	V-9	16	26	16.0	29.5
20	159.3	32.0	45.1	10.9	14.9	24.9	60.4	46.9	II-6	I-9	I-5	V-9	15	24	15.4	26.7
21	174.0	34.4	49.5	11.5	16.0	23.3	66.4	49.6	II-6	I-9	I-5	V-9	17	26	17.0	27.5
22	171.6	31.3	47.4	11.0	14.0	24.2	61.8	47.5	II-6	I-9	I-5	V-9	15	25	15.5	26.5
23	173.6	34.4	48.2	11.6	14.7	23.8	63.3	48.0	II-6	I-9	I-5	V-9	15	25	15.9	29.9
24	182.7	35.2	51.0	11.5	16.3	25.2	66.8	54.1	II-6	I-9	I-5	V-9	15	26	18.6	30.1
25	155.1	30.0	42.6	9.5	13.7	21.5	55.7	43.4	II-6	I-9	I-v	V-9	15	25	14.8	25.0
26	156.5	30.5	42.3	10.0	14.4	22.9	56.6	42.7	II-6	I-9	I-5	V-9	15	24	15.5	25.4
27	168.9	33.1	46.9	11.0	15.2	23.1	62.5	48.4	II-6	I-9	I-5	V-9	15	26	16.5	27.5
28	174.1	29.5	48.0	11.1	14.7	23.1	63.0	48.6	II-6	I-9	I-5	V-9	15	26	15.6	29.0

Oreochromis niloticus August, 1986 Fish Family Cichlidae

Loc.: IAB

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lth.
1	122.3	49.7	42.6	11.4	9.9	15.8	13.0	18.0	21.6	21.3	XVII-12	II-13	I-5	III-9	29	22/12	49.4
2	149.8	64.8	54.5	18.3	11.6	19.6	15.6	23.1	26.0	25.8	XVII-13	II-13	I-5	III-10	31	22/11	56.9
3	119.4	57.4	40.4	11.8	8.5	15.5	13.7	18.8	20.0	19.5	XVII-12	II-13	I-5	III-10	32	21/12	45.1
4	89.2	37.8	29.2	8.4	6.8	10.7	10.7	13.4	15.8	14.9	XVII-13	II-12	I-5	III-9	27	21/13	34.6
5	59.5	23.6	22.5	6.0	6.2	6.9	6.5	8.1	10.8	10.6	XVII-12	II-13	I-5	III-10	26	21/13	23.7
6	69.5	32.0	23.5	6.1	5.7	8.3	7.3	10.8	11.8	11.9	XVII-13	II-13	I-5	III-10	27	22/11	25.2
7	154.9	64.5	52.8	16.0	11.6	19.8	15.4	23.3	26.5	25.7	XVII-12	II-13	I-5	III-10	33	22/11	58.5
8	171.0	70.7	61.3	20.5	13.6	21.7	17.7	26.1	30.3	28.4	XVII-13	II-13	I-5	III-10	33	23/11	67.8

Oreochromis niloticus August 1986 Fish Family Cichlidae

Loc.: Pacific Farm - Mixed Pond (Ghana).

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lth.
1	122.0	47.4	42.3	13.1	9.3	15.1	13.6	18.6	23.1	22.9	XVII-12	II-13	I-5	III-10	27	21/13	52.8
2	81.7	31.4	30.2	9.1	7.9	9.1	11.7	11.0	15.1	14.6	XVII-12	II-13	I-5	III-10	27	22/12	34.2
3	165.2	66.1	59.8	19.4	12.4	22.5	19.6	24.5	29.6	25.8	XVII-13	II-13	I-5	III-10	31	22/13	61.4
4	157.9	70.7	53.4	15.6	10.9	19.4	16.6	23.3	21.5	22.5	XVII-13	II-13	I-5	III-10	29	21/14	52.5
5	160.8	71.3	54.4	17.4	10.8	20.8	16.8	24.5	27.6	25.8	XVII-13	II-13	I-5	III-10	30	22/12	-
6	151.8	60.4	54.6	16.9	11.2	19.6	17.8	22.5	28.2	26.4	XVII-12	II-13	I-5	III-9	30	23/12	56.3
7	166.4	76.4	54.5	17.1	10.9	20.3	16.3	25.3	30.0	25.6	XVII-13	II-14	I-5	III-10	27	22/12	66.3
8	152.5	58.6	52.8	17.0	11.2	19.6	17.2	22.1	28.5	28.1	XVII-13	II-14	I-5	III-10	28	21/12	56.8
9	179.8	77.1	59.5	18.0	11.35	22.8	18.6	25.5	29.4	27.1	XVII-13	II-13	I-5	III-10	29	22/13	71.7
10	119.7	49.4	44.9	13.4	10.0	15.0	12.6	13.6	22.1	19.0	XVII-13	II-13	I-5	III-9	29	22/12	48.0
11	162.7	71.8	57.4	17.3	10.7	20.6	18.5	25.4	27.9	26.4	XVII-13	II-13	I-5	III-10	28	22/12	-
12	161.8	61.8	58.0	17.3	12.0	20.1	19.6	22.3	29.5	27.8	XVII-12	II-13	I-5	III-10	32	22/12	63.8

Fish: S. galilaeus August, 1986. Fish Family - Cichlidae

Loc.: IAB.

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lth.
1	163.8	83.2	56.6	20.5	11.4	23.8	16.6	29.1	29.5	23.5	XVI-14	II-12	I-5	III-11	27	21/12	67.7
2	137.8	71.7	44.8	14.3	9.6	18.2	14.7	24.8	25.2	20.1	XVI-13	II-12	I-5	III-11	27	21/12	56.9
3	119.4	62.6	42.5	13.4	9.2	16.2	11.6	21.3	21.5	17.5	XVI-13	II-13	I-5	III-11	28	21/12	-
4	120.3	63.8	41.7	13.1	8.8	16.5	13.5	21.5	22.6	18.2	XVII-13	II-13	I-5	III-12	28	22/11	53.6
5	114.0	62.1	40.0	13.2	9.0	15.9	11.3	20.7	21.2	17.0	XVI-14	II-13	I-5	III-12	27	19/13	50.5
6	124.7	65.3	42.6	12.5	9.8	17.6	12.5	22.4	23.0	18.5	XVI-13	II-12	I-5	III-12	27	21/12	54.8
7	97.1	49.6	34.3	10.4	8.1	13.3	9.7	17.5	16.0	13.6	XVI-13	II-13	I-5	III-11	27	20/13	43.4
8	98.3	47.8	34.5	10.1	8.3	12.8	10.5	17.5	16.5	14.6	XVI-13	II-12	I-5	III-11	27	19/12	42.3
9	98.6	51.2	33.0	9.0	8.5	13.1	10.3	16.9	17.5	15.0	XVI-13	II-13	I-5	III-11	28	21/11	42.5
10	130.9	70.4	45.5	14.8	10.1	18.6	13.1	24.5	25.2	19.7	XVI-14	II-13	I-5	III-12	29	21/12	58.5
11	115.0	58.0	38.5	12.0	8.4	15.5	12.4	21.1	20.6	17.5	XVI-13	II-13	I-5	III-11	28	20/11	49.2
12	133.6	68.5	43.9	13.5	9.5	17.8	15.2	23.9	23.2	19.2	XVI-13	II-12	I-5	III-11	27	20/12	55.9
13	151.5	77.3	49.9	16.3	9.9	20.5	14.1	27.0	28.1	22.7	XVII-13	II-12	I-5	III-12	30	21/11	66.8
14	105.9	50.9	35.1	10.5	8.8	13.0	11.8	18.5	17.7	15.8	XVII-14	II-13	I-5	III-10	28	21/12	45.1
15	108.5	57.0	38.8	12.5	8.8	16.0	11.6	20.8	19.6	16.7	XVI-13	II-13	I-5	III-11	29	21/11	49.6
15	119.2	66.7	40.5	13.2	9.3	17.3	11.4	21.1	23.0	19.0	XV-14	II-12	I-5	III-10	28	21/10	-
17	102.8	-	35.8	10.5	8.6	14.0	11.4	18.5	19.0	15.5	XVI-14	II-13	I-5	III-12	28	21/11	44.1
18	128.6	70.7	45.1	15.1	9.6	18.5	11.7	23.3	23.2	19.4	XVI-14	II-13	I-5	III-12	29	21/11	58.6
19	106.9	53.4	36.4	11.5	8.5	14.1	11.4	18.4	19.6	14.5	XVI-13	II-12	I-5	III-11	29	19/13	44.2
20	132.5	70.4	45.8	15.4	10.0	18.4	12.6	24.3	25.7	21.5	XVI-13	II-13	I-5	III-11	28	20/12	56.7
21	132.8	71.7	45.5	14.4	9.8	18.2	13.4	24.4	24.4	21.4	XVII-13	II-12	I-5	III-10	30	20/12	58.0
22	116.6	58.4	40.7	12.2	8.9	15.3	12.2	20.4	21.5	15.8	XVI-14	II-12	I-5	III-12	27	20/12	51.4
23	115.9	62.0	41.1	13.0	9.4	15.9	11.5	21.1	21.3	18.8	XVI-14	II-12	I-5	III-12	28	20/13	51.5
24	104.2	49.6	36.5	9.9	8.6	13.2	11.5	17.3	17.7	15.2	XVI-13	II-12	I-5	III-11	28	20/12	-
25	118.0	63.5	41.0	14.5	9.4	15.7	11.5	21.0	22.4	17.6	XVI-14	II-12	I-5	III-12	29	21/11	50.8
26	121.8	61.9	39.3	13.2	8.8	14.4	14.3	21.3	25.4	18.5	XV-14	II-14	I-5	III-10	13	20/10	-
27	114.2	57.7	39.2	11.8	9.0	15.0	13.9	20.3	20.5	17.5	XVI-14	II-13	I-5	III-12	26	21/12	49.5
28	118.3	62.3	41.6	13.0	9.0	15.5	12.5	21.7	21.8	17.6	XVII-13	II-13	I-5	III-12	28	20/12	53.6
29	155.8	83.5	52.0	17.2	10.9	21.5	15.9	27.9	28.0	24.5	XV-14	II-12	I-5	III-12	29	20/12.5	69.9
30	100.6	48.9	35.2	10.5	8.0	13.2	10.6	17.6	17.6	16.4	XVI-14	II-12	I-5	III-12	27	21/11	-

Fish: Seratherodon melanotheron Fish Family - Cichlidae

Loc. River Densu, Ghana.

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lth.
1	153.3	65.5	56.3	20.5	9.8	20.5	18.4	26.6	20.2	20.8	XV-11	II-12	I-5	III-9	22	18/12	64.3
2	167.4	69.5	60.4	21.6	11.7	21.3	21.1	27.1	19.0	18.6	XVI-11	II-12	I-5	III-10	21	19/10	67.0
3	178.6	70.6	64.3	22.8	12.1	21.3	24.6	28.4	20.1	17.8	XVI-11	II-13	I-5	III-10	21	20/10	70.6
4	172.8	74.3	63.7	22.0	11.7	22.6	22.8	29.6	25.0	21.2	XVI-10	II-13	I-5	III-9	20	19/12	72.4
5	99.4	44.3	35.5	10.3	7.5	12.3	12.0	16.5	15.3	13.8	XV-11	II-12	I-5	III-9	21	20/10	39.0
6	161.6	67.5	58.5	20.8	10.7	20.2	19.2	27.2	21.7	20.0	XVI-10	II-11	I-5	III-9	20	20/10	65.5
7	119.0	55.8	38.4	12.3	7.5	14.0	11.4	18.4	17.3	13.8	XV-11	II-12	I-5	III-9	20	20/10	45.8
8	93.7	40.8	33.5	10.0	7.0	11.2	10.8	16.4	14.4	13.2	XV-11	II-12	I-5	III-9	19	19/11	35.5
9	172.8	73.9	59.8	20.8	11.1	21.2	19.5	27.1	24.4	19.4	XV-10	II-12	I-5	III-9	20	19/10	68.9
10	99.2	43.5	36.0	12.8	7.4	12.7	11.5	16.9	16.4	12.8	XIV-11	II-12	I-5	III-9	22	20/9	37.3
11	105.5	43.7	39.9	11.9	7.0	12.3	14.2	18.0	16.9	15.4	XVI-11	II-12	I-5	III-9	20	20/10	43.1
12	161.7	60.7	58.0	21.5	10.2	18.9	20.3	25.8	16.5	19.8	XV-11	II-13	I-5	III-9	21	20/10	63.2
13	106.2	45.5	36.8	12.2	7.9	12.9	12.7	17.1	14.9	13.0	XVI-10	II-12	I-5	III-9	20	20/10	38.7
14	107.6	50.3	39.7	14.2	7.3	13.5	11.3	18.4	16.5	14.5	XVI-11	II-12	I-5	III-9	19	19/10	42.5
15	115.7	51.6	49.6	13.5	8.1	14.0	12.8	20.1	16.9	15.3	XVI-11	II-12	I-5	III-9	20	18/10	-
16	112.5	47.9	40.0	13.1	7.9	14.1	14.0	18.6	15.2	13.6	XV-11	III-11	I-5	III-9	21	20/10	41.0
17	146.2	62.1	52.3	17.8	11.3	18.8	18.8	24.0	20.5	20.1	XV-12	I-13	I-5	III-9	21	20/10	61.4
18	138.2	61.1	51.1	17.8	9.7	17.7	18.4	23.7	19.8	20.0	XV-11	II-12	I-5	III-9	21	17/11	-
19	96.6	42.5	34.5	10.4	7.7	12.3	11.5	15.9	15.4	12.9	XVI-11	II-12	I-5	III-9	22	20/10	33.1

Fish: Tilapia busumana August, 1986. Fish Family - Cichlidae

Loc.: Lake Bosomtwe (Ghana, Aug. 1986).

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lt
1	92.7	37.8	34.8	9.4	9.2	12.6	12.4	15.7	13.6	13.8	XV-13	II-12	I-5	III-10	-	19/12	37.9
2	104.7	37.7	38.8	10.4	9.5	11.9	13.5	16.1	16.1	14.5	XV-13	II-11	I-5	III-10	24	19/12	42.2
3	84.5	34.0	30.9	8.6	9.0	9.9	9.9	13.6	11.8	12.0	XV-13	II-12	I-5	III-10	27	20/10	31.8
4	75.6	31.3	29.0	8.5	8.2	8.7	10.5	13.0	10.5	10.5	XVI-12	II-11	I-5	III-9	24	19/12	30.8
5	84.7	34.2	32.5	8.9	8.5	10.4	10.6	13.7	11.9	10.7	XIV-12	II-12	I-5	III-11	26	20/12	32.4
6	86.3	34.3	32.2	9.2	8.8	10.9	11.6	14.5	-	12.3	XVI-12	II-12	I-5	III-10	27	20/11	35.9
7	86.6	33.5	32.5	8.5	8.9	9.6	10.2	14.1	14.5	13.6	XIV-14	II-12	I-5	III-10	27	19/12	38.4
8	98.8	37.0	36.0	10.3	9.5	11.1	12.5	15.5	14.8	12.1	XV-12	II-13	I-5	III-10	25	19/11	40.9
9	97.9	35.8	35.1	10.4	10.1	11.7	12.0	15.5	15.2	12.5	XVI-13	II-12	I-5	III-11	27	20/11	34.3
10	81.9	29.4	28.0	7.4	7.3	7.2	10.9	13.3	11.7	10.0	XV-12	II-12	I-5	III-8	16	20/10	-
11	80.7	33.0	30.1	7.5	9.3	9.1	12.0	13.5	12.4	11.1	XV-13	II-12	I-5	III-11	27	19/11	33.5
12	86.4	31.4	31.0	8.7	8.5	9.7	11.9	13.4	12.0	11.4	XV-13	II-11	I-5	III-11	26	20/10	37.7
13	80.0	33.4	30.3	7.8	8.8	9.3	10.2	14.0	9.9	9.4	XIV-13	II-12	I-5	III-11	27	20/10	33.2
14	84.5	35.5	31.6	9.0	8.8	10.7	8.9	14.8	13.3	11.8	XV-13	II-13	I-5	III-11	28	20/11	33.9
15	88.5	33.1	32.4	8.9	7.7	9.9	12.1	13.9	13.6	12.4	XV-13	II-12	I-5	III-10	27	20/10	37.5
16	89.3	36.9	32.3	8.9	8.4	11.3	12.8	15.4	12.6	10.8	XV-13	II-12	I-5	III-10	27	20/10	34.5
17	84.7	33.1	30.8	8.2	8.4	10.1	10.8	14.7	12.8	12.9	XV-13	II-11	I-5	III-10	26	19/12	37.4
18	80.0	32.8	29.9	8.0	7.4	9.3	10.5	13.6	11.6	10.7	XV-13	II-12	I-5	III-10	29	20/10	33.5
19	96.5	38.4	35.8	11.2	9.4	12.7	13.7	15.8	16.2	13.8	XVI-11	II-12	I-5	III-11	28	20/12	40.8
20	87.2	34.6	31.3	9.5	7.8	10.1	11.0	14.5	12.3	11.9	XVI-13	II-11	I-5	III-10	28	20/12	-
21	99.4	40.9	36.4	11.4	8.1	12.4	13.6	16.2	15.0	15.5	XV-13	II-12	I-5	III-10	-	20/11	42.4
22	90.5	36.9	33.5	9.2	8.8	11.1	10.8	15.0	14.3	12.5	XVI-13	II-11	I-5	III-10	25	19/11	37.3
23	84.4	34.1	31.1	8.1	8.4	10.5	11.2	13.9	12.4	11.0	XV-13	II-11	I-5	III-10	25	20/11	32.5
24	90.7	31.9	32.5	8.6	8.8	9.8	12.2	14.4	14.0	13.6	XV-13	II-11	I-5	III-10	27	19/12	39.8
25	87.8	29.7	30.8	8.0	8.9	8.5	11.4	13.4	13.7	12.8	XV-13	II-11	I-5	III-10	26	18/14	29.9

Fish: Tilapia zillii August, 1986 Fish Family - Cichlidae

Loc.: River Densu (Ghana)

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L	CP.W	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.I.Sc.	Pect. Fin lth.
1	123.3	52.7	38.3	13.8	9.2	12.7	13.2	21.0	21.4	15.3	XV-14	II-13	I-5	III-10	14	21/11	42.5
2	111.5	50.0	36.5	12.7	8.9	12.3	13.0	19.1	19.4	14.5	XV-13	II-13	I-5	III-10	13	20/12	41.4
3	95.8	45.2	31.3	11.0	7.5	11.1	11.2	16.8	18.7	14.9	XV-12	II-13	I-5	III-10	14	21/11	39.1
4	131.3	61.0	43.1	16.7	9.5	14.3	17.4	22.4	21.9	16.3	XV-14	II-13	I-5	III-10	13	19/12	48.3
5	159.0	71.9	51.3	21.4	10.4	18.1	18.8	28.8	27.3	18.4	XVI-13	II-13	I-5	III-10	13	21/10	61.0
6	100.2	44.4	31.7	10.8	7.4	10.7	10.0	17.2	18.4	13.2	XV-14	II-13	I-5	III-9	13	18/11	37.5
7	121.7	54.1	39.6	13.6	10.0	12.4	14.6	21.4	19.5	14.8	XV-14	II-10	I-5	III-10	14	20/10	44.0
8	107.5	46.3	33.5	11.7	8.4	11.4	10.9	17.6	19.7	14.4	XV-14	II-13	I-5	III-10	14	21/10	40.7
9	99.6	43.7	30.6	10.4	7.9	10.5	11.9	16.4	17.4	13.6	XVI-13	II-13	I-5	III-10	13	21/11	37.1
10	156.1	71.9	49.7	19.0	10.8	17.1	19.9	27.2	27.3	18.8	XV-13	II-13	I-5	III-10	13	21/10	58.2
11	143.9	64.0	46.1	18.4	11.7	16.8	15.1	23.8	24.5	18.5	XV-14	II-14	I-5	III-10	14	21/10	51.4
12	136.4	61.5	42.9	14.6	10.0	15.9	13.9	20.0	26.2	18.8	XVI-13	II-13	I-5	III-10	13	20/11	54.1
13	133.0	58.3	44.0	16.8	9.7	14.8	15.0	23.0	23.6	16.0	XVI-12	II-14	I-5	III-10	13	22/9	47.4
14	115.5	50.7	36.5	12.9	8.3	12.1	11.9	18.7	20.7	14.5	XV-13	II-13	I-5	III-10	14	20/11	45.8
15	90.7	37.9	29.8	10.0	8.0	9.4	10.8	15.5	15.6	13.0	XV-13	II-13	I-5	III-10	13	20/10	32.5
16	150.6	67.3	48.8	17.5	11.4	16.5	16.5	26.5	26.8	19.4	XV-13	II-13	I-5	III-10	13	20/10	54.8
17	118.4	52.4	37.6	13.8	8.8	13.2	12.9	19.6	21.4	16.9	XV-13	II-13	I-5	III-10	13	21/10	42.5
18	108.8	47.8	34.6	12.4	8.4	11.8	12.7	19.4	19.9	14.6	XV-13	II-13	I-5	III-9	13	20/11	41.0
19	126.7	54.8	39.8	13.8	9.2	13.5	14.3	20.9	21.0	16.2	XVI-13	II-13	I-5	III-9	13	21/10	41.2
20	121.0	55.6	37.3	13.8	9.2	14.1	12.8	20.6	19.3	15.8	XV-13	II-13	I-5	III-10	13	21/10	45.1
21	117.1	51.4	37.3	13.3	9.0	12.5	12.0	20.1	22.5	17.5	XVI-12	II-13	I-5	III-10	14	20/10	45.7

Tilapia zillii August. 1986. Fish Family - Cichlidae

Loc.: IAB Ponds

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin Lth
1	73.5	33.9	25.6	7.0	6.8	9.1	8.0	12.2	12.5	10.3	XV-12	II-12	I-5	III-11	28	19/13	27.0
2	120.8	52.0	39.5	15.2	10.4	13.7	13.7	20.1	25.3	17.3	XVI-13	II-12	I-5	III-10	12	21/12	41.6
3	133.3	61.5	46.0	17.4	10.2	15.2	13.8	23.1	27.4	19.6	XV-13	II-14	I-5	III-10	13	22/11	49.8
4	126.9	64.3	41.5	15.8	9.1	14.1	14.0	22.2	25.1	15.4	XV-14	II-14	I-5	III-10	13	20/11	47.8
5	140.8	71.2	46.2	17.9	10.2	15.8	14.9	25.5	30.7	20.7	XIV-15	II-13	I-5	III-10	14	20/12	55.9
6	145.9	70.3	47.5	18.5	10.6	16.4	17.2	26.1	30.4	21.6	XV-14	II-13	I-5	III-10	15	21/11	54.1

Fish: Tilapia zillii August, 1986. Fish Family - Cichlidae

Loc.: Pacific Farms MiXed Pond (MP)

	S.L.	B.D.	H.L.	Sn.L.	E.D.(M)	Iob.W.	CP.L.	CP.W.	D.Sp.L.	A.Sp.L.	D.	Pec.	Pel.	A.	Gill.R.	Lat.L.Sc.	Pect. Fin L
1	82.8	38.8	27.0	8.5	6.2	8.9	11.0	13.9	13.9	12.9	XV-13	II-13	I-5	III-10	15	20/11	28.5
2	59.2	21.5	18.8	6.4	4.9	5.5	6.9	9.8	10.0	7.1	XVI-12	II-13	I-5	III-9	15	21/	20.4
3	83.2	35.7	27.5	8.5	6.6	8.1	9.5	12.6	14.0	11.0	XVI-12	II-14	I-5	III-10	15	21/11	29.5
4	81.3	34.5	27.2	7.9	6.0	8.5	10.1	13.4	15.5	13.9	XV-13	II-14	I-5	III-9	15	21/10	27.5
5	59.4	22.0	18.5	5.8	4.8	5.2	8.4	9.2	9.8	7.1	XV-12	II-13	I-5	III-10	15	21/9	21.4
6	77.8	35.1	25.5	8.0	6.3	8.2	9.5	13.0	-	10.8	XVI-13	II-14	I-5	III-9	15	21/10	26.9
7	81.2	37.2	27.0	8.2	6.3	8.7	9.5	13.7	14.3	11.8	XV-13	II-13	I-5	III-10	15	21/11	26.4
8																	
9	82.7	37.4	28.3	9.0	7.5	8.7	10.7	13.5	15.7	12.5	XV-13	II-14	I-5	III-10	15	21/10	-
10	96.3	46.2	31.3	10.9	7.5	11.0	12.2	17.4	16.8	13.9	XV-13	II-13	I-5	III-10	15	21/10	33.6
11	90.1	39.4	29.2	9.4	6.5	9.2	12.2	15.1	16.4	13.1	XVI-13	II-13	I-5	III-10	15	21/11	32.1
12	84.5	36.4	27.4	8.3	6.0	8.9	13.4	13.9	15.5	12.1	XVI-12	II-13	I-5	III-10	15	21/11	30.5
13	51.5	30.6	17.4	4.6	4.6	5.0	7.0	8.0	9.3	6.9	XIV-12	II-13	I-5	III-9	12	21/10	19.1
14	84.8	38.8	28.0	8.4	7.0	8.4	10.5	14.0	14.5	12.5	XVI-13	II-13	I-5	III-10	13	20/12	31.3
15	85.6	37.4	27.7	9.5	-	8.6	10.8	13.9	14.8	12.3	XV-13	II-14	I-5	III-10	16	20/11	32.4
16	62.4	26.0	21.4	6.4	4.8	6.6	7.5	10.0	10.0	8.7	XVI-13	II-13	I-5	III-10	16	21/10	22.1
17	98.5	45.5	32.2	10.8	7.0	10.4	12.7	17.3	16.6	13.2	XV-13	II-13	I-5	III-10	15	20/11	33.1
18	74.9	31.8	24.9	7.5	6.4	8.4	10.0	12.3	12.8	10.0	XVI-12	II-13	I-5	III-10	14	21/10	26.3
19	71.4	28.9	22.9	5.6	5.0	6.9	9.4	10.8	11.3	8.9	XVI-13	II-13	I-5	III-10	16	22/12	25.5

APPENDIX 3

Table 3.1: Genotype frequency.

Fish: Marcusenius senegalensis (Pellegrin 1904).
Source: River Oti, River Pru - Volta basin (Ghana)

Locus	Genotype	R. Oti	R.Pru
		(8)	(18)
Ada-1	M/M	8	18
Ada-2	M/M	8	18
Adh	M/M	8	18
Ap-1	M/M	8	18
Fum	M/M	8	18
αGpdh	M/M	8	18
G6pdh	M/M	8	18
HK	M/M	8	18
Ldh-1	M/M	8	18
Ldh-2	M/M	8	18
Ldh-3	M/M	8	18
Mdh-1	M/M	8	18
Mdh-2	M/M	8	18
Me-1	M/M	8	18
Me-2	M/M	8	18
6Pgdh	M/M	8	18
Pgi-1	M/M	8	18
Pgi-2	F/F	-	-
	F/S	-	2
	S/S	8	16
Pgm	M/M	8	18
Sdh	M/M	8	18
Sod	M/M	8	18
Xdh	M/M	8	18

Table 3.2: Schilbeidae (fm.) Genotype Frequencies at 28 Loci.

Fish		Schilbe mystus					Eutropius niloticus				Eutropius mandibularis		Siluranodon auritus		
Locality		Asukaw kaw	Black Volta	Pru	Oti	Pooled	White Volta	Oti	Black Volta	Pooled	Ivory	Coast	Black Volta	Nasia	Pooled
Locus	Genotype														
Adh-1					(6)	(6)		(6)		(6)	(5)			(6)	(6)
	F/F				-	-		-		-	-			6	6
	S/S				6	6		6		6	5			-	-
Adh-2					(6)	(6)		(6)		(6)	(5)			(6)	(6)
	F/F				6	6		-		-	-			-	-
	M/M				-	-		6		6	-			6	6
	S/S				-	-		-		-	5			-	-
Ap-1		(6)	(3)	(14)	(7)	(30)	(13)	(6)	(6)	(25)	(5)		(4)	(7)	(11)
	M/M	6	3	14	7	30	13	6	6	25	5		4	7	11
Ap-2		(6)	(3)	(14)	(7)	(30)	(13)	(6)	(6)	(25)	5		(4)	(7)	(11)
	M/M	6	3	14	7	30	13	6	6	25	5		4	7	11
CK		(15)	(9)	(0)	(6)	(30)	(17)	(6)	(0)	(23)	(5)		(0)	(25)	(25)
	F/F	15	9	0	6	30	17	6	0	23	5		0	-	-
	S/S	-	-	0	-	-	-	-	-	-	-		-	25	25
Est-1		(24)	(9)	(51)	(7)	(101)	(21)	(24)	(9)	(54)	(5)		(41)	(21)	(62)
	M/M	24	9	51	7	101	21	24	9	54	5		41	21	62
Est-2		(24)	(9)	(51)	(7)	(101)	(21)	(24)	(9)	(54)	(5)		(41)	(21)	(62)
	F/F	24	9	46	7	96	21	24	9	54	5		-	-	-
	S/S	-	-	5	-	5	-	-	-	-	-		41	21	62
Est-3		(24)	(9)	(48)	(12)	(93)	(21)	(22)	(20)	(63)	(5)		(41)	(21)	(62)
	F/F	-	-	-	-	-	19	21	17	57	5		-	-	-
	M/M	24	8	46	11	89	-	-	-	-	-		41	21	62
	S/S	-	-	-	-	-	-	-	-	-	-		-	-	-
	F/M	-	-	2	-	2	-	1	3	4	-		-	-	-
	M/S	-	1	-	1	2	-	-	-	-	-		-	-	-
	F/S	-	-	-	-	-	2	-	-	2	-		-	-	-
αGpdh-1		(6)			(6)	(12)		(6)		(6)	(5)			(6)	(6)
	M/M	6			6	12		6		6	5			6	6

		(6)			(6)	(12)		(6)			(5)		(6)	(6)
αGpdh-2	F/F	6			6	12		-			5		-	-
	M/M	-			-	-		6		6	-		-	-
	S/S	-			-	-		-		-	-		6	6
		(6)	(3)	(52)	(13)	(74)	(3)	(36)	(5)	(44)	(5)	(30)	(8)	(38)
G6pdh	F/F	6	3	52	12	73	-	-	-	-	5	-	-	-
	M/M	-	-	-	-	3	36	5	44	-	-	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	-	30	8	38
	F/M	-	-	-	1	1	-	-	-	-	-	-	-	-
		(6)	(9)	(40)	(17)	(72)	(3)	(17)	(5)	(25)	(5)	(20)	(9)	(29)
Idh	F/F	6	9	40	17	72	3	17	5	25	5	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	-	19	9	28
	F/S	-	-	-	-	-	-	-	-	-	-	1	-	1
		(15)	(3)	(44)	(17)	(79)	(19)	(46)	(5)	(70)	(5)	(60)	(21)	(81)
Ldh-1	F/F	-	2	29	-	31	-	-	-	-	-	60	21	81
	S/S	15	-	11	17	43	1º	46	5	70	5	-	-	-
	F/S	-	1	4	-	5	-	-	-	-	-	-	-	-
		(15)	(3)	(44)	(17)	(79)	(19)	(47)	(5)	(71)	(5)	(60)	(21)	(81)
Ldh-2	F/F	-	-	-	-	-	-	-	-	-	-	-	-	-
	M/M	15	3	40	16	74	19	47	5	71	5	-	-	-
	S/S	-	-	4	-	4	-	-	-	-	-	-	-	-
	VS/VS	-	-	-	-	-	-	-	-	-	-	60	21	81
	F/M	-	-	-	1	1	-	-	-	-	-	-	-	-
		(13)	(9)	(56)	(17)	(95)	(22)	(50)	(0)	(72)	(5)	(58)	(14)	(72)
Me-1	VF/VF	-	-	-	-	-	-	-	-	-	-	-	-	-
	F/F	-	-	-	-	-	16	36	-	52	5	-	-	-
	M/M	-	-	1	3	4	-	-	-	-	-	-	-	-
	S/S	-	-	-	-	-	3	1	-	4	-	-	-	-
	VS/VS	13	9	47	9	78	1	-	-	1	-	58	14	72
	M/VS	-	-	7	5	12	-	-	-	-	-	-	-	-
	VF/F	-	-	1	-	1	-	-	-	-	-	-	-	-
	F/S	-	-	-	-	-	2	13	-	15	-	-	-	-
		(13)	(9)	(56)	(17)	(95)	(22)	(50)	(0)	(72)	(5)	(58)	(14)	(72)
Me-2	F/F	-	-	-	-	-	1	-	-	1	5	-	-	-
	M/M	13	8	56	16	93	21	47	-	68	-	-	-	-
	S/S	-	-	-	-	-	-	2	-	2	-	-	-	-
	VS/VS	-	-	-	-	-	-	-	-	-	-	58	14	72
	F/S	-	1	-	-	1	-	-	-	-	-	-	-	-
	M/S	-	-	-	1	1	-	1	-	1	-	-	-	-

Table 3.2 Contd.:

Fish		Schilbe mystus					Eutropius niloticus				Eutropius mandibularis	Siluranodon auritus		
Locality		Asukaw kaw	Black Volta	Pru	Oti	Pooled	White Volta	Oti	Black Volta	Pooled	Ivory Coast	Black Volta	Nasia	Pooled
Locus	Genotype													
		(8)	(9)	(47)	(17)	(81)	(8)	(41)	(10)	(59)	(5)	(38)	(14)	(52)
Mdh-1	F/F	-	-	-	-	-	8	41	10	59	-	-	-	-
	M/M	8	8	47	17	80	-	-	-	-	-	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	5	38	14	52
	F/M	-	1	-	-	1	-	-	-	-	-	-	-	-
		(8)	(9)	(47)	(17)	(81)	(8)	(41)	(10)	(59)	(5)	(38)	(14)	(52)
Mdh-2	F/F	-	-	-	-	-	8	41	10	59	-	-	-	-
	M/M	8	8	42	17	75	-	-	-	-	5	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	-	-	-	-
	VS/VS	-	-	-	-	-	-	-	-	-	-	38	14	52
	M/S	-	1	5	-	6	-	-	-	-	-	-	-	-
		(8)	(0)	(10)	(6)	(24)	(17)	(6)	(0)	(23)	(5)	(4)	(16)	(20)
Mpi	F/F	-	-	-	-	-	17	6	0	23	-	4	16	20
	S/S	8	0	10	6	24	-	-	-	-	5	-	-	-
					(6)	(6)		(6)		(6)	(5)		(6)	(6)
Pep-1	F/F				6	6		5		5	5		-	-
	S/S				-	-		1		1	-		6	6
					(6)	(6)		(6)		(6)	(5)		(6)	(6)
Pep-2	F/F				6	6		4		4	5		-	-
	S/S				-	-		2		2	-		6	6
		(8)	(9)	(56)	(17)	(90)	(8)	(50)	(11)	(69)	(5)	(48)	(13)	(61)
Pgi-1	F/F	8	9	56	17	90	-	-	-	-	5	-	-	-
	M/M	-	-	-	-	-	-	-	-	-	-	48	13	61
	S/S	-	-	-	-	-	-	-	-	-	-	-	-	-
	VS/VS	-	-	-	-	-	8	49	11	68	-	-	-	-
	S/VS	-	-	-	-	-	-	1	-	1	-	-	-	-
		(8)	(9)	(56)	(17)	(90)	(8)	(50)	(11)	(69)	(5)	(59)	(13)	(72)
Pgi-2	F/F	-	-	-	-	-	-	1	-	1	-	59	13	72
	M/M	-	-	-	-	-	8	49	11	68	5	-	-	-
	S/S	8	9	56	17	90	-	-	-	-	-	-	-	-

		(5)	(9)	(56)	(17)	(87)	(5)	(50)	(10)	(65)	(5)	(58)	(6)	(64)
Pgm	VF/VF	-	-	-	-	-	-	-	-	-	-	-	-	-
	F/F	5	8	56	16	85	-	-	-	-	-	-	-	-
	M/M	-	-	-	-	-	-	-	-	-	4	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	-	-	-	-
	VS/VS	-	-	-	-	-	5	49	9	63	-	58	5	63
	VS/VVS	-	-	-	-	-	-	1	-	1	-	-	1	1
	VF/F	-	1	-	-	1	-	-	-	-	-	-	-	-
	F/M	-	-	-	1	1	-	-	-	-	-	-	-	-
	S/VS	-	-	-	-	-	-	-	1	1	-	-	-	-
	M/VS	-	-	-	-	-	-	-	-	-	1	-	-	-
		(0)	(0)	(36)	(23)	(59)	(0)	(44)			(5)	(48)	(6)	(54)
6-Pgdh	F/F			-	-	-		-			-	-	-	-
	M/M			-	-	-		43			5	-	-	-
	S/S			-	-	-		-			-	48	6	54
	VS/VS			36	23	59		-			-	-	-	-
	F/M			-	-	-		1			-	-	-	-
					(6)			(6)			(5)		(6)	
Sdh-1	F/F				-			-			-		6	
	S/S				6			6			5		-	
					(6)			(6)			(5)		(6)	
Sdh-2	F/F				-			-			-		6	
	S/S				6			6			5		-	
					(6)			(6)			(6)		(6)	
Sdh-3	F/F				-			-			2		-	
	M/M				-			-			-		-	
	S/S				4			-			-		-	
	VS/VS				-			-			-		5	
	F/M				-			-			4		-	
	M/S				2			6			-		-	
	S/VS				-			-			-		1	
		(20)	(5)	(47)	(17)	(89)	(31)	(38)	(5)	(74)	(5)	(46)	(21)	(67)
Sod	VF/VF	-	-	2	-	2	-	-	-	-	5	-	-	-
	F/F	-	-	4	-	4	-	-	-	-	-	46	21	67
	M/M	19	5	39	17	80	-	-	-	-	-	-	-	-
	S/S	-	-	-	-	-	-	-	-	-	-	-	-	-
	VS/VS	1	-	-	-	1	31	38	5	-	-	-	-	-
	F/S	-	-	1	-	1	-	-	-	-	-	-	-	-
	VF/M	-	-	1	-	1	-	-	-	-	-	-	-	-

APPENDIX 3

Table 3.3: Genotype frequencies in *Crysichthys* (Bleeker, 1858).

Species: *C. nigrodigitatus* and *C. auratus*

Locus	Genotype	C. nigro.	C. auratus
Adh-1	F/F	29	-
	S/S	-	30
		(29)	(30)
Adh-2	F/F	29	-
	S/S	-	30
		(29)	(30)
Adh-3	M/M	29	30
		(29)	(30)
Ap-1	M/M	29	30
		(29)	(30)
Ap-2	M/M	29	30
		(29)	(30)
Apk	M/M	15	14
		(15)	(14)
CK	M/M	29	31
		(29)	(31)
Est-1	F/F	22	-
	S/S	-	22
		(22)	(22)
Est-2	F/F	-	14
	F/S	-	5
	S/S	-	3
	VS/VS	22	-
		(22)	(22)
Est-3	F/F	6	12
	F/S	7	1
	S/S	8	9
		(22)	(22)
Est-4	M/M	21	22
		(21)	(22)
αGpdh	M/M	15	14
		(15)	(14)
G6pdh	M/M	29	31
		(29)	(31)

Table 3.3 Contd.:

Locus	Genotype	C. nigro.	C. auratus
Idh	M/M	29	31
		(29)	(31)
Lap	M/M	16	19
		(16)	(19)
Ldh-1	F/F	29	-
	M/M	-	30
	M/S	-	1
	S/S	-	-
		(29)	(31)
Ldh-2	M/M	29	31
		(29)	(31)
Mdh-1	F/F	-	-
	F/S	1	-
	S/S	28	30
		(29)	(30)
Mdh-2	F/F	10	-
	F/M	6	-
	M/M	-	-
	S/S	-	16
		(16)	(16)
Mdh-3	M/M	28	28
		(28)	(28)
Me-1	F/F	-	3
	F/M	-	1
	M/M	-	6
	M/S	4	7
	S/S	25	12
		(29)	(29)
Odh	F/F	15	-
	S/S	-	14
		(15)	(14)
6Pgdh	M/M	16	16
		(16)	(16)
Pgi-1	M/M	29	31
		(29)	(31)
Pgi-2	F/F	11	-
	F/M	11	-
	M/M	6	31
	M/S	1	-
	S/S	-	-
		(29)	(31)

Table 3.3 Contd.:

Locus	Genotype	C. nigro	C. auratus
Pgm	VF/VF	-	20
	VF/F	-	4
	VF/M	-	4
	F/F	-	1
	M/M	26	-
	M/S	3	-
	S/S	-	-
		(29)	(29)
Sdh	F/F	14	-
	F/M	2	-
	M/M	-	-
	S/S	-	19
		(16)	(19)
Sod	M/M	29	31
		(29)	(31)

APPENDIX 3 TABLE 3.4: Genotype Frequency in Tilapias.

SPECIES	Oreochromis aureus	O. moss	Oreochromis niloticus			Sarotherodon galilaeus			S. macro.	S. mel.	T.busu.	T.rend.	TILAPIA T.zillii			
Source/Locality	Stir./Swan.	Stir./ Swan.	Stir.	I.A.B.	M.P.	Stir.	I.A.B.	I.A.B.	Stir.	Den.	Bosom.	Stir.	Stir.	Den.	I.A.B.	M.P.
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Locus (Buffer) / Genotype																
	(12)	(2)	(12)	(7)	(8)	(3)	(20)		(1)	(19)	(30)	(2)	(3)	(22)	(3)	(10)
Ada F/F	12	2	12	7	8	-	20	-		19	-	2	-	-	-	2
(CIC; M/M	-	-	-	-	-	3	-	-	-	-	-	-	3	22	2	6
Poulik) S/S	-	-	-	-	-	-	-		1	-	-	-	-	-	1	1
F/M	-	-	-	-	-	-	-		-	-	-	-	-	-	-	1
VS/VS	-	-	-	-	-	-	-		-	-	30	-	-	-	-	-
	(4)	(2)	(6)	(6)	(6)	(3)	(4)	(1)	(1)	(19)	(10)	(2)	(3)	(3)	(3)	(5)
Adh-1 F/F	4	-	-	-	-	-	-	-	-	-	10	-	3	3	3	5
S/S	-	2	6	6	6	3	4	1	1	19	-	2	-	-	-	-
	(10)	(2)	(6)	(6)	(6)	(3)	(4)	(1)	(1)	(19)	(16)	(1)	(3)	(9)	(5)	(12)
Adh-2 F/F	10	-	6	6	6	3	4	1	1	19	16	1	3	9	5	12
(CIC) S/S	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(12)	(2)	(9)	(12)	(10)	(3)	(16)	(1)	(1)	(19)	(11)	(1)	(2)	(36)	(3)	(19)
AP VF/VF	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1
(CIC/ F/F	12	2	1	8	3	-	-	-	-	-	1	-	-	-	-	3
Poulik M/M	-	-	2	1	7	3	16	1	1	19	10	1	2	35	-	14
S/S	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	1
VS/VS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F/S	-	-	5	1	-	-	-	-	-	-	-	-	-	-	-	-
S/VS	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	(12)	(2)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(31)	(3)	(3)	(31)	(5)	(19)
Est-1 M/M	12	2	12	12	8	2	30	1	1	19	31	3	3	31	5	19
	(6)	(1)	(12)	(12)	(8)	(2)	(31)	(1)	(1)	(19)	(31)	(3)	(2)	(31)	(5)	(19)
Est-2 F/F	6	1	12	12	8	2	30	1	1	-	-	3	2	31	5	19
S/S	-	-	-	-	-	-	-	-	-	19	31	-	-	-	-	-
	(12)	(2)	(7)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(31)	(1)	(3)	(31)	(5)	(19)
Est-3 F/F	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
M/M	12	-	7	12	7	2	30	1	1	-	-	-	-	-	-	-
S/S	-	2	-	-	-	-	-	-	-	19	-	-	3	27	5	18
VS/VS	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-
M/S	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
S/VS	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-

Table 3.4 Contd.

SPECIES		Oreochromis aureus	O. mossambicus	Oreochromis niloticus			Sarotherodon galilaeus			S. macro.	S. mel.	T.busu.	T.rend.	TILAPIA T.zillii			
Source/Locality		Stir./Swan.	Stir./ Swan.	Stir.	I.A.B.	M.P.	Stir.	I.A.B.	I.A.B‡	Stir.	Den.	Bosom	Stir.	Stir.	Den.	I.A.B.	M.P.
OTU		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Locus (Buffer)	Genotype																
		(12)	(2)	(12)	(12)	(6)	(2)	(30)	(1)	(1)	(19)	(31)	(2)	(3)	(36)	(5)	(19)
Est-4	F/F	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	M/M	-	-	11	-	-	-	-	1	-	19	-	2	3	29	5	19
	S/S	12	2	-	9	5	2	27	-	1	-	31	-	-	1	-	-
	F/S	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
(Poulik)	M/S	-	-	1	2	1	-	3	-	-	-	-	-	-	1	-	-
	M/VS	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-
		(12)	(1)	(12)	(11)	(5)	(3)	(30)	(1)	(1)	(18)	(25)	(2)	(2)	(34)	(5)	(17)
αGpdh	F/F	-	-	-	-	-	-	-	1	-	-	-	2	2	31	4	2
(TEB)	M/M	-	-	-	-	-	-	-	-	-	15	25	-	-	3	-	5
	S/S	12	1	10	11	5	3	30	-	1	3	-	-	-	-	1	7
	VS/VS	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	3
		(12)	(2)	(12)	(12)	(6)	(1)	(25)	(1)	(1)	(19)	(20)	(2)	(2)	(33)	(5)	(17)
G6pdh	F/F	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
(TEB)	M/M	12	-	12	12	6	-	23	-	-	19	20	-	2	30	5	17
	S/S	-	2	-	-	-	-	-	-	1	-	-	2	-	-	-	-
	VS/VS	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	M/S	-	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-
		(12)	(2)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(18)	(30)	(2)	(3)	(27)	(5)	(19)
Idh	M/M	12	2	12	12	8	3	31	1	1	18	30	2	3	27	5	19
(CTC)																	
		(6)	(2)	(6)	(6)	(8)	(2)	(30)	(1)	(1)	(19)	(30)	(2)	(3)	(12)	(5)	(19)
Lap-1	F/F	6	2	6	6	8	2	30	1	1	19	-	-	3	12	5	19
(CTC)	S/S	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
	VS/VS	-	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-
		(6)	(2)	(6)	(6)	(8)	(2)	(30)	(1)	1	(19)	(30)	(2)	(3)	(12)	(5)	(19)
Lap-2	F/F	-	-	-	-	-	2	-	-	-	19	-	-	-	-	-	-
(CTC)	M/M	6	-	6	6	8	-	29	1	-	-	-	-	3	12	5	18
	S/S	-	2	-	-	-	-	-	-	1	-	-	2	-	-	-	-
	F/S	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	VS/VS	-	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-
	M/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 5.4 Cont'd.

SPECIES	Oreochromis aureus	O. mossambicus	Oreochromis niloticus			Sarotherodon galilaeus			S. macrochir	S. melanotheron	T.busumanus	T.rendalli	TILAPIA T.zillii			
Source/Locality	Stir./Swan.	Stir./ Swan.	Stir.	I.A.B.	M.P.	Stir.	I.A.B.	I.A.B†	Stir.	Den.	Bosom.	Stir.	Stir.	Den.	I.A.B.	M.P.
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Locus (Buffer) Genotype																
	(6)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(38)	(3)	(3)	(36)	(5)	(19)
Ldh-1 F/F	-	-	-	-	-	-	-	1	-	-	-	-	3	-	-	12
(CIC) S/S	6	1	12	12	8	2	30	-	1	19	38	3	-	36	5	1
F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
	(6)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(38)	(3)	(3)	(35)	(5)	(19)
Ldh-2 F/F	6	1	12	10	6	2	30	1	1	-	38	3	3	33	5	19
(CIC) S/S	-	-	-	-	-	-	-	-	-	19	-	-	-	1	-	-
F/S	-	-	-	2	2	-	-	-	-	-	-	-	-	1	-	-
	(6)	(1)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(19)	(34)	(3)	(3)	(32)	(5)	(19)
Mdh-1 F/F	-	-	-	-	-	3	30	-	-	-	-	-	-	-	-	-
F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
S/S	6	1	12	12	8	-	-	1	1	19	34	3	3	32	4	19
	(6)	(1)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(19)	(33)	(3)	(3)	(32)	(5)	(19)
Mdh-2 F/F	-	-	-	-	-	3	30	-	1	-	1	-	3	32	5	19
M/M	6	1	12	12	8	-	-	1	-	-	32	-	-	-	-	-
S/S	-	-	-	-	-	-	-	-	-	19	-	3	-	-	-	-
	(6)	(1)	(12)	(12)	(8)	(3)	(30)	(1)	(1)	(19)	(34)	(3)	(3)	(31)	(5)	(19)
Mdh-3 F/F	-	1	-	-	-	-	-	-	1	19	-	3	3	31	5	18
M/M	-	-	-	-	-	3	30	1	-	-	33	-	-	-	-	1
S/S	6	-	12	12	8	-	-	-	-	-	-	-	-	-	-	-
F/S	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	(12)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(19)	(34)	(2)	(1)	(37)	(3)	(5)
Me-1 F/F	-	-	12	12	8	-	-	-	1	-	33	-	1	-	-	-
M/M	12	-	-	-	-	2	30	1	-	19	1	2	-	36	2	5
S/S	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-
F/M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
M/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(12)	(2)	(12)	(10)	(7)	(2)	(30)	(1)	(1)	(19)	(33)	(2)	(3)	(42)	(3)	(19)
Me-2 F/F	-	-	1	-	-	-	-	-	-	-	32	2	-	-	-	16
M/M	12	2	10	-	5	-	30	-	-	16	1	-	3	36	-	-
(CIC) S/S	-	-	-	8	2	2	-	1	-	-	-	-	-	4	3	-
VS/VS	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
F/M	-	-	1	1	-	-	-	-	-	3	-	-	-	-	-	-
M/S	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	3
S/VS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.4 Contd.

SPECIES	Oreochromis aureus	O. moss	Oreochromis niloticus			Sarotherodon galilaeus			S. macro.	S. mel.	T.busu	T.rend.	TILAPIA T.zillii			
Source/Locality	Stir./Swan.	Stir./ Swan.	Stir.	I.A.B.	M.P.	Stir.	I.A.B.	I.A.B‡	Stir.	Den.	Bosom.	Stir.	Stir.	Den.	I.A.B.	M.P.
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Locus (Buffer) Genotype																
	(12)	(2)	(12)	(12)	(6)	(3)	(21)	(1)	(1)	(19)	(14)	(2)	(3)	(27)	(3)	(12)
Pep-1 (CTC) M/M	12	2	12	12	6	3	21	1	1	19	14	2	3	27	3	12
	(12)	(2)	(13)	(10)	(6)	(3)	(21)	(1)	(1)	(19)	(12)	(2)	(3)	(27)	(3)	(12)
Pep-2 (CTC) F/F	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	3
M/M	12	2	10	9	6	2	18	1	1	19	-	-	3	27	2	9
S/S	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-
VS/VS	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
F/M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
M/S	-	-	2	-	-	-	-	3	-	-	-	-	-	-	-	-
	(12)	(2)	(6)	(6)	(6)	(3)	(30)	(1)	(1)	(19)	(8)	(2)	(3)	(15)	(5)	(18)
6Pgdh-2 (TEB) F/F	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	3
M/M	-	-	-	-	-	-	3	-	-	-	8	-	3	12	4	15
S/S	12	2	6	6	6	3	27	1	1	19	-	-	-	2	1	-
M/S	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	(12)	(1)	(12)	(12)	(8)	(2)	(30)	(1)	(1)	(18)	(25)	(3)	(3)	(36)	(5)	(19)
Pgi-1 F/F	-	1	-	-	-	-	-	-	-	-	-	-	-	-	5	-
M/M	-	-	12	12	8	2	30	-	-	-	25	3	3	-	-	-
S/S	12	-	-	-	-	-	-	1	-	18	-	-	-	35	-	19
M/S	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-
	(12)	(1)	(12)	(12)	(8)	(2)	(26)	(1)	(1)	(19)	(25)	(2)	(3)	(38)	(5)	(19)
Pgi-2 VF/VF	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F/F	-	-	-	-	-	-	3	-	1	4	-	-	-	-	1	3
(CTC) M/M	12	-	12	12	8	-	23	-	-	15	25	-	3	1	2	8
S/S	-	-	-	-	-	-	-	-	-	-	-	2	-	35	-	-
VF/M	-	-	-	-	-	2	-	-	-	-	-	-	-	2	-	-
F/M	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	-
F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
	(12)	(1)	(12)	(12)	(8)	(3)	(26)	(1)	(1)	(19)	(34)	(2)	(3)	(36)	(5)	(19)
Pgm (CTC) F/F	-	1	12	12	8	-	-	-	-	-	-	-	-	-	-	-
M/M	12	-	-	-	-	3	26	1	1	16	32	2	3	36	5	13
S/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
VS/VS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
M/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
M/vs	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	1
S/VS	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1

Table 3.4 Contd.

SPECIES		Oreochromis aureus	O. moss	Oreochromis niloticus			Sarotherodon galilaeus			S. macro.	S. mel.	T.busu.	T.rend.	Z I L A P I A T.zillii			
Source/Locality		Stir./Swan.	Stir./ Swan.	Stir.	I.A.B.	M.P.	Stir.	I.A.B.	I.A.B.	Stir.	Den.	Bosom	Stir.	Stir.	Den.	I.A.B.	M.P.
OTU		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Locus (Buffer)	Genotype																
		(12)	(2)	(12)	(11)	(6)	(3)	(27)	(1)	(1)	(19)	(19)	(2)	(2)	(27)	(5)	(11)
Sdh-1 (CIC)	F/F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	9
	S/S	12	2	12	11	6	3	27	1	1	19	19	2	2	27	-	-
	F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
		(12)	(2)	(12)	(11)	(6)	(3)	(27)	(1)	(1)	(19)	(19)	(2)	(2)	(27)	(5)	(19)
Sdh-2	F/F	-	-	-	-	-	-	27	1	-	-	-	-	-	-	-	-
	M/M	12	-	12	11	6	3	-	-	1	19	19	-	2	27	5	19
	S/S	-	2	-	-	-	-	-	-	-	-	-	2	-	-	-	-

R E F E R E N C E S

ABBAN, E.K. (1979). Preliminary observations on the biology of Eutropius mentalis in Northern Ghana. IAB/OCP Hydro.Biol. Mon. Rep., 25-37.

ABBAN, E.K. and SAMMAN, J. (1982). Further observations on the effect of Abate on fish catches. Environmental Pollution (Series A); 245-54.

ABBAN, E.K. and SKIBINSKI, D.O.F. (1988). Protein variation in Schilbe mystus (L) and Eutropius niloticus (Ruppel) (Pisces: Siluriformes) in the Volta Basin of Ghana, West Africa. Aquaculture and Fisheries Management, 19: 25-37.

ALTUKHOV, YUP. (1982). Biochemical population genetics and speciation. Evolution, 36(2); 1168-1181.

ALTUKHOV, YUP, SALMENKOV, E.A., SACHKO, V.T., and SLYNKO, G.D. (1972). Number of mono-and polymorphous loci in the population of tetraploid salmon species Oncorhynchus keta (Walb.) Genetika (Moscow), 8(2):67-78 (RE).

ASPINWALL, N. (1974). Genetic analysis of North American populations of the pink salmon (Oncorhynchus gorbuscha) possible evidence for the neutral mutation - random drift hypothesis. Evolution, 28:295-305.

AVISE, J.C. (1974). Systematic value of electrophoretic data. Systematic Zoology, 23:465-481.

AVISE, J.C. (1977). Genetic heterozygosity and rate of speciation. Paleobiology, 3:422-432.

AVISE, J.C. and AYALA, F.J. (1976). Genetic Differentiation in Speciose versus Depauperate Phylads: Evidence from the California Minnows. Evolution, 30:46-58.

AVISE, J.C. and LANSMAN, (1983). Polymorphism of mitochondrial DNA in populations of higher animals. In: Evolution of Genes and Proteins. (Eds. Nei, M. and Koehn, R.K.). Sinauer Associates, Sunderland Massachusetts, 147-164.

AVISE, J.C. and SELANDER, R.K. (1972). Evolutionary genetics of cave dwelling fishes of the genus Astynax. Evolution Lancester, 26:1-19.

AVISE, J.C. and SMITH, M.H. (1974a). Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, Lepomis macrochirus. Evolution, 28:42-56.

AVTALION, R.R. (1982). Genetic markers in Sarotherodon and their uses for sex and species identification. In Pullin and Lowe-McConnell (Eds.) q.v. 269-277.

AYALA, F.J. (1975). Genetic differentiation during the speciation process. In. Evolutionary Biology, 8. (Eds. Dobzhansky, T., Hecht, M.K. and Steere, W.C.). Plenum Press, New York, 1-78.

AYALA, F.J. (Ed. 1976). Molecular Evolution. Sinauer Associates Inco. Sunderland, Massachusetts.

AYALA, F.J. (1983). Enzymes as taxonomic characters. In: Protein polymorphism: Adaptive and taxonomic significance (Eds. Oxford, G.S. and Rollinson, D.). Academic Press, London, New York, Paris, San Diego, San Francisco, Sao Paulo, Sydney, Tokyo, Toronto, 3-26.

AYALA, F.J., TRACEY, M.L. (1974). Genetic differentiation within and between species of the Drosophila willistoni group. Proc. Natl. Acad. Sci. U.S.A., 71:999-1002.

AYALA, F.J., VALENTINE, J.W., HEDGECOCK and BARR, L.C. (1975). Deep-sea asteroids: high genetic variability in a stable environment. Evolution, 29:203-212.

BADINO, G. and SELLA, G. (1980). Phosphoglucose isomerase variability in sympatric populations of Mediterranean species of Patella (Gastropoda; Prosobranchiata). Mar. Ecol., 2:315-320.

BEARDMORE, J.A. and MORRIS, S.R. (1978). Genetic variation and species coexistence in Littorina. In. Marine organisms; genetics, ecology and evolution (Eds. Battaglia, B. and Beardmore, J.A.) New York, Plenum Press, 123-140.

BECKMAN, L. and JOHNSON, R.M. (1964). Genetic control of aminopeptidase in Drosophila melanogaster. Hereditas, 51:221-230.

BELL-CROSS, G. (1976). The fishes of Rhodesia. Illustrated by Jubb, H.M. Trustees of National Museums and Monuments, Rhodesia, Salisbury, Rhodesia.

BENADO, M., AGUILERA, M., REIG, O.A. and AYALA, F.J. (1979). Biochemical genetics of Venezuelan spiny rats of the Proechimys guairae and Proechimys trinitatis superspecies. Genetics, 50:89-97.

BERGER, E.M. and WEBER, L. (1974). The ribosomes of Drosophila. II. Studies on intraspecific variation. Genetics, 78:1173-1183.

BROWN, W.M. (1983). Evolution of animal mitochondrial DNA. In. Evolution of genes and proteins (Eds. Nei, M. and Koehn, R.K.) Sinauer Associates, Sunderland, Massachusetts, pp. 62-88.

BROWN, W.M., PRAGER, E.M., WANG, A. and WILSON, A.C. (1982). mt. DNA sequences of primates: tempo and mode of evolution. J. Molec. Evolut., 18: 225:239.

BULLINI, L. and SBORDONI, V. (1980). Electrophoretic study of gene-enzyme systems: micro-evolutionary processes and phylogenetic inference. Boll. Zool., 47s:95-112.

CAUGANT, D., SELANDER, R.K. and JONES, J.S. (1982). Geographic structure of molecular and morphological polymorphism in Pyrenean populations of the snail Cepaea nemoralis. Genetica, 57:177-191.

CHITTY, D. (1960). Population processes in the vole and their relevance to geral theory. Can. J. Zool., 38: 99-113.

CHUA, K.E., CROSSMAN, E.J. and GILMOUR, C.A. (1978). Lactate dehydrogenase (LDH) Isozymes in muscle of fresh-water fish by isoelectric focusing in thin-layer polyacrylamide gel. Scientific Tools, 25(1): 9-11.

CRACRAFT, J. (1983). Systematics, comparative biology and the case against creationism. In. Scientists against Creationism. (Ed. Godfrey, L.R.). Norton, New York, 163-191.

CRUZ, T.A., THORPE, J.P. and PULLIN, R.S.V. (1982). Enzyme electrophoresis in Tilapia zillii: A pattern for determining biochemical genetic markers for use in Tilapia stock identification. Aquaculture, 29: 311-329.

DAGET, J. (1954). Les poissons du Niger Superieur. Mem. Inst. fr. Afr. noire, (36):391.

DAGET, J. (1956). Poissons. In Le Parc National du Niokolo-koba. Mem. Inst. fr. Afr. noire, (48): 167-169.

DAGET, J. and DURAND, J.R. (1981). Poissons. In Flore et Faune de l'Afrique Sahelo Soudanienne. (Eds. Durand, J.R. and Leveque, C.). Tome ORSTOM, Paris, 2:287-771.

DAGET, J. and ILTIS, A. (1965). Poissons de Cote d'Ivoire (eaux douces et saumatres). Mem. Inst. fr. Afr. noire, (74): 385.

De VOS, L. (1984). Preliminary data on the African species of the family Schilbeidae (Pisces: Siluriformes). Revue de Zoologie Africaine, 98:2.

De VOS, L. and LEVEQUE, C. (1983). Etude systématique et morphologique du gene Eutropius en Afrique de l'ouest. Revue de Zoologie Africaine, 97:468-533.

DOBZHANSKY, T. (1973). 'Nothing in Biology Makes Sense Except in the Light of Evolution', Amer. Biology Teacher, 35:125-129.

EDWARDS, C.A. and SKIBINSKI, D.O.F. (1987). Genetic variation of mitochondrial DNA in mussel (Mytilus edulis and M. galloprovincialis) populations from South West England and South Wales. Marine Biology, 94:547-556.

F.A.O. (1980). Report of the Ad. Hoc Consultation on aquaculture research. FAO Fisheries Report, 238. FIR/R238. FAO Rome, pp. 26.

FELSENSTEIN, J. (1982). Numerical methods for inferring evolutionary trees. Quarterly Review of Biology. 57(4):379-404.

FERGUSON, A. (1980). Biochemical Systematics and Evolution. Blackie, Glasgow and London.

FERGUSON, A. (1981). Systematics of Irish charr as indicated by electrophoretic analysis of tissue proteins. Biochem. Sys. Ecol., 9:225-232.

FERGUSON, A., HIMBERG, K-JM and SVARDSON, G. (1978). Systematics of the Irish pollan (Coregonus pollan. Thompon): an electrophoretic comparison with other holarctic coregoninae. J. Fish Biol., 12(3):221-233.

FRANKEL, O.H. and SOULE, M.E. (1981). Population genetics and conservation. In. Conservation and Evolution. Cambridge Uni. Press, pp. 31-77.

FROST, W.E. (1965). Breeding habits of Windermere Charr, Salvelinus willughbii (Gunther), and their bearing on speciation of these fish. Proceedings of the Royal Society, Vol. (B) 163:232-284.

FRYER, G. and ILES, T.D. (1969). Alternative routes of evolutionary success as exhibited by African cichlid fishes of the genus Tilapia and the species flocks of the Great Lakes. Evolution, 23:359-369.

FRYER, G. and ILES, T.D. (1972). The cichlid fishes of the Great Lakes of Africa. Oliver and Boyd, Edinburgh.

GAINES, M.S. and KREBS, C.J. (1971). Genetic changes in fluctuating vole populations. Evolution, Lancester, Pa. 24:702-723.

GORMAN, G.C. and RENZI, J.Jr. (1979). Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. COPEIA, (2): 242-249.

GOTTLIEB, L.D. (1975). Allelic diversity in the outcrossing annual plant Stephanomeria exigua ssp., (Carotifera: Compositae). Evolution, Lancaster, Pa. 29:213-225.

GREENWOOD, P.H. (1975). Classification. In. A History of Fishes, 3rd edn. (Ed. Norman, J.R.) London: Ernest Benn, pp. 378-396.

GREENWOOD, P.H. (1983c). The zoogeography of African freshwater fishes: bioaccountancy or biogeography? In. Evolution Time and Space: The Emergence of the Biosphere, (Eds. Sims, R.W., Price, J.H. and Whalley, P.E.S.) London Ac., pp. 197-199.

GREENWOOD, P.H. (1984). African cichlids an evolutionary theories. In. Evolution of fish species flocks. (Eds. Echelle, A.A. and Kornfield, I.) University of Maine at Orono Press, pp. 141-54.

GYLLENSTEN, U. (1985). The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. J. Fish Biol., 26:691-699.

GÜNTHER, A. (1902). Last account of the fishes collected by Mr. R.B.N. Walker, C.M.Z.Z., on the Gold Coast. Proc. Zool. Soc. Lond., :330-339.

HARRIS, H. and HOPKINSON, D.A. (1978). Handbook of Enzyme Electrophoresis in Human Genetics. Supplement, North Holland Publishing Comp. Amsterdam, New York, Oxford.

HENNIG, W. (1966). Phylogenetic Systematics. University of Illinois Press, Urbana.

HILLIS, D.M. (1984). Misuse and modification of Nei's genetic distance. Systematic Zoology, 33:238-240.

HOLDEN, J.M. (1963). The populations of fish in dry season pools of Sokoto river. Collections of Fish Publications. London, (19).

HOPSON, A.J. (1965). Four new species of Barbus (Pisces: Cyprinidae) from Ghana. Revue. Zool. Bot. Afr., 71:245-257.

HUMPHRIES, C.J. (1983). (Review of Joysey, K.A. and Friday, A.E., (eds.), Problems of Phylogenetic Reconstruction), Systematic Zoology, 32:302-310.

HUNTER, R. and MARKERT, C.L. (1957). Histochemical demonstration of enzymes separated by zone electrophoresis. Science, 125:1294-1295.

IHSSEN, P.E., BROOKE, H.E., CASSELMAN, J.M., McLADE, J.M., PAYNE, N.R. and UTTER, F.M. (1981). Stock identification: Materials and methods. Can. J. Fish Aquat. Sci., 38:1838-1855.

IRVINE, F.R. (1947). Freshwater fishes and fisheries of the Gold Coast. London Crown Agents.

JAMIESON, A. and TURNER, R.J. (1978). The extended series of Tf alleles in Atlantic cod, Gadus morhua. In. Marine Organisms (Eds. Baltaglia, B. and Beardmore, J.A.). Plenum Publishing Corp. New York and London, pp. 699-729.

JAMIESON, A. and TURNER, R.J. (1980). Muscle protein differences in two eels, Anguilla anguilla (Linnaeus) and Anguilla rostrata (Le Seuer). Biol. J. Linn. Soc. London, 13:41-45.

JONES, J.S., SELANDER, R.K. and SCHNELL, G.D. (1980). Patterns of morphological and molecular polymorphism in the land snail Copaea nemoralis. Biol. J. Linn Soc. London, 14:359-387.

JUKES, T.H. (1971). Comparison of the polypeptide chains of globins. J. Mol. Evol., 1:46-62.

KIMURA, M. (1983). The neutral theory of molecular evolution. Cambridge University Press. Cambridge, London, New York, Rochelle, Melbourne, Sydney.

KIMURA, M. and OHTA, T. (1973). Mutation and evolution at the molecular level. Genetics (Suppl.), 73:19-35.

KIRPICHNIKOV, V.S. (1981). Genetic Bases of Fish Selection. Translated by G.G. Cause. Springer-Verlag - Berlin, Heidelberg, New York.

KORNFIELD, I.L. (1978). Evidence for rapid speciation in African cichlid fishes. Experientia, 34(3):335-336.

KORNFIELD, I.L., RITTE, U., RICHLER, C. and WAHRMAN, J. (1979). Biochemical and cytological differentiation among cichlid fishes of the sea of Galilee. Evolution, 33:349-369.

KORNFIELD, Irv., SIDELL, B.D. and GAGNON, P.S. (1982). Stock identification in Atlantic herring (Clupea harengus harengus): Genetic evidence for discrete fall and spring spawning populations. Can. J. Fish Aquat. Sci., 39(12):1610-1621.

LAVIE, B. and NEVO, E. (1981). Genetic diversity in marine molluscs: a test of the niche-width variation hypothesis. Marine Ecology, 2:335-342.

LAVIE, B., NOY, R. and NEVO, E. (1987). Genetic variability in the marine gastropod Patella coerulea and Patella aspera: Patterns and problems. Marine Biology, 96:367-370.

LEVEQUE, C. and HERBINET, P. (1979-1980). Characteres meristiques et biologiqie de Schilbe mystus (Pisces: Schilbeidae) en Cote d'Ivoire. Hydrobiologistes. ORSTOM, 13(3-4):161-170.

LEVEQUE, C. and PAUGY, D. (1984). Guides des poissons d'eau douce de la zone du programme de lutte contre l'onchocercose en Afrique de l'Ouest. Convention ORSTOM-OMS, Ouagadougou.

LEWONTIN, R.C. (1974). The Genetic Basis of Evolutionary Change. Columbia University Press, New York.

LEWONTIN, R.C. (1978). Genetic heterogeneity of electrophoretic alleles. In. XIV Int. Congr. Genet. Moscow, (Contrib. Pap. Session Abstr.), 1:467.

LEWONTIN, R.C. and HUBBY, J.L. (1966). A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics, 54:595-609.

LOWE-McCONNELL, R.H. (1972). Freshwater Fishes of the Volta and Kainji Lakes. Ghana University Press, Accra.

LOWE-McCONNELL, R.H. (1987). Ecological Studies in Tropical Fish Communities. Cambridge University Press. Cambridge, New York, New Rochelle, Melbourne, Sydney.

LUNDSTROM, R.C. (1977). Identification of fish species by thin-layer polyacrylamide gel isoelectric focusing. Fishery Bull. Fish Wild.Serv. U.S., 75(3):571-576.

LUNDSTROM, R.C. and RODERICK, S.A. (1979). Fish species identification by thin-layer isoelectric focusing of sarcoplasmic proteins. Science Tools, 26,(3): 38-44.

MAYR, E. (1969). Population, Species and Evolution (An abridgement of Animal Species and Evolution). Cambridge, Massachusetts and London. The Balknap Press.

MAYR, E. (1969b). Principles of Systematic Zoology. McGraw-Hill, London.

MAYR, E. (1981). Biological classification: towards a synthesis of opposing methodologies. Science, N.Y., 214: 510-516.

McANDREW, B.J. and MAJUMDAR, K.C. (1983). Tilapia stock identification using electrophoretic markers. Aquaculture, 30:249-61.

McANDREW, B.J. and MAJUMDAR, K.C. (1984). Evolutionary relationships within three tilapiine genera (Pisces: Cichlidae). Zoological Journal of the Linnean Society, 80:421-435.

McFARLAND, W.N., POUGH, F.H., CADE, T.J. and HEISER, J.B. (1985). Vertebrate Life, 2nd Ed. New York, MacMillan: and London: Collier MacMillan.

MERRELL, D.J. (1981). Ecological Genetics. Longman, London.

MULLER, H.J. and KAPLAN, W.D. (1966). The dosage compensation of Drosophila and mammals as showing the accuracy of the normal type. Genet. Res., 8:41-59.

MURRAY, J. (1972). Genetic Diversity and Natural Selection. Oliver and Boyd, Edinburgh.

NEI, M. (1971). Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. Amer. Natur., 105:385-398.

NEI, M. (1972). Genetic distance between populations. Amer. Natur., 106:283-292.

NEI, M. (1975). Molecular Population Genetics and Evolution. North Holland Publication Co., Amsterdam.

NEI, M. and ROYCHOUDHURY, A.K. (1974). Sampling variances of heterozygosity and genetic distances. Genetics, 76:379-390.

NEVO, E. (1978). Genetic variation in natural population: Patterns and theory. Theoretical population Biology, 13:121-177.

NEVO, E., BEILES, A. and BEN-SHLOMO, R. (1984). The evolutionary significance of genetic diversity. In: Evolutionary dynamics of genetic diversity. (Ed. Mani,G.S.) Lecture notes in Biomathematics, 53:13-213.

NUTTALL, G.H.F. (1901). The new biological test for blood in relation to zoological classification. Proc. Soc. Lon., 69:150-153.

O'MAOILEIDIGH, N., CAWDERY, S., BRACKEN, J.J. and FERGUSON, A. (1988). Morphometric, meristic character and electrophoretic analyses of two Irish populations of twaite shad, Alosa fallax (Laepede). J. Fish Biol., 32:355-366.

OMELCHENKO, V. (1974). Electrophoretic study of haemoglobins of Far East fish species. Genetica, 10:35-43.

OWUSU-FRIMPONG, M. (1987). Breeding behavioural pattern of the lake fish Tilapia discolour (Gunther) (Teleosti: Cichlidae) in captivity. J. Fish Biol., 30:1-5.

PATTINGTON, J.D. and MILLS, C.A. (in press). An electrophoretic and biometric study of arctic Charr, Salvelinus alpinus (L.) from ten British lakes. J. Fish Biol.

PETERS, H.M. and BERNS, S. (1978). Uber die Vorgeschichte der maulbrütenden Cichliden. Aquar. Mag. Stuttgart, (5):211-217 and (7):324-331.

PETERS, H.M. and BERNS, S. (1982a). Die maulbrutpflege der cichliden. Untersuchugen zur evolution eines verhaltensmusters. Zeitschrift fur Zoologische Systemik und Evolutionsforschung, 20:18-52.

POWELL, J.R. (1975). Protein variation in natural populations of animals. In. Evolutionary Biology (Eds. Dobzhansky, Th., Hecht, M.K. and Steere, W.C.) Plenum, New York, 8:79-99.

POWERS, D.A., GREANEY, G.S. and PLACE, A.R. (1979). Physiological correlation between lactate dehydrogenase genotype and haemoglobin function in Killi fish. Nature, Lond., 277:240-241.

PULLIN, R.S.V. (1985). Tilapias: "Everyman's fish". Biologist, 32(2):84-88.

REGAN, T. (1920). The classification of the fishes of the family cichlidae. I. The Tanganyika genera. Annals of the magazine of Natural History Series, (9)5:33-53.

REIZER, C., MATTEL, X. and De VOS, L. (1980). Contribution a l'etude de la faune ichtyologique du bassin du fleuve Senegal. V. Schilbeidae. Bulletin de l'IFAN T. 42 (Ser A) No. 1.

RIDLEY, M. (1986). Ancestral groups and sister groups. In: Evolution and Classification, The reformation of cladism. Longman, London, New York, pp. 138-149.

ROBERTS, T.R. (1975). Geographical distribution of African freshwater fishes. Zoological Journal of the Linnean Society, 57:249-319.

ROMAN, B. (1966). Les Poisson de hauts bassins de la Volta. Musee Royal de l'Afrique Centrale - Tervuren, Belgique.

ROMER, A.S. (1950). The Vertebrate Body. W.B. Saunders Company, Philadelphia and London,

ROMER, A.S. (1966). Vertebrate Paleontology (3rd edn.) University of Chicago Press, Chicago, London, University of Toronto, Toronto.

SAUNDERS, N.C., KESSLER, L.G. and AVISE, J.C. (1986). Genetic variation and geographic differentiation in mitochondria DNA of the horseshoe crab, Limulus polyphemus. Genetics Austin, Tex., 112:613-627.

SAURA, A. (1983). Population structure, breeding systems and molecular taxonomy. In: Protein Polymorphism: Adaptive and Taxonomic Significance (Eds. Oxford, G.S. and Rollinson, D.). Academic Press, London, New York, Paris, San Diego, San Francisco, Sydney, Tokyo, Totonto, pp. 310-324.

SKIBINSKI, D.O.F. (1985). Mitochondrial DNA variation in Mytilus edulis L. and the padstow mussel. J. Exp. Mar. Biol. Ecol., 92:251-258.

SNEATH, P.H.A. and SOKAL, R.R. (1973). Numerical Taxonomy, W.H. Freeman and Co., San Francisco.

SBORDONI, V., ALLEGRUCCI, G., CACCONE, A., CESARONI, D., COBOLLI, S.M. and de MATTHAEIS, E. (1981). Genetic variability and divergence in cave populations of

Troglophilus cavicola and T. andreinii (Orthopera, Rhaphidophoridae), Evolution, Lancester, Pa. 35: 226-233.

SCHNELL, G.D., BEST, T.L. and KENNEDY, M.L. (1978). Interspecific morphologic variation in Kangaroo rats (Dipdomys): Degree of concordance with genetic variation. Systematic Zoology, 27:34-48.

SELANDER, R.K., SMITH, M.H., YANG, S.Y., JOHNSON, W.E., and GENTRY, J.B. (1971). IV. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old field mouse, (Peromyscus polionotus). Uni. Texas Publ., 7103:49-90.

SELANDER, R.K. and JOHNSON, W.E. (1973). Genetic Variation among vertebrate species. Ann. Rev. Ecol. System., 4:75-91.

SELANDER, R.K. and WHITTAM, T.S. (1983). Protein polymorphism and genetic structure of populations. In; Evolution of genes and proteins, (Eds. Nei, M. and Koehn, R.K.) Sinauer Associates Incorp. Sunderland, pp. 89-114.

SEMEONOFF, R. and ROBERTSON, F.W. (1968). A biochemical and ecological study on plasma esterase in natural populations of the field vole, Microtus agrestis. Biochem. Genet., 1:205-227.

SHAW, C.R. and PRASAD, R. (1970). Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genet., 4(2):297-320.

SIMPSON, G.G. (1961). Principles of Animal Taxonomy. Columbia University Press, New York.

SMITH, A.C. (1965). Intraspecific eye lens protein differences in yellow fin tuna, Thumus albacares. Calif. Fish Game, 51(3):163-167.

SMITH, A.C. (1968). Electrophoretic studies of eye lens protein from marine fishes. Diss. Abstr., 28(12):68.

SMITH, P.J. and JAMIESON, A. (1978). Enzyme polymorphism in Atlantic mackerel, Scomber scombrus L. Comp. Biochem. Physiol., 60B:487-489.

SMITH, P.J. and JAMIESON, A. (1980). Protein variation in the Atlantic mackerel, Scomber scombrus. Anim. Blood Grps. Biochem. Genet., 11:207-217.

SMITH, P.J. and ROBERTSON, D.A. (1981). Genetic evidence for two species of sprat (Sprattus) from New Zealand waters. Marine Biology, 62:227-233.

SMITHIES, O. (1955). Zone electrophoresis in starch gel: group variation in the serum proteins of normal human adults. Biochem, J., 61(4):629-641.

TAMARU, C.S. (1986). Population structure. In: Aquaculture of Milkfish (Chanos chanos): State of the Art. (Eds. Lee, C-S., Gordon, M.S. and Watanabe, W.O.). The Oceanic Institute Waimanalo, Hawaii, U.S.A., pp. 37-55.

TANIGUCHI, N., MACARANAS, J.M. and PULLIN, R.S.V. (1985). Introgressive Hybridization in cultured tilapia stocks in Philippines. Bulletin of the Japanese Society of Scientific Fisheries, 51(8):1219-1224.

TAVERNE, L. (1972). Osteologie des genres Mormyrus Linne, Mormyrops Muller, Hyperopisus Gill, Isichthys Gill, Myomyrus Boulenger, Stomatorhinus Boulenger et Gymnarchus Cuvier. Considerations generales sur la systematique des poissons de l'ordre des Mormyriformes. Ann. Mus. Roy. Afr. Centr., Ser. in 8°, Sci. Zool., 200, 194 p.

THORPE, J.P. (1982). The molecular cloak hypothesis: biochemical evolution, genetic differentiation and systematics. Annual Review of Ecology and Systematics, 13:139-168.

THORPE, J.P. (1983). Enzyme variation, genetic distance and evolutionary divergence in levels of taxonomic separation. In: Protein polymorphism: Adaptive and taxonomic significance (Eds. Oxford, G.S. and Rollinson, D.). Academic Press, London, New York, Paris, San Diego, San Francisco, Sydney, Tokyo, Totonto, pp. 131-152.

THYS VAN DEN AUDENAERDE, D.F.E. (1966). Les Tilapia (Pisces: Cichlidae) du Sud-Cameroun et du Gabon. Etude systematique. Annls. Mus., r. Afr. cen. Ser. 8°. Sci. Zool., 153, 98 pp., 12 pls.

THYS VAN DEN AUDENAERDE, D.F.E. (1968b). An annotated bibliography of Tilapia (Pisces; Cichlidae). Documn. Zool. Mus. r. Afr. centr. 14:XI + 406 pp.

THYS VAN DEN AUDENAERDE, D.F.E. (1971). Some new data concerning the Tilapia species of the subgenus Sarotherodon (Pisces; Cichlidae). Revue Zool. Bot. Afr., 84:203-216.

THYS VAN DEN AUDENAERDE, D.F.E. (1988). Natural distribution of tilapias and its consequences for the possible protection of genetic resources. In: Tilapia Genetic Resources for Aquaculture (Ed. Pullin, R.V.S.) ICLAM Publication, Manila, Philippines, pp. 1-12.

TREWAVAS, E. (1966a). Tilapia aurea (Steindachner) and the status of Tilapia niloticus exul, T. monodi and T. lemassoni (Pisces: Cichlidae). Israel J. Zool., 14:258-276.

TREWAVAS, E. (1966b). Fishes of the genus Tilapia with four anal spines in Malawi, Rhodesia, Mozambique and Southern Tanzania. Revue Zool. Bot. Afr., 74:50-62.

TREWAVAS, E. (1966c). A preliminary review of fishes of the genus Tilapia in the eastward-flowing rivers of Africa, with proposals of two new species names. Revue Zool. Bot. Afr., 74:394-424.

TREWAVAS, E. (1973a). On the cichlid fishes of the genus Pelmatochromis with proposals of a new genus for P. congicus; on the relationship between Pelmatochromis and Tilapia and the recognition of Sarotherodon as a distinct genus. Bull. Br. Mus. Nat. Hist. (Zool.), 25:1-26.

TREWAVAS, E. (1980). Tilapia and Sarotherdon? Buntbarsche Bull. (81):1-6.

TREWAVAS, E. (1981b). Nomenclature of the tilapia of Southern Africa. J. Limnol. Soc. Sth. Afr., 7:42.

TREWAVAS, E. (1982a). Genetic groupings of Tilapiini used in aquaculture. Aquaculture, 27:79-81.

TREWAVAS, E. (1982b). Taxonomy and speciation. (Eds. Pullin and Lowe-McConnell), q.v.:3-13.

TREWAVAS, E. (1983). Tilapiine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia. British Museum (Natural History), London.

TREWAVAS, E. and IRVINE, F.R. (1947). Freshwater fishes. In. The Fishes and Fisheries of the Gold Coast Irvine, F.R., Crown Agents, London, pp. 221-282.

UTTER, F.M., ALLENDORF, F.W. and HODGINS, H.O. (1973). Genetic variation and relationships in Pacific salmon and related trout based on protein variation. Systematic Zoology, 22:257-270.

WARD, R.D. and BEARDMORE, J.A. (1977). Protein variation in plaice, Pleuronectes platessa, L. Genet. Res. Camb., 30:45-62.

WEBSTER, T.P., SELANDER, R.K. and YANG, S.V. (1972). Genetic variation and similarity in the Anolis lizards of Bimini. Evolution, 26:523-535.

WHITT, G.S. (1983). Isozymes as probes and participants in developmental and evolutionary genetics. In: Isozymes. Current topics in biological and medical research. Vol. 10: Genetics and Evolution 1-40. Alan R. Liss Incorp., New York.

WHYTE, S.A. (1975). Distribution, trophic relationships and breeding habits of the fish populations in a tropical lake basin (Lake Bosomtwi - Ghana). J. Zool. Lond., 177:25-56.

WILKINS, N.P. (1977). Genetic variability in littoral gastropods: phosphoglucose isomerase and phosphoglucomutase in Patella vulgata and Patella aspera. Marine Biology, 40:151-155.

ZOUROS, E., LOUKAS, M., ECONOMOPOULOS, A., MAZOMENOS, B. (1982). Selection at the Alcohol dehydrogenase locus of the Olive fruit fly Dacus oleae under artificial rearing. Heredity, 48:169-185.