Genetic diversity and stock structure of Lake Tanganyika Kapenta (*Limnothrissa miodon*) introduced to African lakes

Final Report on ODA Research Project
(Fish Genetics Programme: R4801)

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Cover picture: Fishermen returning from beach seining in Kibwesa, Lake Tanganyika, Tanzania. The large canoes in the foreground are used to set the beach seine, the small canoes in the background to spot shoals.

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Executive summary

1. Context

Kapenta (*Limnothrissa miodon*: Clupeidae) fisheries in Central and Eastern Africa provide a major source of affordable protein which, when sun-dried, can be stored and transported with relative ease to inland communities. The high demand for protein, together with social and economic constraints has resulted in heavy exploitation of native and introduced populations, with a consequent need for developing sound management policies to ensure sustainability of resources.

The present project examines levels of genetic diversity and stock separation in kapenta fisheries in Lakes Tanganyika (Burundi, Tanzania, Zambia, Zaïre), where it is endemic, and in Kariba (Zambia and Zimbabwe) and Kivu (Zaïre and Rwanda) where it was introduced in 1959 and 1967-68 respectively. Significant yields are currently obtained, though with the exception of Kariba, scope for markedly enhanced productivity exists. Current estimates are, however, based on a poor understanding of fish movements and stock structure.

2. Project aims and rationale

I. SCIENTIFIC OBJECTIVES

(i) *To compare genetic diversity in Lake Tanganyika with introduced populations in Lakes Kivu and Kariba.* The maintenance of genetic variability is of critical importance for adaptation to environmental changes. Although the numbers introduced (57,400 to L. Kivu and 360,000 to L. Kariba) appear sufficient to prevent loss of genetic variability, high initial mortality and limited reproductive success may reduce breeding populations significantly. Comparison of genetic diversity in source and transplant populations using genetic markers (allozymes, and variation in mitochondrial DNA (mtDNA)), were examined to determine whether such declines have occurred.

(ii) *To determine stock separation within Lakes Tanganyika, Kivu and Kariba.* Knowledge on stock structure is vital for defining the unit of management as well as estimating the potential yield from a fishery. In addition to allozymes and sequence variation in mtDNA, the utility of morphometrics (body shape) as stock markers were explored.

(iii) *To estimate the genetic component of changes in size and life-history.* Kapenta exhibits markedly different life-histories in Kariba compared with other lakes. Genetic differences coinciding with the phenotypic changes would strongly support the hypothesis of genetic changes following introduction into Lake Kariba, providing information on adaptability in relation to exploitation.

(iv) *To examine the relationship between genetic structure, fishing pressure and production of kapenta.* High fishing pressure may reduce genetic variability in exploited populations, thus compromising future scope for adaptation. It was planned to examine the effects of different intensities of exploitation within Lake Kariba.

II. DEVELOPMENTAL OBJECTIVES

Aimed at maintaining the long-term productivity and biodiversity (genetic resources) of kapenta.

(v) *To provide guidelines for preserving genetic diversity for future introductions*. Recommendations are suggested for the conservation of genetic variability during introductions (e.g. numbers introduced, source of parental stock).

(vi) *To provide guidelines for preserving genetic diversity in exploited stocks*. Information on the extent of stock separation in native and introduced populations may reveal the extent of independence of exploited stocks to harvesting, thus identifying the potential effects of local stock extinction on genetic diversity and productivity estimates.

3. Methodology

Considerable delays were experienced in obtaining research permits from respective countries, with no permits obtained for Zimbabwe, Mocambique and Zaïre. Kapenta were therefore obtained from Zambian waters in Kariba, and the E. and S.E. shores of Tanganyika. Duplicate samples of 40-50 kapenta (in alcohol, mtDNA; frozen, allozymes) were obtained from several sites in all lakes (Tanganyika, n = 10; Kivu, n = 4; Kariba, n = 10), for genetic and morphometric analysis. Allozyme variation was screened at 29 loci, of which 16 exhibited variants (polymorphism), and sequence variation was examined in one region of the mtDNA genome ("ND" 5/6 genes) using the polymerase chain reaction.

4. Results

I. SCIENTIFIC

4.1 *Genetic diversity*

Allozyme data demonstrated that there was no reduction in genetic diversity following introductions into Lakes Kariba and Kivu. However, there was some indication of a loss in mtDNA diversity in Lake Kivu, where fewer individuals were introduced in ten separate episodes.

4.2 *Stock separation*

Significant morphometric differentiation was detected among samples in each lakes, which in Lake Kariba exhibited small-scale structuring. In Lake Kariba, these phenotypic differences did not coincide with genetic heterogeneity, indicating the existence of identifiable groups ("harvest" stocks) which either are too recently separated, or experience high levels of exchange. In Lake Tanganyika, a similar small-scale heterogeneity was detected at the allozyme level, supporting the notion of small, distinct groups. However, the movements and boundaries of such putative units, possibly representing shoals, are unclear, and were not clarified by the mtDNA data. The results from lake Kivu support the hypothesis of migrating groups, owing to the apparent persistence of genetic changes following the introduction, and the lack of correspondence between genetic similarity and geographic proximity.

Despite the evidence for heterogeneity among samples in each lake, it was not possible to define stock boundaries.

4.3 Genetic basis of life-history changes

The marked differences in life-history characters and body size in Lake Kariba kapenta did not coincide with genetic differentiation, thus indicating that these differences are most likely due to phenotypic responses to the new environment. Caution is urged against assuming that the kapenta fishery can adapt, in the long-term, to increased fishing pressures through reducing its body size at maturity.

4.4 Effect of harvesting on genetic diversity

Owing to the lack of genetically-defined stock boundaries, and failure to obtain a Zimbabwean research permit, it was not possible to evaluate the effects of varying fishing pressures on genetic diversity.

II. DEVELOPMENTAL

4.5 Guidelines for future introductions

Genetic data from Lake Kivu indicate that the release of 57,000 fish, which may have contained a high proportion of *Stolothrissa*, was not sufficient to preserve the genetic diversity. It is therefore recommended that (i) the size of future founder populations should approach that of Lake Kariba (360,000), (ii) simultaneous release of mass numbers may be preferable to sequential release; (iii) that the species composition of the fry be estimated; (iv) that a representative sample of genetic variability in the parental stock would be obtained from a local population (high degree of within-sample gene diversity).

4.6 Guidelines for management of exploited stocks

The existence of morphometric and genetic differences among some samples underline the importance of examining the degree of temporal heterogeneity in such characters. If units exhibit temporal stability, it would be necessary to consider them as local stocks. Alternatively, temporal changes would suggest a greater mobility of differentiated groups, allowing rapid recolonisation of depleted areas. It is therefore recommended that (i) until such information is available, estimates of potential yields obtained by extrapolating local yields over whole lake should be regarded with caution; (ii) that harvesting practices should avoid overdepletion of localised fishing ground; (iii) it should not be assumed that intense fishing pressure would produce correspondingly high productivities as in the relatively young Kariba fishery; (iv) any directional reductions in size at maturity in Tanganyika or Kivu kapenta be regarded as symptomatic of overfishing, with appropriate relaxation of fishing pressures.

Additional outputs in the form of suggestions for future studies, and dissemination of results and recommendations are provided in the full report.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Aims and scope of final report

The present report presents the findings of an ODA-funded project (Fish Genetics Programme: R4801) to examine genetic diversity and stock structure in the freshwater clupeid, *Limnothrissa miodon* ("kapenta"), in three East African Lakes: Lake Tanganyika, where the species is endemic, and Lakes Kivu, and Kariba, where it has been introduced. *L. miodon* forms a significant pelagic fishery in each of these lakes, so providing one of the most important sources of affordable protein in Central and Eastern Africa.

The work was undertaken between November 1991 and March 1994, and was based in the *Marine and Fisheries Genetics Laboratory* in the School of Biological Sciences (University of Wales, Swansea), with consultancy advice from Professor T.J. Pitcher (*Marine Resource Assessment Group* (MRAG), Imperial College, London: November 1991-December 1992), and since January 1993, the *Fisheries Centre* at the University of British Columbia, Vancouver following the move of Professor Pitcher from MRAG. The project involved four major activities: First, a preparatory phase involving the procurement of written permission (research permits) by the appropriate government institutions to obtain samples, plus planning of sampling logistics with local fishery personnel; Secondly, field work carried out on two sampling trips to collect kapenta samples; Thirdly, laboratory studies involving the genetic analyses (allozyme electrophoresis and mitochondrial DNA analysis (mtDNA)) of preserved kapenta samples, and fourthly, analysis of morphometric, allozyme and mtDNA data.

The report contains a summary of original project aims, together with details of changes since its initial inception. To facilitate the interpretation and significance of data presented, an introduction to the genetic management of capture fisheries, and salient features of *Limnothrissa* biology are provided. Project outputs as estimated by publications, presentations at conferences and recommendations to respective fishery bodies are summarised. Emphasis is placed on how the progress achieved may enhance management and conservation of kapenta fisheries to ensure continued future productivity. Suggestions are provided in practical terms on how this may be achieved, including the identification of priorities for future research.

1.2 Project aims and any changes

Six original objectives were identified:

(1) To compare the genetic diversity of kapenta in its natural state in Lake Tanganyika with introduced populations in Lakes Kivu and Kariba with the aim of describing and conserving kapenta biodiversity;

(2) To determine the extent of stock separation among populations at the northern (Burundi) and southern (Zambia) end of Lake Tanganyika, which have been the sources of introductions to Lakes Kivu and Kariba respectively;

(3) To assess stock structure for three of the five major basins of Lake Kariba where commercial fisheries have been successfully established;

(4) To examine the relationship among genetic structure, life history characters, different fishing pressures and production of kapenta fisheries, in order to estimate the genetic component of variability in kapenta production;

(5) To provide guidelines for preserving genetic diversity for future introductions of kapenta;

(6) To provide management guidelines for preserving genetic diversity in exploited stocks of kapenta.

Three independent, though complementary approaches were chosen to examine genetic diversity and stock structure: morphometric analysis of body shape using the "truss system" (Straus & Bookstein, 1982), allozyme electrophoresis which provides discrete and stable genetic markers for analysis of population structure, and the analysis of sequence variation in the rapidly evolving mitochondrial DNA genome. The use of different techniques increases the opportunities for both detection of stock separation, as well as facilitating the interpretation of any patterns detected.

The outputs of the project were to focus on the description and conservation of kapenta biodiversity, incorporating a knowledge of:

(1) kapenta stock structure;

(2) the impact of introductions on kapenta genetic diversity;

(3) the impact of fishing intensity on kapenta genetic diversity in Lake Kariba.

Details concerning the extent to which the objectives have been met are provided in Chapter 6, but the most significant changes are summarised below.

I. *Changes to objectives:*

(1) All objectives have been addressed except objective 4 which set out to examine the relationship between genetic structure, life-history characters, different fishing pressure and production of kapenta fisheries in Lake Kariba. To meet objective 4 would have required clear genetic differentiation among sites, and detailed information on the fishery in Lake Kariba as well as the execution of an intensive sampling programme from the different basins in the lake. Unfortunately, despite considerable efforts, we were unable to obtain a research permit from the Government of Zimbabwe, so restricting our sampling to exploited areas in Zambian waters. It was also not possible to detect genetically-based differentiation.

(2) The project provides the first genetic data on kapenta in its endemic and introduced populations, thus allowing an evaluation of the extent of phenotypic plasticity in life history characters which are known to differ significantly between artificial (e.g. Kariba, Cahora Bassa) and natural (Tanganyika) lakes. Such information is of considerable importance both to the management of existing fisheries, and in planning any future introductions, and represents an additional component to the study.

(3) Plans to sample from Lake Cahora Bassa (Mocambique), where the kapenta are presently unexploited, were abandoned due to civil unrest following advice from the Foreign Office in London.

(4) It was not possible to sample from the Western shore of Lake Tanganyika (Zaire) due to problems of security. It was, nevertheless, still possible to obtain a meaningful estimate of kapenta stock structure in this lake.

2. *Changes to implementation of the project*:

(1) Professor Tony Pitcher left MRAG in January 1993 to take up directorship of the Fisheries Centre in Vancouver, from where he continued to provide advice.

(2) The original plans for the provision of secretarial support and computing facilities by MRAG were abandoned, and all administrative and scientific duties were undertaken by Swansea staff.

(3) An improved procedure for analysis of sequence variation in mtDNA incorporating the polymerase chain reaction was adopted, so increasing the number of samples that could be analysed.

(4) The considerable delays resulting from attempts to secure research permits resulted in the provision of a three month extension (salary) for the employed research assistant (Mr Lorenz Hauser).

1.3 Genetic management of capture fisheries

Two primary objectives of the present project were to examine levels of genetic diversity in kapenta populations, and to describe stock structure in the respective fisheries. Some background is provided here to facilitate an understanding of the relationship between these scientific objectives and the wider developmental benefits that may accrue.

Genetic management involves the incorporation of information on the levels and distribution of genetic variability into management programmes, with the overall aim of conserving genetic resources (levels of allelic diversity and associated genotypic variance in ecologically significant traits; Carvalho, 1993). The overall aim is to ensure the perpetuation of a sustainable resource for the future. Perpetuation of a natural resource requires continuity over time, which involves necessarily a study of the hereditary mechanism and microevolutionary forces. Since differential survival and reproduction of genotypes may affect the abundance and biological characteristics of a fish population, especially in changing environments, short-term benefits through heavy exploitation for example, may lead to an irreversible loss of biodiversity (Nelson & Soulé, 1987).

The genetic management of resources in African freshwaters are at an early stage of development, as indicated by a recent meeting of on Biological Diversity in African Fresh and Brackish Water Fishes (ORSTOM, 1993). A significant reason for the limited application of such genetic studies arises from the requirement of a generally high degree of specialized expertise and facilities, preferably at the local level (Carvalho & Hauser, 1992). However, a further hindrance is the generally poor appreciation of how such work may contribute in practical terms to the management of a fishery, exacerbated by the restrictions imposed by immediate social and economic demands (Carvalho & Hauser, 1994).

That genetic variability is necessary for adaptive evolutionary change is a dictum of evolutionary genetics. New variation enters a population either on an evolutionary time-scale through mutation, or as a result of gene flow through interbreeding between native individuals and genetically distinct immigrants. The possession of heritable variation in fitness traits provides phenotypic flexibility for adaptation to novel changes such as the introduction of a predator, or sudden contamination with a pathogen or pollutant. The fitness of an individual in one environment may be a poor predictor of fitness in another (Ryman, 1991). It then becomes important to conserve the heritable component of variance in phenotypic response to environmental changes, so maximising the opportunities for survival of particular varieties. Thus the conservation of genetic resources is an important objective of management programmes since the requirement to procure sustainable yields does not only depend on the *numbers* of individuals remaining to breed, but also their *quality*, that is, the genetic and ecological characteristics of those individuals (Ryman, 1981; FAO/UNEP, 1981; FAO/CIFA, 1985; Nelson & Soulé, 1987). Fishery practices that may compromise levels of genetic diversity include introductions (Carvalho, 1993; Carvalho & Hauser, 1995), selective harvesting (Smith *et al.*, 1991), overexploitation and collapse of genetically differentiated local populations (Carvalho & Hauser, 1992; Hilborn & Walters, 1992), and release of cultured fishes into nature (Hindar *et al.*, 1991).

In relation to introductions it is vital to appreciate that it is not the number of individuals ("census" population size) released into a new habitat that determines the genetic structure and adaptability of its residents, but rather the proportion of individuals that survive to contribute to the next generation (Carvalho & Hauser, 1995). In addition, variance in age structure, sex ratio, lifetime family size, and subsequent abundance may further constrain N_e (Frankel & Soulé, 1981; "effective" population size, N_e). The combination of above factors mean that, except in heavily managed hatchery populations, N_e is often an order of magnitude smaller than the census number of individuals. For example, using estimates of survival and homing ability of 21,000 pink salmon fry introduced into the Great Lakes, it was estimated that only 42 individuals survived to reproduce, resulting in a significant reduction in levels of genetic diversity (Gharrett & Thomason, 1987).

The notion that fish constitute a renewable resource is a dangerous one, and is true only in part. The loss of a locally-adapted population or an endemic species clearly cannot be reversed, though in a recently introduced population, a diminution in genetic diversity can, at least in principle, be enhanced through carefully designed supplementary stocking programmes (Krueger *et al.*, 1981). In a newly founded population, there would probably be less relative risk of disrupting local adaptation, a particular threat in native situations (Carvalho, 1993). A decrease in genetic variability within a population can, in the absence of immigration, be compensated only through mutations on an evolutionary time-scale. Such reductions in genetic diversity may render a population less able to adapt to man-made or natural changes in the environment, and thus more likely to undergo severe population fluctuations or eventual extinction (Nelson & Soulé, 1987; Ryman, 1991).

Stock structure analysis is a procedure that determines the extent of population substructuring within an exploited species from a particular geographic region. Ideally the activity comprises several interactive phases: (1) the characterization of each identifiable stock using suitable markers; (2) an estimate of the biological significance of stock separation in terms of differences in patterns of recruitment and mortality (or response to harvesting); (3) the incorporation of such information into stock assessment. Here, we focus on the first phase.

Early studies (reviewed by Sinclair, 1988) demonstrated that few species form single homogeneous populations. Instead, most are composed of discrete subpopulations or stocks, and such stocks may react to harvesting more or less independently. It was the idea of independent responses of different stocks to exploitation that demanded information on stock structure. Hence, the stock concept was linked strongly, at least in theory, with the desire to balance the impacts of harvesting with efforts to ensure continued economic returns. The range of molecular genetic tools now available to explore stock structure have recently been reviewed (Park & Moran, 1994; Carvalho & Hauser, 1994).

Subpopulations may arise from several causes, for example, through individuals occupying different geographical areas; through originating from different spawning areas; through feeding in different nursery grounds; through consisting of different age groups that arise from discrete shoals; or through adults attaining some fidelity to specific spawning grounds. From a fisheries perspective it is not so important whether a species exhibits such structuring at any one time, but whether the structure is transitional and short-lived, followed by a stage of mixing that founds the next generation in a random manner. Alternatively, the persistence of any such structuring may be sufficient to allow the accumulation of genetic differentiation among populations.

In essence, the underlying reasons for examining the extent of stock separation in a commercial species can be summarised thus:

(1) to estimate the extent to which stocks may respond independently to harvesting. An integral component of sustainable management is to ensure sustainable recruitment through forecasts of the likely impact of harvesting. It thus becomes important to assess the extent to which high mortalities due to harvesting in one area influence the abundance of other contiguous populations (Brown *et al.*, 1987; Gauldie, 1988). A rate of interpopulation exchange of say, 10% or more may not be enough for the recovery of an overfished population at the rate or timetable imposed by economic constraints. Thus, rates of gene flow that are large enough to homogenise populations, may still be sufficiently restricted to allow recognition of populations as separate management units.

(2) to estimate the extent to which identifiable stocks share a common gene pool. If a commercially exploited species is fragmented into a series of genetically differentiated stocks, it becomes important to modify the intensity of harvesting in accordance with the relative strengths of each stock (Hilborn & Walters, 1992). Extinction of genetically discrete stocks would result in an overall decline in genetic diversity, which in some cases may produce an associated decline in phenotypic flexibility, and hence a reduction in the ability to adapt to environmental change (O'Brien *et al.*, 1985; Allendorf & Leary, 1986; Soulé, 1986).

The extent of genetic differentiation may also be employed to provide information for the assessment of (1) above.

(3) to provide a biological framework for assessing the extent of differentiation in traits likely to affect patterns of recruitment and mortality (e.g. fecundity, growth rates, size at maturity). If molecular tools detect identifiable stocks, variance in phenotypic traits can be compared among such units (Carvalho & Nigmatullin, 1994). Significant differences in any relevant parameters may then be incorporated into stock assessment.

(4) to estimate the composition of mixed-stock fisheries as in the management of Pacific salmon (Utter & Ryman, 1993), though such applications require detectable population differences, and reliable information on the allele

Table 1.1: Details of release of ndagala larvae (mixture between *Stolothrissa* and *Limnothrissa*) into Lake Kivu from Lake Tanganyika (1959)

Date	quantity caught	quantity released	place of introduction
4.6.59	5,000	3,600	Cyangugu
5.6.59	5,000	3,600	Cyangugu
11.6.59	5,000	3,600	Cyangugu
17.6.59	5,000	3,600	Cyangugu
22.6.59	30,000	20,000	Bukavu
25.6.59	5,000	3,600	Goma
3.7.59	20,000	5,000	Bukavu
10.7.59	10,000	7,200	Bukavu
16.7.59	5,000	3,600	Cyangugu
22.7.59	5,000	3,600	Cyangugu
TOTAL INTRODUCED		57,400	

frequencies in the catch and in the contributing stocks. Those species with persistently separate spawning grounds, either on a spatial (Milner *et al.*, 1985) or temporal (Carvalho & Pitcher, 1989) basis offer the best opportunities for such an approach.

1.4 Aspects of the biology of *Limnothrissa*

Freshwater sardines (family Clupeidae) in Africa are most commonly found in rivers, though some have adapted to lakes. Two of the best known lacustrine species, *Limnothrissa miodon* ("kapenta") and *Stolothrissa tanganicae*, are endemic to Lake Tanganyika, where they support important fisheries (Marshall, 1993). Successful introductions of *L. miodon* to Lakes Kariba and Kivu have been carried out. Kapenta is a valuable resource for pelagic fisheries in most of these lakes, and when sun-dried can be stored for transportation to distant consumers, thus making it one of the most important sources of inexpensive protein for Central and East Africa. The importance of kapenta as a natural resource highlights the necessity to manage it carefully and on a scientifically sound basis.

In view of the commercial importance of *L. miodon*, the species was introduced from Lake Tanganyika into Lake Kivu and the artificial lake, Kariba. In 1959, 57,400 ndagala larvae (mixture between *Stolothrissa* and *Limnothrissa*) were introduced into Lake Kivu (Collart, 1960, 1989), though only the latter became established. The larvae were released sequentially in 10 batches over a period of 2 months (Table 1.1), compared with two major releases over a year in Lake Kariba.

Since the riverine fauna of the Zambezi were unlikely to occupy the open waters of the artificial Lake Kariba, it was suggested to introduce a pelagic zooplanktivore (Jackson, 1961). After a failed attempt in 1963, a total 360,00 *Limnothrissa* fry were introduced at Sinazongwe (Fig. 2.1) in two major airlifts, one in 1967, the other in 1968. The species has quickly dominated pelagic waters of Kariba (Marshall, 1992) and also, via the Zambezi river, has invaded Lake Cahora Bassa, a downstream reservoir. It has contributed to a great increase in the fisheries productivity of Lakes Kariba and Kivu, but the fisheries

of Cahora Bassa are poorly developed because of the civil war in Mocambique. More recently it has been introduced to Lake Itezhi-tezhi (Zambia), though details are not yet available.

The lakes in which kapenta now occur are very different from each other and its success in each of them has attracted much interest (Marshall 1993). One of the most striking features of kapenta is the marked differences in size and life-history characters between the lakes (Table 1.2). These differences are interesting both practically for fisheries management and in ecological/evolutionary terms. Several possible causes for the small size of kapenta in the artificial lakes, such as genetic change, slower growth, environmental unpredictability, food deficiency and higher predation pressure have been proposed (Marshall, 1993). None of these theories could be unequivocally proven hitherto, although high predation pressure seems a likely candidate (Marshall, 1993). However, the question is not only "why?", but also "how?".

There are generally two mechanisms by which a population can adapt to a new environment after an introduction. Either the extent of individual phenotypic plasticity is sufficient to allow persistence in the new environment (Thompson, 1991) or "population" responses elicit changes in genetic structure (e.g. Vuorinen *et al.*, 1991). This question is not merely academic, but has also important management implications: if the change is genetic, it is likely to be irreversible (Ryman, 1991, Ricker, 1972), whereas phenotypic responses are not. In addition, if the smaller size of kapenta in the artificial lakes is caused by a higher predation pressure, similar responses may arise from harvesting. A genetic decrease in size due to exploitation would be an irreversible loss of the resource's commercial and food value, whereas a phenotypic response could be used as an indicator for overfishing. In this sense the resolution of the question as to why and how the kapenta are smaller in the artificial lakes is of vital importance.

Furthermore, as pointed out by Marshall (1993), if the smaller size of kapenta in Lake Kariba has arisen from high mortalities due to predation, early maturation enables the fish to maintain a constant proportion of mature adults in spite of intense predation on adults (Fryer & Iles, 1972). This is of relevance to *L. miodon* because it means that the smaller individuals invest more energy into reproduction than the larger ones since their relative fecundity is higher (Marshall, 1993). The consequence is that production is maximised, and when considered in relation to water volume, rather than lake area, Kariba supports a pelagic biomass comparable to that in Lake Tanganyika (Marshall, 1993). Experimental evidence supports such assertions. For example, predation on adult guppies, *Poecilia reticulata*, by the cichlid, *Crenicichla alta*, caused them to mature earlier, increase their reproductive effort and reduce the size of their offspring (Reznick *et al.*, 1990).

Small body size is of significance to fisheries management because of the implication that fishing effort could be increased until the fish accelerate their life cycle and reproduce earlier, provided of course that sufficient phenotypic flexibility and recruitment could be maintained. Thus intensive fishing, a form of predation on adults, might increase clupeid productivity and allow greatly enhanced productivities from respective fisheries. It is therefore of considerable practical importance to ascertain the contribution of genetic factors to such body size reductions.

1.5. Salient features of environmental background

The present study was restricted to Lakes Tanganyika, Kivu and Kariba. Lake Cahora Bassa was inaccessible due to civil unrest in Mocambique and the introduction of kapenta to Lake Itezhi-tezhi was too recent for a sizeable population to develop, and thus the collection of samples was impossible. Subsequently the most important features of Lakes Tanganyika, Kivu and Kariba are described, as far as they are relevant to the present investigation.

1.5.1. *Lake Tanganyika*

Lake Tanganyika is a long narrow lake (length 650 km, mean width 50 km, mean depth 570 m) in the East African rift valley. The lake can be divided into two major basins, a northern and southern basin (Tiercelin & Mondeguer, 1991), which may have been originally two separate lakes (Beadle, 1981). These two basins differ in their hydrodynamic and thus in their biological properties.

Despite high water temperatures, thermal stratification is well marked and varies seasonally above a permanently anoxic hypolimnion (Coulter & Spigel, 1991). Consistent southerly winds during the dry season (May - August) cause an upwelling of nutrient rich hypolimnetic water, which is slowly pushed northwards. Thus, during the dry season, the hydrodynamic regimes vary considerably between the basins: in the south, stratification is weak with mixing occurring down to 300 m, while in the north the thermocline stays sharp at 50-80 m (Coulter & Spigel, 1991). Due to upwelling, the rate of nutrient supply from the hypo- to the epilimnion is an order of magnitude higher in the south than in the north (Hecky *et al.*, 1991). Therefore the biomass of phytoplankton is higher in the south than in the north during the dry-season, possibly the cause for the higher abundance of pelagic fish therein (Coulter, 1991a).

The pelagic fisheries are based on four *Lates* species and two clupeids (*Stolothrissa tanganicae* and kapenta, *Limnothrissa miodon*). Of the clupeids, *Stolothrissa* is the commercially more important species, comprising between 50 and 80 % of the pelagic catch (Coulter, 1991a, Marshall, 1992). Thus research effort on Lake Tanganyika has concentrated on the latter species. There is evidence for limited mobility of *Stolothrissa* from their localised response to changes in predator abundance and fishing pressure, as well as from time lags in peak spawning or survival of the major annual cohort (Coulter, 1991a), possibly also indicating the presence of several stocks in Lake Tanganyika (Roest, 1985). Although such evidence is sparse for kapenta, it is believed that its migrations are as limited as those of *Stolothrissa* (Coulter, 1991b), thus, in principle, allowing the development of differentiated stocks within the lake.

The current yield from the pelagic fisheries is about 85,000 t per year and is concentrated in the extreme northern (Burundi) and southern (Zambia) tips of the lake. There are reports of overfishing (decrease in catch per unit effort) from these regions (Pearce, 1985a, Coulter, 1991a), which may, however, be due in part to pedator-prey interactions (Pearce, 1985b, Roest, 1985). The potential sustainable yield has been estimated to be between 380,000 and 460,000 tons (Coulter, 1977), based on extrapolating existing yields to the whole lake. This method depends very much on the extent of stock separation, as fishing in a localised area may rely on a stock extending far beyond the range of the actual fleet (Coulter, 1991b). By extrapolating the yields from such localised areas, the potential yield of the whole lake would be grossly overestimated. Therefore

information on stock structure and fish movements is vital for the future management of kapenta.

1.5.2. *Lake Kivu*

Lake Kivu is located in the western rift valley about 100 km north of Lake Tanganyika, into which it discharges via the Ruzizi River. The lake is deep (mean depth 240 m) and it has a highly dendritic shoreline with numerous islands, especially in the south (Beadle, 1981). With its 20,000 year old origin, it is much younger than Lake Tanganyika. Lake Kivu is deoxygenated below 50-70 m and the extent of mixing between the two layers is not clear.

The vulcanic history of Lake Kivu is associated with a very species-poor fish community (Dumont, 1986). In particular, there are no pelagic zooplanktivores. Thus, in an attempt to introduce *Stolothrissa tanganicae* from Lake Tanganyika, about 57,400 ndagala larvae (mixture between *Stolothrissa* and *Limnothrissa*) were introduced in 1958-60 sequentially in 10 batches (Collart, 1960, 1989; Table 1.1). Instead of *Stolothrissa,* kapenta became established, which after establishment of an infrastructural basis to the fishery (FAO project), has become an important protein resource in Rwanda and Zaïre.

Size and other life-history characters of the Lake Kivu kapenta are similar to those in Lake Tanganyika (Table 1.2), though it has a different appearance having relatively larger eyes and a lower condition factor (Cayron, 1979). The current annual catch is estimated to 2,700 tons, with a potential Maximum Sustainable Yield of 6,000 tons (Marshall, 1992).

1.5.3. *Lake Kariba*

Lake Kariba is a man-made lake on the Zambezi River on the border between Zambia and Zimbabwe, which was filled to capacity in 1962. With a mean depth of 30m, Lake Kariba is considerably shallower than the other two lakes. As in most man-made lakes, the inflow is very large in relation to its volume, and the renewal time is around three years (Marshall, 1992). Because of this, productivity depends to a large degree on the nutrient input, and thus on the water level, of its tributaries. The lake is now mesotrophic because the Zambezi is relatively nutrient poor (Marshall, 1992). The lake can be divided into five basins which differ in limnological characteristics like turbidity, water flow, thermal and oxygen stratification, shore characteristics, and productivity (Bourdillon *et al.*, 1985).

Soon after the introduction of kapenta it became clear that they grew only to about half the size of fish in Lakes Tanganyika and Kivu (Marshall, 1992). This, together with higher mortality and earlier maturation, makes kapenta an essentially annual species in Lake Kariba, as compared to a biennial life cycle in the other lakes (Table 1.2). The current kapenta catch is about 28,500 t, with an estimated Maximum Sustainable Yield of 25,000 t (Marshall, 1992)

1.6 A re-evaluation of project aims

I. Scientific objectives

Having highlighted the underlying rationale of genetic management, and salient features of the kapenta fisheries, such information can be related to the original project aims.

1) *To compare the genetic diversity of kapenta in its natural state in Lake Tanganyika with introduced populations in Lakes Kivu and Kariba.* The maintenance of genetic variability is of critical importance for adaptation to environmental changes (Soulé, 1986), which in the case of introduced species involves adaptation to a new habitat. Although the numbers introduced (57,400 to L. Kivu and 360,000 to L. Kariba) seem sufficient to prevent any loss of genetic variability due to founder effects, high initial mortality and limited reproductive success may reduce the effective population size by several orders of magnitude (Gharrett & Thomason, 1987). Comparison of genetic diversity in source and transplant populations using allozymes and mtDNA will determine whether any such declines have occurred.

(2) *To determine the extent of stock separation within Lakes Tanganyika, Kivu and Kariba.* Knowledge on stock structure is vital for defining the unit of management as well as estimating the potential yield from a fishery. Although the major emphasis to reveal stock structure of kapenta was aimed at Lake Tanganyika, several samples were collected also from Lakes Kivu and Kariba, where kapenta has only recently been introduced. In these lakes, the time since introduction may be insufficient for genetic differences to accumulate due to genetic drift and mutations. However, different ecological conditions in various parts of the lakes may exert differential selection pressures, leading quickly to genetically identifiable stocks (Vuorinen *et al.*, 1991). The application of the reputedly more sensitive analysis of sequence variation in mtDNA should provide a valuable comparison with allozyme data. In addition, the utility of morphometric variation among samples as stock markers will be explored.

(3) *To estimate the genetic component of changes in size and life history.*

Table 1.2: Life history parameters of *Limnothrissa miodon* in the lakes investigated. L_m and A_m are length and age at first maturity, respectively. L_∞ and k are von Bertalanffy growth parameters, Z is the total annual mortality rate. From Marshall (1993).

		Tanganyika	Kivu	Kariba
L_m (mm)	males	64	61	35
	females	75	62	35
A_m (mo)		8	8	3
L_∞ (cm)		16.63	15.00	8.10-13.58
k		1.13	1.45	0.95- 1.74
Z		5.73	8.89	11.79
Life cycle		biennial	biennial	annual
Z		5.73	8.89	11.79

While direct and unequivocal conclusions on the genetic basis of phenotypic differences are only possible from transplantation or laboratory experiments, the susceptibility of kapenta to handling stress (Matthes, 1965-66) virtually prohibits such investigations. However, genetic differences coinciding with the phenotypic changes would strongly support the hypothesis of genetic changes following introduction into Lake Kariba.

(4) *To examine the relationship between genetic structure, fishing pressure and production of kapenta.* High fishing pressure may reduce the genetic variability of an exploited population (Smith *et al.*, 1991). The different degrees of exploitation in Kariba offers an opportunity to assess the genetic effects of exploitation on a small, short-lived pelagic species. However, such a comparison depends on the existence of geographically separated stocks.

II. Deveolpmental objectives

(5) *To provide guidelines for preserving genetic diversity for future introductions of kapenta.* The results of the study will be used to provide management guidelines (e.g. numbers introduced, single or sequential events, source of parental stock) for the conservation of genetic variability of kapenta during introductions.

(6) *To provide guidelines for preserving genetic diversity in exploited stocks of kapenta.* Information on the extent of stock separation in native and introduced populations will reveal whether there is any evidence for geographic structuring of populations. The extent of independence of exploited stocks to harvesting in different regions of the same lake will be assessed. Data on the extent of genetic differentiation will reveal useful information on the distribution of genetic variability, thus identifying the potential effects of local stock extinction on genetic diversity.

CHAPTER 2: SAMPLING OF KAPENTA

2.1. Application for research permits

The bureaucratic procedures involved in applying for research permits unfortunately caused unforeseen problems. Table 2.1 gives a timetable of events, which show the considerable administrative delays caused. The often difficult communication with African countries, whose government offices generally have no faxes and unreliable telephone connections, further delayed the onset of the first sampling expedition. Particular problems were encountered in Zimbabwe, Mocambique and Zaïre where research permits were not forthcoming, and in Tanzania where there was a 9 month delay. Due to the problems encountered in Zimbabwe, Lake Kariba was sampled only from the Zambian side. Furthermore, access was obtained only to the Eastern shoreline of Lake Tanganyika. Unfortunately, it was not possible to collect fish from Lake Cahora Bassa, both because of the civil war in Mocambique and the logistic problems of travelling to the lake.

2.2. General sampling procedure

As kapenta supports major fisheries in most areas where it occurs, the collection of samples was generally carried out with the support of commercial or artisanal fishermen. Four different fishing methods were employed in the present study . Firstly, with commercial purse seine units, which consist of a main fishing vessel and several small boats carrying high pressure kerosene lamps. These boats are distributed on the lake at night, where they wait until sufficient fish are attracted around the light. The purse seine is then set around the light boat. After hauling the net, the procedure is repeated around another light boat. Usually one haul per hour can be achieved using this method. As the preparation of tissue samples took some time, it was possible only to obtain a sufficient sample size by using fish from several hauls of the purse seine. Light boats are usually set in close proximity to each other, they thus catch fish from the same area but most likely from different shoals. Therefore some of the samples consist of several shoals from the same area, a feature important to the analysis of the allozyme data. Fish were collected with purse seines only on Lake Tanganyika in Hore Bay (HB1, HB2), Camerone Bay (CB1) and Muguruka (MUG).

The second type of commercial units employed were kapenta rigs on Lake Kariba. These are large rafts with circular lift nets. Fish are attracted by high pressure mercury lamps both above and in the water. Again some samples from

Table 2.1: Timetable (1992) of applications for research permits.

Country	first contact	application submitted	permit granted
Burundi	19.2.	20.3.	10. 5.
Mocambique	10.3.	-----	------
Rwanda	4.2.	20.3.	22. 4.
Tanzania	17.1.	16.7.	24.10.
Zambia	17.1.	10.3.	10. 4.
Zaïre	19.2.	12.3.	------
Zimbabwe	17.1.	24.2.	------

these units were collected from several hauls, however, as the fishing units generally are kept stationary for the whole night, all samples are from the same location. Kapenta rigs are used only on Lake Kariba, where submerged trees would hamper the use of purse or beach seines. It is unclear whether samples here would comprise fish from separate shoals, though the chances are certainly less than with mobile purse seines. All samples from Lake Kariba were collected from kapenta rigs.

Another type of gear used were beach seines. These are usually used in connection with canoes carrying paraffin lamps. These canoes move inshore slowly to attract fish close to the shore within reach of the beach seine. Either a whole fleet of canoes arrives simultaneously at the shore in the morning (Camerone Bay CB2), or the light canoes arrive successively in intervals of about an hour, so allowing several hauls per night (Kipili). Sub-samples were collected only with the latter method.

An alternative to catching light-attracted fish is to locate shoals of clupeids, and catch these with beach seines, the only fishing technique of kapenta carried out during day time. This method was observed only in Kibwesa on Lake Tanganyika. Due to the limited success of day-time beach seining, the sample from Kibwesa consists of two sub-samples collected on consecutive days, one from beach seines, the other from catamarans.

The most widely used fishing method involves the use of catamarans with liftnets, especially in the north of Lake Tanganyika and on Lake Kivu. These units are two (L. Tanganyika) or three (L. Kivu) canoes tied together with poles, with a lift net fixed between them. Again fish are attracted by lamps and caught by lifting the net. The number of lifts per night on these units depends both on the abundance of fish and the enthusiasm of the crew. Therefore the samples were collected from several hauls (Kigoma, Karongo, Kadjaga, Kibuye South) or from a single haul (Gisenyi, Kibuye North, Cyangugu), generally from within the same respective areas.

At three sites, the division of samples into sub-samples is of particular importance: in Kigoma, the first attempt to collect kapenta was of only limited success with 8 fish caught. Therefore the sampling was repeated one week later, collecting 44 fish from two hauls on a catamaran. There is thus a temporal aspect in sub-sampling here. In Kibwesa, fish were collected over a two day period, 10 fish by daytime beach seining on day one, 35 fish on a catamaran during the night following the next day. At these two sites, sub-sampling was caused by logistical constraints rather than through planning; they are thus of only very limited value in the investigation of small scale genetic variation. In contrast, five sub-samples of reasonable size (21-43) were collected in Hore Bay, representing the most extensive, albeit not ideal, set of sub-samples for exploring microgeographic genetic differentiation.

2.3 Collection of tissue samples

Tissue samples were obtained using a standard procedure. Upon catching the fish, a small piece of muscle tissue was cut along the lateral line and preserved in ethanol for DNA analysis. Another piece of muscle from the same fish was frozen in liquid nitrogen for allozyme electrophoresis. Although several different tissues are normally used for allozyme electrophoresis, the present study was confined to muscle, partly because other tissues did not reveal many additional loci in preliminary analyses (*pers. obs.*), and partly because we were keen to preserve the sample as quickly as possible to avoid tissue deterioration.

Fish for morphometric analysis (Chapter 3) were generally collected on the last haul, thus minimising the time between death and the collection of truss data.

2.4. First sampling expedition to Zambia

Between 13 August and 25 September 1992, the first sampling trip was undertaken to collect kapenta from Lakes Tanganyika and Kariba in Zambia. Despite some logistical problems, mainly with transport, we collected all anticipated samples.

The Zambian section of Lake Tanganyika consists of two main bays, Hore Bay and Camerone Bay (Figure 2.1). With the help of Dr. Martin Pearce of the local branch of the Department of Fisheries and Mr. Roger Bills, the manager of a commercial fishing company, we were able to collect two samples in each of these bays (Figure 2.1, Table 2.2). On Lake Kariba, we were able to obtain samples from all five basins, due to the support of local fishery operators who also provided transport to the kapenta fishing rigs. From each site we took frozen and ethanol-preserved fish as well as samples for morphometric analysis. Thus the sampling trip to Zambia was highly successful.

Table 2.2: Location and size of kapenta (*Limnothrissa miodon*) samples from Lakes Tanganyika, Kivu and Kariba.

sample	date	locality	B	latitude	longitude	no. of fish F	E	M
Lake Tanganyika								
KAD	23.9.93	Kadjaga, 10 km W Bujumb.		S 3°25'	E 29°16'	40	40	48
KAR	24.9.93	Karongo, 52 km S Bujumb.		S 3°53'	E 29°23'	50	50	0
MUG	20.9.93	Muguruka, 110km S Bujumb.		S 4°12'	E 29°35'	46	46	40
KIG	14.9.93	Kigoma, Tanzania		S 4°55'	E 29°55'	68	68	50
KIB	17.9.93	Kibwesa, Tanzania		S 6°33'	E 29°55'	47	47	40
KIP	10.9.93	Kipili, S Tanzania		S 7°25'	E 30°35'	50	50	40
HB 1	23.8.92	E of Hore Bay		S 8°41'	E 31° 9'	52	52	50
HB 2	24.8.92	W of Hore Bay		S 8°44'	E 30°58'	101	101	12
CB 1	26.8.92	S of Camerone Bay		S 8°29'	E 30°33'	50	50	0
CB 2	26.8.92	NE of Camerone Bay		S 8°24'	E 30°28'	40	44	32
Lake Kivu								
GIS	30.9.93	Kigufi Bay, 4km S Gisenyi		S 1°45'	E 29°16'	50	50	45
KYN	30.9.93	Syiki, N Kibuye district		S 1°57'	E 29°19'	50	50	46
KYS	1.10.93	Rugari Bay, S Kibuye district		S 2°15'	E 29°11'	50	50	62
CYA	2.10.93	Muhumba Bay, Cyangugu		S 2°29'	E 28°51'	50	50	50
Lake Kariba								
SV 1	1.9.92	15km SW of Siavonga	4	S 16°34'	E 28°32'	50	50	52
SV 2	2.9.92	6km SW of Siavonga	4	S 16°32'	E 28°37'	50	50	49
SV 3	2.9.92	6km SW of Siavonga	4	S 16°32'	E 28°37'	60[2]		
K 1	7.9.92	8km SE of Kariba	5	S 16°35'	E 28°50'	45	45	51
K 2	7.9.92	5km S of Kariba	5	S 16°35'	E 28°47'	45	45	45
C	4.9.92	Chipepo Harbour	3	S 16°48'	E 27°54'	60	60	18
SZ 1	17.9.92	16km W of Sinazongwe	3	S 17°18'	E 27°36'	60	49	50
SZ 2	18.9.92	35km SE of Sinazongwe	2	S 17°34'	E 27°19'	50	50	50
JO 1	19.9.92	Basin I of Lake Kariba	1	S 17°52'	E 27°07'	60	30	40
JO 2	19.9.92	Top of basin II	2	S 17°47'	E 27°08'	50	30	49

Table 2.3: Location and size of *Stolothrissa tanganicae* samples from Lake Tanganyika.

sample date	locality	B	latitude	longitude	no. of fish F	E	M
Lake Tanganyika							
S/KAD 23.9.93	Kadjaga, 10 km W Bujumb.		S 3°25'	E 29°16'	50	50	0
S/KAR 24.9.93	Karongo, 52 km S Bujumb.		S 3°53'	E 29°23'	50	50	36
S/MUG 20.9.93	Muguruka, 110km S Bujumb.		S 4°12'	E 29°35'	40	40	13
S/KIG 14.9.93	Kigoma, Tanzania		S 4°55'	E 29°55'	45	45	42
S/KIB 17.9.93	Kibwesa, Tanzania		S 6°33'	E 29°55'	50	50	12
S/KIP 10.9.93	Kipili, S Tanzania		S 7°25'	E 30°35'	50	50	31
S/HB 1 29.8.92	E of Hore Bay		S 8°41'	E 31° 9'	12	10	0

Legend
B: basin number (Lake Kariba only)
F: frozen
E: ethanol preserved
M: used for morphometrics
1: these fish were used to screen for usable allozymes

2.5. Second sampling expedition to Tanzania, Burundi and Rwanda

The second sampling trip was carried out between 3 September and 10 October 1993, to complete the sampling programme for kapenta. Three sites in Burundi were sampled in the northern part of Lake Tanganyika and at 3 sites in Tanzania along the eastern shore of the lake (Figure 2.1, Table 2.3). Including the four samples from the Zambian part of the lake, samples were therefore obtained from 10 localities of Lake Tanganyika. The lower sampling density in Tanzania reflects the lower human population density in this part of the lake and the corresponding logistical problems. However, they also reflect the fishing intensity, which is much lower in the central part of the lake than at its southern and northern ends.

Following a suggestion of Dr Frits Roest, *Stolothrissa tanganicae* were also sampled on L. Tanganyika (Table 2.3). Results from the second commercially exploited clupeid species, which has a more pelagic life-style than *Limnothrissa,* should provide valuable comparative information on the relation between ecology, life-history and population differentiation. These samples have been analysed morphometrically for the present report, but allozyme data will not be available prior to completion of the final report.

Fortunately, the political situation in Rwanda allowed us to sample on Lake Kivu as well. Due to the excellent support by the local fisheries institute (Projet Pêche, Gisenyi, Rwanda), especially by Mr Augustin Mutamba, it was possible to sample from four sites covering the whole lake (Figure 2.1).

In contrast to Rwanda, the safety situation in Zaïre, on both L. Tanganyika and Kivu, was not satisfactory, and following advice from the Foreign Office, the trip to Zaïre was cancelled. The absence of samples from the western shore of L. Tanganyika, although unfortunate, should not pose major problems, as the lake is only about 70 km wide at its northern end, and thus any significant stock differentiation is unlikely. The same holds true for Lake Kivu.

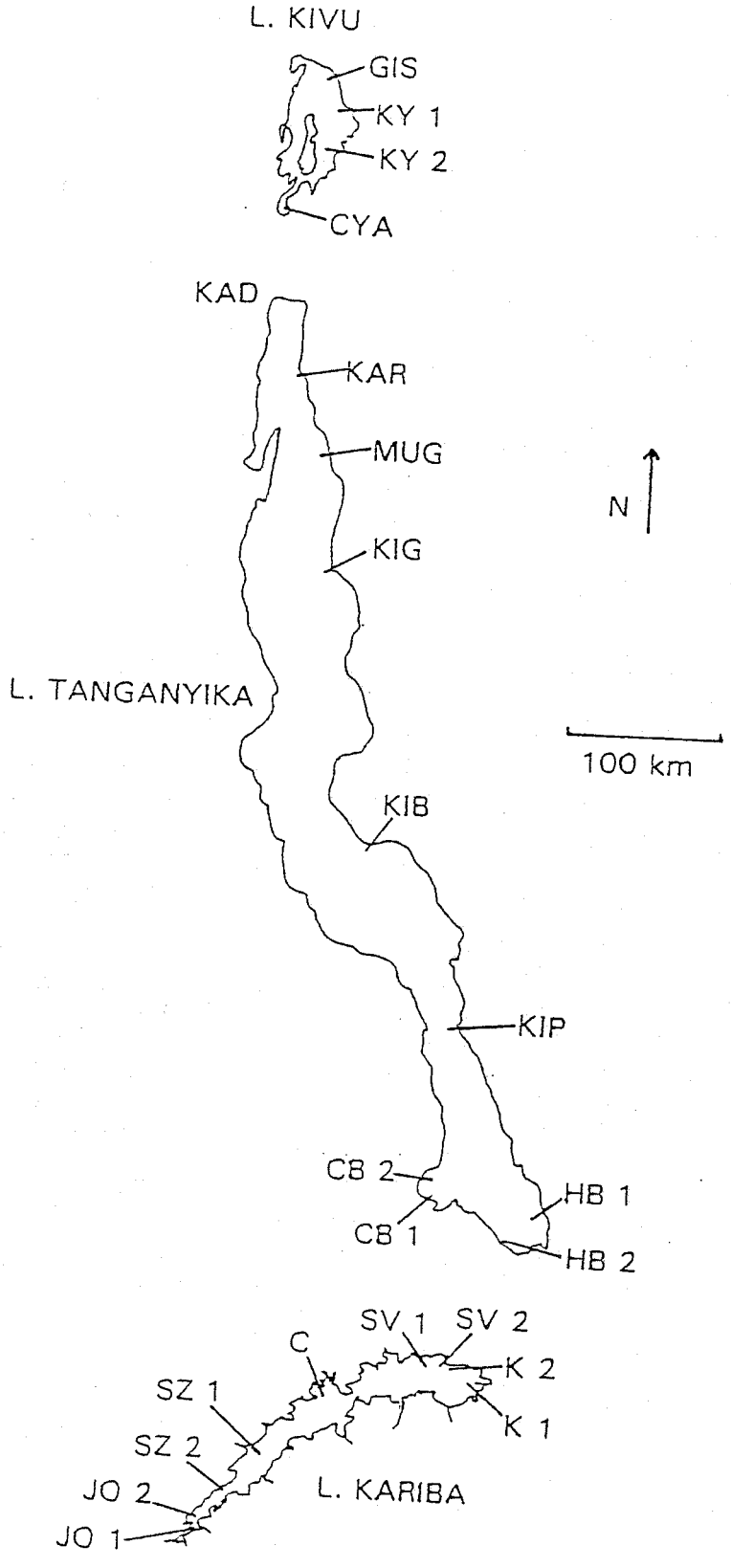


Figure 2.1: Simplified map of Lakes Kivu, Tanganyika and Kariba, showing the sample sites. The distance between Lake Kariba and the other two lakes is not drawn to scale. For further information on sample sizes and sites, see Tables 2.2 and 2.3.

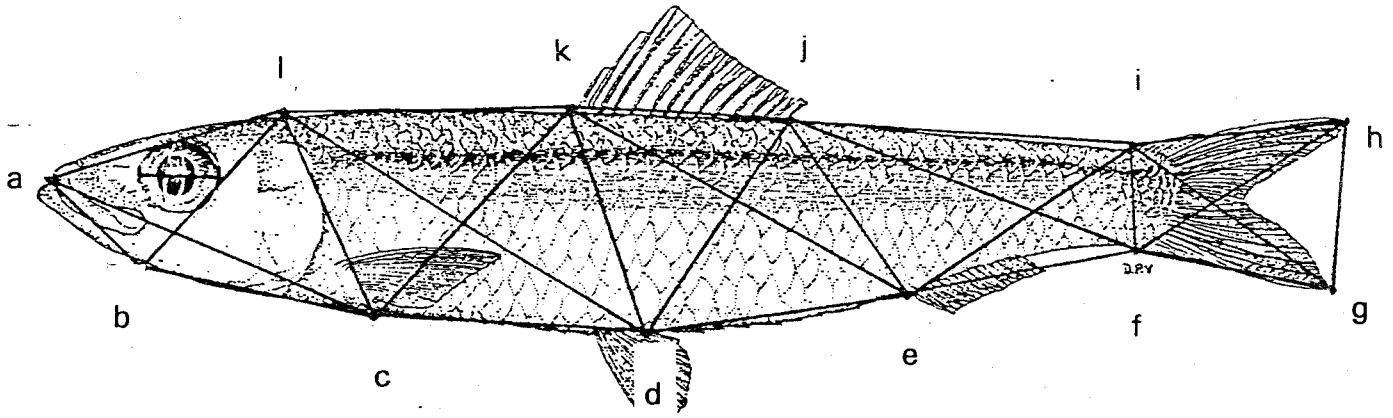


Figure 3.1: Location of landmarks for the truss network used for kapenta and *Stolothrissa*. See Table 3.1 for a description of the landmarks.

1982) which covers the shape of the fish in an uniform network (Figure 3.1) and theoretically should increase the likelihood of extracting morphometric differences between groups (Winans, 1987). Although, the mathematical advantages of the truss system have recently been questioned (Bookstein, 1991), several studies have shown the superior sensitivity of the truss system over conventional measurements (Humphries *et al.*, 1981; Strauss & Bookstein, 1982; Roby *et al.*, 1991).

Upon sampling, individual fish were placed on acetate sheets and the landmarks (Figure 3.1, Table 3.1) were marked using a dissection needle. On return from Africa, XY coordinates were obtained using an image analyzing system (OPTIMAS). From these data, truss measurements were calculated using Pythagoras' law. Distances on the caudal fin were excluded from the analysis, because they depended to a large degree on the positioning of the fish rather than to actual morphological differences. Sample sizes varied between 12 and 52 and are given in Figure 3.4.

On the first sampling expedition, a remarkably higher variance in eye diameter was observed in the fish of Lake Kariba compared to those from Lake Tanganyika. Therefore, on the second sampling expedition to the northern part

Table 3.1: Anatomical landmark points used for the truss system

a	tip of snout
b	posterior end of premaxilla
c	base of pectoral fin
d	base of ventral fin
e	anterior end of anal fin
f	ventral base of caudal fin
g	ventral tip of caudal fin
h	dorsal tip of caudal fin
i	dorsal base of caudal fin
j	posterior end of dorsal fin
k	anterior end of dorsal fin
l	posterior end of cranium
m	posterior end of vertebra column
n	fork of the caudal fin

of Lake Tanganyika and Lake Kivu, eye-diameter was recorded in addition to the truss landmarks. In addition, eye diameter data from Lake Kariba were collected by Tony J. Pitcher during a visit to Zimbabwe.

The main problem in most morphometric studies, especially those involving organisms with indeterminate growth like fish, is that the shapes of individuals differing in size are usually compared. In the present study the problem is exacerbated by the small size of the fish from Lake Kariba, which were only about half the size of those from the other lakes (Figure 3.2). In addition, there were significant differences in size between the samples from one lake (Nested ANOVA: $F_{between\ lakes} = 2360.4$, $df = 2$, $p < 0.001$, $F_{within\ lakes,\ among\ samples} = 128.0$, $df = 19$, $p < 0.001$; $N = 914$). Therefore, the absolute measurements have to be transformed to size-independent shape variables.

Several univariate methods for size adjustment of the truss measurements are available (Reist, 1985; Thorpe & Leamy, 1983) ranging from logarithms, ratios, logarithms of ratios, allometric adjustment and regression techniques. After preliminary tests of the effectiveness of size adjustment of different methods (First Annual Report), it was decided to adopt an allometric formula using the pooled within-sample slope (Thorpe, 1976):

$$M_{adj} = \log M - b(\log SL - \log SL_{avg})$$

where:

- M: original truss measurement or eye diameter
- M_{adj}: size adjusted truss measurement
- SL: standard length
- SL_{avg}: overall mean of standard length
- b: pooled within-sample slope of regressions of logM against logSL

The pooled within-groups slope was used because the overall regression slope would have obscured geographic variation, where samples differ in specimen size. As a test for the effectiveness of the method, a sample of small fish was collected from Lake Kivu (Figure 3.2) and compared with the fish from Lake Kariba.

The transformed data were standardized, that is, transformed to a data set with the mean 0 and the standard deviation 1, and submitted to canonical variates analyses (CVA). CVA transforms the data in a way that the ratio of between-group to within-group variance is maximised (Bookstein, 1991). It thus requires *a priori* grouping of the samples. On the basis of the calculated functions discriminating between groups, individuals can then be classified into certain groups. The percentage of correctly classified individuals is one of the measures for the morphological distinctness of the samples.

Another measure for the effectiveness of the analysis in discriminating groups of fish is Wilk's Lambda, which provides a significance test for the analysis. Plots of the first canonical variates gave an impression of overlap between groups. Canonical loadings, that is, correlations between the canonical variates and the original variable identify the most significant truss measurements in distinguishing groups.

In addition to CVA, a cluster analysis was carried out on the standardised adjusted truss measurements. Since a cluster analysis on the scores of the canonical variates yielded almost identical results, these data are not presented here. In addition, it was considered preferable to perform two completely independent sets of analyses. Clustering was undertaken using Euclidean

Table 3.2: Sets of analysis carried out to investigate the morphological differentiation of kapenta (*Limnothrissa miodon*) and *Stolothrissa tanganicae*. Wilk's lambda (+ significance level), the percentage of correctly classified fish, and the number of variables with significant differences are also shown. Analyses which included the eye diameter have 22 instead of 21 univariate comparisons.

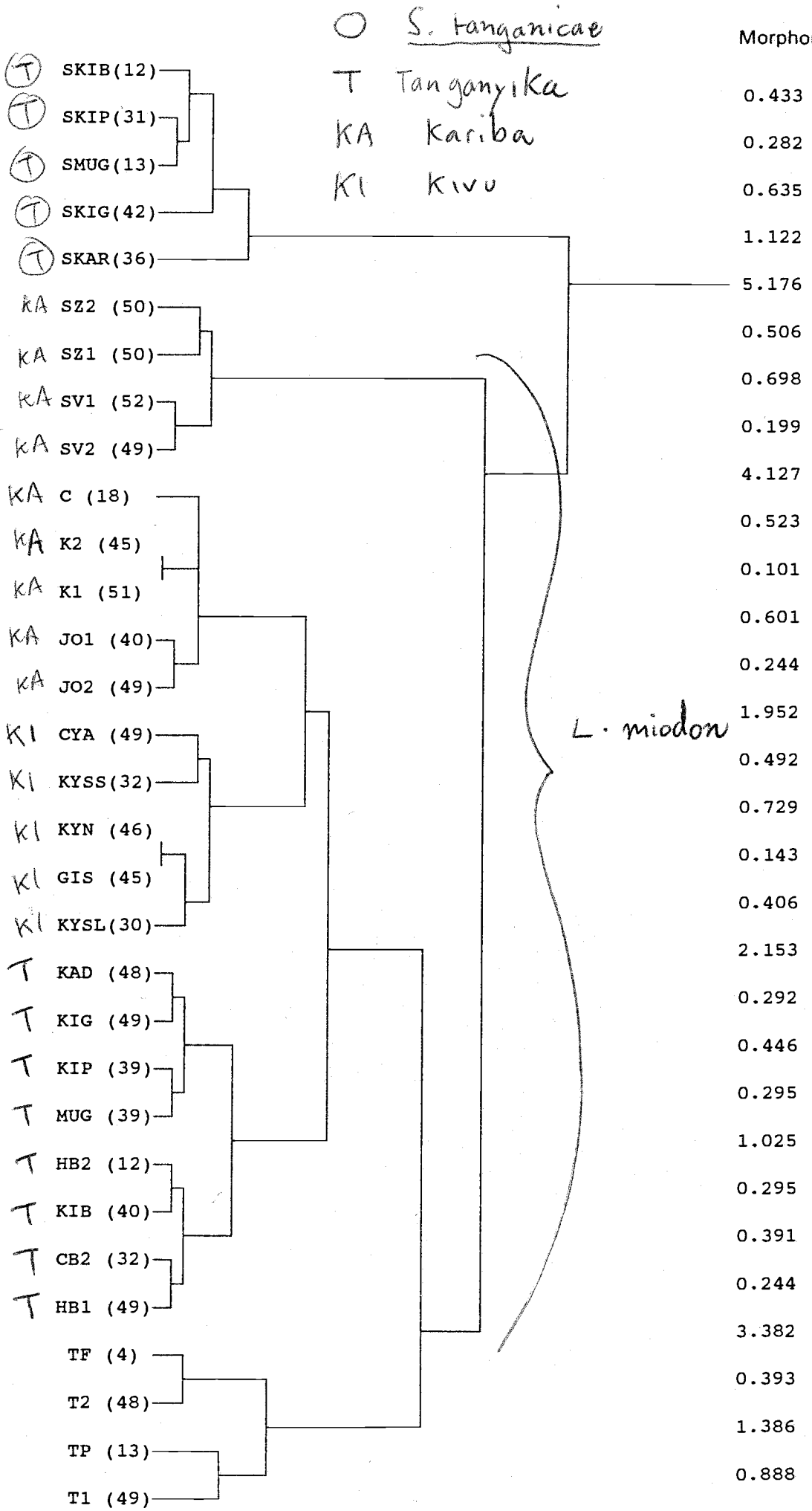
Data set	Wilk's lambda L	F	p	% corr	sign.diff
1.Overall					
a. All samples	0.001	14.36	0.000	61.1	21/21
b. All kapenta, males	0.007	5.98	0.000	74.1	21/21
c. All kapenta, females	0.003	6.17	0.000	79.6	21/21
2.Within lakes					
a. L. Tanganyika	0.015	11.38	0.000	80.8	21/21
c. L. Tanganyika, males	0.057	3.38	0.000	93.0	13/22
d. L. Tanganyika, females	0.025	5.29	0.000	92.2	20/22
e. L. Kivu	0.085	6.775	0.000	74.6	21/22
f. L. Kivu, males	0.077	3.948	0.000	81.1	17/22
g. L. Kivu, females	0.108	2.462	0.000	80.0	8/22
h. L. Kariba	0.099	6.08	0.000	57.8	21/21
3. *Stolothrissa*					
a. *Stolothrissa*	0.061	5.03	0.000	85.8	16/22
b. *Stolothrissa* males	0.006	2.45	0.002	100.0	13/22
c. *Stolothrissa* females	0.018	2.98	0.000	98.4	12/22
4. Randomised sample					
a. L. Kariba	0.660	0.96	0.628	23.8	0/21

distances between group centroids (i.e. the group means at all variables) and Ward's minimum variance method (Ward, 1963).

Univariate comparisons for each truss variable were also performed. The number of significant differences between groups is an additional indication of the degree of group separation.

All analyses were carried out on several sets of data (Table 3.2). In addition, the samples of Lake Kariba were randomised, that is, the fish were randomly distributed among samples of the same size as the real samples.

Figure 3.4 (NEXT PAGE): Cluster tree of all samples, using Euclidean distances and Ward's (1963) minimum variance method of clustering. Numbers in parentheses show sample sizes. Values on the right hand side show the amalgamation distances of clusters.


O S. tanganicae
T Tanganyika
KA Kariba
KI Kivu
SKIB(12)
SKIP(31)
SMUG(13)
SKIG(42)
SKAR(36)
SZ2 (50)
SZ1 (50)
SV1 (52)
SV2 (49)
C (18)
K2 (45)
K1 (51)
JO1 (40)
JO2 (49)
CYA (49)
KYSS(32)
KYN (46)
GIS (45)
KYSL(30)
KAD (48)
KIG (49)
KIP (39)
MUG (39)
HB2 (12)
KIB (40)
CB2 (32)
HB1 (49)
TF (4)
T2 (48)
TP (13)
T1 (49)
L. miodon
0.433
0.282
0.635
1.122
5.176
0.506
0.698
0.199
4.127
0.523
0.101
0.601
0.244
1.952
0.492
0.729
0.143
0.406
2.153
0.292
0.446
0.295
1.025
0.295
0.391
0.244
3.382
0.393
1.386
0.888

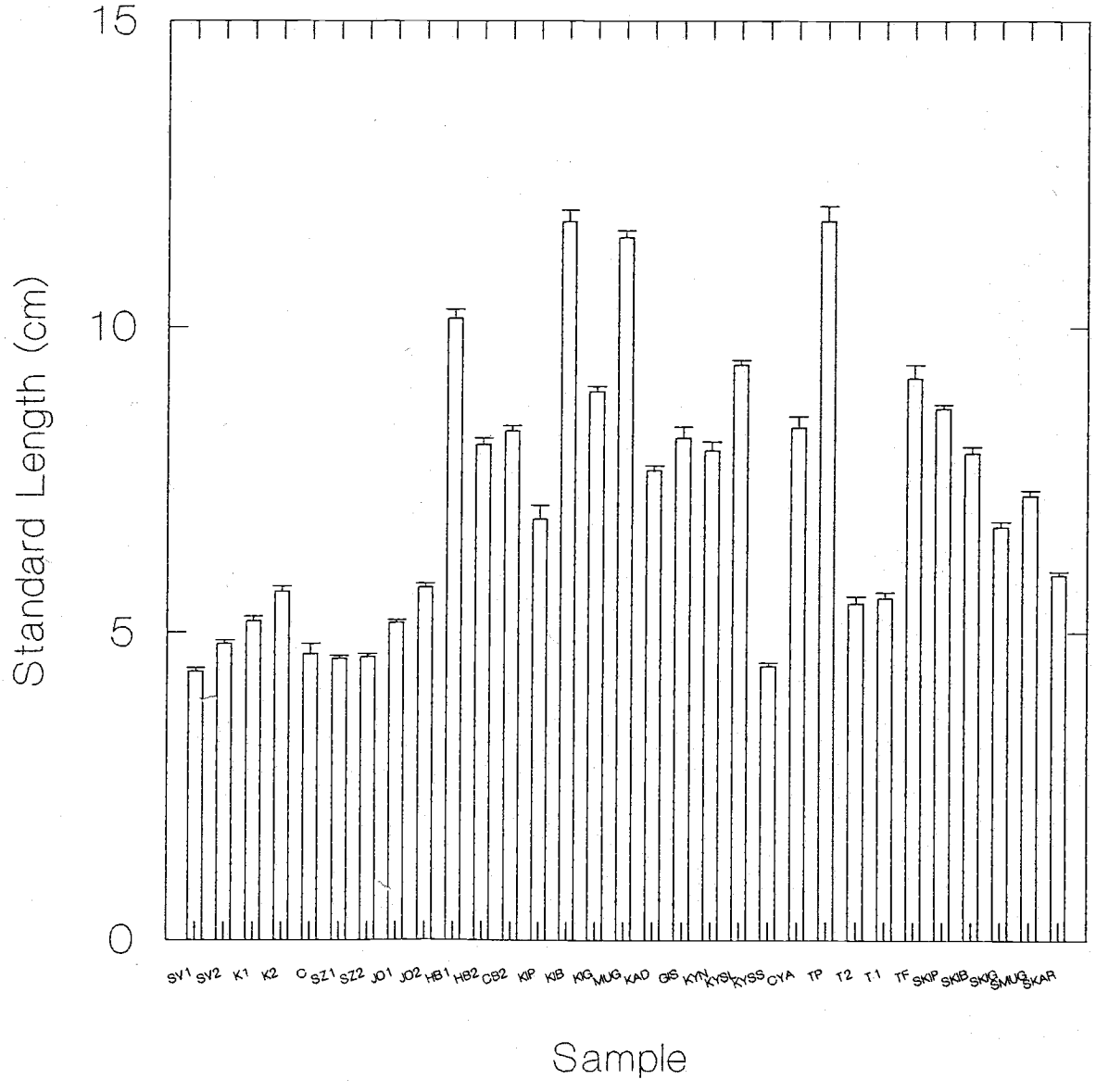


Figure 3.2: Means and standard errors of the standard length of kapenta and *Stolothrissa* samples collected in Lakes Tanganyika, Kivu and Kariba.

3.3. Results

There was a good correspondence between the two multivariate methods employed in this investigation, canonical variance analysis and cluster analysis. Both methods showed the species and lakes as separate clusters: in the CVA, kapenta from Lakes Tanganyika, Kariba and Kivu, *Stolothrissa* and the kapenta measured by TJP are clearly and significantly separated (Figure 3.3, Table 3.2). The cluster analysis gave a similar result, with the only difference that Lake Kariba was divided into two major clusters (Figure 3.4).

The large differences between the Lake Kariba fish measured by TJP and those collected by LH emphasizes the importance of consistent recorder collection of measurements. Because the grouping identified in Figure 3.4 was an artifact, the fish collected by TJP were not analysed further.

Species were mainly separated on the second canonical variate, which was highly correlated with dorsoventral measurements, that is, kapenta has greater body depth than *Stolothrissa*. Although this difference may have been partly due to different collectors (*Stolothrissa* was measured by Mr Stuart Piertney), it corresponds to the visual impression of species differences (pers. obs.).

Separation into sexes improved the percentage of correctly classified fish (Appendix I: Table I.1) but did not change the interpretation of results. As unfortunately not all fish were sexed, the analysis concentrated on pooled samples. Data are presented for the sexes separately in Appendix I.

The kapenta samples of the three lakes were clearly separated into clusters, with 91.3% of the fish classified to the correct lake. In order to improve the intra-lake resolution of the CVA, analyses for each lake were carried out.

Samples of Lake Tanganyika were clearly separated by the CVA (Figure 3.5), 80.8 % of the fish being correctly classified (Table I.1). However, they were not obviously clustered into geographic regions (Figure 3.6). In contrast, there was a much larger overlap between the samples from Lake Kariba (Figure 3.7, 57.8 % correct classification), but the cluster analysis identifies clearly groups of adjacent samples (Figure 3.8, SV1 & SV2, K1 & K2, SZ1 & SZ2). These results indicate the usefulness of morphometrics for assessment of fish distribution on a small geographic scale.

Table 3.3: Sample designation of symbols shown in Figures 3.2, 3.4, 3.6, 3.8, 3.9, 3.11.. Number in parentheses are samples sizes.

Lake Tanganyika		Lake Kivu		Lake Kariba	
HB1 (49)	⬠	GIS (45)	✳	SV1 (52)	·
HB2 (12)	|	KYN (46)	✹	SV2 (49)	○
CB2 (32)	–	KYSL (30)	♂	K1 (51)	△
KIP (39)	⅄	KYSS (32)		K2 (45)	▽
KIB (40)	+	CYA (49)	♀	C (18)	◁
KIG (49)	×			SZ1 (50)	▷
MUG (39)	✶			SZ2 (50)	□
KAD (48)	✳			JO1 (40)	◇
				JO2 (49)	☆

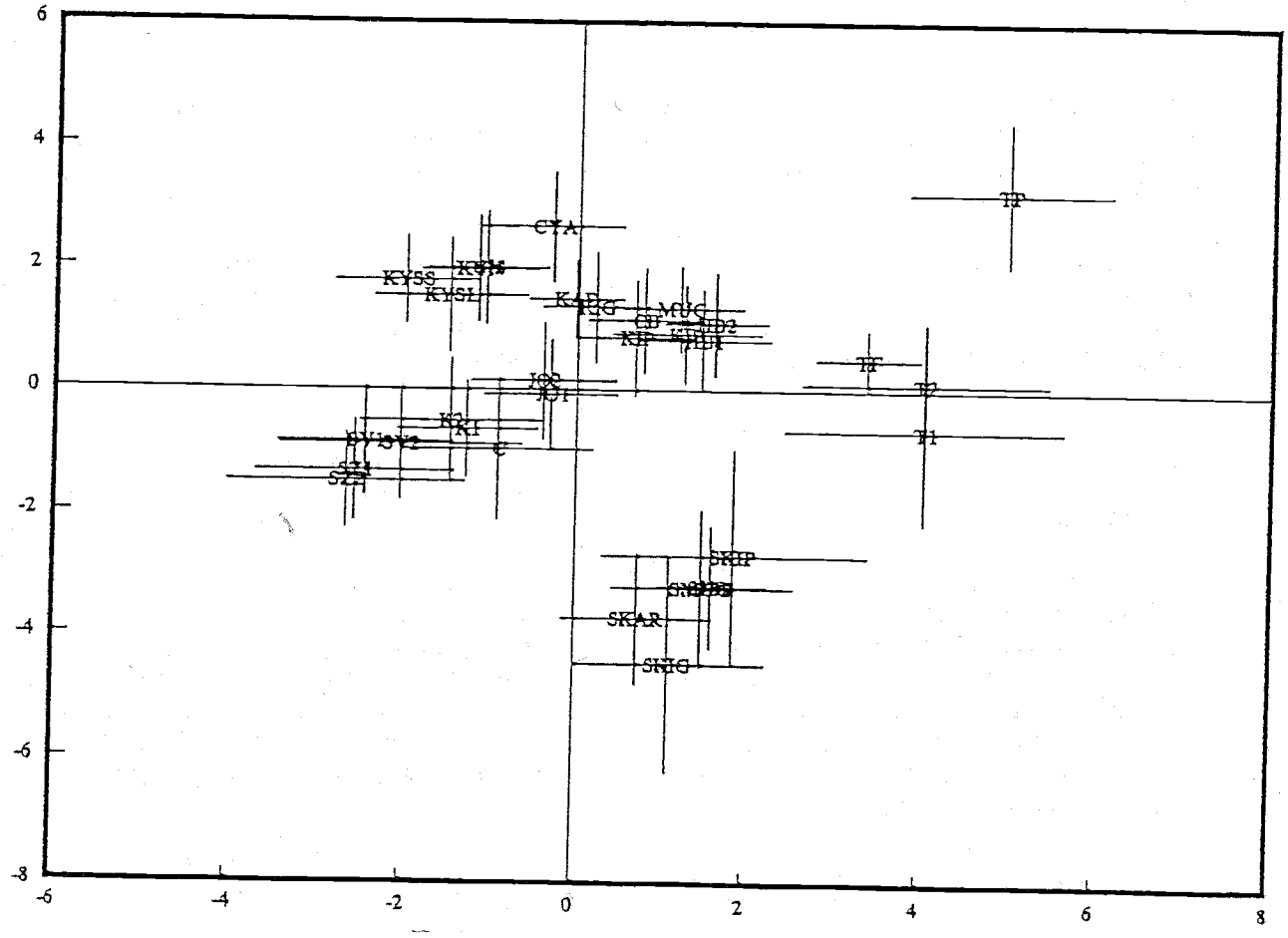


Figure 3.3: Sample means and standard deviations of the canonical variate scores obtained with a CVA on truss measurements of kapenta and *Stolothrissa*. Labels at the crosses indicate samples as shown in Table 2.2. Sample sizes are given in Figure 3.4.

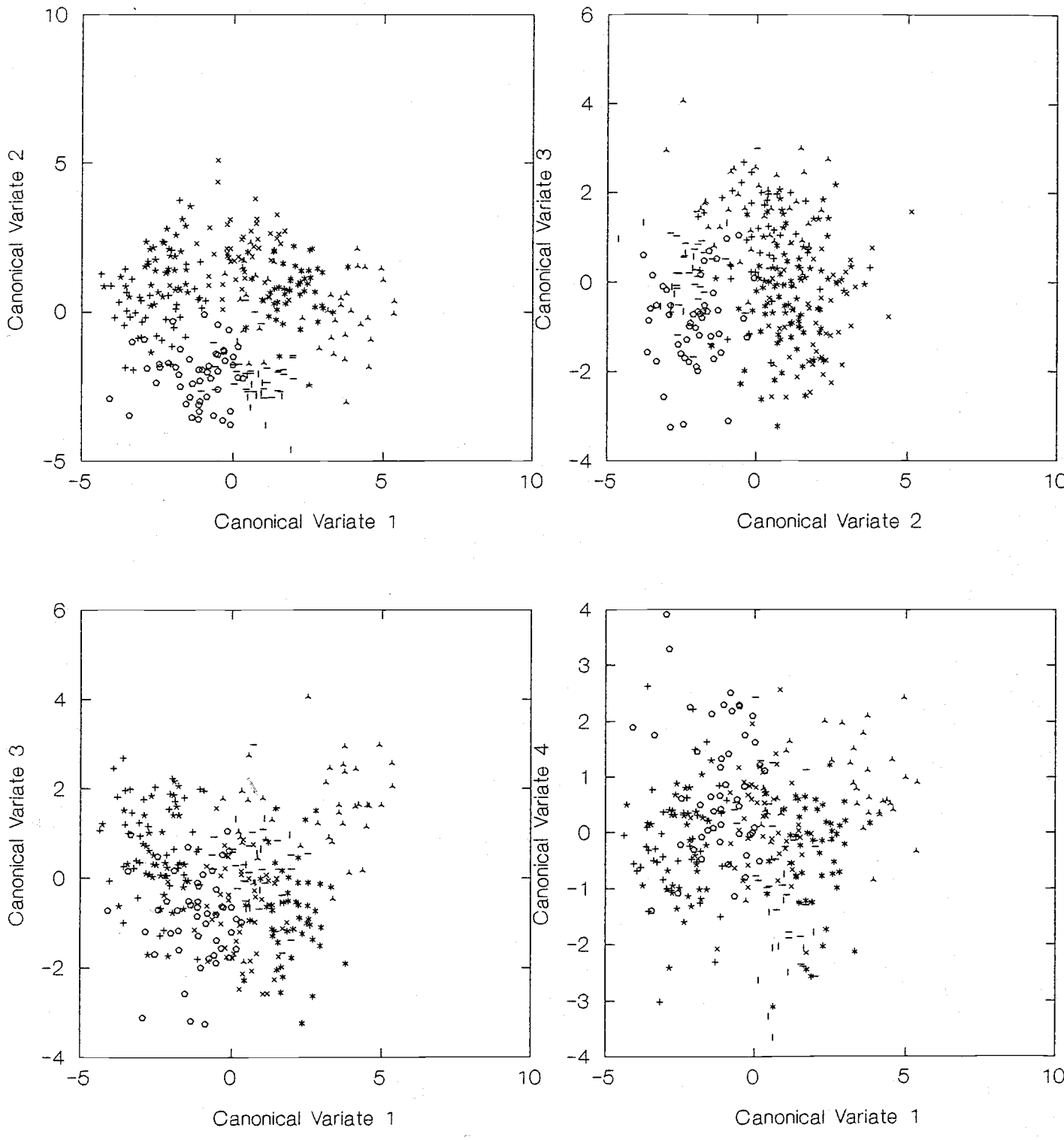


Figure 3.5: Scatterplots of canonical variates 1 - 4 of Lake Tanganyika kapenta. For symbol legends and sample, see Table 3.3.

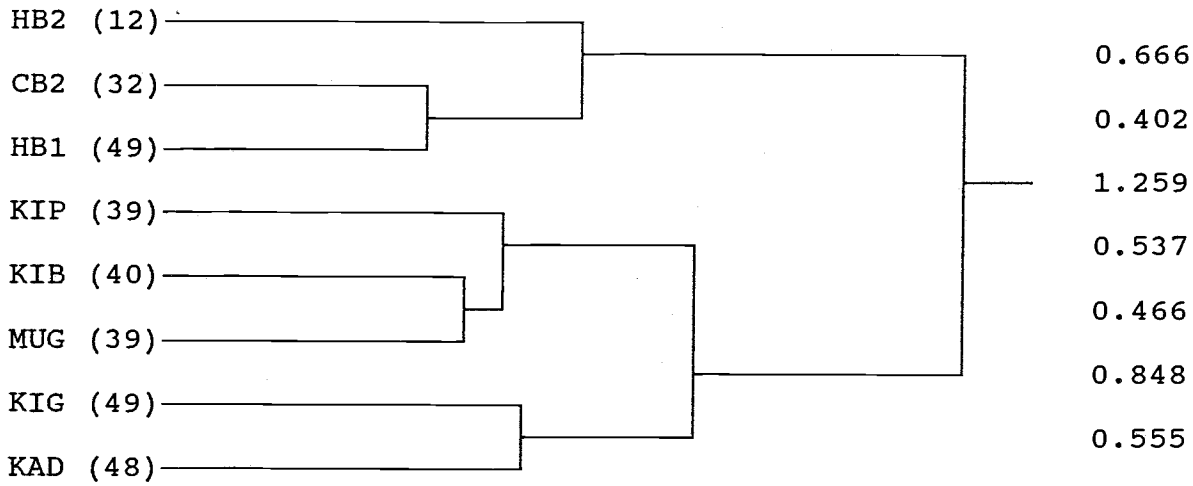


Figure 3.6: Cluster tree of the samples from Lake Tanganyika, constructed using Euclidean distances and Ward's (1963) minimum variance method of clustering. Numbers in parentheses are sample sizes. Values on the right hand side show the amalgamation distances of clusters.

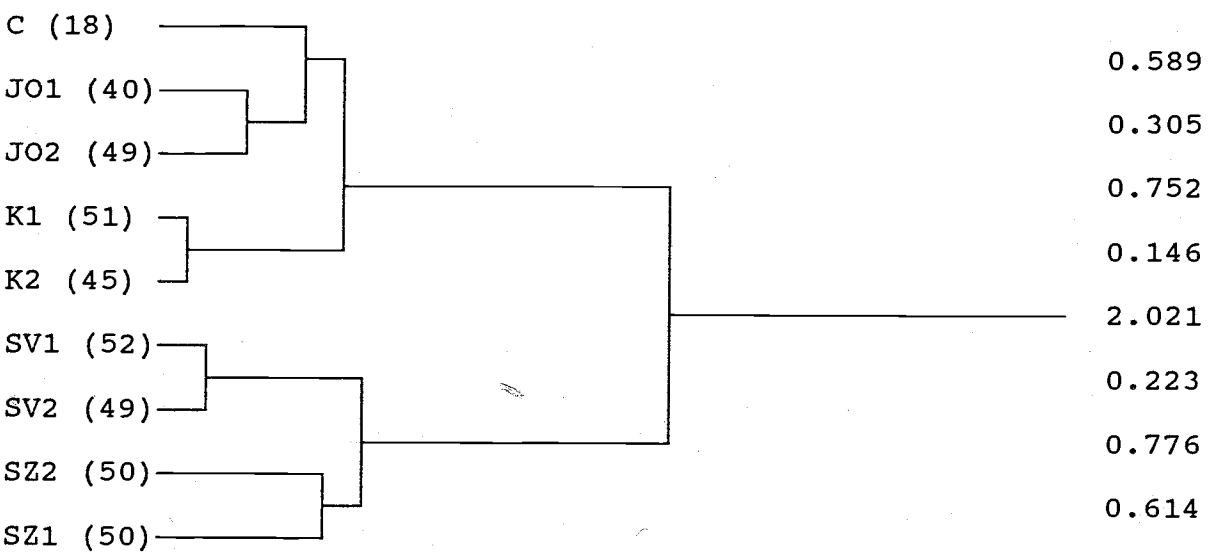


Figure 3.8: Cluster tree of the samples from Lake Kariba, constructed using Euclidean distances and Ward's (1963) minimum variance method of clustering. Numbers in parentheses are sample sizes. Values on the right hand side show the amalgamation distances of clusters.

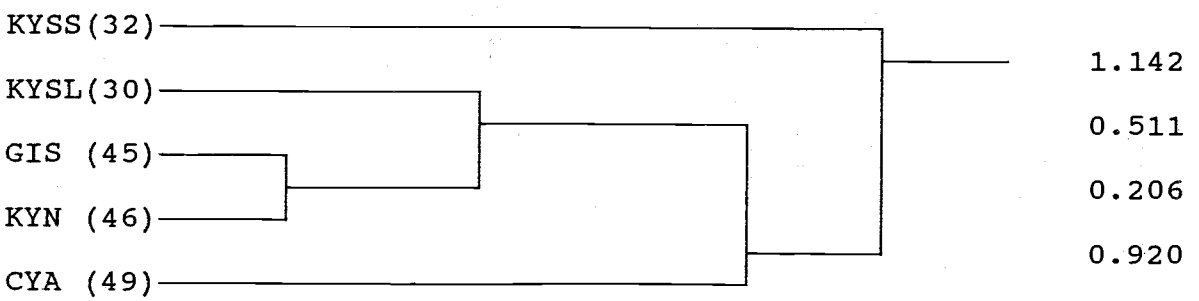


Figure 3.10: Cluster tree of kapenta samples from Lake Kivu, constructed using Euclidean distances and Ward's (1963) minimum variance method of clustering. Numbers in parentheses indicate sample sizes. Values on the right hand side show the amalgamation distances of clusters.

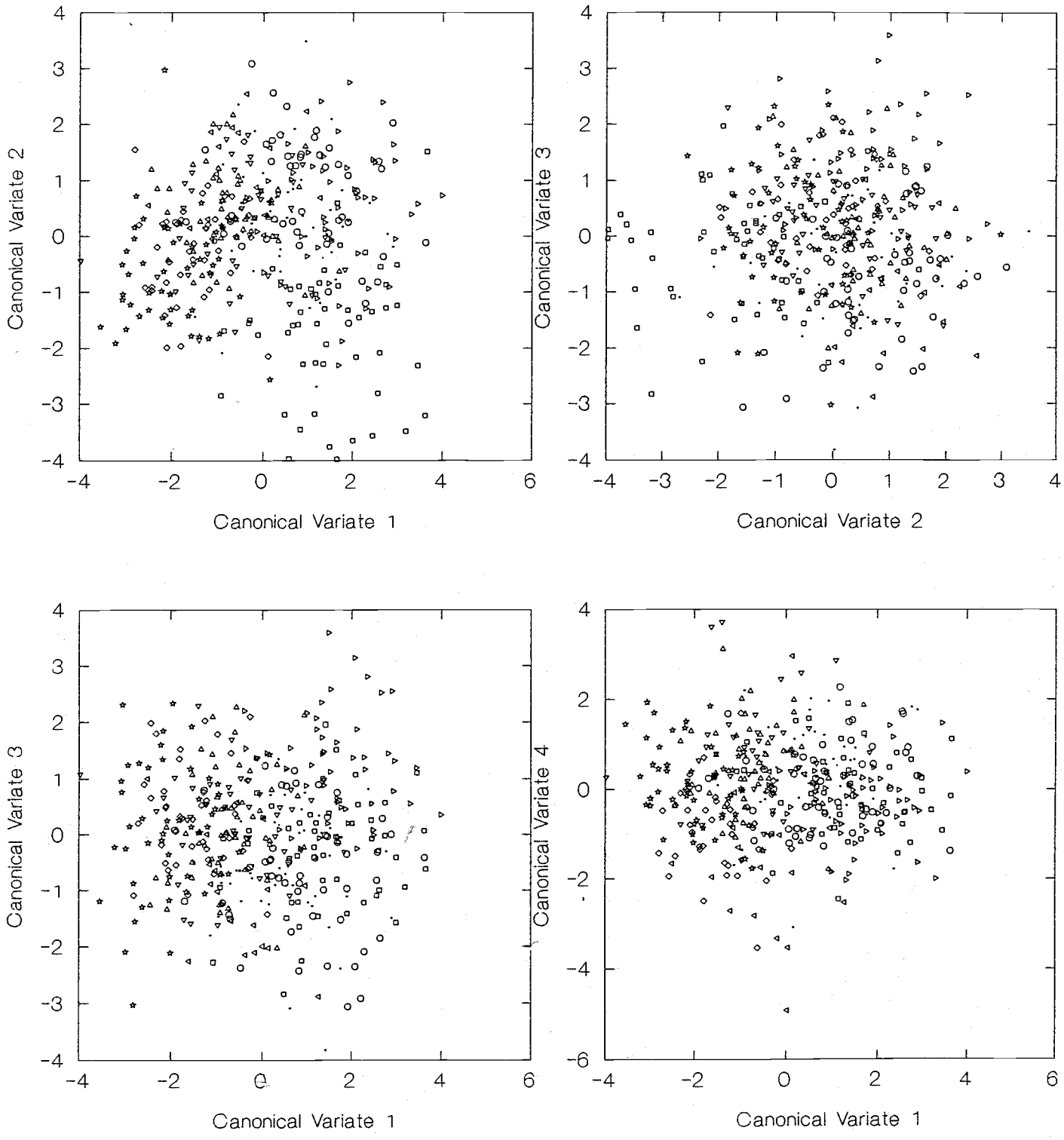


Figure 3.7: Scatterplots of canonical variates 1 - 4 of Lake Kariba kapenta. For symbol legends and sample sizes, see Table 3.3.

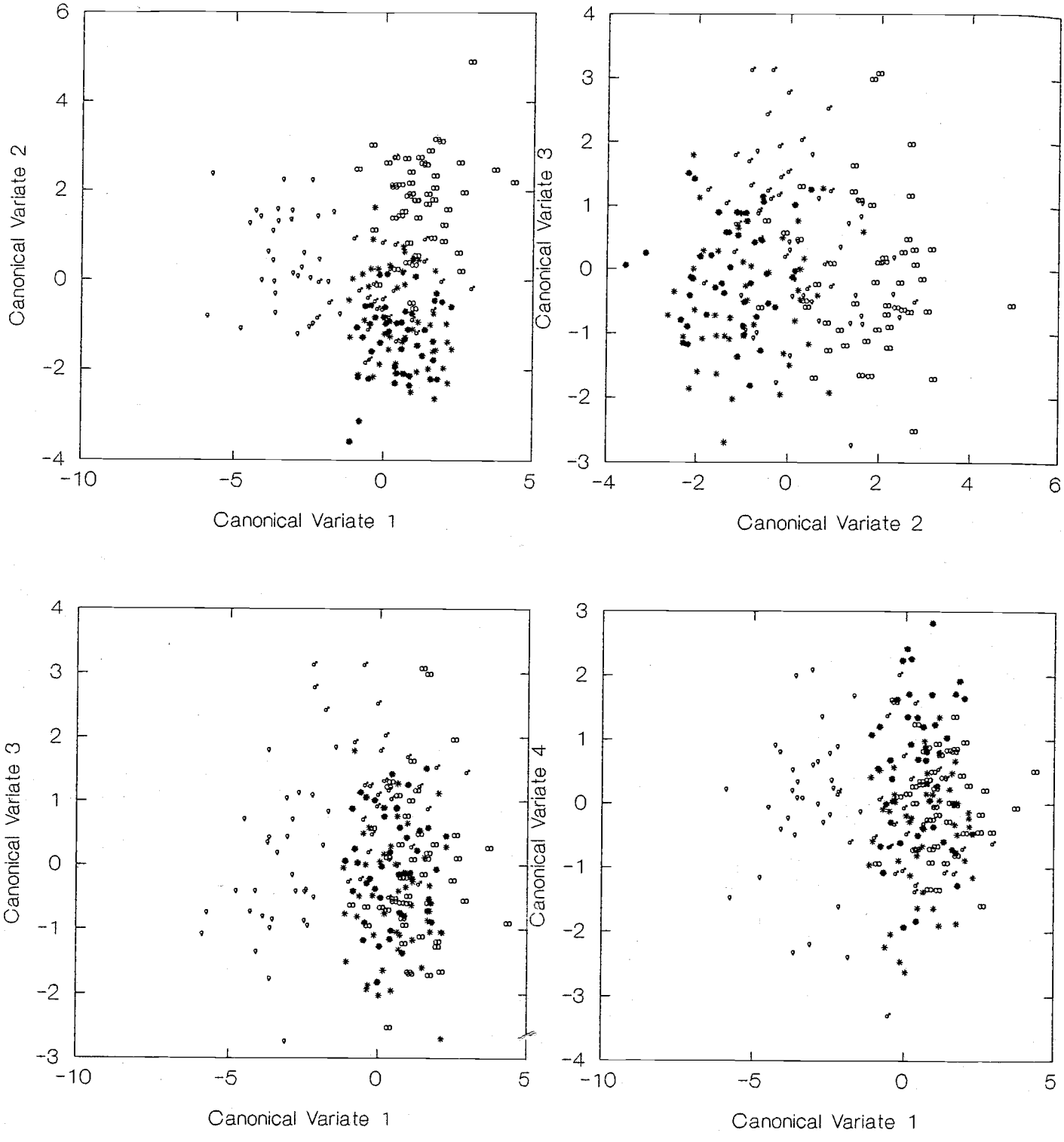


Figure 3.9: Scatterplots of canonical variates 1 - 4 of Lake Kivu kapenta. Symbol legends and sample sizes are shown in Table 3.3.

In Lake Kivu, there is strong separation between the sample of small fish and the other samples (Figure 3.8, 3.10). This is probably due to the fact that these were the only immature fish caught in Lake Kivu. Exclusion of these immature fish shows the Cyangugu sample separated from the other fish, which cluster together closely (Figure 3.9).

In both lakes were kapenta was introduced, the overlap between samples shown by the CVA was considerably larger than in Lake Tanganyika (Figures 3.5, 3.7 and 3.9), and the percentage of correctly classified individuals was considerably lower (Appendix I, Table I.1: Kariba 57.8 %, Kivu after exclusion of small fish 72.4 %, Tanganyika 80.8 %). This may reflect the larger geographic distances between samples on Lake Tanganyika, but could also be due to a higher degree of population subdivision in Lake Tanganyika.

The analysis of randomised samples yielded the expected results: Wilk's Lambda was not significant (Table 3.2), the percentage of correctly classified individuals was considerably lower than in the other analyses (23.8%, Table 3.2), and the CVA plot showed no separation at all (Figure 3.11), thus demonstrating that the results presented here are not artefacts caused by the statistical methods used.

Plots of the eye diameter against standard length showed clear differences between lakes. Whereas the eye diameters showed a linear increase with standard length in Lake Tanganyika, in Lake Kivu this was only the case up to fish of 9 cm length (Figure 3.12). Fish larger than that have highly variable eye diameters (Figure 3.13). In Lake Kariba, the eyes of kapenta were apparently about 1 mm larger than in the other lakes. However, as these fish were measured by TJP, this difference was most likely due to error resulting from different observers. However, it is apparent that the fish in Kariba have slightly more variable eye sizes than in Lake Tanganyika.

3.4. Discussion

Kapenta occupy habitats with little apparent spatial heterogeneity, and like most pelagic species, are highly mobile, presumably due to adaptations to avoid predators or seek food. Since it is widely accepted that a large component of morphometric differentiation arises from environmental variation (Winans, 1987), a likely source of morphological differentiation may occur during development of juvenile stages (Pepin & Carr, 1992). Thus, the existence of detectable differences between nearby groups of the same age can be accepted as evidence of environmental differences during development and therefore the occupation of different territories. The persistence of identifiable groupings would, however, depend on the extent and fidelity of individuals among shoals during adult movements (Pitcher & Parrish, 1993). Support for a degree of integrity of shoals is provided by the existence of size-specific shoals in the associated clupeid, *Stolothrissa* (Coulter, 1991a), and in *L. miodon* (Figure 3.2) indicating the possible association of individuals which have experienced similar environments. Below, some comments are provided on methodological aspects of the procedure, followed by an interpretation of what the data may reveal about kapenta stock structure.

Both multivariate analyses provided strong evidence for the success of the size adjustment of the original variables: First, the sample of small fish from Lake Kivu (KYSS) clustered with the other, large fish from the same lake rather than the similarly-sized Kariba fish Figures 3.3., 3.4). Secondly, the above mentioned differences of fish collected by TJP and LH were found in similar sized fish. Therefore the results appear to represent genuine shape differences between fish.

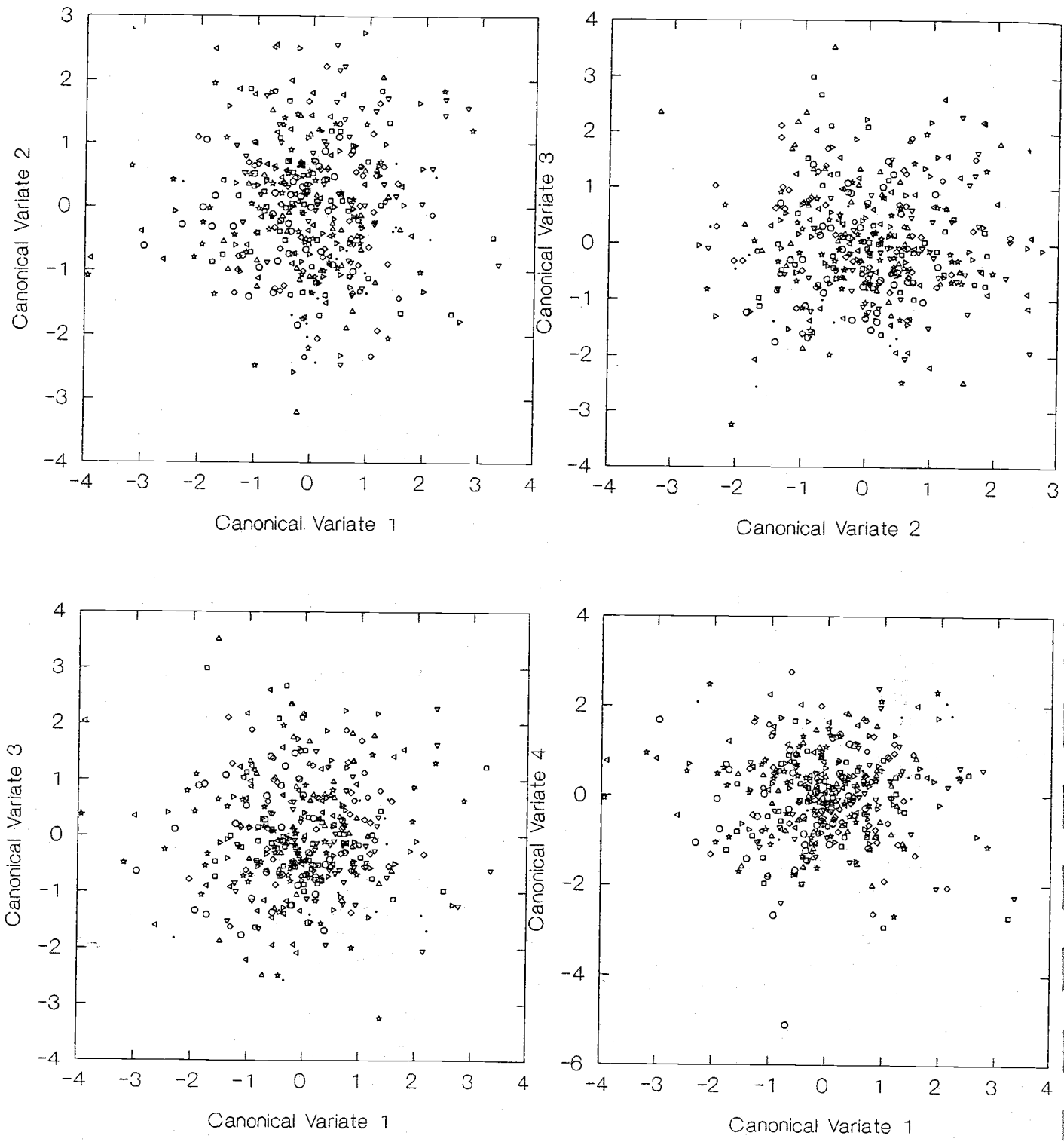


Figure 3.11: Scatterplots of canonical variates 1 - 4 of a CVA on randomised samples from Lake Kariba (see text for details). Symbols denote different randomised samples of the same size as the ones actually collected in Lake Kariba.

In addition, the detection of differences between lakes confirms the applicability of morphometric methods for stock structure investigations in kapenta. The truss measurements and statistical analyses employed here appear to be sufficient to detect subtle shape differences among fish from different sources.

Unfortunately, sex data were not available for all samples. Comparing the percentage of correctly classified fish from pooled data with those obtained from sexes separately (Appendix I) shows that separation between samples was generally better when the sexes where treated separately. Although the pooling of sexes has not created major drawbacks in the present context, it may be important to analyse sexes separately in future studies investigating fine-scale temporal and spatial variation.

The cluster analysis of the samples of Lake Kariba showed a clear grouping of adjacent samples from Siavonga (SV1, SV2), Kariba (K1, K2), Sinazongwe (SZ1, SZ2) and from the western end of the lake (JO1, JO2) (Figure 3.8). This demonstrates the ability of the truss system to reveal not only differences, but also similarities between samples on a small geographic scale. As a high proportion of the fish caught in Kariba were mature, it also suggests that fish may be remaining within close proximity to their nursery area, and thus exhibiting some degree of site fidelity. Wide-scale movements of large numbers of fish might be expected to result in mixing of local populations, thus reducing the integrity of such assemblages. Such an assertion does, however, depend on the extent and persistence of social cohesiveness among shoaling individuals (Pitcher & Parrish, 1993).

The fisheries implications of such apparent small-scale morphometric patchiness depends on the extent to which structuring persists over time. If for example, fish from an area revealed repeatedly consistent differences from distant sites, then irrespective of the underlying causes of such differentiation, the morphometric characters may be employed to characterize individuals from particular areas. Such a stock structure could reveal information on the extent of migration, and hence estimate the exchange of individuals among regions. However, the detection of morphometric differentiation which differs qualitatively over time would indicate more extensive fish movements, so complicating any estimates of putative stock interactions. Clearly, a more intensive sampling programme over time is required to determine the significance of the differentiation detected, though seasonal changes may confound patterns if the variance detected among characters are significantly affected by the environment.

On a larger scale, the cluster analysis did not correspond well to expectations based on proximity, as two groups from opposite ends of Lake Kariba (Siavonga SV and Sinazzongwe, SZ) clustered together (Figure 3.8). Furthermore the cluster tree of Lake Tanganyika showed no geographic pattern, which probably reflects the larger geographic distances between samples on Lake Tanganyika. There are two possible reasons for this deviation of the cluster trees from expectations: first, it may be that there is morphological variation, say, between the northern part and the southern part of the lake, but that the methods employed were not able to detect them. Alternatively, it may be that fish are growing up in a certain nursery area, showing distinct morphological features of genetic or environmental origin, but then move around in the lake as highly distinct groups. The weight of presently available evidence, however, leads to the conclusion that Tanganyika clupeids do not migrate extensive distances around the lake, and in fact that individuals in their short life times are probably limited to fairly small geographic sectors (Matthes, 1965; Coulter, 1991a). Indeed, there is evidence that juveniles may move less than

adults (Coulter, 1970), providing greater opportunities for regional differences in morphology to arise. It is possible, therefore, that beyond a certain geographic scale, that regional differences may become obscured. If similar patterns apply to *Limnothrissa* in Lake Kariba, then regional differentiation may also be enhanced by the similarly annual life cycles, as suggested by the small-scale differentiation described above. The relationship between regional differentiation, and fish movements could be examined by taking a time- series of samples from several locations in a lake and comparing temporal and spatial morphological variation (Winans, 1987).

In summary, the analysis of morphometric variation reveals a degree of spatial differentiation among kapenta samples, which in Kariba, were evident on a small scale, though, the geographic patterns are complex overall. It was, however, not the intention of the present study to utilise morphometric data in isolation from genetic observations: a more useful examination of stock structure is obtained when data from several independent sources are integrated, especially when the genetic basis of markers is uncertain (Winans, 1987; Spanakis *et al.*, 1989; Carvalho & Hauser, 1994).

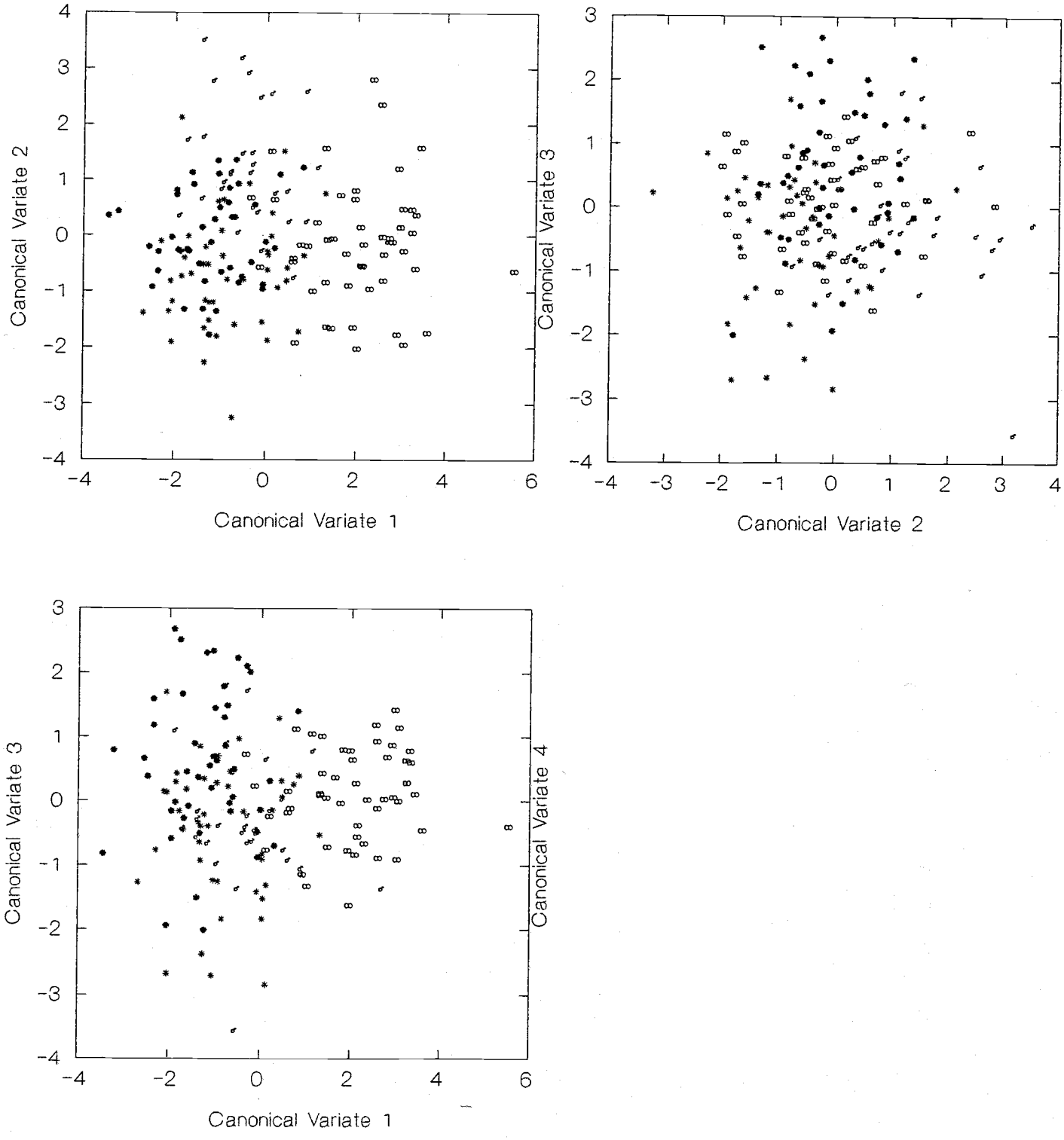


Figure 3.10: Scatterplots of canonical variates 1 - 4 of a CVA on Lake Kivu kapenta excluding the sample of small fish (KYSS). For symbol legends and sample sizes, see Table 3.3.

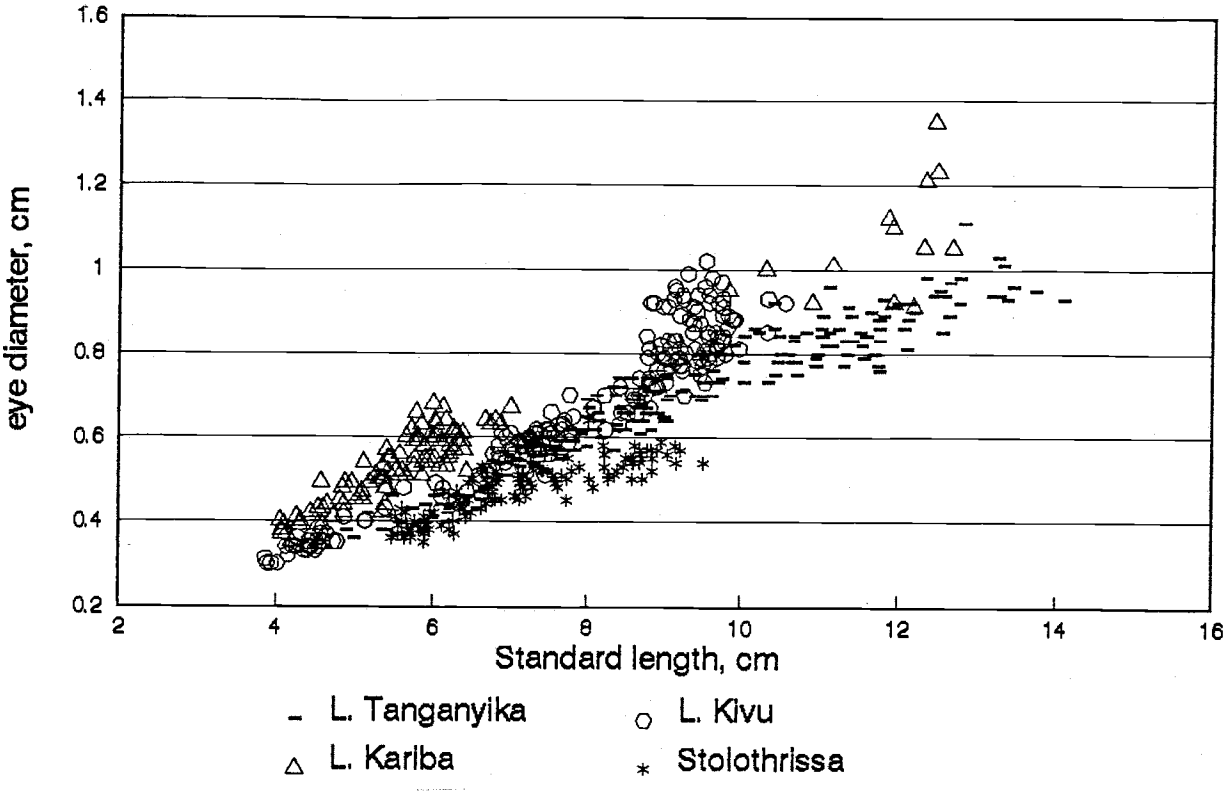


Figure 3.12: Scatterplot of horizontal eye diameter against standard length for kapenta from Lakes Tanganyika, Kivu and Kariba, and *Stolothrissa*.

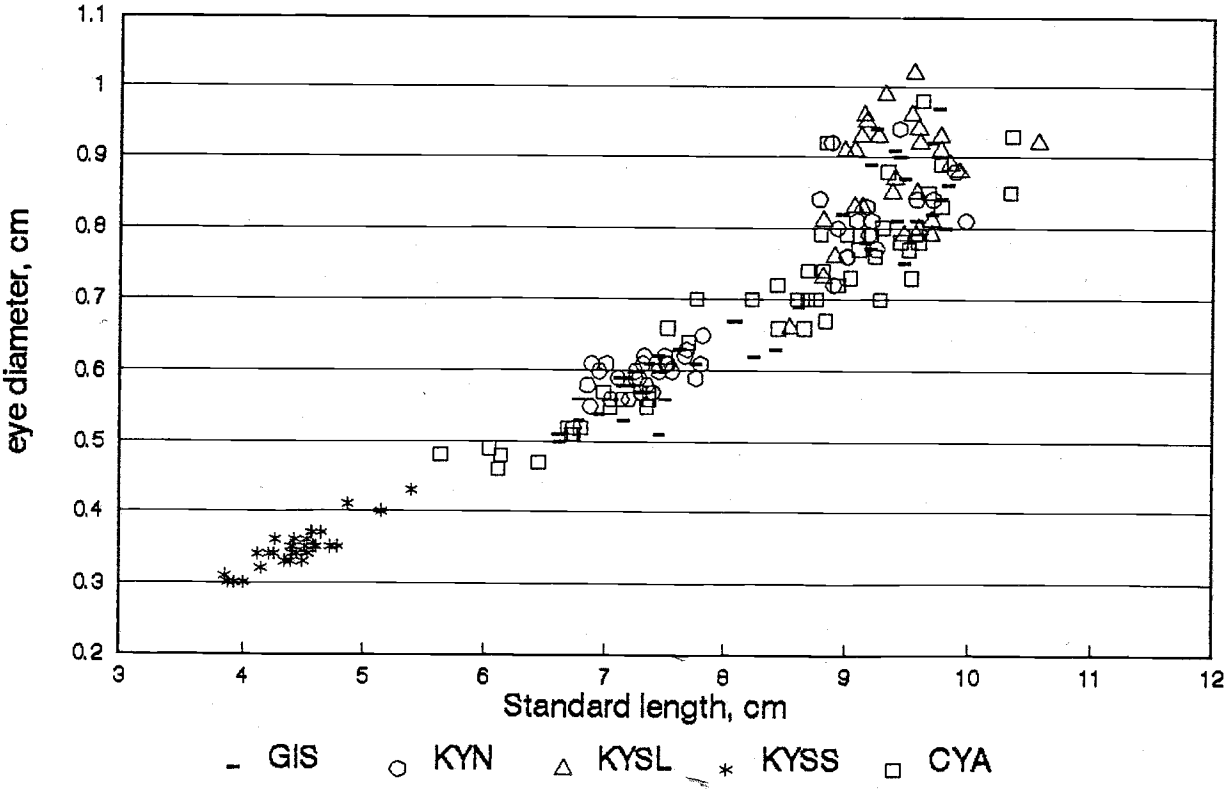


Figure 3.13: Scatterplot of horizontal eye diameter against standard length for kapenta from Lake Kivu.

CHAPTER 4: ALLOZYME ANALYSIS

4.1 Introduction

Various population parameters and physiological, morphometric, meristic, calcareous, biochemical and cytogenetic characters have been used to identify fish stocks (Ihssen *et al.*, 1981). Although traditional methods of stock identification have provided valuable information on stock structure, they often entail the collection of vast amounts of data which exhibit extensive variance requiring sophisticated multivariate analyses. Moreover, the genetic basis of many of the characters measured remain poorly understood, especially if they are subject to environmental modification.

The past 20 years have witnessed the extensive application of allozyme electrophoresis to stock structure analysis in many commercially important species (Richardson *et al.*, 1986; Allendorf *et al.*, 1987). Allozymes arise from heritable, electrophoretically-detectable differences in the amino acid composition of enzymes sharing a common substrate, producing data consisting of arrays of gene frequencies obtained for each sample under study. Where significant genetic differences exist between samples, it may be possible to characterize each by the possession of unique alleles at a locus, their frequencies, or more often a combination of both. Allozymes not only provide estimators of genetic differentiation and reproductive isolation, but can also provide data on mating patterns in relation to the Hardy-Weinberg paradigm. Gene frequencies are obtained by subjecting enzymes to an electric current (electrophoresis), and comparing relative mobilities of allozymes on a gel from individuals in different samples. Differences in electrophoretic mobility usually indicate genetic difference, and so by examining many animals representative of each group, it is possible to determine their degree of genetic divergence.

The effective use of allozymes, as with all markers, depends on their selective neutrality (Smith *et al.*, 1990), such that allele frequencies respond primarily to mutation, gene flow and drift, rather than to selection due to contrasting environments. There is evidence, however, that some allozymic differentiation is determined by locus-specific selection, arising from forces that may be independent of mating patterns and gene flow (reviewed in Utter, 1991; Powers *et al.*, 1991). The problem of selective constraints on allozyme variation has led to the suggestion that protein variation has limited value in the elucidation of stock structure (Gauldie, 1988; 1991). Such an assertion is, at best, only partially true.

Since neutrality can never be proven, only evidence for the absence of strong selection can indicate whether most protein polymorphisms are neutral (e.g. Aspinwall, 1974; Ryman *et al.*, 1979; Ferguson and Mason, 1981). Three observations suggest that most allozyme markers are indeed neutral: first, the general relationship between dispersal capacity, gene flow and degree of genetic differentiation, whereby those taxa with higher vagility tend to exhibit greater genetic homogeneity (Waples, 1987; Ward, 1989). Secondly, the differences in extent of genetic differentiation within marine, freshwater and anadromous fishes (Gyllensten, 1985; Ward *et al.*, 1994). Both patterns underline the importance of population size and migration rate, as predicted by neutral theory (Kimura, 1968). Thirdly, since selection causes changes in genotype frequencies, the generally good fit to Hardy-Weinberg expectations provides indirect evidence for a lack of significant selective forces. Selection may also be detectable through examining the distribution of differentiation across loci: genetic divergence arising from restricted gene flow tends to affect all loci simultaneously, whereas selectively-determined divergence is typically

observed at one or only a few (Lewontin & Krakauer, 1973; Slatkin, 1987). It will remain difficult to interpret differences at single loci, highlighting the importance of utilizing information not only across numerous loci, but also from independent sources such as DNA markers (Ovenden, 1990), tagging studies (Gauldie, 1988; Brodziak *et al.*, 1992) or differentiation at other biological levels (Ihssen *et al.*, 1981; Leslie and Grant, 1990).

While we cannot exclude the selective maintenance of at least some allozyme polymorphisms, empirical and theoretical evidence (Utter, 1991) does justify "a null hypothesis of neutrality rather than selection as a first approximation of reality" (Ihssen *et al.*, 1981).

The extensive allozyme studies undertaken on fish stocks have not only proven valuable for estimating population divergence, and thus identifying discrete fish stocks, but have also focused attention on the underlying evolutionary forces that promote differentiation (Ward & Grewe, 1994). Such information is important when addressing short- (depletion and production models, catch forecasting and effort regulation), and long-term management objectives (long-term sustainability and genetic conservation).

Allozyme data have been particularly valuable in exploring the rates and nature of genetic changes following introductions, with some examples demonstrating significant and rapid allele frequency shifts (Baker & Moeed, 1987), occasionally in a matter of decades (Gharrett & Thomason, 1986; Vuorinen *et al.*, 1991). In addition to comparisons of allele frequencies between native and transplant populations, information on the respective levels of genetic diversity may prove useful in disentangling the roles of stochastic and deterministic forces. For example, data on the number of alleles per locus may be sensitive indicators of population bottlenecks and founder effects (Nei *et al.*, 1975; Mayuyama & Fuerst, 1988), and the distribution of genetic differentiation among loci can indicate the relative effects of selection and genetic drift (Lewontin & Krakauer, 1973; Slatkin, 1987; Baker *et al.*, 1990). Furthermore, where the new environment differs clearly in selection pressures, it may be possible to explore adaptive variation in traits such as morphology, life history and behaviour (Endler, 1980; Reznick *et al.*, 1990; Magurran *et al.*, 1992).

Here, we utilise allozymes to explore: (1) levels of genetic variability in endemic and introduced populations of kapenta, (2) genetic differentiation since kapenta introductions, and (3) stock structure in commercially-exploited populations. The data presented here are not only the first detailed allozyme surveys of an African clupeid, but also one of the few which concentrates on intraspecific genetic variation (Kornfield, 1991).

4.2 Materials and methods

Standard methods of horizontal starch gel electrophoresis (Harris & Hopkinson, 1976) were employed to investigate genetic differentiation at the allozyme level. The initial screening of 53 enzyme systems on 5 buffer systems (Appendix II, Table II.1) revealed 20 enzymes with sufficient activity and resolution (Table 4.1). However, of these systems, four had to be dropped from the analysis due to inconsistent staining and problems with scoring (ACP (EC 3.1.3.2), FBA (EC 4.1.2.13), FK (2.7.1.4), GDA (3.5.4.3)). We therefore examined 16 enzyme systems encoded for by 29 loci (Table 4.2), 16 of which were polymorphic. Table 4.3 gives the relative mobility of the variant alleles at each polymorphic locus.

Table 4.1: Enzymes scored routinely, the buffer system used, number of loci (loci), number of loci polymorphic at the 0.99 level (poly) and the number of variant alleles (all).

enzyme	EC no.	buffer	loci	poly	all
AAT	2.6.1.1	CM 6.2	3	3	8
AK	2.7.4.3	CM 6.2	1		
CK	2.7.3.2	CM 6.2	2		
EST-D	3.1.1.1	TC 8.0	3	1	1
FH	4.2.1.2	CM 6.2	2		
G3PDH	1.1.1.8	TC 8.0	1		
G6PDH	1.1.1.49	TM 8.9	1	1	2
GAPDH	1.2.1.12	CM 6.2	2	1	1
IDH	1.1.1.42	CM 6.2	2	2	2
LDH	1.1.1.27	CM 6.2	2	2	4
MDH	1.1.1.37	CM 6.2	3	1	1
ME	1.1.1.40	CM 6.2	3	3	12
MPI	5.3.1.8	TC 8.0	1		
PGDH	1.1.1.44	TM 8.9	1	1	2
PGI	5.3.1.9	TC 8.0	1		
PGM	5.4.2.2	CM 6.2	1	1	6
Total			29	16	39

Table 4.2: Relative mobilities of variant alleles found in kapenta from Lakes Tanganyika, Kivu and Kariba

Locus	alleles					
AAT-1	*-82*					
AAT-2	*-125*	*-75*				
AAT-3	*0*	*20*	*50*	*200*	*275*	
EST-D-2	*112*					
G6PDH-1	*65*	*85*				
GAPDH-2	*107*					
IDH-1	*10*					
IDH-2	*60*					
LDH-1	*-800*					
LDH-2	*59*	*75*	*140*			
MDH-2	*41*					
ME-1	*-100*	*0*	*125*	*200*		
ME-2	*85*	*112*	*120*	*140*		
ME-3	*70*	*85*	*95*	*113*		
PGDH-1	*92*	*110*				
PGM	*-120*	*-110*	*-90*	*-80*	*-70*	*-30*

Chi-squared tests were used to test for deviations from Hardy-Weinberg equilibrium and for differences between populations. Genetic variability of the populations was estimated by calculating average heterozygosity, percentage of polymorphic loci and mean number of alleles per locus.

For the estimation of stock structure within the lakes, UPGMA dendrograms of Roger's genetic similarity were constructed. As an alternative, neighbouring samples were compared and if not significantly different, pooled together. Thus the sample sizes could be increased, theoretically leading to more sensitive statistical analysis.

Small scale genetic differentiation between sub-samples from the same site was investigated using tests for differences in genotype frequencies as well as a G_{st} analysis (Nei 1973).

4.3. Results

4.3.1. Degree of polymorphism

Although a large proportion of loci were polymorphic (16 out of 29), the frequency of variant alleles was generally low (Table 4.3, Appendix II). Six loci had a common allele frequency of less than 0.90, and were thus most useful for stock structure analysis. The other 10 loci were only weakly polymorphic, which although useful in detecting the loss of rare alleles in population bottlenecks or founder events, have limited value in detecting genetic differentiation of stocks.

4.3.2. Genetic variability of the introduced populations

When comparing genetic diversity between lakes, no obvious difference could be found (Table 4.4). Mean heterozygosities were 0.42-0.56, which is in the range reported for marine clupeids (Smith & Fujio, 1982). There is no reduction of mean heterozygosity evident in either introduced population. In addition, the mean number of alleles per locus, and the percentage of polymorphic loci were similar for natural and introduced populations, suggesting that there was no reductions in allelic diversity during the introductions.

Table 4.3: Frequencies of the most common allele observed in kapenta from Lakes Tanganyika, Kivu and Kariba

	alleles	<1.00	<0.95	<0.90	<0.70
AAT-1	2	+			
AAT-2	3	+			
AAT-3	6	+	+		
EST-D-2	2	+	+	+	+
G6PDH-1	3	+	+		
GAPDH-2	2	+	+	+	
IDH-1	2	+			
IDH-2	2	+			
LDH-1	2	+			
LDH-2	4	+	+	+	
MDH-2	2	+			
ME-1	5	+	+		
ME-2	5	+	+	+	+
ME-3	5	+	+		
PGDH-1	3	+	+	+	
PGM-1	7	+	+	+	+
Loci		16	10	7	3

4.3.3 Distribution of genetic variability

A gene diversity analysis (Nei, 1973) with sub-samples, samples, local areas, lake sections (e.g. north and south), and lakes as hierarchical levels (Figure 4.1) showed that the largest proportion of genetic diversity (98.86%, Table 4.5) occurred within sub-samples. By far the largest between-group component of genetic variability was between sub-samples within samples (1.48 %). Genetic differentiation on a larger scale (area, parts, lakes) contributed much less to the overall genetic variability (0.29 - 0.47 %). Therefore sub-samples were considered separately as far as possible. However to avoid any sampling bias, sub-samples were excluded from the final analysis if they were smaller than 20 individuals, and were pooled if they were not significantly different from each other (Table 4.6).

Table 4.4: Measures of genetic diversity observed in natural and introduced kapenta populations.

Pop	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity Direct-count	HdyWbg expected
LAKE TANGANYIKA					
MP1	30.8	1.3	13.8	0.057	0.057
MP2	21.0	1.5	20.7	0.067	0.067
MP3	28.0	1.5	17.2	0.065	0.068
MP4	30.0	1.6	24.1	0.080	0.076
MP5	41.8	1.4	17.2	0.076	0.071
CB1	47.0	1.5	17.2	0.064	0.064
CB2	41.0	1.4	10.3	0.063	0.063
KIP	50.0	1.6	24.1	0.075	0.073
KIB	38.0	1.4	13.8	0.063	0.063
KIG	34.0	1.3	10.3	0.053	0.056
MUG	44.8	1.4	13.8	0.064	0.067
KAR	46.0	1.5	17.2	0.065	0.066
KAD	37.9	1.3	13.8	0.069	0.064
LAKE KIVU					
GIS	49.6	1.4	17.2	0.062	0.060
KYN	49.9	1.5	20.7	0.067	0.067
KYS	47.7	1.5	17.2	0.068	0.071
CYA	50.0	1.5	17.2	0.062	0.064
LAKE KARIBA					
SV1	51.0	1.5	13.8	0.064	0.064
SV2	57.9	1.4	17.2	0.070	0.067
K 1	48.0	1.5	13.8	0.063	0.064
K 2	48.0	1.6	17.2	0.064	0.064
C	51.0	1.4	20.7	0.069	0.066
SZ1	56.0	1.5	13.8	0.068	0.065
SZ2	59.8	1.7	20.7	0.080	0.073
JO1	45.7	1.5	17.2	0.058	0.063
JO2	46.0	1.6	17.2	0.073	0.071

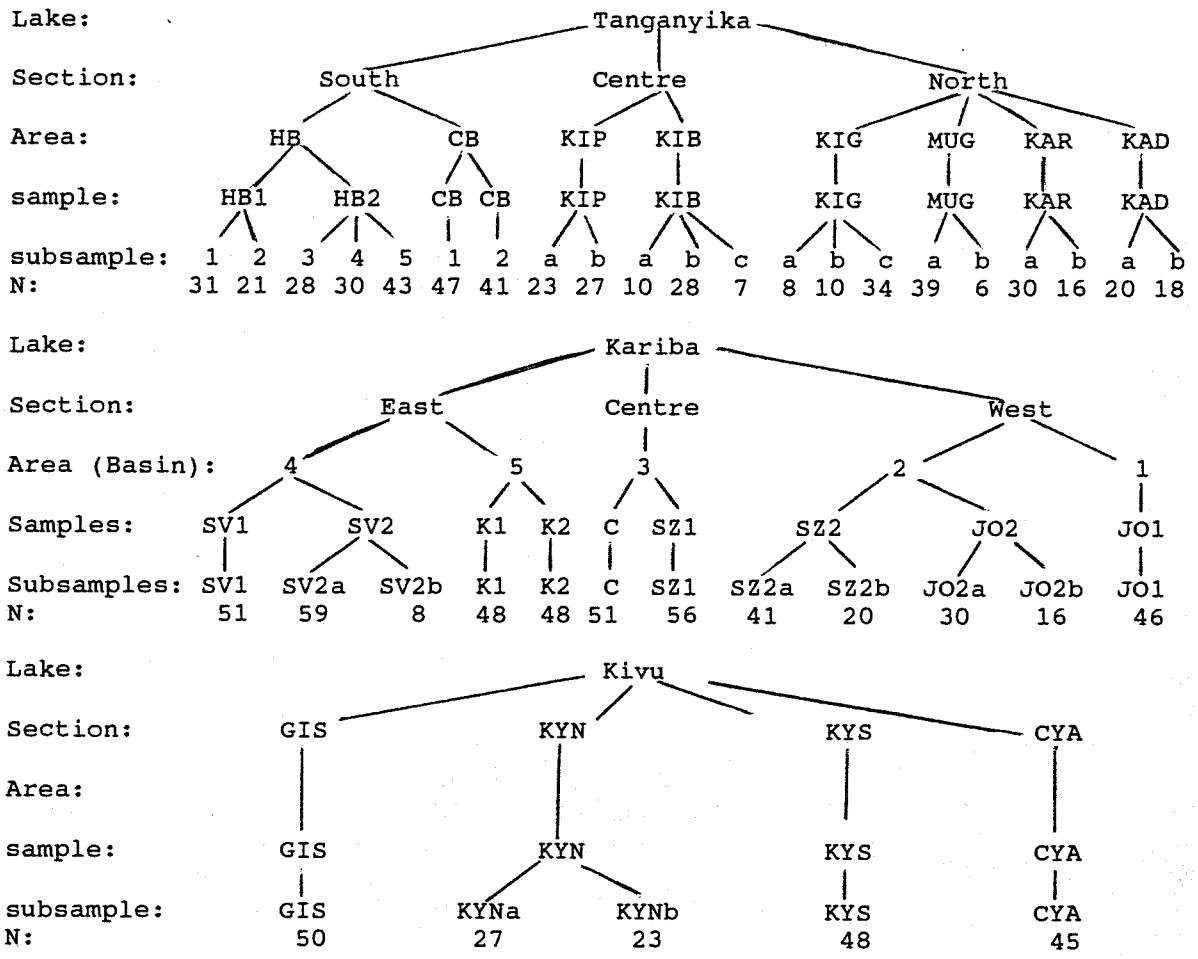


Figure 4.1: Hierarchical levels used in the G_{ST} analysis after Nei (1973).

Table 4.5: Hierarchical analysis of genetic variability (Nei, 1973) using the hierarchical levels shown in Figure 4.1.

	Overall	L.Tang.	L.Kivu	L.Kariba
within samples	96.86	96.12	98.57	98.33
between sub-samples within samples	1.48	2.30	0.20	0.58
between samples within areas	0.43	0.41	----	0.65
between areas within parts	0.47	0.74	----	0.21
between parts within lakes	0.47	0.44	1.23	0.23
between lakes	0.29			

Table 4.6: Results of chi-squared tests for differences in allele frequencies between sub-samples collected in Lakes Tanganyika, Kivu and Kariba. The significance level over all loci (ns-not significant, *-$p<0.05$, ** $p<0.01$, *** $p<0.001$) and the loci with significant differences are shown.

Sample (N)	sub-samples (N)	overall	loci
HBI (53)	MP1 (32), MP2 (21)	ns	*Est-D-2*
HB2 (101)	MP3 (28), MP4 (30), MP5 (43)	*	*ME-3, G6PDH-1*
KIP (50)	I (23), II (27)	ns	---
KIB	I (11), II (28), III (7)	ns	*GAPDH-2*
KIG	I (8), II (10), III (34)	***	*LDH-2, PGM-1, GAPDH-2,G6PDH-1*
MUG	I (39), II (6)	ns	----
KAR	I (30), II (16)	ns	----
KAD	I (20), II (18)	ns	----
KYN	I (27), II (23)	ns	----
SV2	I (59), II (8)	ns	*AAT-3*
SZ2	I (41), II (20)	ns	----
JO2	I (30), II (16)	ns	----

Analysis of genotypic distribution using chi-squared tests showed that most loci in populations were in Hardy Weinberg equilibrium (Appendix II, Table II.1), with deviations showing no consistent patterns. There was no obvious correspondence between the extent of genotypic equilibria, and the nature of sample collection (section 2.2).

4.3.4 Stock structure within the lakes

Lake Tanganyika

In Lake Tanganyika, the proportion of genetic variability between sub-samples within samples was the highest of all lakes (Table 4.5). Chi-squared tests of differences in allele frequencies between sub-samples were significant in the samples from Hore Bay (HB1, HB2), Kigoma (KIG) and Kibwesa (KIB), but not in Kipili (KIP), Muguruka (MUG), Karongo (KAR) and Kadjaga (KAD) (Table 4.6). It is important to note that the samples showing significant heterogeneity between sub-samples were either taken on different days (Kigoma, Kibwesa) or from different localities (HB1, HB2). In contrast, samples in Kipili, Karongo and Kadjaga were taken at the same locality on the same night. The failure to detect any heterogeneity between the sub-samples from Muguruka, which were collected by a purse seine at two different light boats, could be due to the small size of the second subsample (Table 4.6).

In the final analysis of heterogeneity within Lake Tanganyika, sub-samples with less than 20 fish were excluded, and sub-samples with no significant differences were pooled into single samples; thus only the sub-samples from Hore Bay (HB1: MP 1,2; HB2: MP 3,4,5) were considered. The chi-squared analysis testing for heterogeneity in allele frequencies was highly significant, with 4 out of 12 polymorphic loci showing significant heterogeneity (Table 4.7). However, an UPGMA tree shows no correspondence between geographic

location and Roger's genetic similarity (Figure 4.2). In particular, the sub-samples from Hore Bay (Mpulungu 1-5) are widely separated on the UPGMA tree, and show significantly different allele frequencies (Table 4.8). Thus the approach of pooling neighbouring samples, as proposed by Richardson *et al.*, (1986) and carried out in the second annual report was not employed here.

Table 4.7: Results of chi-squared tests to examine the extent of genetic heterogeneity among kapenta samples from Lake Tanganyika. The samples compared are shown in Table 4.4. Significant probabilities are indicated in bold.

Locus	No. of alleles	Chi-square	D.F.	P
AAT-1	2	22.451	12	**.03276**
AAT-2	3	26.384	24	.33399
AAT-3	5	42.814	48	.68470
EST-2	2	14.676	12	.25966
G6PDH	3	44.282	24	**.00707**
GAP-2	2	18.666	12	.09691
LDH-2	4	39.177	36	.32921
ME-1	4	29.096	36	.78593
ME-2	4	52.368	36	**.03820**
ME-3	5	73.825	48	**.00971**
PGDH	2	19.270	12	.08223
PGM	4	50.550	36	.05449
Totals		433.559	336	**.00025**

Table 4.8: Genetic heterogeneity between the sub-samples from Hore Bay near Mpulungu (HB1 (MP1, MP2), HB2 (MP3, MP4, MP5)). The significance over all loci (- not significant, + $p < 0.05$, + + $p < 0.01$) as well as the loci, where a significant difference in allele frequencies were found, are shown.

	MP1	MP2	MP3	MP4	MP5
MP1		-	-	-	-
MP2	*EST-D*		+ +	-	+ +
MP3	*G6PDH*	*LDH-2,ME-2,EST-D*		-	-
MP4	*ME-3*	*ME-2,EST-D*	*ME-3*		-
MP5	-	*ME-2,EST-D*	*G6PDH*	*ME-3*	
overall	+ + / ME-2, ME-3, G6PDH				

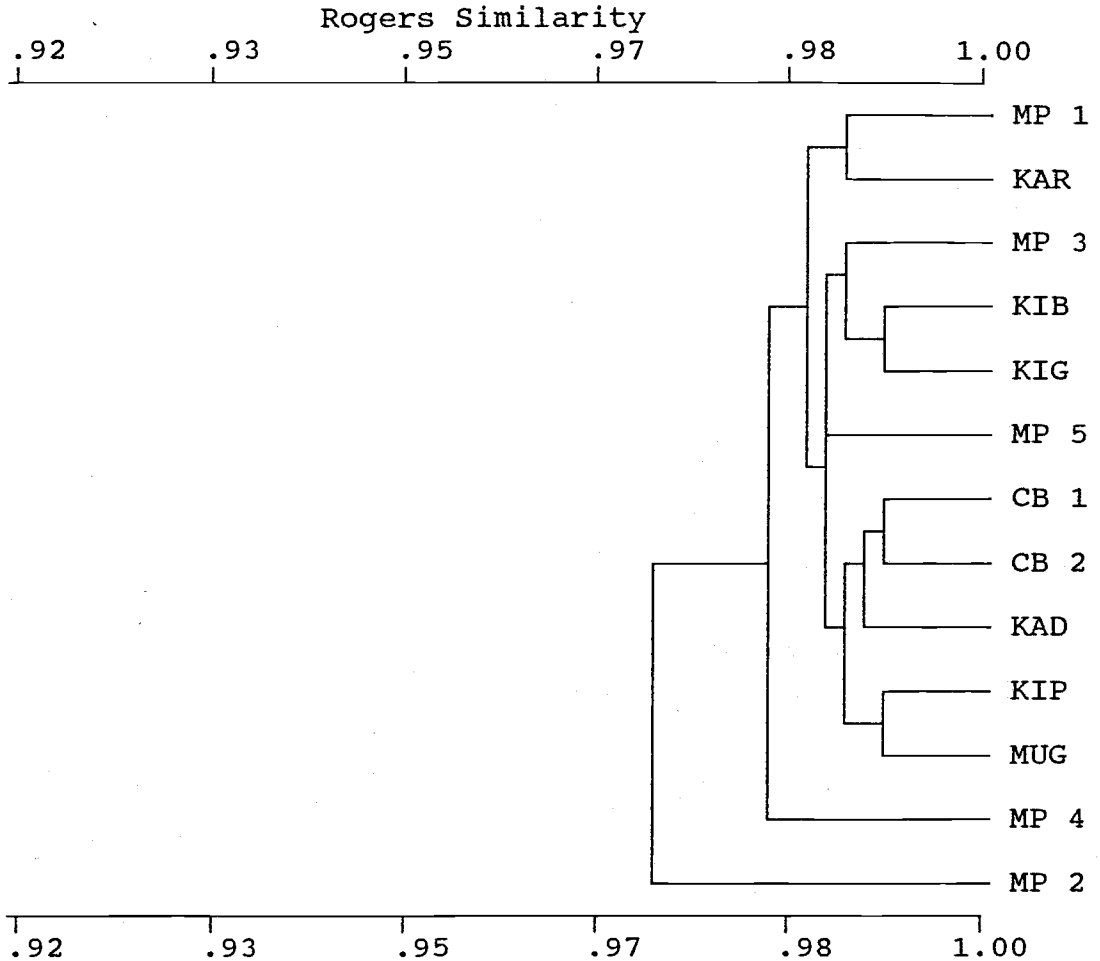


Figure 4.2: UPGMA dendrogram of Rogers genetic similarity between kapenta samples collected in Lake Tanganyika.

Lake Kivu

In Lake Kivu, sub-samples were collected at one site (KYN), and since their allele frequencies were not significantly different, they were pooled (Table 4.6). Genetic differences between the four samples in Lake Kivu were significant (Table 4.8). The samples from the northern (GIS) and the southern end (CYA) of

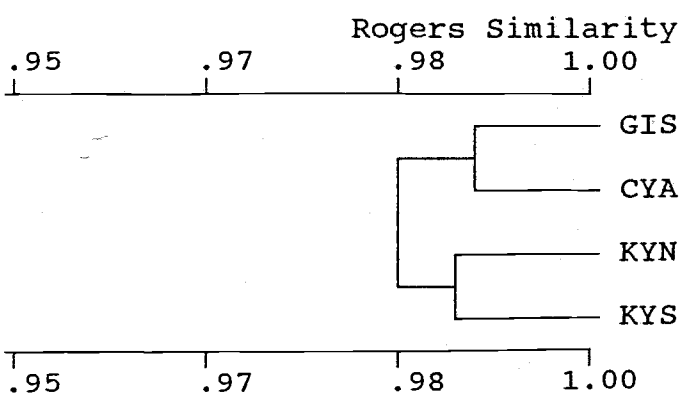


Figure 4.3: UPGMA dendrogram of Rogers genetic similarity between kapenta samples collected in Lake Kivu.

Table 4.8: Results of chi-squared tests to examine the extent of genetic heterogeneity among kapenta samples from Lake Kivu. The samples compared are shown in Table 4.4. Significant probabilities are indicated in bold.

Locus	No. of alleles	Chi-square	D.F.	P
AAT-2	2	7.311	3	.06263
AAT-3	2	2.289	3	.51465
EST-2	2	13.029	3	**.00457**
G6PDH	3	5.175	6	.52153
GAP-2	2	6.417	3	.09298
IDH-2	2	3.133	3	.37159
LDH-2	2	6.568	3	.08704
ME-1	3	5.971	6	.42650
ME-2	4	9.761	9	.37020
ME-3	4	11.524	9	.24148
PGDH	2	2.122	3	.54756
PGM	4	9.352	9	.40542
Total		82.65	6	**.02792**

the lake were similar to each other, but different from the two central samples (KYN, KYS; Figure 4.3). Differences in Lake Kivu were due to only one locus (EST-D-2, Table 4.8), which may suggest different selection pressures rather than restricted gene flow between localities.

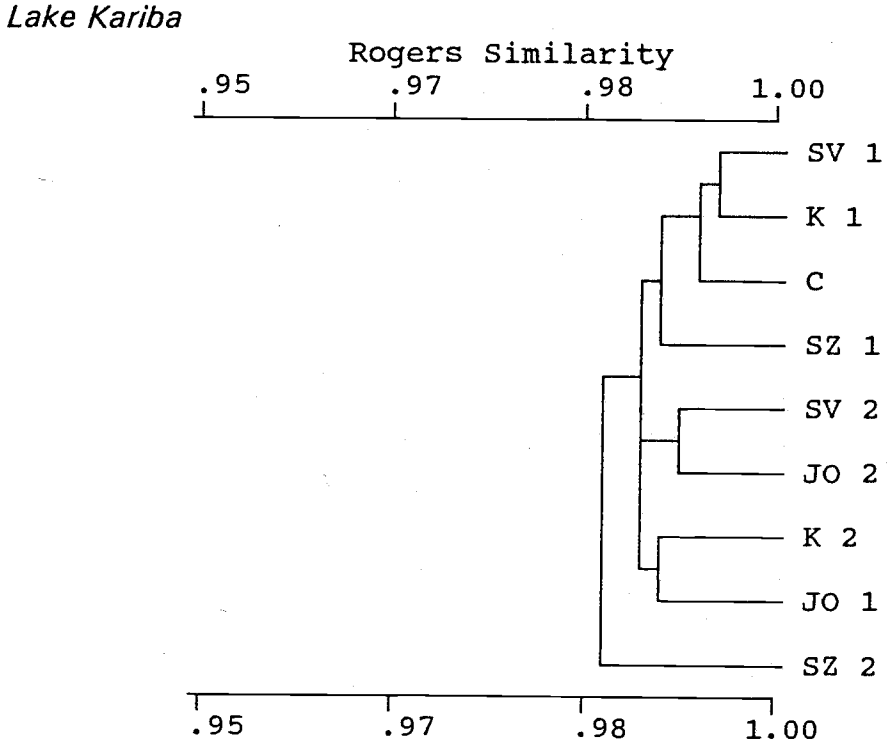


Figure 4.4: UPGMA dendrogram of Rogers genetic similarity between kapenta samples collected in Lake Kariba.

Sub-samples in Lake Kariba were not significantly differentiated (Table 4.4). The only significant difference is due to one heterozygote at the *AAT-3* locus in sample SV2, which does not result in any overall significance. Chi-squared tests among samples did not reveal significant genetic heterogeneity within the lake (Table 4.9), and the dendrogram shows no correspondence between geographic location and genetic similarity (Figure 4.4). Thus there is no evidence for the existence of genetically identifiable stocks.

4.3.5 Genetic differentiation after the introductions

Genetic differentiation after the introduction of kapenta was estimated by comparing the kapenta of Lakes Kariba and Kivu with their respective source populations in Lake Tanganyika. The fish introduced to Lake Kivu were caught in Kabezi, 15 km south of Bujumbura, at the northern end of Lake Tanganyika. Most were released in and around Cyangugu in the south of Lake Kivu, although some were released in Gisenyi in the north (Collart, 1989). For the present analysis, the sample from Kadjaga (10 km west of Bujumbura) was pooled with the sample from Karongo (52 km south of Bujumbura). This pooled sample "Bujumbura" was compared with each sample from Lake Kivu.

Both the Gisenyi (GIS) and the Cyangugu (CYA) sample proved to be significantly different in their allele frequencies from their putative source population in Bujumbura (Table 4.10) at four loci. However, the allele frequencies of the samples from the middle of Lake Kivu (KY1, KY2) were not different from those of the Bujumbura sample.

Table 4.9: Results of chi-squared tests to examine the extent of genetic heterogeneity among kapenta samples from Lake Kariba. The samples compared are shown in Table 4.4. Significant probabilities are indicated in bold.

Locus	No. of alleles	Chi-square	D.F.	P
AAT-1	2	9.140	8	.33060
AAT-2	3	15.724	16	.47237
AAT-3	5	44.433	32	.07078
EST-2	2	6.142	8	.63136
G6PDH	3	13.101	16	.66533
GAP-2	2	7.425	8	.49152
IDH-1	2	5.930	8	.65510
LDH-1	2	9.118	8	.33240
LDH-2	3	10.494	16	.83961
MDH-2	2	8.718	8	.36667
ME-1	3	12.092	16	.73760
ME-2	5	43.892	32	.07847
ME-3	5	33.414	32	.39845
PGDH	3	23.578	16	.09915
PGM	7	54.937	48	.22848
(Totals)		298.139	272	.13241

Table 4.10: Results of chi-squared test to investigate the genetic differentiation of kapenta since their introduction to Lake Kivu. Fish were introduced in Gisenyi (GIS) and Cyangugu (CYA), the putative source population is Bujumbura (KAD + KAR). The significance over all loci are shown (- not significant, + $p < 0.05$, ++ $p < 0.01$), as well as the loci, where a significant difference in allele frequencies was found.

	Bujumbura compared with	
GIS	+	*ME-3, AAT-2, PGM-1, GAPDH-1*
KYN	-	-
KYS	-	-
CYA	++	*ME-3, EST-D-2*
Overall	+	*ME-3, EST-D-2*

In the case of Lake Kariba, kapenta was airlifted from Mpulungu in the south of Lake Tanganyika to Sinazongwe at the north-western shore of Lake Kariba (Marshall, 1992). As the sub-samples from Mpulungu proved to be genetically heterogeneous, the allele frequencies of each subsample was compared with that of both samples from Sinazongwe (Table 4.11). The chi-squared tests show some degree of heterogeneity which was mainly due to two enzyme systems (*ME, G6PDH*). However, the extent of differentiation observed in the introduced Lake Kariba fish depends very much on the subsample chosen to represent the source population, with genetic heterogeneity ranging from high overall significances (Mp2) to no significant differentiation at all loci (Mp3).

4.4 Discussion

A primary criterion for the effective use of allozymes in describing population structure is the presence of polymorphism yielding sufficient variants to compare among samples. Although here, many enzyme loci were weakly polymorphic (i.e. with a common allele frequency of > 0.95), six had a frequency of < 0.90, and three < 0.7, so providing adequate genetic markers. Thus, at the outset it is possible to state that the patterns detected were not compromised by a lack of polymorphism.

The contribution of allozymes to stock structure analysis is, however, influenced by a limitation inherent in the application of the procedure; the

Table 4.11: Results of chi-squared tests to investigate the genetic differentiation of kapenta since their introduction to Lake Kariba. Fish were introduced from Hore Bay, L. Tanganyika (HB1 (MP1, MP2), HB2 (MP3, MP4, MP5)) to Sinazongwe, Lake Kariba (SZ1, SZ2). The significance over all loci are shown (- not significant, + $p < 0.05$, ++ $p < 0.01$), as well as the loci, where a significant difference in allele frequencies was found.

		MP1	MP2	MP3	MP4	MP5
SZ1	-/-	++/*ME-2*	-/-	+/*ME-3*	-/-	
SZ2	-/*G6PDH*	+/*ME-2*	-/-	-/*G6PDH*	+/*G6PDH*	
overall	+++ / *ME-2, ME-3, G6PDH*					

oversensitivity of genetic markers to low levels of gene flow (Carvalho & Hauser, 1994; Ward & Grewe, 1994). A small number of migrants per generation is sufficient to homogenise a gene pool, at least empirically given the relatively small sample sizes usually employed. Therefore, two populations may be effectively self-recruiting yet not be genetically distinct. Gene flow rates of 1%, 5%, 10% and 50% would all probably mean that populations cannot be distinguished genetically and appear panmictic, yet as far as a fishery manager is concerned, gene flow rates of 10% or less may justify treatment as separate stocks. Therefore, molecular methods are generally only useful to the fisheries manager if differences are detected, but there is no robust method that can be applied to predict patterns *a priori* based on vagility (Ward, 1994). If there are no genetic differences, a possible separation into several "harvest stocks" (locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource) has to be investigated by alternative methods like tagging or parasite analysis.

The effect of kapenta introductions on genetic diversity was examined in view of the lack of correspondence between census and effective population size in introduced samples where massive mortality among fry may result during adaptation to a new environment (Gharrett & Thomason, 1987). Allozyme data did not detect any significant reductions of genetic diversity in kapenta from Lakes Kivu or Kariba as estimated by mean heterozygosities and the mean number of alleles per locus. Following an introduction, the most likely indicator of founder events is the loss or rare alleles (Baker & Moeed, 1987) which contribute relatively little to average heterozygosity (Lewontin, 1965). In contrast to allelic diversity, heterozygosity is not very strongly affected by a founder effect, unless the founder population is extremely small, or the rate of population increase is slow (Leberg, 1992; Carvalho & Hauser, 1995).

In Kariba, evidence suggests that fish populated their respective habitats rapidly. For example, after the 1967-1968 releases, the first evidence of sardines occurred when some were found in the stomachs of tigerfish (*Hydrocynus forskahlii*, in Sinazongwe in July 1969 (Marshall, 1992). Sardine shoals were then detected using an echosounder, and were subsequently captured using light attraction and a scoop net. The fish spread rapidly throughout the lake, and were present both in open and shallow waters by 1970. A striking example of the speed of spread is illustrated by Kenmuir (1971) who examined the stomach contents of tigerfish: in early 1969, no sardines were recorded in any tigerfish stomachs, but in 1970 they made up 75% of food items recorded. The events in Lake Kivu have not been so well documented, mainly because of political problems.

Thus, based on allozymes there was no indication of differences in genetic diversity between native and introduced populations of kapenta, indicating sizeable founder populations and/or rapid population growth. Information from the mtDNA data, are however, not so clear-cut (Chapters 5 & 6).

Allozymic data for patterns of kapenta stock structure differ among the lakes. In Kariba, there was no significant genetic heterogeneity, with apparent homogeneity of allozyme frequencies throughout the areas sampled. In Lakes Kivu and Tanganyika, significant divergence was detected among samples examined. In Lake Kivu, the dengrogram of Roger's similarity shows no correspondence between the extent of genetic differentiation and geographic proximity of the four samples: fish form the northern (GIS) and southern (CYA) ends are similar, but different from the two central samples (KYN, KYS; Figure 4.3). Such differences are difficult to interpret without additional, preferably temporal data from fish in the same areas, but are surprising in view of the recency of the introduction (34 years). Such genetic patchiness may arise

either among founder fish (founder effect), or in their descendants, due to localised population bottlenecks, with restricted gene flow, or selection.

The associated allozyme data on diversity do not support the operation of stochastic forces, since the levels of genetic diversity were comparable in Kivu and the source Tanganyika fish. Furthermore, if genetic drift was the primary differentiating force, it would be expected to affect several loci simultaneously (Slatkin, 1987). The confinement of genetic divergence to a single genetic locus (*EST-D-2*) is more indicative of natural selection, where individual loci are affected to differing degrees.

It is appropriate to present some aspects of the limnology and structure of Lake Kivu when considering genetic changes in the resident kapenta populations. Kivu is a volcanic barrier lake formed by volcanic damming of a valley previously draining into the Edward basin, and with a generally impoverished fauna and unusual water chemistry (Beadle, 1981). There is permanent stagnation of the high density water below 70m, preventing recycling of most organic matter in bottom mud, so restricting productivity. The waters habitable by fauna are similarly restricted to the oxgenated waters above 70 m, which during severe storms may become contaminated with stagnant oxygen-free and hydrogen-sulphide-charged deeper waters. In addition to the unusual limnological conditions, the topography of the lake is atypical in that there are expansive areas in the south where numerous islands exist, so disrupting the open pelagic waters characteristic of the north.

Although it must be emphasized that genetic data are preliminary, the nature of genetic heterogeneity observed, taken together with the extraordinary features of the lake, support the possibility of selective changes occurring since the introduction. It is highly unlikely of course that selective responses would be occurring at the enzyme locus itself, but rather that it is acting as a marker for genetic changes at associated loci (Maynard Smith & Haigh, 1974).

The effects of selection may be accelerated by restricted exchange among local populations, or persistent integrity of migrating shoals. The observed similarity at the *EST-D-2* locus between the north and south is puzzling, especially since these same samples were taken from the sites where kapenta were introduced to Lake Kivu, and which are genetically different from their source population at four loci. Clearly, genetic changes have occurred since the time of introduction, which may in part reflect differential adaptation to different regions of the lake. The role of gene flow in determining genetic structure within the lake is difficult to assess based on allozyme data; the apparent similarity between the north and south could have arisen either by chance, due to sampling artefacts, by adaptation to similar environments, or by migration between areas. Although kapenta are normally not expected to travel across extensive areas (Coulter, 1991a), the movement of shoals over distances of the magnitude involved here in Lake Kivu (c. 200 km) may be more feasible. Perhaps the only clear picture to emerge is that genetic heterogeneity exists within the lake, that it has occurred rapidly, and that its determinants may, in part, be selectively-based. The divergence between the two central samples and the others certainly suggest some restriction to exchange of fish on a local scale, though additional samples are required to ascertain the persistence and spatial relationships of such patterns.

Significant genetic heterogeneity was found also among Lake Tanganyika samples, though, similarly to Lake Kivu, there was no correlation between the extent of genetic divergence and geographic separation. Unlike Lake Kivu results, genetic differences were observed at 4 out of 12 loci, suggesting the operation of differentiating forces more related to isolation (e.g. restricted gene

flow), than selection (Lewontin & Krakauer, 1973; Slatkin, 1987; Baker & Moeed, 1987). Evidence of small-scale genetic heterogeneity was provided by the comparisons between sub-samples, which were taken either on different days from the same location, or from slightly different sites. Samples collected at the same locality on the same night were allozymically homogeneous. Such observations strongly support the existence of mobile genetically discrete assemblages, perhaps shoals, moving between sites. Given the shoaling habit of these sardines, the light-attracting fishing method would on some occasions capture many individuals from a single shoal, and on others, a mixture of several shoals that were in the vicinity. Variability in the degree of genetic divergence among samples may be explained by the serendipitous nature of shoal collection if such assemblages were indeed genetically distinct.

Evidence for genetic differentiation among shoals exists in other species (Ferguson & Noakes, 1981; Avise & Shapiro, 1986; Carvalho *et al .*, 1992), and the existence of size-specific shoals in both *L. miodon* (Marshall, 1992; present study, Fig. 3.2) and the other freshwater sardine, *Stolothrissa tanganicae* (Coulter, 1991a) supports the non-random aggregation of individuals. If the stock structure of kapenta is to be understood, the dynamics and determinants of shoaling are clearly a fundamental area requiring research, especially since such social structure imparts unique features relevant to fisheries management (Pitcher, 1992).

The genetic divergence of Lake Kivu kapenta from its source in Tanganyika provides one of the few examples showing allozymic differentiation in a matter of decades (Gharrett & Thomason, 1987; Vuorinen, 1991; Golubtsov *et al.*, 1993), and is thus also of general evolutionary interest. Conventional ideas purport that such time spans would be insufficient to allow the accumulation of genetic differences through mutation and drift. Such predictions ignore the effects that adaptation to a new environment may impose, which may accelerate evolutionary rates, especially where founder populations are relatively small (Barton & Charlesworth, 1984). The Kivu findings not only demonstrate the sensitivity that allozymes can display to novel genetic changes in a matter of decades, but reinforces the evidence for rapid evolutionary change within the time-scale relevant to fisheries management (Carvalho & Hauser, 1995).

Such considerations are relevant to the extreme life-history and size differences recorded in Kariba kapenta. One of the theories proposed for the phenotypic divergence from source populations was that of genetic changes occurring in founder individuals (Marshall, 1993). Despite the large number of fry released into Kariba, mass mortality may have reduced the effective population size dramatically, with consequent selection for a short life cycle and small size. Three lines of evidence, based on allozyme data, suggest that no major genetic rearrangements have accompanied the introduction into Lake Kariba: First, the comparable levels of genetic diversity in Kariba and Tanganyika kapenta; secondly, the lack of any detectable allozymic divergence from Tanganyika fish, and thirdly, the genetic divergence detected between some Kivu and Tanganyika samples. The latter is important because it demonstrates the potential of the allozymes employed to detect genetic changes between recently separated populations, even though the introduction to Lake Kariba happened 9 years later.

It appears likely that the gross phenotypic divergence of *L. miodon* in Kariba is a form of phenotypic plasticity, which in theory at least, may be a reversible phenomenon. Unfortunately, it may prove impossible to test this theory by transfer experiments since adult kapenta die with the minimum of handling (Marshall, 1992). Indirect evidence demonstrates that these sardines

are especially flexible in their habitat requirements: the ability to colonise lakes with widely contrasting limnologies, and its survival as a normally lacustrine species in the Zambezi River are cases in point (Marshall, 1993). As emphasized in Chapter 6, the fisheries implications of such apparent phenotypic plasticity does not preclude artificial selection by harvesting (Ryman, 1993).

Genetic variability, as estimated by heterozygosity using allozymes, is distributed within and between populations to varying degrees depending on species and local factors (Gyllensten, 1985; Hershberger, 1992). One effective way of describing the distribution of genetic variability using electrophoretic data is through an analysis of gene diversity (Nei, 1973; Chakraborty, 1980), which is analogous to a nested analysis of variance where the components of genetic diversity are partitioned according to a hierarchy. Here, the levels consisted of: sub-samples, samples, local areas (grouping of local samples), lake sections (e.g. north and south of a lake), and different lakes. The results of the analysis showed clearly that the majority of genetic variability (98.86%) was contained within sub-samples, that is, within populations. Thus, a single sample of kapenta is likely to contain a representative range of genetic variability, underlining the low incidence of genetic heterogeneity among sites.

The distribution of genetic variability differs markedly among species. For example, in Trinidadian guppies, *Poecilia reticulata*, 66% of the total diversity is attributable to divergence between drainage basins, 32% among rivers within basins, and only 2% within rivers (Carvalho *et al.*, 1991). Such contrasting population structures when linked to the evolutionary significance of genetic variability makes it important to describe the distribution of genetic diversity to ensure conservation of within- and between-population heterogeneity. Such patterns are undoubtedly determined to a large degree by mechanisms of behavioural isolation (e.g. site fidelity vs. high mobility) and the incidence of physical barriers. In kapenta, the pelagic habit appears to ensure overall dispersion of genetic diversity.

In summary, the allozyme data provides four major pieces of information relevant to the original objectives: First, the lack of any detectable reduction in genetic variability during the introductions of kapenta into Lakes Kariba and Kivu; secondly, the existence of some genetic divergence between Tanganyika and Kivu sardines since colonisation; thirdly, the significant genetic heterogeneity among some samples within Tanganyika and Kivu, indicating deviations from panmixia, and fourthly, the presence of small-scale genetic patchiness probably arising from localised aggregations or shoals. The lack of geographic patterning in genetically differentiated samples complicates attempts to formulate models of migration or stock boundaries within lakes. It is clear, nevertheless, that harvesting is unlikely to remove random samples of the gene pool, and that more than a single genetic stock exists within Lakes Tanganyika and Kivu. The implications for fisheries management are discussed in Chapter 6.

CHAPTER 5: MITOCHONDRIAL DNA ANALYSIS

5.1 Introduction

Mitochondrial DNA (mtDNA), a small circular molecule (14,000-26,000 base pairs (bp)) is homologous among widely divergent taxa, and has several characteristics that render it particularly useful for population studies. The rate of nucleotide substitution is approximately 5-10 times that of nuclear DNA, thus enhancing the sensitivity for detecting differences between recently separated populations. The smaller effective population size for mtDNA, which is approximately one-quarter that for nuclear DNA, means that there is an increased chance of population bottlenecks with a consequent reduction in mtDNA variability and greater divergence between mtDNA genotypes (Avise *et al.*, 1987). In fish species where females are philopatric (Shields, 1982), but where spawning males disperse, genetic divergence at the mtDNA level may still be detected, in contrast to allozymic data (Billington & Hebert, 1991). Moreover, the maternal transmission of mtDNA allows relatively easy reconstruction of historical events without the complications arising from recombination. The net result is that mtDNA analysis typically reveal differentiation where allozymes display spatial homogeneity.

Analysis of genetic structure using sequence variation in mtDNA has had variable success, with many marine fishes exhibiting generally low levels of haplotype diversity and population divergence (Avise, 1986; Ovenden, 1990). Initial applications of mtDNA to population genetics (Avise, 1987) were dominated by studies of terrestrial species where surveys often revealed individual mtDNA genotypes confined to single populations. It was thereby possible to detect significant population heterogeneity with sample sizes generally smaller than those employed in allozyme studies. However, in many fishes, common genotypes are frequently distributed widely (Graves *et al.*, 1984; Smith *et al.*, 1990), and thus the analysis of frequency distributions demands larger sample sizes (Ovenden, 1990; Naish, 1993; O'Connell, unpublished data, R.D. Ward, C.S.I.R.O., Hobart, Tasmania, pers. comm.). In contrast to the largely independent sources of information from different allozyme loci, the mtDNA molecule is effectively a single locus, with composite genotypes equivalent to alleles.

The neutrality of mtDNA haplotypes is debatable (MacRae and Anderson, 1988; Nigro and Prout, 1990), since selective constraints may operate on mutations in coding regions. However, the value of mtDNA as a marker in population genetics studies has been shown in number of studies (see Ovenden 1990 for a review), and similar to allozyme, neutrality may be assumed if there is no evidence to the contrary.

The recent application of PCR to restriction analysis of mtDNA (Kocher *et al.*, 1989; Carr and Marshall, 1991; Martin *et al.*, 1992; Whitmore *et al.*, 1992; Karl and Avise, 1993) removes the need to isolate mtDNA by extended ultracentrifugation, and the availability of universal primers (Kocher *et al.*, 1989), together with accumulating sequence data (Brown *et al.*, 1993; Karl and Avise, 1993; Prodöhl, 1993), has greatly advanced the detection of nucleotide diversity in specific regions of the mtDNA genome (e.g. D-loop, ND genes).

Here, we analyse sequence variation in the ND genes of the mitochondrial DNA genome, by employing a universal primer (ND5/6; Avise, 1994). ND genes are sequences of DNA that code for enzymes in the NADH dehydrogenase complex, and have been shown to exhibit polymorphism in several fish species (Hall, 1993; Meyer, 1993; O'Connell, 1994; Chapman *et al.*, 1994). In addition

to adding to our estimates of genetic diversity, comparisons will be made among samples within and between lakes.

5.2. Materials and Methods

As a modification to our original plans to use radioactively labelled herring mtDNA probes to visualise kapenta mtDNA, we pursued the easier and faster approach of amplifying certain regions of kapenta mtDNA with universal primers using the polymerase chain reaction (PCR). This method enabled the more rapid analysis of samples than the conventional whole genomic approach (Chapman *et al.*, 1994).

DNA was extracted from tissue samples following a standard protocol (Taggart *et al.*, 1992, Appendix III) with a modified buffer. Extractions from ethanol-preserved samples yielded better quality DNA than from frozen samples, which could usually not be amplified successfully.

PCR of the ND 5/6 gene was carried out using universal primers (Hall, pers comm.) supplied by Appligene Ltd. The primer sequences were:

A: 5' - AAT AGT TTA TCC AGT TGG TCT TAG - 3'

B: 5' - TTA CAA CGA TGG TTT TTC ATA GTC A - 3'

The reaction conditions are given in Appendix III. The PCR product, which was about 2.5 kbp long, was checked on an 0.8% agarose minigel with ethidium bromide staining for nonspecific products and sufficient yield. The amplified DNA was cut with six restriction enzymes (endonucleases, *Hinf*I, *Cfo*I, *Rsa*I, *Taq*I, *Dde*I, *Msp*I), which cut the DNA at specific four or five base sequences. Restricted samples were run on 6% polyacrylamide gels together with Boehringer marker VI, and visualised using standard silver staining protocols (Appendix III). 17-20 individuals per sample were analysed per sample.

Upper case letters were assigned to each digestion profile for each endonuclease and a composite haplotype for each individual fish was constructed from these data.

Nucleotide diversity was calculated between each pair of haplotypes using the method of Nei and Tajima (1981) and Nei and Miller (1990). The mean amount of sequence divergence between each pair of samples was then calculated using these nucleotide data and haplotype frequencies. An UPGMA tree was constructed from nucleotide divergence data using PHYLIP.

The extent of geographic heterogeneity in composite haplotype frequencies was tested using a Monte Carlo approach of chi-squared tests (Roff and Bentzen, 1989): the chi-squared value for the actual haplotype frequencies are calculated and then compared with a large number of random rearrangements of the contingency table. The result is an estimate of the probability of obtaining a higher chi-squared value than the actual one by chance alone.

Genetic diversity of the mtDNA was estimated in two ways: first, by calculating haplotype diversity, which is equivalent to heterozygosity obtained from allozyme data, and secondly by nucleotide diversity, which is the average number of nucleotide substitutions per site for a group of DNA sequences

sampled (Nei & Tajima 1981; Nei, 1987). All analyses were carried out using the Restriction Enzyme Analysis Package (REAP, Vers. 4.1, McElroy *et al.* 1992).

5.3: Results

5.3.1. Genetic variability of kapenta mtDNA

The six endonucleases used in the present study cut the ND 5/6 gene into an average of 14.78 fragments, meaning that an average of 64.15 bases were surveyed. That corresponds to about 2.57 % of the ND 5/6 gene or about 0.37 % of the total mtDNA genome. Nevertheless, a large number of haplotypes were found: with six restriction enzymes we observed 57 composite haplotypes in 219 fish (Appendix III). This high polymorphism resulted in a large number of very rare haplotypes; only two haplotypes were present in all populations, while 34 haplotypes were observed only once. Because of this high number of rare haplotypes, tests for heterogeneity between samples were also carried out for each endonuclease separately. A Monte Carlo simulation on composite haplotypes in all samples proved significant heterogeneity among samples (Table 5.1.). When testing data from individual endonucleases, only *Hinf*I and *Rsa*I revealed significant heterogeneity among all samples (Table 5.1).

The mean sequence divergence between haplotypes was 1.78 $\pm$ 1.005% ($x \pm$ Sd, range 0.13-4.69%). The average within-population haplotype diversity was 84.92% (Table 5.2), and the average nucleotide diversity 1.85%.

5.3.2. Genetic stock structure within the lakes

No samples taken from within any lakes showed significant heterogeneity in composite haplotype frequencies among samples (Table 5.1). Furthermore, if samples from the southern and the northern basin of Lake Tanganyika were pooled, no differences in haplotype frequencies were evident (N: KAD, MUG, KIG; S: KIB, MP4, CB2; $p = 0.716$).

Table 5.1: Results of Monte Carlo comparisons among-samples within lake for heterogeneity in haplotype frequencies. Composite haplotypes as well as haplotypes obtained from each endonuclease were used as input data. For individual enzymes, within-lake tests were only performed if the overall test was significant.

	All lakes	L.Tanganyika	L.Kivu	L.Kariba
Composite	0.016	0.256	0.530	0.352
*Hinf*I	0.036	0.058	0.981	0.674
*Rsa*I	0.024	0.056	0.322	0.907
*Cfo*I	0.053			
*Taq*I	0.240			
*Dde*I	0.317			
*Msp*I	0.330			

Table 5.2: Haplotype and Nucleotide Diversity within kapenta populations

Sample	Haplotype Diversity x +/- SE	Nucleotide Diversity
L. Tanganyika		
HB 4	0.8770 +/- 0.03793	0.018528
CB 2	0.9179 +/- 0.02143	0.018408
KIB	0.9079 +/- 0.02382	0.021376
KIG	0.9340 +/- 0.01570	0.019948
MUG	0.8770 +/- 0.03793	0.019300
KAD	0.8000 +/- 0.05105	0.017652
L. Kivu		
GIS	0.8364 +/- 0.03689	0.016442
KYN	0.6872 +/- 0.07789	0.017296
KYS	0.8913 +/- 0.02747	0.017627
CYA	0.6769 +/- 0.07508	0.016802
L. Kariba		
SV2	0.8952 +/- 0.03379	0.019563
JO2	0.8889 +/- 0.03455	0.019650
Average	0.8492 +/- 0.00061	0.018549

Tests on data from individual endonucleases failed to show significant heterogeneity within lakes (Table 5.1). However, the results of *Hinf*I and *Rsa*I were close to the significance level, which suggests that these markers may offer some potential for detecting stock separation within the lake. However, haplotype frequencies showed no common trend as to which samples are likely to be different (Table 5.3). *Hinf*I appeared to separate KIB from the other samples, whereas *Rsa*I showed the two bays in south of Lake Tanganyika to be different (MP4, CB2).

In Lake Kivu, there was significant nucleotide divergence between two populations (KYN, CYA) and the other samples (Table 5.2, Figure 5.1).

Table 5.3: Haplotype frequencies obtained using the restriction enzymes *Hinf*I and *Rsa*I, respectively. Although there are differences among samples they are not consistent between the two enzymes.

	*Hinf*I								*Rsa*I								
	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	I
MP4	5	6	9	0	0	0	0	1	10	6	1	0	0	0	0	0	0
CB2	8	3	9	0	0	0	0	0	11	6	0	2	0	0	0	1	0
KIB	10	2	6	0	0	0	0	0	11	1	0	5	0	1	1	0	0
KIG	7	2	8	0	0	0	0	0	12	1	0	3	0	0	1	0	0
MUG	5	6	7	0	0	0	2	0	15	1	0	1	0	0	0	0	0
KAD	5	0	12	0	0	1	0	0	14	1	0	2	0	0	0	0	0

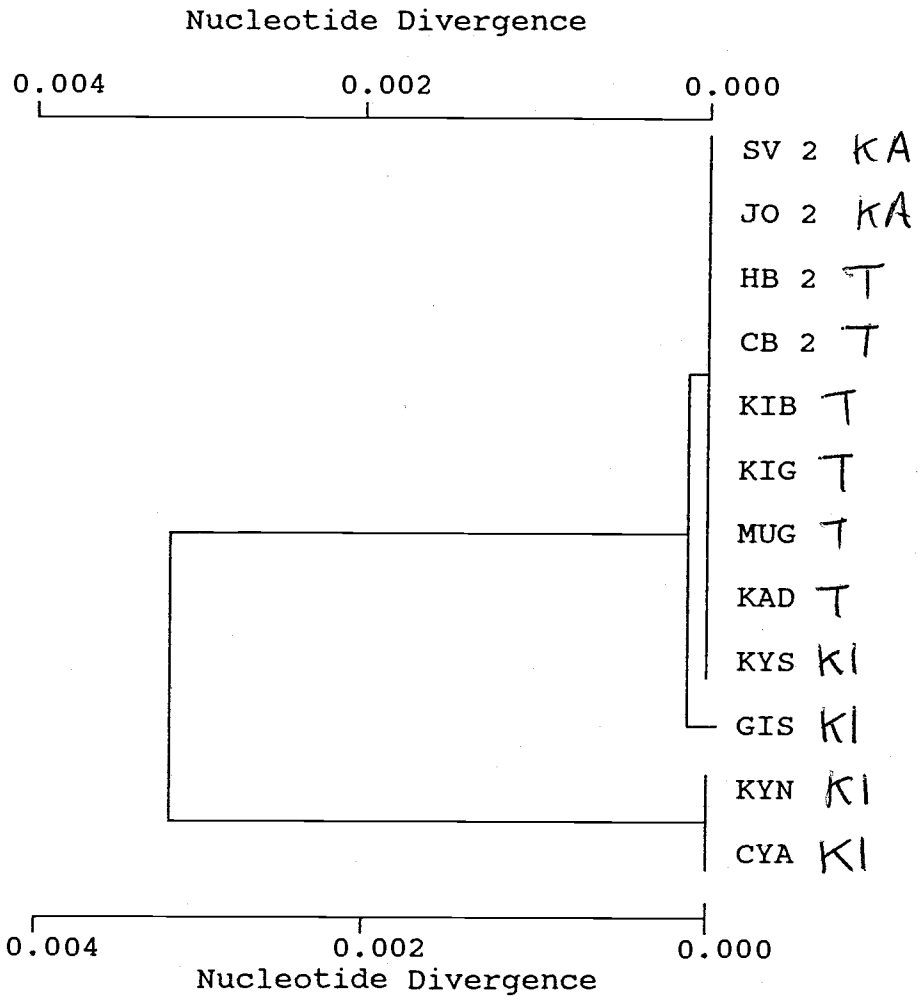


Figure 5.1: UPGMA dendrogram of nucleotide divergence between kapenta samples from Lakes Tanganyika, Kivu and Kariba.

Table 5.4: Nucleotide Divergence between kapenta populations. Positive values indicate significant heterogeneity

	MP4	CB2	KIB	KIG	MUG	KAD	GIS	KYN	KYS	CYA	SV2
L. Tanganyika											
CB2	-0.0599										
KIB	-0.0938	-0.0550									
KIG	-0.0820	-0.0281	-0.0774								
MUG	-0.0904	-0.0084	-0.0784	-0.0578							
KAD	-0.0936	-0.0685	-0.0706	-0.0597	-0.0565						
L. Kivu											
GIS	-0.0518	-0.0633	-0.0403	-0.0167	0.0122	-0.0647					
KYN	-0.0022	0.1305	0.0409	0.0419	-0.0573	0.0326	0.1503				
KYS	-0.0779	-0.0699	0.0620	-0.0508	-0.0173	-0.0872	-0.0752	0.1051			
CYA	0.0346	0.1817	0.0585	0.0867	-0.0449	0.0891	0.1973	-0.0605	0.1638		
L. Kariba											
SV2	-0.0970	-0.0662	-0.1000	-0.0643	-0.0834	-0.0803	-0.0398	0.0172	-0.0583	0.0460	
JO2	-0.0851	-0.0387	-0.0929	-0.0726	-0.0734	-0.0596	-0.0461	0.0389	-0.0449	0.0411	-0.0831

5.3.3. Genetic variability of the introduced populations

Haplotype diversity proved to be high (80.0 - 93.4 %, Table 5.2), except in two populations from Lake Kivu (KYN, CYA). Nucleotide diversity was in the range of 1.64 - 2.13 % for all populations (Table 5.2). Therefore there were weak indications for a reduced genetic diversity in two populations from Lake Kivu, whereas intra-population genetic variability appeared to be unchanged in the introduced population in Lake Kariba.

5.3.4. Genetic differentiation after the introduction.

None of the introduced populations proved to be significantly different in their composite haplotype frequencies from their putative source populations (Table 5.5). However, when samples from each lake were pooled, the haplotype frequency in L. Kivu proved to be significantly different from the other two lakes (Table 5.6).

Table 5.5: Monte Carlo probabilities for significant differences in haplotype frequencies between the introduced kapenta population and their putative sources.

	L. Kivu		L. Kariba	
	KAD		MP4	CB2
GIS	0.739	SV2	0.634	0.832
KYN	0.820	JO2	0.836	0.351
KYS	0.732			
CYA	0.250			

Table 5.6: Results of Monte Carlo simulations to test for significant differences in haplotype frequencies between kapenta from Lakes Tanganyika, Kivu and Kariba. All samples from each lake were pooled for this comparison.

All lakes	0.001
Tanganyika - Kivu	0.000
Tanganyika - Kariba	0.474
Kivu - Kariba	0.005

5.4. Discussion

The present study showed an extremely high variability of the kapenta ND 5/6 region of the mtDNA (56 haplotypes in 219 fish using six endonucleases). Other studies investigating this region found either no variation (Atlantic salmon, *Salmo salar*, O'Connel *et al.*, 1994), intermediate variation (brown trout, *Salmo trutta*: 19 haplotypes, 7 enzymes, 219 fish; Hall, 1993) or a high variability comparable to kapenta (anchovies, *Engraulis encrasicolus*: 40 haplotypes, 6 enzymes, 100 fish; Bembo, pers.comm). Considering the small percentage of the mtDNA genome actually surveyed (0.37 %), these data show clearly that the ND 5/6 gene is a highly variable region in many fish species.

The percentage sequence diversity, a measure for the evolutionary distance between individual haplotypes was, together with that of other clupeiformes, on the top end of the range reported for fish (Table 5.7). Together with the high haplotype diversity observed in a preliminary study on anchovies (*Engraulis encrasicolus*, Bembo, pers. comm.) this is an indication for a generally highly variable mtDNA in the order clupeiformes.

Most of the studies in Table 5.7 investigated the whole mtDNA genome, nevertheless, their results are comparable with the 1.78 % sequence diversity obtained in the present study. Therefore the evolutionary distance between haplotypes in the ND 5/6 gene seems to be comparable to the rest of the mtDNA genome.

The high number of rare haplotypes may have been one of the causes for the lack of any clear-cut differences found in the chi-squared Monte Carlo simulations on haplotype frequencies. While an overall test showed significant

Table 5.7: Percentage nucleotide sequence diversity between haplotypes in fishes. Sequences may be estimated by comparisons between restriction fragments or sites of the whole mtDNA or specific amplified regions, or by direct sequencing. Clupeiformes are shown separately on the bottom of the table. Modified from Ovenden (1990) and Naish (1993).

Fish species	% Sequence Divergence	Reference
Amia alva	0.09%	Bermingham & Avise 1986
Lepomis punctata	0.62	
Lepomis microlophus	0.87	
Lepomis gulosus	0.63	
Lepomis macrochirus	0.85	Avise *et al.* 1984
Salvelinus spp.	0.21-1.63	Grewe *et al.* 1990
Salvelinus fontinalis	0.26-0.47	Danzmann *et al.* 1991
Galaxis auratus	0.08	Ovenden *et al.* 1993
Galaxis tanycephalus	0.15	
Arius felis	1.00	Avise *et al.* 1987
Bagre marinus	1.00	
Merluccius capensis	1.3	Becker *et al.* 1988
Merluccius paradoxus	0.57	
Phoxinus phoxinus	0.89	Naish 1993
Clupeiformes:		
Clupea harengus	1.66	Kornfield & Bogdanowicz 1987
Brevoortia tyrannus	2.4	Avise *et al.* (1989)
Brevoortia prtronus	2.4	
Engraulis encrasicolus	2.13	Bembo, (unpubl. data)
Limnothrissa miodon	1.78	Present study

heterogeneity among all samples, this overall heterogeneity could neither be clearly attributed to population differentiation within lakes nor to differences between introduced and source populations. However, if all samples from each lake were pooled, L. Kivu proved to be significantly different from the other lakes. These results suggest that, with the high variability of the ND5/6 gene, sample sizes may not have been sufficiently large to detect population differentiation.

An attempt to reduce the genetic variability by considering the data of each endonuclease separately, was only partly successful. While two of the six enzymes showed significant heterogeneity between samples, the results of the Monte Carlo simulation approached significance only in L. Tanganyika. This may suggest some differentiation within the lakes, however, the haplotype frequencies exhibited no common pattern.

One of the main objectives of the present study was to investigate the effects of the introductions on the genetic variability of the introduced populations. MtDNA is a sensitive marker for population bottlenecks or founder events, as its effective population size is a quarter of that of nuclear DNA (Billington & Hebert, 1991). The only indication for a reduction in mtDNA variability are the slightly lower haplotype diversity values in two populations from Lake Kivu. It can therefore be concluded that the introductions had no major effect on the diversity of the nuclear genome; some loss of mtDNA variability may, however, have occurred in the Lake Kivu samples.

Although most of the literature concerned with the loss in genetic variability in population bottlenecks and founder events is related to nuclear DNA (Carvalho & Hauser 1994 and references therein), it should be stressed here that the ND5/6 gene investigated in this study encodes for enzymes in the NADH dehydrogenase complex, which are important for the physiology of cells. Therefore in principle, any reduction in variability in this region may have effects on the physiological adaptability of the species. While the reduction in variability observed in Lake Kivu is only suggestive and thus no major reason for concern, it should be remembered that mtDNA variability, in addition to being an important marker for population studies, may also be an important component in the total genetic variability of a species.

In many aspects, the results of the mtDNA analysis are a striking parallel to the allozyme results: highly significant overall genetic heterogeneity which cannot be attributed to clearly defined stocks within or between lakes. Another similarity between the results of the two techniques is the genetic differentiation of kapenta in Lake Kivu. The implications of these findings are discussed in detail in chapters 4 and 6).

CHAPTER 6: GENERAL DISCUSSION

6.1 Overview

The aim of this final section is to provide a synthesis of data obtained, and to relate this to the original project aims as described in Chapter 1. The implications in terms of genetic management will be highlighted, with a view to presenting an exposition of outputs as recommendations for incorporation into management programmes, together with priorities for further research.

6.1.1 *Major obstacles to addressing aims*

Undoubtedly one of the major limitations to undertaking a stock structure analysis are the constraints imposed by coordinating sampling from a distance, and to working in areas with unpredictable security. The collection of samples was dictated by the fishing activities in local regions, though it was possible to obtain largely comparable collections. The conclusions presented below, although based on several independent lines of evidence, would have been strengthened considerably by the inclusion of temporal sampling at several sites. Such data would not only have provided valuable comparisons between temporal and spatial patterns of differentiation, but also increased opportunities for monitoring shoal movements and putative stock boundaries. Although the programme has been hindered by the lack of a temporal element, it has still been possible to indicate the most likely scenarios regarding stock structure, with suggestions for appropriate tests of their validity.

Since the opportunities for sample collection were restricted to short time periods, it was not possible to collect widely distributed juveniles or spawning females. The incorporation of such material in samples would have maximised opportunities for detecting genetic isolation if distinct spawning periods or sites existed within lakes.

The problems relating to stock structure analysis do not, however, apply to the objectives focusing on estimates of genetic diversity, where the large number of spatially separated samples provide an effective indication of patterns within and between lakes.

6.1.2 *Management of pelagic fisheries*

Before considering the interpretation and significance of data obtained, it is necessary to point out some special features of pelagic fishes which affect their management.

First, the high mobility of pelagic fishes make it difficult to relate the distribution of adults to their original spawning grounds. It is therefore important to appreciate at the outset that the spatial dispersion of fish may represent feeding, rather than breeding units, and that ideally the analysis of juveniles or spawning adults are required to provide direct information on the latter. High mobility may therefore affect the opportunities for genetic differentiation to develop, especially since the exchange of relatively few individuals among sites may be sufficient to homogenise allele frequencies (Slatkin, 1987).

Genetic stock structure will, however, depend on hydrographic factors which may serve to restrict individuals to distinct regions. For example, *Limnothrissa* apparently move most readily against water currents (Coulter, 1991a), which leads to a net movement of individuals in Tanganyika southwards. The distribution of planktonic biomass may impart a wide variance in food availability, which is known both in *Stolothrissa* and *Limnothrissa* to affect their local abundance, both horizontally and vertically in the water column (Coulter, 1970). Hence, pelagic waters may exhibit heterogeneity likely to reduce random mixing resulting from wide-scale movements.

Secondly, shoaling may impose a degree of local structure to fish distribution which may persist over time (Carvalho *et al.*, 1992; Pitcher *et al.*, 1986; Pitcher & Parrish, 1993), which, depending on the distribution of genotypes therein, may lead to microgeographic genetic differentiation (Avise & Shapiro, 1986; Carvalho *et al.*, 1992). Such localised aggregations of individuals, if susceptible to complete removal during harvesting could affect overall levels of genetic diversity if variance between shoals was reduced. Although there is no direct empirical demonstration of such impacts, the net effect of shoaling would be to reduce overall levels of population mixing, though local hydrographic and biotic factors may greatly modify the extent of integrity (Pitcher & Parrish, 1993).

In addition, shoaling fish are especially vulnerable to overfishing because the catch-per-unit-effort (CPUE) of vessels equipped with fish finding gear does not decrease with declining stock size as assured by conventional assessment methods. For such shoals that are easily located by fishing vessels, catchability stays constant with decreasing stock size (Pitcher, 1992), leading to unstable depensatory mortality and risk of stock collapse. Models suggest that even a slight overshoot of the optimal fishing effort could engender a rapid catastrophic collapse. In relation to the most commonly employed light attraction-based methods of the studied lakes, intact shoals could be enticed into hauls, imparting inflated estimates of CPUE (Pitcher, 1992), with the greater risk of local collapse. The occurrence of such population declines would clearly depend on the extent of exchange with neighbouring shoals.

The mobility and shoaling behaviour of kapenta thus imparts a complexity to monitoring migrations, stock boundaries, the degree of reproductive isolation, and forecasts of yield. In such circumstances, especially where stock structure is unclear, it is appropriate to exercise caution when predicting sustainable yields.

6.2 Results in relation to scientific and developmental objectives

Below, the results from each of the approaches are integrated, and considered in relation to the original objectives set out in Chapter 1. The fisheries implications of the patterns that emerge are summarised as a suite of recommendations in the final two sections (6.3.5 and 6.3.6) which focus on the developmental objectives.

I. SCIENTIFIC OBJECTIVES

6.2.1 *To compare the genetic diversity of kapenta in its natural state in Lake Tanganyika with introduced populations in Lakes Kivu and Kariba.*

Evidence from allozyme data indicate clearly that, as predicted from the numbers of individuals released (Chapter 1), that there was no significant reduction in genetic diversity following introductions into Lakes Kariba and Kivu. The levels of heterozygosities and mean number of alleles per locus were comparable between native and introduced populations, and representative of marine clupeids. In Kariba, haplotype diversity, an analogous estimate of allozyme heterozygosity, was comparable between descendants of introduced fish and native Tanganyika samples.

In contrast, there was some indication of a reduction in haplotype diversity in two of the samples from Lake Kivu. The same two samples were also most genetically distinct as shown by the UPGMA tree of nucleotide divergences. The apparent disparity between allozyme and mitochondrial DNA data is not unusual, and follows conventional theory on the consequences of a smaller (one-quarter) effective population size of the mtDNA genome, relative to nuclear DNA (Billington & Hebert, 1991). The observation that a reduction was detected in only two out of four samples from Lake Kivu is difficult to explain, but could be an effect arising from the nature of the introductions. Since kapenta was introduced on ten different occasions, each with only a few thousand fish (Table 1.1), the extent of founder events may have differed among introductions. Such an effect would, however, depend on the extent of exchange among founders following release. There is some evidence from allozymes of the existence of small-scale genetic heterogeneity within the lake suggesting some degree of group integrity.

Unfortunately the events following the introduction of kapenta into Kivu were not monitored owing to civil unrest, and so it is unclear whether they flourished in this unusual environment as they did in Kariba. Evidence presented below does provide circumstantial evidence for strong selection, suggesting high initial mortality rates. The disruption of the pelagic waters by numerous islands in the south, taken together with the effects of mass mortality consequent upon colonisation may have led to localised population bottlenecks and genetic drift with some reduction in diversity. It is rather difficult to envisage a scenario that retains the genetic integrity of any fragmentated introduced populations in a pelagic species, though fidelity of juveniles near to spawning grounds (Coulter, 1991a), and social cohesiveness during shoaling may contribute. Before fully assessing the significance of the lower haplotype diversity observed in mtDNA, additional samples and analyses using other regions of the molecule are required.

6.2.2 *To determine the extent of stock separation within Lakes Tanganyika, Kivu and Kariba.*

An important characteristic of kapenta in Lakes Kariba and Kivu is the recency of their introductions. In the absence of strong selection pressures, a lack of genetic differentiation within either of these lakes could arise from two sources: first, insufficient time for the accumulation of significant heterogeneity among isolated populations through drift and mutations, and secondly, the exchange between populations may be too great to allow divergence to develop. It was therefore important in the present study not only to rely on genetic approaches, but also to examine phenotypic traits.

Furthermore, genetic and phenotypic characteristics differ in their sensitivity to gene flow among populations (Carvalho & Hauser, 1994): morphometric differentiation indicates phenotypic variation among stocks that may persist even in presence of significant levels of migration. In contrast, genetic methods usually define stocks of very high integrity, with low levels of gene flow, except where strong selection pressures cause divergence (Ward & Grewe, 1994). The comparison of these approaches can thus provide estimates of the exchange among stocks.

In the present study, there was strong evidence for morphological divergence among kapenta samples within all lakes sampled, though in general this divergence did not coincide with genetic heterogeneity. The geographic patterning of morphometric variability in Kariba was different to other lakes, in that proximate samples on a small scale were most similar to each other. Such homogeneity in morphology among contiguous samples has been attributed to the occupation of similar territories, demonstrating the existence of population integrity, though this need not be reflected in genetic isolation. When considering the relevance of such heterogeneity to the stock concept, the unit of management relevant to harvesting does not necessarily have to be genetically discrete. It may merely describe a group of individuals whose abundance depends to a very much larger degree on recruitment and mortality, especially that caused by fishing, than on immigration and emigration ("harvest stock", Carvalho & Hauser, 1994). Thus in Kariba, it is possible that such harvest stocks exist, and that such integrity is relevant to stock assessment. Thus, based on these data, it would be incautious to assume that localised overfishing would be quickly compensated by immigration, especially if harvest stocks differed in aspects of their biology such as spawning times, age and size at maturity or fecundity. Such characters are known to show wide variance throughout the lake (Marshall, 1992), but it is not known to what extent such heterogeneity arises from spatial patterning of local populations. It is at least clear that kapenta populations do not represent the generally mixed sample of individuals that may be predicted for a pelagic fishery (Carvalho & Hauser, 1994).

It is not possible to decide whether the lack of correspondence between morphometric and genetic differentiation in Kariba is due to gene flow among local populations, inadequate sample sizes, or due to insufficient time for genetic divergence since the introduction. The congruent lack of genetic heterogeneity in allozymes and mtDNA haplotypes certainly does support the absence of any clear genetic structuring, especially since some such heterogeneity was detected in other lakes. Nevertheless, since individuals may be assigned to their respective sample populations with high probability using morphometric variation, the integrity of samples certainly warrants closer examination to assess temporal stability and putative stock boundaries.

In native Lake Tanganyika kapenta, small scale genetic differentiation was detected using allozymes, indicating genetic integrity of assemblages. Unfortunately, it was not possible to delineate major areas of the lake, such as northern and southern basins, which others (e.g. Marshall, 1992) have suggested may represent isolated stocks. Allozyme data presented here do support a degree of genetic isolation in these much older, native populations, but not as much as might be expected if gene flow among such areas was very low. Morphometric differentiation was once again detected, though without any obvious geographic patterns.

It is worthwhile considering here, what factors may give rise to such apparent genetic homogeneity between kapenta from widely separated geographic regions within the same lake. A lack of genetically detectable stock

separation may arise from five sources: (i) sufficient gene flow to maintain panmixia; (ii) occasional "sweepstake" events such as sporadic recruitment from distant, non-neighbouring areas which could produce the appearance of panmixia; (iii) stabilising selection arising from exposure to similar environments; (iv) recent divergence of the compared populations, or (v) failure to detect genetic variants either due to the technique employed or to insufficient sample sizes. With the exception of (v), it may be impossible to distinguish between the various possibilities using molecular genetics alone and it becomes especially important to consider data from other methods.

The weight of evidence for Lake Tanganyika *L. miodon* suggest the presence of mobile discrete assemblages, possibly representing migrating shoals, which on occasion might lead to periodic gene flow. The maturity of the lake's fauna would argue against insufficient time for genetic differences to accumulate, and the existence of some significant differentiation on a local scale demonstrates the ability of the technique to detect deviations from panmixia.

It may be hypothesized that if genetically discrete shoals exist, that their mixing during capture may obscure spatial differences, resulting from a greater within-sample variance. However, there is no clear evidence to support this assertion especially since there was no consistent deficit of heterozygotes within samples, as predicted due to the Wahlund effect (Richardson *et al.*, 1987). Furthermore, the gene diversity analyses demonstrated that most genetic diversity was contained within local samples, so reducing the overall effects of combining heterogeneous groups.

The fisheries implications for Tanganyika kapenta are similar to those of Kariba, in that it is likely that more than a single stock exists, though in the absence of geographic structuring, it is impossible to identify putative stocks. Such an objective might not be within the capabilities of the two molecular approaches employed here because of the high within-sample variance. Although improved information would undoubtedly be obtained through temporal sampling, and analyses of spawning groups, the use of either an increased sample size and/or a less variable region of mtDNA may improve stock delineation. It may of course be that the mobility of discrete assemblages confounds attempts to separate fish by region. Until such further work can be carried out, the recommendation would be to exercise restraint when extrapolating local yields over the whole lake to estimate potential yield, and to avoid overexploitation of any localised areas. With the degree of heterogeneity detected, it is unclear whether recruitment would be sufficiently rapid to prevent local stock collapse. The latter is significant because localised depletion would enhance the variance in regional productivity, thus complicating forecasts based on stock-recruitment relationships (Hilborn & Walters, 1992). The removal of discrete shoals, if continued may also reduce the genotypic diversity of the gene pool, though the rate of loss would depend on the extent of spatial heterogeneity, which available evidence indicates is relatively low.

Application of the polymerase chain reaction (PCR) to stock structure analysis has increased significantly in recent years (Park & Moran, 1994; Wright & Bentzen, 1994). The technique employed here (Chapter 5) revealed a high level of haplotype diversity which failed to clearly discriminate any within-lake samples. Such findings are unusual, since it is expected that the more rapidly evolving mtDNA molecule would accumulate mutational variants more rapidly than nuclear DNA (Avise, 1987). Here, findings are to the converse: the allozymic heterogeneity observed among some samples in Tanganyika and Kivu were not shown at the mtDNA level. When individual restriction enzymes were analysed, however, some approached the level of significance (Chapter 5), but

it is unwise to comment on the importance of such patterns. Thus, the mtDNA data in their present form do not support the presence of genetic stocks of kapenta.

In view of the novelty of PCR-based analysis of mtDNA variability, it is worth noting that the sample size used may have been too small to detect divergence owing to the large number of distinct haplotypes detected. It would be useful to increase sample size to say 50 fish to see whether similar patterns emerge.

The genetic data from Kivu demonstrated some local genetic heterogeneity, but again failed to delineate stocks of kapenta by region. The evolutionary basis of divergence detected in such recently founded populations is unexpected, and discussed elsewhere (Chapter 4). In the absence fo additional data, the implications for management of the Kivu fishery are similar in principle to that for Tanganyika.

In summary, the morphometric approach appears to be a potentially powerful tool for monitoring harvest stocks of kapenta, whereas the utility of the genetic approach depends on the tool employed: allozymes demonstrated that at least a proportion of samples were genetically distinct, whereas the mtDNA approach largely failed to detect any such units. Although it was not possible to delineate stock boundaries, the value of the present study is that it demonstrates that sufficient morphometric and allozymic variation exists to discriminate units of kapenta. The basis of any related future studies must incorporate a meaningful and comprehensive sampling strategy, especially by using time-series sampling designed to determine the persistence and mobility of stocks detected.

6.2.3 To estimate the genetic component of changes in size and life-history.

A characteristic features of *L. miodon* is its apparently high degree of phenotypic plasticity (Marshall, 1993), which has allowed these fish to colonise lakes markedly different from their native conditions in Tanganyika. The observed distinction of Lake Kariba populations were notable upon introduction, before fishing began, but it has remained unclear whether the small body size and changes in life history (Chapter 1) have arisen from genetic change, or a phenotypic response to environmental conditions in the lake. Indeed, similar features are observed in the artificial lake of Cahora Bassa (Marshall, 1992), where again, small body size is a feature of the introduced kapenta. However, since the founders of Cahora Bassa were derived from Kariba kapenta migrating down the Zambezi river, it is possible that the genetic characters of the latter were merely passed on by gene flow. Indeed, this may seem a strong argument in favour of a genetic component to the patterns observed.

The various alternative explanations are discussed in detail by Marshall (1993), who concludes that in view of the lack of any genetic data to the converse, the most likely explanation to account for biological differentiation was high predation in both Kariba and Cahora Bassa, where predators make up a high proportion of the fish stock. Elucidation of the genetic vs. phenotypic origin of differences from the native stock is important from a fisheries perspective because if the differences are largely phenotypic, they are in principle reversible. Indeed, the additional artificial predation imposed by harvesting might be expected to accelerate any strong selection for small size (Reznick *et al.*, 1990), possibly compromising future productivity. If, on the

other hand, variance in life history characters is retained through phenotypic plasticity (Thompson, 1991), adaptability to varying predation and harvesting intensity would exist.

The genetic data presented here provides no evidence of major genetic rearrangements in Kariba kapenta since their introduction from Tanganyika. It has to be stated that the lack of genetic differentiation between the lakes does not exclude the possibility of genetic changes having occurred, though the existence of both allozymic and mtDNA homogeneity between native and introduced fishes does make it less likely. Further, the comparability in genetic diversity between native and introduced populations discounts a stochastic contribution to genetic changes resulting from a founder effect. Despite the recency of the kapenta introductions, genetic divergence (allozymic and mtDNA) was detected between Tanganyika and Kivu, demonstrating the potential for such effects to be detected using these tools. Examples from the literature also show the ability of allozymes to disclose divergence between source and transplant populations over similarly short time spans (Gharrett & Thomason, 1987; Golubstov *et al.*, 1993; Shaw *et al.*, 1992).

If, as the present data suggests, kapenta are phenotypically adaptable to intense predation, size-selective harvesting may have less long-term impact on productivity than in other fisheries (Ricker, 1972; Nelson & Soulé, 1987; Smith *et al.*, 1991). Indeed, Marshall (1993) suggests that the small size of kapenta in Kariba is a distinct advantage, since by maturing early and at small size, fish are able to maintain a constant proportion of mature adults in the stock despite intense predation on adults (Fryer & Iles, 1972). Small body size thereby redistributes more energy to reproduction, resulting in relatively higher fecundities. When considered in relation to water volume, rather than area, Kariba and Cahora Bassa support a pelagic biomass comparable to that of Lake Tanganyika (Marshall 1993). Marshall (1993) then argues that fishing effort could be increased in the latter until the fish accelerate their life cycle and reproduce earlier, so increasing clupeid productivity, allowing greatly increased yields from the fisheries they support.

Although such reasoning may be attractive in economic terms, it should be stressed that evidence for the basis of Marshall's (1993) assertions are presently circumstantial. In view of the young age of the Kariba and Cahora Bassa fisheries, it would be incautious to extrapolate much from their current productivities in order to manage Tanganyika fish.

One way of examining the extent of any directional genetic changes in Kariba kapenta would be to instigate a seasonal pattern of allozyme screening (Carvalho & Pitcher, 1989) to examine temporal stability of allele frequencies. Predominantly stable allele frequencies, especially in the absence of any spatial patterning, would further support the current evidence that the fish within Kariba have not undergone major genetic changes following introduction.

6.2.4 To examine the relationship between genetic structure, fishing pressure and production of kapenta.

In order to examine the relationship between fishing pressure and genetic diversity requires *a priori* the existence of identifiable genetic boundaries among kapenta within a lake. This original objective was aimed at studying *L. miodon* in Lake Kariba where it was known that harvesting differed definably among regions. However, two major forces prevented attainment of this objective: (i) the genetic homogeneity among all samples examined using allozymes and

mtDNA polymorphisms, and (ii) the lack of a research permit from the Zimbabwe government, so hindering access to samples and data on fishery practices.

It is interesting, nevertheless, to record the possession of similar levels of heterozygosity in Kariba kapenta, despite their small size. There is in many species, a positive correlation between body size and heterozygosity as estimated by allozymes (for recent review, see Zouros & Pogson, 1994). One of the few studies to examine the effects of fishing pressure on genetic diversity (Smith *et al.*, 1991) detected an overall decline commensurate with a reduction in body length of the fishes captured. In kapenta, there is either no correlation between body length and heterozygosity (presently not examined), or, as the current genetic data indicates, there has been no directional genetic changes in the introduced fish.

II. DEVELOPMENTAL OBJECTIVES

6.2.5 To provide guidelines for preserving genetic diversity for future introductions.

The effective population size for kapenta introduced into Lake Kariba was sufficiently large to prevent any detectable genetic erosion, and may be considered a successful strategy, especially in view of the rapid spread of kapenta throughout the lake. In Lake Kivu, on the other hand, the slight reduction in mtDNA haplotype diversity in two of the samples, taken together with some genetic divergence from the parental stocks, suggests that some genetic depletion may have occurred, though it is difficult to separate such effects from selective changes. Indeed, the latter may be accelerated in populations of small effective size, resulting in rapid alterations in genetic structure. The strategy of releasing, on separate occasions, only a few thousand fry into Kivu, with an unknown proportion of *L. miodon*, would appear less effectual than a mass release of larger numbers.

It is recommended therefore, based on available data, that in any future introductions (i) numbers approaching the magnitude of those released into Kariba should be employed, (ii) that the simultaneous mass release of fry may be preferable to release of smaller numbers on several occasions, (iii) that the species composition of fry be estimated prior to release, (iv) that owing to the high degree of within-sample gene diversity, that the parental stock need only be collected from a local population. The latter strategy would greatly simplify the logistics and coordination of sample collection and release.

6.2.6 Guidelines for management of exploited stocks

No evidence of genetic stocks within Kariba, or between geographically distant regions within the other two lakes was revealed using the molecular tools employed. If the samples being compared were truly panmictic, then treating the fishery as a unit stock would have no consequences in terms of recruitment from overfished locales. If, however, some undetected isolation existed, the unit stock hypothesis may result in local overexploitation diminishing the overall fishery yield. Furthermore, undetected stocks may become eliminated, with a corresponding reduction in interpopulation genetic variance. Thus the lack of significant genetic heterogeneity should be interpreted with caution, and where feasible, additional sources of information

should be sought (e.g. Jennings and Beverton, 1991), or conservative measures deployed (Leslie and Grant, 1990).

The detection of putative harvest stocks in Lake Kariba, and morphometric and genetic heterogeneity in lakes Tanganyika and Kivu suggest the presence of more than a single stock within each lake. Such differentiation is likely to be relevant to management since the integrity detected demonstrates sufficient restriction to movements to influence rates of recruitment and biological differentiation in life-history parameters. Further stock structure studies are urgently required to determine the persistence and dynamics of putative stocks. If units exhibit temporal stability, it would be necessary to consider them as local stocks, with appropriate partitioning within stock assessment models. Alternatively, temporal changes would suggest a greater mobility of differentiated groups, allowing rapid recolonisation of depleted areas. It is thereby recommended that (i) until such information is available, estimates of potential yields obtained by extrapolating local yields over the whole lake should be regarded with caution; (ii) that harvesting practices should avoid overdepletion of localised fishing ground; (iii) that although kapenta appear to exhibit marked phenotypic plasticity, it should not be assumed that intense fishing pressure would produce correspondingly high productivities as in the relatively young Kariba fishery. Thus, (iv) evidence of any significant directional reductions in size at maturity in Kapenta of the Tanganyika and Kivu fisheries should be seen as an indication of overfishing, which should be responded to by a relaxation of fishing pressures in combination with close monitoring of age and size structure. It is premature, and potentially dangerous, to suggest that kapenta resources can maintain high productivity under such circumstances.

6.3 Future priorities for research

An important component of the outputs includes the identification of priorities for future associated studies. The existence of a complex, and possibly dynamic stock structure within native and introduced populations of kapenta raises the urgent need to undertake a more comprehensive and detailed examination of populations. Suggestions are not given in order of priority, but are arranged beginning with those studies which appear most feasible for commencement in the short-term. In particular, it is suggested to study:

(i) ***the dynamics of putative harvest stocks in Lake Kariba more fully using morphometrics***.

This would require cooperative effort between Zambia and Zimbabwe in an effort to search for stock boundaries. The technique has the advantage of being inexpensive and requiring non-specialist facilities, except the availability of computers and appropriate statistical software. The sampling design must incorporate temporal analyses over several seasons.

(ii) ***migration patterns and the integrity of kapenta shoals***.

A important characteristics of these pelagic fisheries is the shoaling habit of *L. miodon*. Little information is currently available on the integrity and movements of such entities apart from scattered data on the distribution of length-frequencies and some anecdotal observations. A more rigorous approach to

examining within haul variance in size and morphometric and life-history characters (e.g. sex, maturity, fecundity) is required. The use of tagging, either artificial or natural (e.g. parasites) would provide more equivocal estimates of shoal mobility.

If suitable samples could be obtained, it would be worthwhile examining directly the genetic relatedness within shoals using appropriate DNA markers.

(iii) ***spawning and reproductive patterns of kapenta***.

Little is known about the locations and spawning activity of kapenta in the various lakes. Since they are known to spawn over protracted periods, especially in Kariba, it is important to ascertain the extent of genetic isolation in spatially and temporally separated assemblages. The initial focus should be on studying reproductive behaviour (timing, gravidity, distribution of reproductive stages including mature adults, fry and juveniles) to examine more rigorously the partitioning of such events within lakes.

(iv) ***further applications of molecular tools***.

The present work demonstrates both the power and limitations of the molecular approach. The power, through detecting genetic heterogeneity, but the limitations, in not ascribing a meaningful spatial pattern to the heterogeneity that can be used by fishery managers (e.g. separation of northern and southern stocks in Lake Tanganyika). If it were possible to coordinate a meaningful sampling programme, possibly in combination with the collection of data in any of the above, then further molecular studies would prove useful providing the following criteria were satisfied: (1) the availability of temporally separated samples; (2) the availability of more samples within each of the areas examined here; (3) an increase in the sample size analysed, say to 50 (mtDNA) and 100 (allozymes) fish; (4) the employment of a less variable mtDNA region with a view to identifying area-specific haplotypes, providing of course that migration patterns of shoals enable their detection; (5) the support of local fishery personnel to make data available on population parameters.

6.4 Assessment of outputs in relation to project aims

Having discussed each of the project aims in turn, it remains to give some assessment of how effective the project has been. One objective way of carrying out this assessment is to consider the nature of outputs produced. Ultimately, however, the adoption of recommendations by the respective fishery authorities and bodies for developmental aid will serve as a valuable indicator of success.

The outputs may be grouped into four major sections: the data produced, the fishery recommendations based on the data, identification of future priorities which take into account local facilities and expertise available, and the dissemination of findings and recommendations through participation at conferences and through publications.

(i) ***the data produced***: each of the techniques employed provided useful information that could be related to the project aims, which on this basis can be

deemed successful. Within the constraints imposed by organising a sampling programme at a distance, and which was dependent upon cooperation and the availability of commercial fishermen, the comparison of parental stocks and introduced populations, and the within-lake analysis of stock structure produced moderately clear results. A representative geographic spread of samples was obtained in each lake. The main short-coming was the failure to identify any clear spatial patterning to the putative stock heterogeneity detected, though it is unclear whether this was due to short-comings of sample collection, or because such patterning does not exist. Thus, the data produced did tackle the aims effectively, based on the ability of approaches to detect stock separation/levels of diversity (e.g. polymorphism, discrimination of morphometric variance).

It was not, however, possible to address the relationship between genetic diversity and fishing pressure, though this deficiency was balanced by the additional information provided on the genetic component of phenotypic differentiation in Kariba kapenta (see **6.3.3**).

(ii) ***the fishery recommendations based on the data***: as originally intended, it was possible to construct a suite of recommendations aimed at conserving the biodiversity and fisheries production of kapenta resources.

(iii) ***identification of priorities for future work***: these were successfully produced and ordered in relation to those that were logistically most likely to be adopted in the short-term.

(iv) ***dissemination of findings***: several presentations of aspects of the work have been given, including:

Carvalho, G.R. & Hauser, L. The genetic impacts of fish introductions in Africa. ODA sponsored Symposium on *Impact of Species Changes in African Lakes*. Imperial College, London. April 1992.

Hauser, L., Carvalho, G.R. & Pitcher, T.J. The genetic basis of phenotypic variation in the clupeid, *Limnothrissa miodon*, following introduction to a man-made lake. FSBI Symposium on *Factors Affecting the Distribution of Fish*. Conwy, North Wales. July 1993. (poster)

Hauser, L., Carvalho, G.R. & Pitcher, T.J. Morphometric and genetic variation in natural and introduced populations of kapenta, *Limnothrissa miodon*. ORSTOM Symposium on *Biological Diversity in African and Brackish Water Fishes*. Dakar, Senegal. November 1993.

Carvalho, G.R. Genetic management of African freshwater fishes. ORSTOM Symposium on *Biological Diversity in African and Brackish Water Fishes*. Dakar, Senegal. November 1993.

Hauser, L., Carvalho, G.R. & Pitcher, T.J. Phenotypic and genetic variation in kapenta, *Limnothrissa miodon*, after introduction to two African lakes. PGG meeting, Reading. December 1993 (poster).

The ORSTOM meeting in Senegal was a particularly valuable venue to present data, not only because it was possible to discuss implications with representatives of some of the respective fisheries bodies, but also because we were able to make a case for the role of genetic management in fisheries biology. Members of the Symposium organising committee identified several key themes from the symposium as priorities for future work. The importance of genetic management and knowledge of the within-species component of genetic diversity was adopted as one such key area.

Reports and publications which incorporate aspects of the present study are indicated below, though it is proposed to publish the findings in full separately.

Carvalho, G.R. & Hauser, L. (1992) Species changes in African lakes: genetic impacts of fish introductions and exploitation. Consultancy report for the *Impact of Species Changes in African Lakes* project, funded by the ODA.

Carvalho, G.R. & Hauser, L. (1994) Molecular genetics and the stock concept. In: *Molecular Genetics and Fisheries* (ed. G.R. Carvalho & T.J. Pitcher). Special issue of *Reviews in Fish Biology and Fisheries*. To be published in September 1994

Carvalho, G.R. & Hauser, L. (1995) Genetic impacts of fish introductions: an African perspective. In: *Species Changes in African Lakes* (ed. T.J. Pitcher & P. Hart). Chapman & Hall, *In press*.

The following publications are planned, and should be submitted by the end of the year:

1. Phenotypic variation of native and introduced kapenta (*Limnothrissa miodon*) populations as described by morphometrics.

2. Rapid genetic divergence of kapenta (*Limnothrissa miodon*) following introduction into Lake Kivu as revealed by allozymes. sub to J. Fish. Biol.

3. Stock structure of exploited kapenta populations (*Limnothrissa miodon*) in African lakes: evidence from allozymes and PCR-amplified mitochondrial DNA.

4. Genetic stock structure of the exploited clupeid, *Stolothrissa tanganicae*, in Lake Tanganyika.

5. Relevance of stock structure data to the management of pelagic fisheries in African lakes.

The aims to disseminate the information both to the relevant personnel in African fisheries, and wider scientific community will be completed once the publications have been produced. Attendance at the Senegal ORSTOM meeting was particularly timely and served to identify the genetic management of biodiversity as an important component of future research in African freshwater fisheries.

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GLOSSARY OF TERMS

Allele One or more alternative forms of a gene, each possessing a unique nucleotide sequence. In diploid cells, a maximum of 2 alleles will be present, each in the same relative position or locus on homologous chromosomes of the chromosome set.

Allozymes Enzymes differing in electrophoretic mobility as a result of allelic differences at a single gene (cf. **Isozyme**).

Amplify (in the molecular sense) To increase the copies of a nucleotide sequence using the **polymerase chain reaction**.

Base pairs (bp) The bases Adenine (A) and Thymine (T) or Cytosine (C) and Guanine (G) linked by hydrogen bonds (A=T; C=G) binding complementary strands of DNA.

Complementary sequence A sequence of nucleotides related by the base-pairing rules. For example in DNA, a sequence A-G-T in one strand is complementary to T-C-A in the other. A given sequence defines the complementary sequence.

D-loop A non-coding region of (vertebrate) **mitochondrial DNA** (mtDNA) that serves as the initiator of mtDNA replication and is often more variable than the coding regions of mtDNA.

Denature To break the hydrogen bonds between two complementary strands of DNA, separating them into two single-stranded molecules.

DNA fingerprinting In original usage, the use of multilocus probes to reveal hypervariability (see **Hypervariable sequence**) at many loci in the human genome. More generally used to refer to the characterization of an individual's genome by developing a DNA fragment band (allele) pattern. If a sufficient number of different sized fragments are revealed, these banding patterns which resemble a bar code profile, will usually be unique for each individual except identical twins.

Effective population size (N_e) The effective size of a population is defined as the size of an idealised population (a random mating population of self-compatible hermaphrodites, with no selection, mutation or genetic migration occurring) which behaves in the *same way* as the real population under consideration. It is important to understand that effective population size does not mean something as simple as that fraction of the population able to reproduce (e.g. it does not mean total population minus juveniles and senescent adults), but incorporates information on mating patterns and the extent of population subdivision.

Electrophoresis The separation of macromolecules (e.g. enzymes or DNA) in the presence of an electric current. In molecular genetics, differences in charge, size or shape (i.e. differences in electrophoretic mobility) of the macromolecules are used to estimate genetic differentiation.

Endonuclease See **Restriction enzyme**.

Fishery stock A group of individuals exploited in a particular area or by a specific method. The definition takes no account of the biological basis of

operating. For two alleles (*A* and *a*) with frequencies *p* and *q*, there are three genotypes *AA, Aa,* and *aa*; and the expected Hardy-Weinberg ratio for the three is p^2 *AA* 2*pq Aa* q^2 *aa*. Genotypic frequencies obtained from molecular genetic analysis of natural populations can be compared with predicted frequencies calculated from allele frequencies to determine whether samples are drawn from large, randomly mating populations.

Harvest stock Locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource. This definition does not imply any genetic nor phenotypic differences between stocks, but describes a group of individuals whose abundance depends to a very much larger degree on recruitment and mortality, especially that caused by fishing, than on immigration and emigration. C.f. **Stock, Genetic and Fishery stock.**

Heritability In the "narrow sense", the ratio of the additive genetic variance (differences that will be inherited consistently by the offspring) to the total phenotypic variance.

Heterozygosity Proportion of individuals in a population that are heterozygous (see **Heterozygote**) at a given locus. Can be calculated: $H_L = 1 - \text{sum. } x_i^2$, where x_i is the frequency of the *i*th allele at a locus. The mean heterozygosity per locus, H_L is the sum of H_L over all loci (including loci with two identical alleles where $H_L = 0$), divided by the total number of loci examined. An observed heterozygosity (H_O) can be determined from a direct count of the frequency of heterozygous genotypes in a sample. H_L and H_O can be compared statistically to determine whether genotypic ratios are in accordance with Hardy-Weinberg expectations (see **Hardy-Weinberg equilibrium**).

Heterozygote The presence of two dissimilar alleles at a given genetic locus.

Homology Common ancestry of two or more genes or gene products (or portions thereof).

Homozygote Two identical alleles at a genetic locus.

Hybridization (in the breeding sense) Crossing of inbred lines or individual organisms of differing genetic constitution or species.

Hybridization (in the molecular sense) To induce, experimentally, the pairing of complementary nucleic acid strands, often from different individuals or species, to form a DNA-DNA or RNA-DNA hybrid molecule.

Hypervariable sequence A segment of a chromosome characterized by considerable variation in the number of tandem repeats at one or more loci. See **Tandem array.**

Introgression Incorporation of genes from another species or population into an existing gene pool.

Isozyme Enzymes differing in electrophoretic mobility but which share the same substrate. Isozymes may arise from genetic (multiple loci or alleles) or epigenetic (post-translational) sources. It is therefore essential to exclude epigenetic variability if isozymes are to be used as genetic markers.

Linkage A measure of the degree to which alleles of two genes do not assort independently at meiosis or in genetic crosses. Those loci on different

chromosomes are non-linked, whereas those close together on the same chromosome are closely linked and are usually inherited together.

Linkage disequilibrium Departure from the predicted frequencies of multiple locus gamete types assuming alleles are randomly associated. When there is no deviation, the population is said to be in linkage equilibrium.

Local adaptation A process that increases the frequency of traits which enhance the survival or reproductive success of individuals in a particular environment.

Locus A physical position of a gene on a chromosome.

Marker Any diagnostic feature (e.g. allozyme, mtDNA haplotype, meristic, morphometric) of an individual, population or species.

Microsatellite (= simple sequences) Tandem array of short (1 - 6 base pairs) repeated sequences, with a total degree of repetition of five to about one hundred at each locus, and usually scattered randomly throughout the genome. For example, the repeat unit can be simply "CA", and might exist in a tandem array of, for instance 50 repeats, denoted by $(CACACACACA...)_{50}$. The number of repeats in an array can be highly variable giving rise to extensive **polymorphism**.

Minisatellite Tandem array of from two to several hundred copies of a short (9 - 100 bp) sequence of DNA, usually interspersed, but often clustered in telomeric regions of the chromosome. Arrays generally have different numbers of copies on different chromosomes, which when cut by restriction enzymes produce DNA fragments of differing lengths, thereby potentially giving rise to a **DNA fingerprint**.

Mitochondrial DNA (mtDNA) DNA located in the mitochondrion. In animals it is generally a small circular molecule with 16,000 to 18,000 base pairs long, and is, with rare exceptions, solely maternally inherited.

Mixed stock analysis (MSA) The use of markers to determine the relative proportions of identifiable stocks in a mixed-stock fishery. MSA is widely employed in the management of Pacific salmon.

Multilocus probe Used typically to refer to probes used in **DNA fingerprinting** (e.g. minisatellites) where many loci are visualized simultaneously producing a banding pattern comprising many DNA fragments. In such cases it is usually not possible to identify loci or assess levels of heterozygosity.

ND genes Sequences of DNA in the mitochondrial genome that code for enzymes in the NADH dehydrogenase complex.

nDNA Nuclear DNA, the DNA contained in the chromosomes within the nucleus of eukaryotic cells, and inherited from both maternal and paternal parents.

Nucleotide One of the monomeric units from which DNA molecules are constructed, consisting of a purine (adenine and guanine) or pyrimidine (thymine and cytosine) base, a pentose sugar, and a phosphoric acid group.

Oligonucleotide Short DNA fragment typically of 10-20 nucleotides. Generally refers to single-stranded, synthetic DNA molecules used as a probe or primer.

Polymerase An enzyme that assembles the subunits of macromolecules. DNA polymerases have the ability to synthesize the complementary strand of single-stranded DNA template. Synthesis only extends from existing double-stranded sequences across a single-stranded template.

Polymerase chain reaction (PCR) The amplification of particular regions of DNA using primers (which flank the region of DNA to be amplified) and the DNA polymerase of the thermophilic bacterium *Thermus aquaticus*. PCR involves a cycle of denaturation to single strands (around $94^{o}C$), primer annealing ($37\text{-}60^{o}C$), and primer extension (around $70^{o}C$). Thirty or more cycles are typically carried out to create a large number of copies of the target DNA sequence.

Polymorphism Existence at the same time of two or more different classes of a morph within a population, that is, individuals with discrete phenotypic differences. In the molecular sense, polymorphism may be detected as alternative forms of a gene (e.g. allozymes or nucleotide sequence), and is sometimes defined as variants with a frequency of > 1% or 5% in the population. The latter criterion is employed more often to exclude the incidence of rare mutations.

Primer A short single-stranded sequence of DNA which binds to a complementary sequence and initiates the extension of adjacent DNA regions (DNA strand synthesis, e.g. in **PCR**) using DNA polymerase. Primers can be designed so that they will bind to a very specific region of the DNA, and will thus initiate synthesis of a targeted sequence (as in **PCR** or DNA sequencing).

Probe A length of RNA or single-stranded DNA radioactively (or otherwise) labelled and used to locate complementary sequences by base-pairing in a heterogeneous collection of sequences. The probe therefore **hybridizes** with the target sequence (one or many repeat copies) making it visible to the naked eye (e.g. **autoradiography**), so allowing the degree of variability to be assayed.

Random Amplified Polymorphic DNA (RAPD) A technique allowing detection of DNA polymorphisms by randomly amplifying multiple regions of the genome by PCR using single arbitrary primers. The primers are generally between 10 and 20 base pairs long, of an arbitrary but known sequence.

Repetitive DNA Nucleotide sequences occurring repeatedly in chromosomal DNA. Repetitive DNA can belong to the highly repetitive (sequences of several nucleotides repeated millions of times) or middle repetitive (sequences of 1 - 500 base pairs in length, repeated 100 to 10,000 times each) categories.

Restriction enzyme An enzyme that cleaves double-stranded DNA. Type I are not sequence-specific; type II (the type used routinely in molecular genetic analyses) cleave DNA at a specific sequence of nucleotides known as **restriction** or **recognition sites**. The enzymes are named by an acronym that indicates the bacterial species from which they were isolated, followed by a Roman numeral that gives the chronological order of discovery when more than one enzyme came from the same source.

Most restriction enzymes currently employed in fish studies recognise sequences of either four, five or six bases.

Restriction fragment length polymorphism (RFLP) Variations occurring within a species in the length of DNA fragments generated by a specific restriction enzyme. Such variation is generated either by base substitutions that cause a gain or loss of sites, or by insertion/deletion mutations that change the length of fragments independent of restriction site changes.

Restriction site (= recognition site) A specific sequence of nucleotide bases which is recognised by a restriction enzyme. The enzyme will cleave both DNA strands at a specific location within that sequence. Variation in the presence and absence of restriction sites among individuals generate restriction fragment length polymorphisms (RFLPs).

Satellite DNA DNA from a eukaryote that separates on gradient centrifugation as a distinct fraction. The separation results from differences in the base composition of the distinct fraction and main band of genomic DNA (i.e. the A + T or G + C content is higher in the satellite than in the main band). Many satellites consist of highly repetitive DNA, usually millions of tandem repeats of a relatively short sequence.

scnDNA (single copy nuclear DNA) Sequences that occur once, or very few times, in a genome.

Single-locus probe (SLP) A probe consisting of short repeat sequences (e.g. minisatellites) that identify allelic products at a single locus, thus producing banding patterns typically consisting of either one (homozygote) or two (heterozygote) DNA fragments. C.f. Multilocus probe.

Southern blot A membrane (e.g. nitrocellulose or nylon) onto which DNA has been transferred directly from an electrophoretic gel. The transfer is facilitated by simple diffusion of salts across the membrane, or using automated vacuum blotters. The membrane can then be exposed to a labelled probe that will bind to the specific fragments of interest, allowing their visualization independent of thousands of other fragments from the gel.

Stock Unit of an exploited species which is employed in stock assessment. Definition depends on management aims and time-scale of interest (see Fishery, Genetic and Harvest stock).

Stock assessment The use of various statistical and mathematical calculations to make quantitative predictions about the reactions of exploited populations to alternative management options.

Stock structure analysis (SSA) A procedure that determines the extent of population substructuring within an exploited species from a particular geographic region(s). Ideally comprises several interactive phases: (i) the characterization of each identifiable stock using suitable markers; (ii) an estimate of the biological significance of stock separation in terms of differences in patterns of recruitment and mortality (or response to harvesting); (iii) the incorporation of such information into stock assessment.

Table I.2: Percentage of correctly classified fish obtained from a CVA on *Stolothrissa* data only.

	All	males	females
Overall	85.8	100	98.4
SKIP	87.1		100
SKIB	83.3	100	100
SKIG	85.7	100	100
SMUG	69.2	100	83.3
SKAR	91.7	100	100

APPENDIX II - ALLOZYME ELECTROPHORESIS

Table II.1: Buffer systems used

Buffer	Electrode (g/l)	Gel (g/l)	Current & duration of run
Citrate morpholine (pH 6.2) CM 6.2	8.4 citric acid adjust to pH 6.2 with N-(3-aminopropyl)-morpholine	Dilute 1:10	35 mA / gel 8 h
Tris citrate (pH 8.0) TC 8.0	30.3 g Tris 11.98 g citric acid	Dilute 1:25	35 mA / gel 8 h
Tris maleic (pH 7.4) TM 7.4	12.1 g Tris 11.6 g maleic acid 3.7 g EDTA 4.05 g $MgCl_2$	Dilute 1:10	15 mA / gel 17 h
Tris maleic (pH 8.9) TM 8.9	12.1 g Tris 11.6 g maleic acid 3.7 g EDTA 4.05 g $MgCl_2$	Dilute 1:10	15 mA / gel 17 h
mod. Ridgeway's (pH 6.8) TCB 8.6	18.6 g boric acid 4.2 g lithium hydroxide	9.2 g Tris 1.05 g citric acid 53 ml electrode buffer	25 mA / gel 20 h

Table II.2: Enzymes used for screening kapenta

Enzyme	Abbrev.	EC number	A	R
Aspartate aminotransferase	AAT	2.6.1.1	1	2
Aconitase hydratase	ACOH	4.2.1.3	5	
Acid phosphatase	ACP	3.1.3.2	2	2
Adenosine deaminase	ADA	3.5.4.4	5	
Alcohol dehydrogenase	ADH	1.1.1.1	5	
Adenylate kinase	AK	2.7.4.3	1	3
Alanine aminotransferase	ALAT	2.6.1.2	5	
Aldehyde dehydrogenase	ALDH	1.2.1.5	5	
Aldehyde oxidase	AO	1.2.3.1	5	
Alkaline phosphatase	AP	3.1.3.1	5	
Carbonate hydratase	CAR	4.2.1.1	2	3
Creatine kinase	CK	2.7.3.2	1	2
Diaphorase	DIA	1.6.4.3	5	
Enolase	ENO	4.2.1.11	5	
Esterase-D	EST-D	3.1.1.1	1	1
Fructose biphosphate aldolase	FBA	4.1.2.13	2	2
Formaldehyde dehydrogenase	FDH	1.2.1.1	5	
Fructose 1,6 diphosphatase	FDP	3.1.3.11	5	
Fumarate hydratase	FH	4.2.1.2	1	1
Fructokinase	FK	2.7.1.4	4	2
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	1	2
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	2	2
ß-Galactosidase	ß-GAL	3.2.1.23	5	
ß-Glucosidase	ß-GLUS	3.2.1.21	5	
Glyceraldehyde-3-phosphate dehydrog.	GAPDH	1.2.1.12	1	3
Guanine deaminase	GDA	3.5.4.3	3	2
Glucose dehydrogenase	GDH	1.1.1.47	5	
Glutamate pyruvate transaminase	GPT	2.6.1.2	5	
Glutamate dehydrogenase	GTDH	1.4.1.2	5	
Glutamate dehydrogenase NADP	GTDH-P	1.4.1.4	5	
(S)-2-Hydroxy-acid phosphatase	HAOX	1.1.3.15	5	
ß-N-Acetylglucosaminidase	HEX	3.2.1.30	2	5
Hexokinase	HK	2.7.1.1	5	
Iditol dehydrogenase	IDDH	1.1.1.14	5	
Isocitrate dehydrogenase	IDH	1.1.1.42	1	2
Lactate dehydrogenase	LDH	1.1.1.27	1	1
Malate dehydrogenase	MDH	1.1.1.37	2	1
Malic enzyme	ME	1.1.1.40	1	1
Mannose phosphate isomerase	MPI	5.3.1.8	2	2
Purine-nucleoside phosphorylase	PNP	2.4.2.1	5	
Octanol dehydrogenase	ODH	1.1.1.73	5	
Peptidase	PEP	3.4.-.-	2	4
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	1	2
Phosphoglucose isomerase	PGI	5.3.1.9	1	1
Phosphoglycerate kinase	PGK	2.7.2.3	5	
Phosphoglucose mutase	PGM	5.4.2.2	1	2
Pyruvate kinase	PK	2.7.1.4	1	3
Shikimate dehydrogenase	SKDH	1.1.1.25	5	
Superoxidase dismutase	SOD	1.15.1.1	5	
Succinate dehydrogenase	SUDH	1.3.99.1	5	
Triose phosphate isomerase	TPI	5.3.1.1	1	2
Xanthine dehydrogenase	XDH	1.2.1.37	5	
Xanthine oxidase	XO	1.2.3.2	5	

Legend
A: activity; R: resolution
1 excellent, 2 good, 3 moderate, 4 bad, 5 insufficient

Table II.2: Allele frequencies of kapenta in Lakes Tanganyika, Kivu and Kariba. Allelic designations (A, B....) are given at the bottome of the table. x^2 is the chi-squared value of tests for deviations from Hardy Weinberg equilibrium. Significant deviations are shown in bold.

	Population								
Locus	SV1	SV2	K1	K2	C	SZ1	SZ2	JO1	JO2
LDH-2									
(N)	51	59	48	48	51	56	61	46	46
A	.971	.932	.958	.969	.951	.964	.926	.957	.957
B	.029	.068	.042	.031	.049	.036	.066	.043	.043
C	.000	.000	.000	.000	.000	.000	.008	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
x^2	.031	.271	.067	.033	.107	.057	.028	.071	**14.156*****
ME-1									
(N)	51	59	48	48	51	56	61	46	46
A	1.000	.975	.990	.990	1.000	.991	.992	1.000	.989
B	.000	.017	.010	.000	.000	.009	.008	.000	.011
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.008	.000	.010	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
x^2		.027	.000	.000		.000	.000		.000
ME-2									
(N)	51	41	48	48	50	56	61	46	46
A	.578	.622	.563	.677	.540	.571	.533	.663	.554
B	.402	.317	.406	.292	.430	.411	.410	.283	.326
C	.020	.061	.021	.021	.030	.018	.049	.054	.120
D	.000	.000	.010	.010	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.008	.000	.000
x^2	1.538	4.355	2.493	.859	2.720	**112.959*****	2.036	**14.073****	5.442
ME-3									
(N)	51	59	48	48	51	56	61	46	46
A	.961	.966	.990	.969	.941	.982	.926	.989	.924
B	.039	.034	.010	.031	.059	.009	.041	.011	.054
C	.000	.000	.000	.000	.000	.009	.016	.000	.022
D	.000	.000	.000	.000	.000	.000	.008	.000	.000
E	.000	.000	.000	.000	.000	.000	.008	.000	.000
x^2	.063	.054	.000	.033	.164	.009	.341	.000	.265
MDH-2									
(N)	51	59	48	48	51	56	61	46	46
A	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.010	.000	.000	.000	.000	.000	.000
x^2			.000						
AAT-1									
(N)	51	59	48	48	51	56	61	46	46
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.989
B	.000	.000	.000	.000	.000	.000	.000	.000	.011
x^2									.000
AAT-2									
(N)	51	59	48	48	51	56	61	46	46
A	.980	1.000	.990	.990	1.000	.991	.992	1.000	.989
B	.000	.000	.000	.000	.000	.009	.008	.000	.011
C	.020	.000	.010	.010	.000	.000	.000	.000	.000
x^2	.010		.000	.000		.000	.000		.000

AAT-3									
(N)	51	59	48	48	51	56	61	46	46
A	.951	.966	.958	.927	.941	.973	.885	.913	.978
B	.049	.034	.042	.063	.039	.027	.049	.043	.011
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.010	.020	.000	.057	.022	.011
E	.000	.000	.000	.000	.000	.000	.008	.011	.000
F	.000	.000	.000	.000	.000	.000	.000	.011	.000
x^2	.107	.054	.067	.252	.164	.028	**21.611****	.361	.011
PGM									
(N)	51	59	48	48	51	56	61	46	46
A	.422	.559	.458	.521	.461	.518	.582	.500	.554
B	.549	.432	.531	.458	.529	.482	.418	.489	.435
C	.000	.008	.000	.000	.010	.000	.000	.000	.000
D	.010	.000	.000	.010	.000	.000	.000	.000	.011
E	.000	.000	.010	.000	.000	.000	.000	.011	.000
F	.000	.000	.000	.010	.000	.000	.000	.000	.000
G	.020	.000	.000	.000	.000	.000	.000	.000	.000
x^2	6.846	1.017	2.977	2.509	1.844	1.025	**8.501****	.995	1.328
GAP-2									
(N)	51	59	48	48	51	56	38	45	46
A	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	.989
B	.000	.000	.000	.010	.000	.000	.000	.000	.011
x^2				.000					.000
EST-2									
(N)	51	57	48	48	51	56	61	46	46
A	.784	.719	.760	.729	.775	.705	.689	.793	.761
B	.216	.281	.240	.271	.225	.295	.311	.207	.239
x^2	.058	.162	1.765	1.347	.167	.644	.013	.641	**4.304***
PGDH									
(N)	51	59	48	48	51	56	61	39	46
A	.980	1.000	.979	1.000	1.000	.973	.984	1.000	1.000
B	.020	.000	.021	.000	.000	.009	.016	.000	.000
C	.000	.000	.000	.000	.000	.018	.000	.000	.000
x^2	.010		.011			.028	.008		
G6PDH									
(N)	51	47	48	48	51	56	55	46	46
A	.912	.894	.906	.948	.892	.911	.964	.891	.891
B	.059	.085	.063	.042	.069	.071	.009	.054	.087
C	.029	.021	.031	.010	.039	.018	.027	.054	.022
x^2	.421	.594	.452	.115	3.674	5.749	.058	**8.159***	.610
IDH-1									
(N)	51	59	48	48	51	56	61	46	46
A	.990	.992	1.000	.979	1.000	.973	.992	.989	.989
B	.010	.008	.000	.021	.000	.027	.008	.011	.011
x^2	.000	.000		.011		.028	.000	.000	.000
IDH-2									
(N)	51	59	48	48	51	56	61	46	46
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-1									
(N)	51	59	48	48	51	56	60	46	46
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.989
B	.000	.000	.000	.000	.000	.000	.000	.000	.011
x^2									.000

	Population								
Locus	MP1	MP2	MP3	MP4	MP5	CB1	CB2	KIP	KIB
LDH-2									
(N)	31	21	28	30	43	47	41	50	38
A	.984	.929	1.000	.917	.965	.989	.963	.940	.974
B	.016	.071	.000	.067	.035	.000	.037	.060	.026
C	.000	.000	.000	.000	.000	.011	.000	.000	.000
D	.000	.000	.000	.017	.000	.000	.000	.000	.000
x^2	.000	.081	.000	.195	.037	.000	.039	.168	.014
ME-1									
(N)	31	21	28	30	43	47	41	50	38
A	1.000	.976	.982	.950	.977	.989	1.000	.980	1.000
B	.000	.024	.018	.017	.023	.000	.000	.010	.000
C	.000	.000	.000	.017	.000	.011	.000	.000	.000
D	.000	.000	.000	.017	.000	.000	.000	.010	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
x^2		.000	.000	.055	.012	.000		.010	
ME-2									
(N)	31	21	28	30	43	47	41	50	38
A	.694	.857	.482	.600	.581	.628	.610	.550	.539
B	.274	.119	.429	.383	.407	.340	.341	.390	.408
C	.016	.000	.071	.017	.012	.021	.049	.060	.053
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.016	.024	.018	.000	.000	.011	.000	.000	.000
x^2	3.682	2.949	2.437	1.594	.720	.839	.643	1.415	3.834
ME-3									
(N)	31	21	28	30	43	47	41	50	38
A	.984	.929	.946	.817	.953	.936	.951	.990	.961
B	.016	.071	.018	.167	.047	.064	.024	.010	.026
C	.000	.000	.018	.017	.000	.000	.012	.000	.013
D	.000	.000	.000	.000	.000	.000	.012	.000	.000
E	.000	.000	.018	.000	.000	.000	.000	.000	.000
x^2	.000	.081	.059	.289	.076	.180	.080	.000	.042
MDH-2									
(N)	31	21	28	30	43	47	41	50	38
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
AAT-1									
(N)	31	21	28	30	43	47	41	50	38
A	1.000	.976	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.024	.000	.000	.000	.000	.000	.000	.000
x^2		.000							
AAT-2									
(N)	31	21	28	30	43	47	41	50	38
A	.984	.976	1.000	.983	1.000	.989	1.000	.980	1.000
B	.016	.024	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.017	.000	.011	.000	.020	.000
x^2	.000	.000		.000		.000		.010	
AAT-3									
(N)	31	21	28	30	43	47	41	50	38
A	.952	.952	.964	.983	.930	.968	.963	.910	.947
B	.048	.024	.018	.000	.070	.021	.037	.040	.013
C	.000	.000	.000	.000	.000	.000	.000	.010	.000
D	.000	.024	.018	.017	.000	.011	.000	.030	.039

	E	.000	.000	.000	.000	.000	.000	.000	.000	.000
	F	.000	.000	.000	.000	.000	.000	.000	.010	.000
	x^2	.053	.026	.019	.000	4.342	.034	.039	.431	**24.334*****
PGM										
	(N)	31	21	28	30	43	47	41	50	38
	A	.597	.619	.518	.583	.535	.500	.463	.520	.605
	B	.403	.357	.482	.400	.465	.500	.537	.480	.395
	C	.000	.000	.000	.017	.000	.000	.000	.000	.000
	D	.000	.000	.000	.000	.000	.000	.000	.000	.000
	E	.000	.000	.000	.000	.000	.000	.000	.000	.000
	F	.000	.000	.000	.000	.000	.000	.000	.000	.000
	G	.000	.024	.000	.000	.000	.000	.000	.000	.000
	x^2	2.055	2.194	.219	1.119	.520	.048	.689	.050	2.237
GAP-2										
	(N)	31	21	28	30	8	47	41	50	38
	A	1.000	.976	.982	1.000	1.000	.989	1.000	.950	.974
	B	.000	.024	.018	.000	.000	.011	.000	.050	.026
	x^2		.000	.000			.000		.110	.014
EST-2										
	(N)	31	21	28	30	43	46	41	50	37
	A	.774	.548	.768	.750	.767	.739	.720	.690	.757
	B	.226	.452	.232	.250	.233	.261	.280	.310	.243
	x^2	.281	1.065	2.901	.590	1.136	.001	.009	.747	3.577
PGDH										
	(N)	31	21	28	30	43	47	41	50	38
	A	1.000	1.000	.982	1.000	.965	.989	.963	1.000	.974
	B	.000	.000	.018	.000	.035	.011	.037	.000	.026
	C	.000	.000	.000	.000	.000	.000	.000	.000	.000
	x^2			.000		.037	.000	.039		.014
G6PDH										
	(N)	31	21	28	30	43	47	41	50	38
	A	.903	.929	.875	.900	.826	.904	.951	.920	.961
	B	.097	.071	.036	.083	.151	.064	.037	.050	.026
	C	.000	.000	.089	.017	.023	.032	.012	.030	.013
	x^2	2.748	.081	.482	.304	1.775	3.807	.080	.328	.042
IDH-1										
	(N)	31	21	28	30	43	47	41	50	38
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	.000	.000	.000	.000	.000	.000	.000	.000	.000
IDH-2										
	(N)	31	21	28	30	43	47	41	50	38
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-1										
	(N)	30	21	28	30	43	47	41	50	38
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	.000	.000	.000	.000	.000	.000	.000	.000	.000

	Population							
Locus	KIG	MUG	KAR	KAD	GIS	KYN	KYS	CYA
LDH-2								
(N)	34	45	46	38	50	50	48	50
A	.971	.967	.989	.961	1.000	.950	.990	.970
B	.029	.033	.011	.039	.000	.050	.010	.030
C	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000
x^2	.015	.035	.000	.042		.110	.000	.032
ME-1								
(N)	34	45	46	38	49	50	48	50
A	.985	.989	.989	.987	.990	1.000	1.000	.990
B	.015	.000	.000	.013	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.011	.011	.000	.010	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.010
x^2	.000	.000	.000	.000	.000			.000
ME-2								
(N)	34	45	46	38	50	50	48	50
A	.515	.544	.620	.566	.600	.640	.573	.560
B	.485	.411	.315	.355	.320	.310	.344	.420
C	.000	.044	.065	.079	.070	.050	.083	.020
D	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.010	.000	.000	.000
x^2	.591	.329	1.378	2.385	3.801	1.077	.979	1.558
ME-3								
(N)	34	45	46	38	50	50	48	50
A	.956	.967	.989	.974	.930	.930	.938	.940
B	.029	.033	.011	.026	.070	.040	.052	.030
C	.015	.000	.000	.000	.000	.010	.010	.030
D	.000	.000	.000	.000	.000	.020	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000
x^2	.048	.035	.000	.014	.241	**15.560***	.176	.168
MDH-2								
(N)	34	45	46	38	50	50	48	50
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.000	.000	.000
AAT-1								
(N)	34	45	46	38	50	50	48	50
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.000	.000	.000
AAT-2								
(N)	34	45	46	38	50	50	48	50
A	1.000	.978	.989	1.000	.960	1.000	1.000	.980
B	.000	.022	.011	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.040	.000	.000	.020
x^2		.011	.000		.064			.010
AAT-3								
(N)	34	45	46	38	50	50	48	50
A	.971	.978	.957	.987	.990	1.000	.979	.980
B	.029	.022	.033	.013	.010	.000	.021	.020
C	.000	.000	.000	.000	.000	.000	.000	.000

	D	.000	.000	.011	.000	.000	.000	.000	.000
	E	.000	.000	.000	.000	.000	.000	.000	.000
	F	.000	.000	.000	.000	.000	.000	.000	.000
	x^2	.015	.011	.071	.000	.000		.011	.010
PGM									
	(N)	34	45	46	38	50	50	48	49
	A	.588	.500	.565	.421	.340	.470	.406	.347
	B	.412	.500	.435	.579	.650	.500	.573	.622
	C	.000	.000	.000	.000	.000	.020	.010	.031
	D	.000	.000	.000	.000	.010	.010	.010	.000
	E	.000	.000	.000	.000	.000	.000	.000	.000
	F	.000	.000	.000	.000	.000	.000	.000	.000
	G	.000	.000	.000	.000	.000	.000	.000	.000
	x^2	.064	.449	2.125	.167	.776	6.803	1.748	1.428
GAP-2									
	(N)	34	40	46	38	50	50	48	50
	A	1.000	.962	.935	.974	1.000	.990	.958	.990
	B	.000	.038	.065	.026	.000	.010	.042	.010
	x^2		.040	.185	.014		.000	.067	.000
EST-2									
	(N)	34	44	46	36	50	48	47	50
	A	.765	.716	.826	.736	.870	.760	.691	.860
	B	.235	.284	.174	.264	.130	.240	.309	.140
	x^2	.041	3.617	.111	1.485	2.418	1.140	.190	1.220
PGDH									
	(N)	34	45	46	38	50	50	48	50
	A	1.000	1.000	.957	1.000	.980	.960	.979	.990
	B	.000	.000	.043	.000	.020	.040	.021	.010
	C	.000	.000	.000	.000	.000	.000	.000	.000
	x^2			.071		.010	.064	.011	.000
G6PDH									
	(N)	34	45	46	38	40	50	39	50
	A	.985	.911	.880	.908	.875	.880	.872	.830
	B	.015	.033	.098	.092	.112	.100	.103	.170
	C	.000	.056	.022	.000	.013	.020	.026	.000
	x^2	.000	.371	.764	2.190	.727	.986	1.532	**7.157****
IDH-1									
	(N)	34	45	46	38	50	50	48	50
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	.000	.000	.000	.000	.000	.000	.000	.000
IDH-2									
	(N)	34	45	46	38	50	50	48	50
	A	1.000	1.000	1.000	1.000	1.000	1.000	.990	1.000
	B	.000	.000	.000	.000	.000	.000	.010	.000
	x^2							.000	
LDH-1									
	(N)	34	45	46	38	50	50	48	50
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	.000	.000	.000	.000	.000	.000	.000	.000

Designation of alleles: capital letters refer to alleles with the following relative mobilities:

Locus	A	B	C	D	E	F	G
LDH-2	100	140	59	75			
ME-1	100	0	200	125	-100		
ME-2	100	112	120	140	85		
ME-3	100	85	95	113	70		
MDH-2	100	41					
AAT-1	-100	82					
AAT-2	-100	-75	-125				
AAT-3	100	0	50	200	275	20	
PGM-1	-100	-70	-120	-30	-80	-110	-90
GAPDH	100	107					
EST-2	100	112					
PGDH	100	110	92				
G6PDH	100	65	85				
IDH-1	100	10					
IDH-2	100	60					
LDH-1	100	-800					

APPENDIX III - MITOCHONDRIAL DNA

Table III.1: Protocol for extracting DNA from kapenta. Modified from Taggart et al. (1990)

1. To 1.5 ml microfuge tubes add 375 μl extraction buffer (0.1 M Tris, 0.01 M EDTA, 0.1 M NaCl, 2% SDS, pH 8.0), and about 100 mg of muscle tissue, which has been cut into small pieces. Be careful to avoid cross-contamination.

2. Add 20 μl of Proteinase K solution (10mg/ml).

3. Mix briefly and incubate overnight at 37^{o}C.

4. Add 400 μl phenol (pH 8.0 equilibrated). Shake vigorously for 20 seconds, followed by more gentle mixing for 10 min.

5. Add 400 μl chloroform:isoamylalcohol (24:1). Again, shake vigorously for 20 s, followed by gentle mixing for 10 min.

6. Spin tubes in microcenttrifuge for 3 min.

7. Using a wide bore pipette tip, transfer the top aqeous layer to a new tube. Do not disturb tissue debris on the inetrface.

8. Repeat steps 5, 6 and 7 on the top aqueous layer.

9. Add 1 ml of ice cold absolute ethanol and invert several times. The DNA should precipitate in most samples as white fluff. If no DNA is visible the sample has to be spinned for 3 min.

10. Decant off the absolute ethanol and add 1 ml of 70% ethanol. Wash overnight by gentle mixing.

11. Decant off the 70% ethanol, removing all the liquid with a pipettes. Leave tubes open, but covered on the bench for the samples to dry for about 1hr. Resuspend DNA in 100 μl TE (10mM Tris, 1 mM EDTA, pH 8.0).

Table III.2: Conditions for Polymerase Chain Reaction (PCR).

In 500 µl tube add:

5 µl Boehringer PCR buffer
5 µl dNTP solution (200µM final concentration)
2 µl $MgCl_2$ (final concentration 2.5mM)
1 µl of each primer (1 unit)
0.2 µl Taq polymerase (Boehringer)
0.5 µl template DNA
36.3 µl sterile, filtered water

Overlay with 2 drops of sterile mineral oil. Put tubes into PCR machine.

Amplification cycle

°C	time	function	
95	5'	Initial denaturation	
51	1'	Annealing of primers	┐
72	1'30"	Extension	├ repeat 25 times
94	30"	Denaturation	┘
51	1'	Annealing	
72	10'	Final extension	
25	30"	Cool down samples	

Table III.3: Silver staining protocol

Solutions (to be prepared fresh)
Buffer A: 10% ethanol/0.5% acetic acid
Buffer B: 0.1% Silver nitrate
Buffer C: 1.5% NaOH, 0.1% $NaBH_4$, 0.15% formaldehyde - disolve NaOH first as $NaBH_4$ is explosive with water!!!

Protocol:
1) Remove gels from rig and wash separately in sandwich boxes on a belly-dancer.

a) 5 min in 100 ml buffer A
b) discard and wash for another 5 min in 100 ml new buffer A
c) discard buffer and wash for 10 min in 100 ml buffer A
d) wash twice with distilled water
e) wash in 150 ml buffer C until bands are visible (about 10-15 min)
f) heat seal into a plastic bag.

Table III.3: Composite haplotype frequencies observed in kapenta populations from Lakes Tanganyika, Kivu and Kariba.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
HB 4	5	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0
CB 2	3	2	4	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	2	0	1	0	0
KIB	4	2	1	0	1	0	0	2	0	0	2	0	0	1	0	0	0	1	0	0	0	0	1	0	2	0	0	0	0
KIG	1	2	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	0	0	0	2	0	0	0	0
MUG	5	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	3	0	0	1	1
KAD	7	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
GIS	6	3	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
KYN	11	2	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KYS	4	3	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CYA	11	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
SV2	5	1	2	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
JO2	5	1	0	3	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
HB 4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
CB 2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
KIB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
KIG	2	2	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
MUG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
KAD	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
GIS	0	0	0	0	0	0	0	4	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
KYN	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
KYS	2	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
CYA	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
SV2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	2	1	0	0	0	0	0	0	0
JO2	0	1	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0