

**An Investigation into the Allozyme Genetic Variation Patterns Among
Populations of Freshwater Fish from Different River Systems of
Southern Africa.**

M. Sc. (Physiology)

S. K. Mpherwane

2011

**An investigation into the allozyme genetic variation patterns among populations of freshwater
fish from different river systems of Southern Africa.**

BY

SALOME KEDIBONE MPHERWANE

DISSERTATION

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL HEALTH

In the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Molecular and Life Sciences)

At the

UNIVERSITY OF LIMPOPO, TURFLOOP CAMPUS

SOUTH AFRICA

SUPERVISOR: Prof. P. F. S. Mulder

CO-SUPERVISOR: Prof. G. D. Engelbrecht

2011

DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science has not previously been submitted by me for a degree at this or any other University; that it is my own work in design and in execution, and that all material contained therein has been duly acknowledged.

Signed: _____

26-08-2011

S K Mpherwane (Mrs)

DEDICATION

This study is dedicated to my husband, Kgatedi, our sons, Tlou, Maphophosha, Nkosinathi, to our parents, to our brothers and sisters who persevered much of my absence and gave words of support to this effect.

ACKNOWLEDGEMENTS

Upon completion of this study, I wish to express my deepest and sincerest indebtedness to the following people and institutions who contributed to the success and completion of this study:

To God my Creator, for having spoken into existence; the universe and nature upon which this study was based.

To my husband, Broer Kgatedi, our sons Tlou, Maphophosha and Nkosinathi for their endurance, support and persuasion in the study.

To my parents Macleod and Annah Mpya, and all in their family for taking care of our sons while the study lasted and for their encouragement and support.

To my sister Lizzy, and my niece Nkele, for being mother over the kids throughout time.

To my in-laws, for having not refused me the privilege to study.

To all my brothers, my sisters, and my sisters-in-law including Motlalepule Maleka for all the support.

To all my friends, my husband's friends, our pastors Jack Hlambisa, Mortimer Mannya, Moses Dolo, Magowa and their families for encouragement and support.

To Masepekwa, a friend and a daughter who always pestered me to complete.

To my precious neighbours and their children for just saying, "Go on."

To Prof. P. F. S. Mulder, my supervisor, and Prof. G. D. Engelbrecht, my co-supervisors, for their professional leadership, sacrifice, courage, enthusiasm and unyielding assistance throughout the course study.

To Mr. I. M. Campbell for technical assistance, supervision of laboratory equipment and practices, and for words of encouragement.

To Dr. Johann Engelbrecht, the Director of the Mpumalanga Parks Board for the ready availability, sacrifice, assistance and for providing safety measures during collection of samples.

To Mmane Charlotte Malatji, Papa Willy Makwela, Mmalsaka Sethemane and Mokgotsi Rachel Makwela of the School of Molecular and Life Sciences for all the support and encouragement.

To the personnel of Department of Physiology both academic, technical and support services for their interest and support in the study; for, even though they were not all supervising this study, I was their responsibility in terms of being a student in the Department.

To the Director of the School of Molecular and Life Sciences, Prof. R. Becker for being able to listen to us as students.

To the NRF (National Research Fund) for financially supporting this project.

To God, be the Glory, forever and ever.

CONTENTS

Page

Declaration.....	ii
Dedication	iii
Acknowledgements	iv
Contents	vi
List of tables	ix
List of figures	xi
List of abbreviations.....	xii
Abstract	xv

CHAPTER 1. INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 The environment.....	3
1.3 Surface water	5
1.4 Rivers	7
1.4.1 Transportation and dilution of pollutants.....	7
1.4.2 Partial temporary innate water purification.....	8
1.4.3 River bank stabilization.....	8
1.4.4 Soil wetting, fertilization of floodplains and deltas.....	8
1.5 Dam construction.....	9

1.6 Biodiversity and genetic consideration	10
1.7 Morphology.....	12
1.8 Ecology.....	14
1.9 Distribution	16
1.10 Pollution.....	18
1.11 Hybridization.....	20
1.12 Genetic structure analysis	22
 CHAPTER 2. Review of genetic variation of fresh water fish from randomly selected water bodies in the southern African Development Communities.....	25
2.1 Introduction.....	25
2.2 Inter-catchment or inter-basin water transfers	27
2.3 Impacts of inter basin water transfers.....	29
2.4 Materials and methods	31
2.5 Results	41
2.6 Discussion	46
2.7 Conclusion and recommendations	53
2.8 Summary	54

CHAPTER 3. An investigation into the allozyme genetic structure of four populations of <i>Labeo cylindricus</i> Peters, 1852 (Pisces: Cyprinidae) from the Okavango, the Upper Zambezi, the Olifants (Transvaal), and the Incomati (Tonga WEIR), river systems.	56
3.1 Introduction.....	56
3.2 Relationship between <i>Labeo</i> and other organisms	60
3.3 Distribution	64
3.4 Habitat	66
3.5 Pollution.....	68
3.7 Reproductive ecology	71
3.8 Objectives of the study.....	61
3.9 Materials and methods	76
3.10 Results	77
3.11 Discussion	87
3.12 Conclusion and recommendations	96
CHAPTER 4. References	97

List of tables

Table	Table title	Page
Table 1:	Genetic variability in different localities of southern African fresh water fish from the region. Fields include river systems, rivers and/or sampling localities, species and sample size, number of loci, levels of heterozygosity and reference sources.....	34
Table 2:	Average expected heterozygosity values of freshwater fish from southern Africa, grouped according to catchments or river systems.....	43
Table 3:	Inter-catchments, Bonferroni correction tests for mean differences between the Orange-Vaal-Zambezi and Orange-Vaal-Limpopo catchments areas.....	45
Table 4:	Some pathogens infecting <i>Labeo</i> and other freshwater fish.....	61
Table 5:	Sampling localities from which the fish were collected.....	75
Table 6:	Proteins, Locus Abbreviations and Enzyme Commission (EC) Numbers used in the genetic analysis of <i>Labeo cylindricus</i> populations.....	78
Table 7:	Polymorphic loci, relative mobility (R_M) of alleles, mean number of alleles per locus (N_A) percentage of polymorphic loci using 0.95 criterion ($P_{0.95}$), average expected heterozygosities (H_E) with standard error (S.E.) and X^2 values for the loci which deviated significantly ($p < 0.05$), from expected Hardy-Weinberg proportions, for five populations of <i>Labeo cylindricus</i> . OKAV: Okavango, ZAMB: Zambezi, OLIF: Olifants: INCOM: Incomati system.....	80

Table :8	Coefficients of genetic distance (D) for various taxonomic levels as defined by literature. Nei's theoretical genetic distance of D=1 is equivalent to five (5) million years of divergence (Grant <i>et al.</i> , 1988).	84
-----------------	---	----

Table :9	Matrix of pairwise allelic fixation index values (F_{ST}) (above the diagonal) and effective number of individuals exchanged between populations (N_{em}) (below the diagonal) Values for four <i>L. cylindricus</i> populations on the basis of polymorphic loci.	85
-----------------	---	----

List of figures

Figure	Title	Page
Figure 1:	Average expected heterozygosities values of fresh water fish from southern Africa river catchments population sizes (in parentheses) of ten (10) or more.....	44
Figure 2:	Average expected heterozygosities (H_E) for four populations of <i>Labeo cylindricus</i> . OKAV: Okavango, ZAMB: Zambezi, OLIF: Olifants: INCOM: Incomati river systems with their sample numbers in the parentheses.....	83
Figure 3:	Genetic distance coefficients (D) between pairs of populations of <i>L.cylindricus</i> from four river systems, according to the methods of Nei (1978).....	84

List of abbreviations

AK:	Adenylate kinase
AMD:	Acid mine drainage
ANOVA:	Analysis of variance
BBC:	British Broadcasting Company
CK:	Creatine kinase
CMS:	Conservation of Migratory Species (The Bonn convention)
DDT:	Dichloro-diphenyl-trichloroethane
D:	Genetic distance coefficient
EC number:	Enzyme Commission Number
EIA:	Environmental impact assessment
EST:	Esterases
Fig.	Figure
F _{ST} :	The measure of genetic differentiation (F-statistics) between populations.
G3PDH:	Glucose-3-phosphate dehydrogenase
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
GL:	Glycine-Leucine
GPI:	Glucose-6-phosphate isomerase
GSI:	Gonado-somatic index
H:	Average Heterozygosity
H _E :	Expected Average heterozygosity values
HK:	Hexokinase
IBT:	Interbasin (water) transfer
IDH:	Isocitrate dehydrogenase
IMERCSA:	India Musokotwane Environment Resource Centre for Southern Africa

INCOM:	Incomati River system population
IUCN:	The International Union for Conservation of Nature and Natural Resources
<i>L.</i> :	<i>Labeo</i>
LDH:	Lactate dehydrogenase
LHWP:	Lesotho Highlands Water Project
MDH:	Malate dehydrogenase
ME:	Malic enzyme
MPI:	Mannose-6-phosphate isomerase
n. sp.:	New species
N:	population sample size/number of individuals analysed per population
NAD:	Nicotinamide adenine dinucleotide
N _{em} :	The effective number of individuals exchanged between populations each generation.
OKAV:	Okavango River system population
OLIF:	Olifants River system population
P:	Percentage of polymorphic loci
p:	The measure of significance of values between observations, the values are significant when $p \leq 0.05$.
PEP-A:	Peptidases: (Glycine-Leucine)
PGDH:	Phosphogluconate dehydrogenase
PGM:	Phosphoglucomutase
SADC:	Southern African Development Community
SADCC:	Southern African Development Coordination Conference
SARDC:	Southern African Research and Documentation Centre
SARS:	Severe acute respiratory syndrome
SD:	Standard deviation
SE:	Standard error
SOD:	Superoxide dismutase

SPSS:	Statistical Package for Social Sciences
WHO:	World Health Organization
X^2 :	Chi-square test
ZAMB:	Zambezi River system population

Abstract

Running water is the most impacted ecosystem on the planet and is exploited heavily for water supply, irrigation, electricity generation and waste disposal. Running or lotic water systems have very intimate contact with their catchments and, as such, land use alterations affect the waters greatly and directly. Catchment management becomes complicated by numerous links between different forms of anthropogenic influences and toxicity pressure is constantly exerted on the aquatic fauna and flora of the river catchments. Planning and implementation of conservation strategies can only be promoted effectively if there is sufficient data on the fundamental knowledge of the biology, gene pools and ecology in the water bodies.

Allozyme genetic variation data from literature was assembled and analysed statistically. The results revealed that river catchments south of the Limpopo River exhibited higher heterozygosity values than river catchments north of the Limpopo. Significant differences existed between the Orange-Vaal and Zambezi and between the Orange-Vaal and the Limpopo River systems. The former pair was more significant different ($p=0.002$) than the latter pair ($p=0.023$). This initial study was followed by a population genetic study of *Labeo cylindricus* from southern African river systems.

The genus *Labeo*, is a group of fishes with sucker-like mouths adapted for bottom feeding and is widely distributed in Asian and African river systems. *Labeo cylindricus* has a potential to be commercially exploited for the ornamental aquarium trade. They are relatively small, attractive and adapt very well in an aquarium and, as a result of their feeding behaviour, they are capable of contributing to the hygiene and health of their habitat and are in great demand to keep aquaria algae-free. Taking their ornamental potential into account, it has become a matter of priority to document the genetic variation and differences between populations of this species.

In an attempt to assess the genetic structure, genetic differentiation and relationship between *Labeo cylindricus* populations, samples from the Okavango, Zambezi, Olifants and Incomati River systems were collected. Morphometrics and meristics were taken on site as means to facilitate identification, while muscle, heart and liver tissue samples were collected and stored in liquid nitrogen (-196 °C) for on-site storage and transportation purposes. In the laboratory they were transferred to a freezer (-40°C) to await electrophoresis.

The following were assessed: allelic frequencies, average heterozygosity, percentage of polymorphic loci, goodness of fit to Hardy-Weinberg equilibrium, heterozygosity deficits or excesses, the F-statistics and the genetic distances between the populations and between the species. The results revealed low levels of genetic variation in all the populations.

Of the 27 protein coding loci best resolved, polymorphism was displayed by the EST 2 and 3 loci, the MDH-1 and the PEP-A-1 locus only. Genetic variability estimates exhibited low levels of heterozygosity (0.014 to 0.042) and the percentage polymorphism ranged from (3.57% to 14. 29%) and there were an average number of 1.07-1.18 alleles per locus. These values are much lower than the average reported for southern African fish species. The results suggest that *L. cylindricus* from South Africa may be differentially negatively affected by catchment disturbances. Fixation for monomorphic loci for same alleles, in conjunction with genetic distance values obtained for the populations (0.018-0.078), suggest conspecificity in these populations. The smaller genetic distances of 0.018, between the Zambezi and the Olifants populations coupled by low F_{ST} value of 0.245 and moderate magnitude of gene flow ($N_{em}=0.433$) suggest a relatively recent divergence between the two populations.

The project was conducted with the assistance of Mpumalanga Parks Board (Research Division) and will assist them with their conservation management plans.

CHAPTER 1. INTRODUCTION

1.1 Introduction

The voice cries out amidst the global environmental chaos: “What and why, the global pollution; what and why global warming?” (Mathews and Zimmerman 1990). And the World Health Organization (WHO) weeps bitterly: “What, why and how long, the impacts of global pollution and global warming on human health? (WHO, 2002: Scally, 2011)” The entire biota groans with pain: “What, why and how long, the fears; how long the tears?”

In all the spheres of life, fear infiltrates. Every sector of the population has fear of a unique focus; unique to its own needs and niches. To the politician the fear revolves around power struggle, coups and wars. To the World Health Organization (WHO), the fear is centred on human health, human welfare and poverty alleviation. To the environmentalist; the fear is focussed on desertification, soil erosion and land degradation. To the conservationist and population geneticists; fear rules as well. In as much as we fear the extinction of species, so we fear the genetic contamination, genetic erosion, and genetic loss in these resources.

For every change or adventure, fear of the unknown exists and spreads. Whether the change is within the social or the physical realms, fear exists. It is worse when change occurs over which we, as humans, have little or no control. People fear change, and the unknown change carries with it elements of fear and of risks into the abstract world. So, there is justifiable reason for fear to some or to all. We fear things in proportion to the predictable enlightenment about them, and we fear the future in terms of our ignorance about it.

When every sector in the universe sweats and pants under individualistic niche fears, WHO suffers the biggest of all fears, in a quest for human health and life (WHO, 2006 a, b).

For many of us, there is a distinction between human health and life, and environmental health. For the World Health Organization (WHO) the unifying perspective is that good human health is dependent entirely on the stability and operation of the entire biosphere.

“More than 80% of all global morbidities and more than 25% of all global mortalities are attributable to environmental hazards and mismanagement” (WHO, 2006 b). Because of the complexity of interdependence between human life and all fields of the biosphere, human life and health are negatively impacted by the any hazard in any field. A loss to one is a loss to all; an injury to one is an injury to all (WHO, 2006 b).

Man has always been experiencing profound problems of health from time indefinite. In the quest to solve such problems, industrial development came into being. However, in the process, the environment got impacted upon negatively to the extent of its own ill-health. Unfortunately the unhealthy environment impacts more on human health and natural resources.

Accepting it or denying it, man is in authority to rule; and to rule the environment globally. Whether compelled by urgent physiological needs to survive, or luxurious exploration and curiosity invention, it boils down to one thing; the environment (air, water, land) suffers silently, with no one advocating its course. Once bruised, however, the environment lashes out at human health.

Pollution is a multi faceted disorder of nature and/or of human behaviour. It ranges from the mere drop of a match stick in the soil to serious contamination of the environment which results in cancer deaths and hereditary disorders, e.g. subatomic radiation pollution of the atmosphere (Freedman,1995). Pollution or toxicity of any sort has become the greatest threat to the life of all biota. Whilst we acknowledge that most of the toxicity on the planet has anthropogenic sources, natural sources cannot be left blameless. Wild fires, floods and epidemics pollute the remaining pieces of the world to an extent that life and health can no longer be sustained fully (Science Daily, 2006 a, b).

1.2 The environment

The environment consists of the entire physical space of external biotic and abiotic influences that act on organisms, or populations of organisms, in mutual relationships consistent with the term ecosystem (Freedman, 1995; Udo de Haes and Klijn, 1995). This complex is capable of changing the behaviour of organisms and is also subject to being changed by the organisms it is supporting. The environment of different organisms, man inclusive, is complex and very difficult to investigate and to assemble. This complexity therefore requires fragmentation, classification and taxonomy, in order to utilize, manage, protect, develop or change (Haber, 1995). Therefore a need emerges for sound environmental planning to prevent environmental problems.

Environmental problems are also complex, and intensify reciprocally in complexity as humans develop the environment for better living (health, food security, shelter, transport) and industrialization. The human population increases, as our ability to solve natural problems like epidemics increases (Shugart, 1998). Southern Africa is still in its infancy of economic and industrial development, and has to take into

consideration that the environmental crisis not only has social and economic sources, but ecological, biological and evolutionary consequences and responses as well.

The environment in its complexity is a major water user (Mpande and Tawanda, 2004). Man is central as a water user and man, as a cause of the majority of environmental problems, is expected to respond accordingly to solve and alleviate these problems, because he can survey and foresee the consequences of his actions (Udo de Haes and Klijn, 1994), but his efforts are subject to other external influences like poverty and desperation for use of natural resources for survival sake, among others. The competition on the environmental resources, mismanagement or/and management of the environment at an expense of addressing certain socio-economic fates like poverty and/or unfair land distribution may be the cause of conflict (Moncel, 2004).

Conflict between riparian countries of shared river systems over water or hydropolitics of river catchments, is not uncommon or new. Since after the cold war poverty, diseases, population explosion, food insecurity and water shortage has been experienced as hot spot issues and the reasons for conflict and stress in Third World countries. Water conflicts have emerged as prominent in the last few decades (Meissner, 2004) and water has become the cause for concern, conflict and potential war within and between states on the African continent (Country Reports, 2004; Meissner, 2004). For example, in 1978, Anouar El-Sadat, then president of Egypt, uttered a life threatening statement to the Ethiopians who were planning to build a dam on the Nile. He stated that, Egypt won't hold back going to war, since the water issue is a question of life and death to them (Moncel, 2004).

Military activities, however, are harmful to the environment. Not only do they absorb a large share of the country's economy (through training, infrastructure destruction, logistic supplies, research and loss of

human lives), but they also affect ecosystems badly (Freedman, 1995). During the three decades of civil war in Angola thousands of refugees, who fled to neighbouring countries, exerted pressure on environmental property of those countries through poor sanitation, unplanned refuse disposal and utilization of ecological resources for shelter and for energy. Post war returning refugees then put more pressure on their native land and river resources (TVE's Earth Report, 2004).

Freedman (1995) claims that, despite the ecological damage that wars have caused so far, no scientific inquest and dispensation of information on adverse ecological impacts of warfare had been documented up to 1995. Some wildlife species have become almost extinct as a result of civil wars and rebellion, political instability and laxity or inadequacy of environmental law enforcement, widespread availability of automatic weapons and explosives to poachers and the use of buried explosives like landmines (Freedman, 1995).

Some causes for water crisis are the fast growing human populations and the luxurious lifestyle of some. The wealthy may waste water, because they can afford the cost, while the poor and the rural economize on it until it is not safe for use any more. Moreover, water is inefficiently used in industry and agriculture, while much of the water is being lost through leakages (Kirby, 2004). Inefficient water use also causes environmental changes by intensifying land degradation, soil erosion and water pollution (Country Reports, 2004).

1.3 Surface water

Water is a natural resource of significant value. It is an indispensable ingredient of life and of the environment, determining the existence and sustainable development of the country (Zumdaht 1997;

Singh *et al.*, 2003; Mohammed, 2003). It occurs in either one of the three phases; gas (vapour), solid (ice) and liquid. Water is a constituent of cells of living organisms, and of crystals and minerals. It has many uses in science and industry as a catalyst, diluent, coolant, waste disposal and transport agent. It distributes heat and it can be used to generate electricity (Zumdahl, 1997).

On the other hand, water, either naturally or through anthropogenic induction, may become catastrophic to humans and to the environment (Singh *et al.*, 2003; Kambole, 2004). As a result of anthropogenic activities, rivers contain structures such as dams, hydroelectric power plants and irrigation canals which affect the environment (Brismar, 2002).

Surface water comprises only the visible part of a continuous fresh water ecosystem, which includes ground water, alluvial/floodplains and riparian systems (Hancock, 2002; Young, 2002). There exists an area of exchange called the hyporheic zone, between the surface zones and underground zones of water. It occurs in the sediments of gravel or sandbed streams. It is a porous ecotone between the surface and ground water. It hosts diverse and unique invertebrate fauna called hyporheos and it is often a site of intense biochemical activities. It contains surface and subsurface species, including fungi and other microbes which transfer, release and stabilize some forms of nutrients. The capability of the hyporheic zone to act as a physico-chemical filter should not be taken for granted or as a leeway for complacency, as the filter may break down if excessively overused (Hancock, 2002).

Water quality management addresses the spatial and temporal heterogeneous nature of the environment and in particular, its effect on water quality. Terrestrial processes have an enormous influence on the state of the recipient body of water. Environmental variables affecting water quality are not uniformly distributed across the landscape and may not change at the watershed. Variables such as

physiogeography, soil characteristics and anthropogenic land use, are among the most critical when determining water quality (Jenerete *et al.*, 2002).

1.4 Rivers

A river is a large natural water passage running through the terrestrial land. It is a conveyer that channels rainwater back to the sea/ocean or to large water bodies like lakes, in an almost closed cycle. A large river system naturally comprises of water basins or river channels, banks, floodplains, streams, inland and coastal deltas and estuaries and is associated with groundwater aquifers (Brismar, 2002; Meissner, 2004). The main water flow is confined to the river channel, bordered by river banks and the river bed at the bottom (Meissner, 2004). Streams flowing through urban areas receive substantial amounts of effluents (industrial and domestic) and storm water (Hancock, 2002).

Rivers have several functional commitments and services to the welfare of the ecosystems. These include:

1.4.1 Transportation and dilution of pollutants

The river has a limited native capacity to dilute polluted discharges and transport pollutants downstream. This depends on the rate of flow and the suspended particles in the water to which pollutants can adsorb (Brismar, 2002).

1.4.2 Partial temporary innate water purification

Pollutants are diluted, suspended, adsorbed onto particles, assimilated into living organisms, embedded into the sediments and/or decomposed by biochemical reactions to least toxic agents. Sedimentation and assimilation into organisms remove pollutants from the water, while biochemical decomposition transforms and possibly detoxifies pollutants (Brismar, 2002). This phenomenon occurs even in wetlands and other aquatic ecosystems, thus leading to improved water quality (Twinch and Grobler, 1986; Brismar, 2002). This is not an indefinite performance, however, and it has its limits.

1.4.3 River bank stabilization

The riparian vegetation has the ability to reduce bank erosion and to arrest silt (Brismar, 2002). Riparian zones also serve as chemical, physical/mechanical and biological boundaries between the rivers, streams and the watershed they drain. They regulate temperature and silt flux as a means to ensure riparian zone protection (Carignan *et al.*, Serveiss, 2002).

1.4.4 Soil wetting, fertilization of floodplains and deltas

A river can overflow onto floodplains as a means of reducing downstream flooding. The floodplains then become a nutrition rich bed and breeding ground for some aquatic fauna, rich grazing land for cattle and highly fertile agricultural land. The floodplains also constitute groundwater aquifer recharge areas and buffer the downstream destructive flooding (Brismar, 2002), just as dams are purposed for.

1.5 Dam construction

South Africa is renowned for aridness or semi-aridness. The low rainfall and the low mean annual runoff have led to the construction of dams and the planning, designing and construction of inter-basin transfer systems in the country (Allanson, 1995). In developing countries, the infrastructure around dam construction as a means to protect the land against flooding and to achieve large scale agricultural irrigation, industrialization, hydroelectricity generation and overall socioeconomic development has been established (Brismar, 2002).

There is growing awareness however of the environmental risks associated with building of large dams. Therefore, in the process, consideration must be given to potential eco-environmental adverse impacts of dams during planning and decision making (Brismar, 2002; Meissner, 2004).

The guidelines of a comprehensive environmental impact assessment (EIA) must be followed. The EIA is a major tool for sound management practices in dam erection and any other socio-economic and environmental development. Thus impoundment should be performed with due consideration of its ecological impacts. EIA is a procedure to predict, assess, estimate and communicate consequences, both profitable and detrimental, of any proposed development, and to ensure the consequences are taken into consideration in planning, design, implementation and management (Brismar, 2002).

Water retained from rivers in the dam lower downstream water flow. This, more frequently, decreases the volume of hyporheic zones by decreasing areas of sediment covered with water at a channel-aquifer interface (Hancock, 2002).

Dam related manipulations of natural water flow threaten the biodiversity of native aquatic faunal populations in many ways and obstruct migratory spawning species (Brismar, 2002; Asinar.Net Global Encyclopaedia, 2004; Meissner, 2004), degrading the quality and quantity of downstream floodplains (Brismar, 2002). Dams have a localised beneficial effect on fish survival in drought, but impede migration to a certain extent and therefore inhibit breeding. Migratory fish survival in dams may be sustained by building large WEIRs (Kleynhans, 1980; Olivier, 1994). The water in the reservoir undergoes changes in temperature, turbidity, oxygen content, silt and nutrients. As water gets released from the reservoir, these changes get transmitted downstream, extensively affecting biological diversity (Brismar, 2002).

1.6 Biodiversity and genetic consideration

Biology is a continuous entity and physiology is not in any sense a solitary component of the organism. It is inseparable from morphology, behaviour, ecology (Bailey, 1984) and most of all, genetics and evolution, which greatly constitute what we observe physiologically and morphologically (Callow and Forbes, 1998). The genetic differences therefore constitute all the diversity we observe in life forms (Spellerberg, 1996; Yokogawa, 1997; Yoshiyama and Sassaman, 1998)).

Biological diversity refers to the immense variability among individuals within species and with different species (Andrews and Chilton, 1999; Cutter and Renwick, 1999; Spellerberg, 1996). It includes variability in their abundance and habitats (Spellerberg, 1996; Swanson, 1997). To break it down to comprehensible levels, it must be properly systematised. It is therefore arrayed into several levels, from genetic levels to varieties of ecosystems (Spellerberg, 1996). Systematizing gets clearer when both morphological and ecological data are validated by the molecular data to describe biological diversity (Buth, 1984; Moritz and Hillis, 1990). This makes genetic diversity the central focus of all biological

diversity and evolutionary processes and also a necessity for all kinds of biological diversity (Spellerberg, 1996).

There is a national and global awareness in the decline of biodiversity (Swanson, 1997). The International Union for Conservation of Nature and Natural Resources (IUCN) has advanced the creation of parks and protected areas, their use and management. The Convention on Migratory Species (CMS) also called the Bonn Convention as part of IUCN was created for protection of migratory species (Swanson, 1997) of which some of the species in this study and *L. cylindricus* form part (Skelton, 1993).

Natural diversity among populations or species is not at all times ascertained in terms of size, shape, morphology and colour of individuals (Spellerberg, 1996). There have been several arguments on the relationship between morphological variation and heterozygosity (Mitton and Grant, 1984). Moritz and Hillis (1990) settled the dispute by stating that there is no strict interdependence between the evolution of morphological characteristics and molecular evolution. Different codes are being followed in each, and manoeuvring by different evolutionary pressures is encountered by each (Moritz and Hillis, 1990).

In the light of the above mentioned statements, conservation managers require, therefore, loyal estimates of the genetic structure of populations and the risk of genetic diversity erosion based on ecological background and pollution endurance, in order to manage, conserve or protect natural populations objectively (Heithaus and Laushman, 1997). Conservation of fish populations is also important to the local people living around water bodies, both nutritionally and in splendour (Merron and Bruton, 1993).

Population genetic studies, describe the genetic variation within and between populations and species and events producing or hampering such variation. Population genetic studies examine the genetic behaviour of specified or model populations and even make empirical investigations of genetic variability in and among the populations and species possible (Crozier, 1997). Furthermore, genetic studies experiment and/or make attempts to understand the adaptive significance of genetic diversity (Grant, 1993; Stuart and Gaugler, 1996). To address the above concerns, population genetics studies incorporate conservation biology, ecological dynamics and systematics (Crowe, 1989; Crowe *et al.*, 1989; Jain, 1983; Grant, 1993). Population genetics and ecology are particularly valuable in studying evolutionary processes, both in the short term and long term, and also for predicting the fate of populations (Jain, 1983).

In as much as the environments are changing in form and size, so is the magnitude of genetic variation (Spellerberg, 1996; Dobson *et al.*, 1997). Genetic consideration is, therefore, very crucial for management of both wild and captive populations and for their environments. Furthermore, low genetic variation threatens small populations with their rapid extinction (Spellerberg, 1996; Dobson *et al.*, 1997).

1.7 Morphology

Some species can be recognised at a glance because they are morphologically distinct, even if they occupy the same geographical area. Others are alike but vary in that they occupy different climatic zones, whilst others still are ecologically and morphologically similar (Clausen *et al.*, 1947). Hubby and Throckmorton (1967) maintained that it has been a long-term phenomenon that many different genotypes can and do produce indistinguishable phenotypes. The authors further explained that a high degree of morphological similarity that exists in sibling species is often a result of developmental

homeostasis between them, but not significantly of genetic similarity. Therefore one cannot assert similar morphologies as evidence of similar or dissimilar genotypes.

The morphology of the fish corresponds to their habitat preferences, e.g. small eyes, well developed nasal tentacles and broad united gill membranes are designed for species which live in swiftly flowing water (Reid, 1985). Living fish as a part of aquatic fauna and biological diversity are so diverse that they are complex to define at a glance (Bone *et al.*, 1995). Fish exhibit higher phenotypic variability than any other vertebrates. Such phenotypic variations are environmentally related due to the plasticity that fish have in relation to response to their environmental conditions (favourable or unfavourable). Genotypic diversity is important in the maintenance of the phenotypic diversity among populations, because phenotypic diversity has a genetic basis (Carvalho, 1993). Genetic structure within the populations of all organisms determines their diversity as it is seen (Spellerberg, 1996). Genotypic variation determines the organism's ability to migrate, tolerate pH variations and toxicants. It also determines immunity/resistance to diseases and general stress tolerance (Carvalho, 1993).

Fish are the oldest, most numerous and most diverse group among the vertebrates. They exhibit morphological, physiological and behavioural adaptations with which they occupy diverse water bodies and habitats (Lowe-McConnell, 1988; Moyle and Cech, 2000). Fish face great difficulties in all attempts of moving from one aquatic system to another. Often they stick to one habitat range; compelled to adapt to changes right there, or die (Lévêque, 1997). As a result of this feature, fish are rated among the most appropriate and significant indicators of environmental variables and ecological conditions and subjects for studying trends in aquatic diversity generally. They are selective and sensitive to specific habitat conditions e.g. pollution (Heithaus and Laushman, 1997; Lévêque, 1997; Scheimer, 2000). They

have influence on the biodiversity and biodiversity distribution of other aquatic organisms through predation, competition, mechanical impacts and being preyed upon (Lévêque, 1997).

In order to manage or protect natural populations successfully, conservation managers require reliable estimates of the genetic structure of populations, as well as information on the risk of genetic variation erosion based on ecological impact history and their pollution tolerance (Spellerberg, 1996; Heithaus and Laushman, 1997). Genetic consideration is therefore crucial for management of wild, as well as captive populations, since erosion of genetic variability of small populations threatens the species with possible extinction (Spellerberg, 1996). It is therefore important that managers should plan river resource development with careful consideration of onsite and downstream effects (Brismar, 2002).

1.8 Ecology

Ecology includes the integration of population dynamics, genetics, natural selection and evolution. Ecology and life history are related to the level and the distribution of genetic variation in a population (Lincoln *et al.*, Smith, 1996). Ecological attributes have a direct effect on the genotypic frequencies and therefore genetic variation of populations. Toxicity in the environment selects against certain heterozygotes (Heithaus and Laushman, 1997) and reduced genetic variation is in itself a threat to survival, growth rate (Mitton and Grant, 1988; Andrews and Chilton, 1999), reproductive success and ability to adapt to stress or disturbances in environmental variables (Heithaus and Laushman, 1997). Population genetics and ecological studies become valuable tools, among others, in studying evolutionary processes (both short term and long term) and also in foreshadowing the fate of populations (Jain, 1983).

Species do not exist in pure isolation. Organisms have diversified to be complementary or symbiotic to one another as parasites, prey species, predators, commensals or mutualists (Thompson, 1999). Multitudes of biotic and abiotic attributes are so interconnected that any change in any part of the ecosystem affects the whole to a larger or lesser extent. The stability of ecosystems is enhanced when the diversity (genetically and at all levels of organization) of organisms is maintained (Cutter and Renwick, 1999).

Jackson (1961) indicated that predation can have a profound effect on the evolution of the population. The author demonstrated that predators can kill such large numbers of its prey that there are only a few left for gene transmission to next generations. Inbreeding then becomes imperative and genotypic frequencies become greatly changed. The same perspective is also echoed by Emlen (1977).

A parasite on the other hand is described by Smith (1980), as a miniature predator since it leaves its host either dead or incapacitated. This view is also shared by Barber *et al.* (1995), and Barber and Huntingford (1996). Thus parasitic infection mortalities have an influence on the genotypic frequencies of the population. There are also empirical views that certain parasites can even cause cancer with very high metastasis, thus increasing morbidity and mortality in populations through changes in the composition, the structure and function of the DNA (Campbell, 1997).

Environments are forever changing (Emlen, 1977; Stuart and Gaugler, 1996; Smith, 1996; Spellerberg, 1996). With anthropogenic activities, the changes are more speeded up than they would occur naturally (Gaigher and Pott, 1973; Gaigher *et al.*, 1980; Spellerberg, 1996). Environmental factors affecting populations relate directly to the gene-determined physiological responses (Nei, 1978; Stuart and Gaugler, 1996; Calow and Forbes, 1998) and to gene diversity (dynamics of genes or alleles) (Nei,

1978; Carvalho, 1993; Stuart and Gaugler, 1996; Calow and Forbes, 1998). Adaptation to changing environments is imperative if survival is to be insured. Adaptation itself is a product of natural selection, which results in changes of relative genotypic frequencies, in an attempt to suit the new environment (Smith, 1980).

Environments should therefore not be ignored when formulating, interpreting and comparing studies of organisms (Stuart and Gaugler, 1996). Factors such as poor nutrition, water pH, pollution and genetic factors such as inbreeding depression, genetic drift, or founder effects have adverse genetic and microevolutionary consequences in natural populations (Nei, 1978; Stuart and Gaugler, 1996). All these factors have the potential to reduce the genetic variation in a population. Lack of genetic variation is in itself a constraint violating the population's adaptation capacity in changing environments (Stuart and Gaugler, 1996).

1.9 Distribution

The distribution of organisms gives an account of major historical events or changes in the earth's surface which effected barriers of species distribution and/or migration (McKeown, 1984; Carvalho, 1993; Bone *et al.*, 1995).

Geographical barriers play an important role in shaping patterns of ecological and genetic diversity within and between species. River barriers act as significant impediments to gene flow among populations of aquatic species and act as major drivers of aquatic fauna diversification. These barriers achieve the development of geographical or physical isolation between populations (Carvalho, 1993).

Geographical isolation is one of the major forces affecting differentiation of gene pools in populations (Mayr, 1959; Ribbink and Eccles, 1988). It may be irreversible depending on the ultimate attainment of reproductive or sexual isolation in populations (Dobzhansky, 1937; Mayr, 1959; Jameson, 1977; Smith, 1992; Andrews and Chilton, 1999). Sexual isolation is the product of gene divergence (Dobzhansky, 1937; Koopman, 1950; Selander and Johnson, 1973), and an essential feature of speciation (Wilson *et al.*, 1974). It is acquired during the course of building up of adaptive gene complexes in populations (Dobzhansky, 1937; Koopman, 1950; Selander and Johnson, 1973). Once evolution of sexual isolation has been attained, the gene pools become protected against contamination by other gene pools (Mayr, 1959).

All these mechanisms tend to prevent hybrids from occurring (Dobzhansky, 1937) and promote the process of speciation without any disturbances from hybridization (Wright, 1940).

Different environmental variables, lack of gene flow, or gene exchange through only few migrants substantially enhance population differentiation (Carvalho, 1993). Geographically isolated populations of species collected in similar habitat types (or connected through migration and gene flow) often exhibit greater phenotypic and genetic homogeneity than those collected from dissimilar environments within the same geographical area (Carvalho, 1993; Kyle and Boulding, 1998). Such parallel ecophenotypic characters have been described as resulting from parallel evolution, since such populations' adaptive characters are brought about by similar localized selective pressures (Lavin and McPhail, 1993; Kyle and Boulding, 1998). Responses to environmental factors are governed by genotypic-environment interactions (Carvalho, 1993).

Distribution of organisms may also be brought about by a complex of eco-genotypic factors. These factors include environmental variables such as food availability, diseases, parasites, predators, pH, O₂, temperature, salinity, genetic drift and selective pressures. Furthermore levels of variability in ecologically significant traits, population size, genetic drift, number of founders and historical events causing barriers may also have influence on spatial dissimilarities among and within the species (Carvalho, 1993).

1.10 Pollution

Droughts are very common in the SADC states and water scarcity can be very severe. Due to aridness, more than 400 million Africans in the Sub-Saharan region are without access to pure water (Kirby, 2004; TVE's Earth Report, 2004; Country Reports, 2004; Moncel, 2004; Mpande and Tawanda, 2004). Compounding water scarcity is pollution, making the very scantily available water in most river systems not suitable for use. Agriculture and demographic pressure increase tension or stress on the resource (Country Reports, 2004; Moncel, 2004). While ground water may be used in times of severe surface water shortage, depleting it is disastrous, since rivers, wetlands and lakes which depend on it for a refill can dry out. Salty sea water may then flow in, to replace ground water and make the terrestrial and freshwater zones more unfit for life (Kirby, 2004).

Human activities, through agriculture and urbanization have constituted very dramatic transformation of the landscapes and ecosystems. These activities have resulted in habitat destruction, which adversely affects species survival through various ways (Jain, 1983; Lafferty, 1997; Cutter and Renwick, 1999). It has also been observed that through such interventions, many river systems have been intensely affected by toxic pollutants and that such toxicity is directly correlated with reproductive problems of the species (Cutter and Renwick, 1999; Weis *et. al.*, 1999). Pollution is among the major anthropogenic

influences on populations, leading to greater stress and increasing dispersal (translocations and introductions). Dispersal ultimately results in greater intermixing of gene pools between species/hybridization (Jain, 1983; Allendorf, 1991) and may even form new taxa (Jain, 1983). Introductions are also harmful to the native fish and other organisms through predation, disease introduction and competition (Allendorf, 1991).

Gaigher (1973) reported that hazards of river pollution due to agriculture, industry and drought, compounded by extraction of water to dams and manmade lakes, pose a very serious threat to the fish survival in east flowing rivers of the Transvaal (now Mpumalanga and Limpopo Provinces). Aquatic pollutants incorporate nutrients, which contribute to eutrophication problems (Weis *et al.*, 1999). Toxicity influences the aquatic populations holistically. It handicaps genetic expression in populations (Heithaus and Laushman, 1997) as well as all physiological, morphological, growth, reproductive and behavioural activities (Weis *et al.*, 1999). The most vulnerable of the species are those which depend on well oxygenated water for existence (Gaigher 1973; Weis *et al.*, 1999).

Pollution correlates directly with changes in genotypic frequencies (Jain, 1983; Heithaus and Laushman, 1997). Prolonged exposure to pollution has an eroding effect on genetic variation at the *PGM*, *MDH*, *IDHP*, *GPI*, and other related loci (Heithaus and Laushman, 1997). These authors stated that acid and heavy metal poisoning are closely linked to lowered heterozygosity in most fish populations, a perspective echoed by Henry *et al.*, (1999).

Not only does pollution affect the organism's chemical metabolism, but it also has profound effects on the cultivation of certain pathogens (Lafferty, 1997) and on algal blooms, thus lowering the oxygen tension in water (Weis *et al.*, 1999). Pollution has differential life span effects on a variety of organisms.

It may cause some to thrive while being fatal to others (Lafferty, 1997). Pollution can cause invertebrate prey populations to decline thereby decreasing the protein supplement for the fish (Heithaus and Laushman, 1997). It may also impair both prey capture in predatory species and predator escaping mechanisms in the prey (Weis *et al.*, 1999). Furthermore effects of pollution involve evolutionary processes (both short term and long term) and population genetics and ecology are an effective machinery to study such processes, providing valuable data for projecting the fate of populations (Jain, 1983).

1.11 Hybridization

Isolating mechanisms (mechanisms preventing the interbreeding of species complexes) responsible for attainment of reproductive isolation and genetic integrity exist in nature in order to prevent disintegration of gene patterns due to unlimited hybridization. Such mechanisms are necessary to allow uninterrupted molecular evolution (Dobzhansky, 1937; Speith, 1949).

Fossil records suggest that hybridization has always occurred in natural populations (Smith, 1992; Billington *et al.*, 1996), and that it is a widespread occurrence among fresh water fishes (Smith, 1992; Moyle and Cech, 1996). Hybridization is even more common among cyprinid species (Buth *et al.*, 1991). Intrageneric and interfamilial hybridization was also noted to be even possible between Claridae and Cyprinidae. A typical example of such hybridization is interbreeding between *Labeo rohita*, *Clarias mrigala* and *Clarias catla* which occurs naturally (Padhi *et al.*, 1998).

Hybridization often brings about an unstable genetic situation in the population (Templeton, 1980). The instability can lead to rapid speciation (Templeton, 1980; Moyle and Cech, 1996) through evolution of

systems that conserve hybrid states and restrain hybrid breakdown (Templeton, 1980). The new species formed can be, for example unisexual mollies or tetraploids in fish (Moyle and Cech, 1996; King and Stanfield, 1997).

Interspecific hybridization is influenced primarily by the gene regulatory systems between parental genomes (Wilson *et al.*, 1974). The degree of similarity of gene pools of parental species plays a major role in the successful development of an interspecific zygote (Wilson *et al.*, 1974; Smith, 1992).

Various speculative ideas have been advanced for introgression and hybridization (Smith, 1992). There are speculations that fertile hybrids and their backcrosses (hybrids and parental species) are a source of genetic variation among evolving fish populations (Smith, 1992). Padhi *et al.* (1998) however indicated that F₁ hybrids that backcross with the parental species impose a threat of reduction of genetic variation in these species through introgression. The authors investigated this through DNA, Mbo II satellites.

Hybridization, which sometimes occurs between similar African *Labeo* species confuses the taxonomy within the genera (Reid, 1985) and because it is often very difficult to distinguishing hybrids from parental species on morphological basis (Billington, 1996), molecular studies are a necessity for the detection of hybrids (Buth *et al.*, 1991; Billington, 1996).

There are various ways of characterizing hybrids between species. Smith (1992) and Billington (1996), indicated that fixed diagnostic electromorphs in parental species and hybrids may be considered good indicators of introgression. Wilson *et al.* (1974) stated that fibrinopeptides do not differ much within hybridisable species pairs. The authors also discovered that in fish, glucose-6-phosphate

dehydrogenase is encoded exclusively by the paternal alleles and that expression of the maternal alleles is completely repressed. The allelic repression was more common in extreme hybrids and directly correlated with greater taxonomic distance between parental species.

1.12 Genetic structure analysis

Electrophoresis has been the method of choice in most population genetics studies (May and Marsden, 1992; Grant, 1993; Ramshaw *et al.*, 1996; Sabeh *et al.*, 1996; Baillie *et al.*, 1998). It is among the most objective tools in identifying, comparing and discriminating between cryptic species (Selander and Johnson, 1973; Smith, 1976; Grant *et al.*, 1988; Menezes and Naiks, 1993; Oosthuizen *et al.*, 1993), in detecting hybrids (Moritz and Hillis (1990) and hybrid zones (Grant, 1993) and in quantifying gene flow (Buth *et al.*, 1991, Lewis *et al.*, 2000). It can be highly valuable too in assigning proper taxonomic status in populations (Scobie and Mackie, 1995). Variation in a large number of loci can be studied, even if only a few individuals are available. This becomes of utmost importance when we consider the scarcity of some of the populations in question (Gorman and Renzi, 1979).

Within the limitations of the technique, electrophoresis accompanied by staining techniques for specific enzymes and other proteins allows for the determination of the genotype of each individual for a selection of specific enzymes (Keenleyside, 1991). Data so obtained is useful for the investigation of genetic variability within and among natural populations (Grant, 1984; Grant *et al.*, 1988; May and Marsden, 1992). It can be used for differentiating species and populations from a large variety of taxa (Shaklee and Tamaru, 1981; May and Marsden, 1992) and in the description of genetic relationships among closely related, geographically isolated species (Grant 1984; Grant *et al.*, 1988). However the success of its use depends on the proper interpretation of the results (Quicke, 1993). It is worth noting

that not all allelic variation is detectable through electrophoresis (Selander and Johnson, 1973). Nevertheless the results of a moderate number of enzyme loci can be extended to the entire genome of the organism though, since loci studied may be assumed to represent a random sampling of the genome in terms of allelic frequencies (Ayala *et al.*, 1974).

Grant (1993) has indicated that there is limited allozyme data available for the animal populations in southern Africa. With little more than representative genetic research done on the Cyprinids (Family Cyprinidae) (Buth, 1984), and far less on the genus *Labeo*, it generates reason to investigate the biodiversity of these resources at a genetic level. The fundamental question is: “what is the relative contribution of genetic and environmental influences to morphological, ecological and behavioural differences in species from this genus?”

Generally there exists very little genetic differentiation between local populations. Therefore, to quantify genetic differentiation between populations, two statistical parameters have been used; the Nei's (1978) genetic distance coefficients (D) and F-statistics (F_{ST}).

According to Nei (1978) the average genetic distance per locus is:

$$D = -\log I$$

Where I represents the mean genetic similarity per locus between the given populations x and y , and

$$I = I_{xy} / (I_x \cdot I_y)^{1/2}$$

$$= \text{means of } 3x_i y_i, 3x_i^2 \text{ and } 3 y_i^2$$

x_i and y_i being the frequencies of the i th allele in the populations x and y , respectively.

At most loci (92%), populations in the same geographical area have essentially identical allelic frequencies ($I = 0.95-1.00$). Only at few loci do they have considerably different genetic frequency (Ayala *et al.*, 1974).

The average genetic distance (D) represents a measure of the average number of codon substitutions per locus (those that are electrophoretically detectable) which have accumulated since populations have separated from the common ancestor (Ayala *et al.*, 1974).

The degree of differentiation among populations is represented by the following equation:

$$F_{ST} = 1 / (1 + 4 N_{em})$$

Where $N_{em} = n / (n - 1)^2$, n is the number of populations analysed and N_{em} is the effective number of individuals exchanged between populations each generation.

CHAPTER 2: Review of genetic variation of fresh water fish from randomly selected water bodies in the Southern African Development Community.

2.1 Introduction

The Southern African Development Community (SADC) was born from Southern African Development Coordination Conference (SADCC) following the Lusaka declaration on economic liberation in 1980 and established by only nine states. The SADCC was replaced in 1992 by the SADC in a declaration and a treaty signed at the summit in Namibia (SADC Facts, 2004). Currently the Southern African Development Community comprises 14 countries; Angola, Botswana, Democratic Republic of Congo (DRC), Lesotho, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe (SADC Countries database SADC/FSTAU & FAO/GIEWS, 2004; SADC Facts, 2004; Shoko, 2004).

Each member state has the responsibility to coordinate one or more sectoral activities on behalf of SADC; for example, Lesotho is responsible for environment, land and water management; Malawi, inland fishery, forestry and wildlife; South Africa, finance and investments (SADC Sectoral Responsibilities, 2004). According to the treaty signed, each member state is legally bound to coordinate their respective policies and plans for sustainable development in all areas of human activity (SADC facts. 2004).

Seven river basins, i.e. the Congo, Zambezi, Okavango, Limpopo, Orange-Vaal, Olifants, Ruvuma and Cunene, with catchment areas of up to 6.76 million km² maximally, are shared by at least two of the twelve countries (Mauritius and Seychelles excluded). The Zambezi River is shared by Angola, Botswana, Malawi, Mozambique, Namibia, Tanzania, Zambia and Zimbabwe and has therefore become the focus of most attention over years (Mpande and Tawanda, 2004; Shoko, 2004).

Environmental ailments affecting the catchments are deforestation, soil erosion and silting and air and land pollution through agriculture and mining activities. The mining industry consists of coal, chromium, asbestos, gold, copper, nickel, iron ore, vanadium, lithium, tin and platinum, producing effluents which have highly detrimental effects on the environment, in particular rivers and water bodies (Country Reports, 2004).

The discharge of high levels of metal waste over decades has resulted in siltation and destruction of aquatic habitats in the tributaries of the Zambezi River. Pollution control measures in most mines are either lacking or inadequately designed and consequently sulphates, heavy metals and pH unstabilized mine sludge are discharged into the river basin. The by-products of hydroelectrification, e.g. excessive heat energy, also get discharged into the river basin (Dixon *et al.*, 2004).

Pollution in the Zambezi River is further generated actively by the rapidly increasing population growth, intensive urbanisation and industrial and agricultural activities. Industry in particular is a source of massive pollution with its effluents discharged into the water bodies, illegally dumped wastes washed into the river and pollutants emitted into the air, affecting the quality of rain and of respired air. Many industrial pollutants include pulp-and-paper mills, the steel industry and abattoirs, all producing noxious chemical effluents. It must be borne in mind that these effluents affect even the groundwater (SARDC-IMERCSA. 2004) Pollution leads to changes in the ecosystems. A changed ecosystem may so become unstable or detrimental to the environment and to the populations sustained by the environment (Klijn, 1995).

The Limpopo River catchment was affected by severe drought between the years 1980 and 1990 and this situation was intensified by upstream water extraction (Mpande and Tawanda, 2004). The Incomati and the Limpopo are in semi arid riparian regions of the SADC. The aridness is worsened by

increasing population growth that has lead to the erection of a water reservoir infrastructure and irrigation schemes in the catchments (BBC News, 2004).

South Africa and Mozambique embarked on an investigation into the pollution that affects the river systems shared by these two states. This investigation was held because of the reported industrial pollution into the Limpopo River catchment area that led to birth defects and killing of animals (terrestrial and aquatic) inhabiting the catchment region. Humans suffered renal and gastrointestinal ailments, stomatitis and other severe physical defects, while plants/crops and riparian vegetation died as well (WildNet Africa News Archive, 2004).

The Okavango River system remains one of the few undeveloped systems to date. However this is for the good of the world heritage site, the Okavango Delta (TVE's Earth Report, 2004), which hosts some of the highest biodiversity in the Sub-Saharan region. The biodiversity in this river basin is endangered by the use of its inflowing water for unsustainable development purposes and by lack of adequate management of tourism, local participation and sustainable use of the resource (Monna, 2004).

2.2 Inter-Catchment or Inter-Basin Water Transfers

Inter-basin water transfer (IBT) is generally defined as water transfer from one geographically distinct river catchment or basin to another, or from one river reach to another even within the same river system (Snaddon, *et al.*, 1999).

In most arid environments, inter-basin water transfers (IBTs) are frequently perceived as the most workable solution to problems associated with available aquatic resources in relation to human population needs and demands. In most parts of the world, water transfer schemes have become the

life blood, rescue and resuscitation of the developing and existing human settlements, for which no alternative seems to exist (Snaddon, *et al.*, 1999). In countries with very high rainfall the IBTs are primarily used to augment the power generation potential of hydro-electric schemes, while in arid and semi-arid countries, IBTs supply potable and irrigation water (Snaddon, *et al.*, 1999).

There are several water transfer schemes in South Africa, e.g. the Tugela-Vaal, Caledon-Modder and Mooi-Mgeni River schemes (Snaddon, *et al.*, 1999), just to mention a few. Of much and more recent interest to South Africans is the Lesotho Highlands Water Project IBT.

South Africa is renowned for its semi aridness and complete aridness in certain parts of the country. Lesotho on the other hand has water as a relatively abundant natural resource. The Republic of South Africa has shown some interest in acquiring and utilizing the water from Lesotho Highlands to meet the growing demand of industry and domestic life. A joint venture between Lesotho and South Africa led to the building of networks and channels in the remote parts of Lesotho to channel rain water to South Africa, for both industrial and domestic purposes. The dams trap water that normally runs through the Orange River basin (which originates in Lesotho) and within its catchment and turn it north into the Vaal River system towards Gauteng Province (International Rivers, 2005; TED Case studies, 2002).

The Lesotho Highlands Water Project (LHWP) has dual objectives: to turn Lesotho's water into export revenue and to generate enough hydro-electricity to supply Lesotho's power needs and reduce its dependence on South Africa for power supply. This development however noble and beneficial to both Lesotho and South Africa as it appears was not without eco-environmental detriments. The project carries with it cause for environmental damage, in terms of populations dislocation, grazing land flooding and degradation of the site by tourists (TED Case studies, 2002).

2.3 Impacts of Inter-Basin Water Transfers

Prior to deliberation on the impacts of any particular project, consideration must be given to the fact that the project has both beneficial and adverse effects. It all depends on which one outweighs the other. The outweighed project falls off if compliance with the EIA is maintained.

For development of proposed IBT pre-and post-transfer ecological assessment surveys have rarely been undertaken to date (Snaddon, *et al.*, 1999). Proposals for IBTs, world-wide, have been made despite lack of knowledge of ecological and environmental effects of such schemes (Snaddon, *et al.*, 1999; Pottinger, 2003). The LHWP was no exception.

Impacts of IBTs include earthquakes or Reservoir-Induced Seismicity (McCully, 2003; International Rivers, 2005), conflict over water transfers e.g., guerilla warfare occurred in Lesotho over selling Lesotho's water to South Africa (Pottinger, 2003) and the Zambezi's Cahora Bassa project, which led to habitat destruction for humans, fauna and flora and became the source of FRELIMO guerilla warfare (Bourgeois *et al.*, 2003/4). In Lesotho the effects of IBTs also included effacement of species from their native habitats e.g., the apparent disappearance of the umbraculate frog (*Rana vertebralis*) which was an important indicator species for water quality from Lesotho waters (Pottinger, 2003). The Maloti minnow (*Pseudobarbus quathlambae*), rock catfish (*Austroglanis sclateri*), the renowned rare and endangered species of bearded vulture, (*Gypaetus barbatus*), also lost habitat to the project and the threatened endemic spiral aloe (*Aloe polyphylla*) and various bird species which nest in the area were affected by the project (Pottinger, 2003).

Changes in the water quality below the dam include alterations in sediments, oxygen levels, nutrients and water temperature. In addition, the initiation of a transfer out of the donor catchment may result in

a decrease or permanent loss of water in that catchment, to the detriment of its future development, since water is a finite resource within any catchment. Substantial reductions and fluctuations of water flow in donor catchments due to decreases in riparian vegetation, erosion and transport of sediment down the river, may also occur. There may also be changes in the riverine flora and fauna (Snaddon, *et al.*, 1999).

The TED Case studies (2002) claims a small likelihood that Katse reservoir water may cause major problems with transfer of water weeds or water borne disease vectors into the recipient system. With the LHWP in particular, no major impacts on the ecology of the Vaal River were expected to result from the transfer of water from the Sequ catchment of the Orange River system, since the waters of both systems originate from the same geological base (TED Case studies, 2002).

The river systems in the SADC host a high diversity of flora and fauna. Among these are a variety of fish species. Fish in the SADC are subject to different environments and habitats. Organisms almost always respond genetically to changing environments (Hoffman and Parsons, 1997). Together with extrinsic barriers, environmental variables have profound effects on the integrity of genetic structure for each population (Templeton, 1980; Kirpichnikov, 1992). Among these organisms, fish represents the oldest and most diversified of the vertebrates (Love and Calliet, 1979; Heithaus and Laushman, 1997) and it therefore becomes most relevant in studying evolutionary or genetic structures of populations. Sensitivity of aquatic fauna's genetic variation to pollution also augments the suitability of genetic variation to most environmental research (Heithaus and Laushman, 1997). For conservation purposes, genetic variation of a population must be preserved as far as possible, since it maximises the population's potential to adapt to, or withstand, unpredictable environmental changes and stresses (Hoffman and Parsons, 1997).

Genetic variation can be characterised by two measures:

1. Proportion of polymorphic loci.
2. Mean heterozygosity levels in a population.

It must be emphasised though, that even if it is true that high genetic variation is protective of the population integrity for survival and reproduction, the high genetic variation strategies of a population will have very little impact on the survival of the population if the entire ecosystem is severely compromised by pollution. Extreme environmental change has an impact on the expression of phenotypic and genotypic variation in a population. Environmental changes potentiate periods of intensive natural selection and this selection may lead to characteristics which may or may not be favoured by the environment. These characteristics, when interacting with the environmental variables may influence the population size, resulting in maladaptation, speciation or extinction (Hoffman and Parsons, 1997).

The changing environmental variables prompt for their impact on the fauna and flora depending on the environment. This review attempts to disseminate the information on patterns of genetic structure of fish across the riparian zones of major river basin catchments in the SADC regions. The genetic structure used in this study consists of allozyme genetic variation.

2.4 Materials and methods

A variety of bibliographies and reference sections of retrieved articles for studies on genetic structure of freshwater fishes from major river basins and their tributaries in southern Africa, were assembled and tabulated.

Consideration was given to populations for which information on allozyme genetic variation (specifically average heterozygosity values) was available. From each study, information extracted comprised of river systems, rivers and/or sampling localities, species, number of loci, number of individuals per population, average heterozygosity values and source references.

The data was initially grouped for river systems, for their tributaries and for populations of species. This data was analyzed using SPSS software for statistical analysis. SPSS (Statistical Package for Social Sciences) contains a comprehensive range of statistical procedures and extensive facilities for file manipulations and recoding transformation of data (Everitt, 1998).

The grouping was then done according to regions in which river systems form catchments. The descriptive analysis of variance data was therefore processed from the mean heterozygosity values using One Way Analysis of Variance (One Way Anova). Repeated measures of Anova to assess significant relationship (differences or similarities) in genetic variation between the populations in river systems were used. Where population sizes analyzed were not equal in number, the significant differences in the mean heterozygosity values observed between populations necessitated more statistical qualification.

The data was finally pooled according to river basin catchments. To compromise between having sufficient number of studies (for comparison sake) and ensuring that several populations have been considered, only studies providing information from at least ten (10) populations per river system were considered and the rest were not considered.

What remained significantly different from the variance of means was further qualified using the Bonferroni correction test for pairwise intercatchment comparison. It allowed comparison between the major river basin catchments.

ANOVA (analysis of variance) is a total variation displayed by a set of observations, as measured by the sums of squares of deviation from the mean. It may in certain circumstances be separated into components associated with defined sources of variation as criteria of classification for observation (Marriott, 1991).

Table 1. Genetic variability in different localities of Southern African fresh water fish from the SADC region. Fields include river systems, rivers and/or sampling localities, species and sample size, number of loci, levels of heterozygosity and reference sources.

River system	River &/ Sampling locality	Species	Number		H _E	Source
			Loci	Individuals		
Breë	Brandvleidam	<i>Barbus andrewi</i>	31	50	0.05	Mulder (1989)
Budzi	Budzi	<i>Tilapia rendalli</i>	38	13	0.00	Feresu-Shonhiwa & Howard (1998)
Budzi	Budzi	<i>Oreochromis mossambicus</i>	38	15	0.04	Feresu-Shonhiwa & Howard (1998)
Cowie	Bloukrans	<i>Barbus anoplus</i>	25	10	0.07	Engelbrecht, G. D. (2001)
Cowie	Cowie	<i>Barbus pallidus</i>	29	40	0.05	Engelbrecht, G. D. (2001)
Incomati	Crocodile	<i>Barbus pallidus</i>	25	30	0.02	Engelbrecht, J. S. & Van der Bank (1996, a)
Incomati	Incomati	<i>Barbus polylepis</i>	31	23	0.04	Mulder (1989)
Incomati	Usuthu	<i>Barbus polylepis</i>	31	50	0.11	Mulder (1989)
Incomati	Crocodile	<i>Barbus anoplus</i>	25	31	0.04	Engelbrecht, J. S. (1996)
Incomati	Marite	<i>Barbus brevipinnis</i>	25	30	0.01	Engelbrecht, J. S. & Van der Bank (1996, b)
Incomati	Sands	<i>Barbus brevipinnis</i>	25	30	0.06	Engelbrecht, J. S. & Van der Bank (1996, b)
Incomati	Crocodile	<i>Barbus neefi</i>	29	36	0.03	Engelbrecht, G. D. (2001)
Incomati	Crocodile	<i>Barbus brevipinnis</i>	29	50	0.02	Engelbrecht, G. D. (2001)
Incomati	Crocodile	<i>Chiloglanus anoterus</i>	32	27	0.11	Engelbrecht, G. D. & Mulder (2001)
Limpopo	Letaba	<i>Barbus viviparus</i>	21	42	0.05	Engelbrecht, G. D. (2001)

CHAPTER 2

Limpopo	Letaba	<i>Labeo congoro</i>	29	50	0.03	Van Vuuren (1989)
Limpopo	Crocodile	<i>Barbus mattozi</i>	31	35	0.22	Mulder (1989)
Limpopo	Crocodile	<i>Tilapia sparmanii</i>	40	34	0.03	Van der Bank <i>et al.</i> (1989)
Limpopo	Mogalakwena	<i>Labeo ruddi</i>	29	50	0.01	Van Vuuren (1989)
Limpopo	Mogol	<i>Schilbe intermedius</i>	10	50	0.00	Engelbrecht, J. S., <i>et al.</i> (1994)
Limpopo	Origstad	<i>Barbus motebensis</i>	25	33	0.05	Engelbrecht, J. S., & Van der Bank (1996, a)
Limpopo	Origstad	<i>Barbus neefi</i>	25	30	0.03	Engelbrecht & Van der Bank (1996, b)
Limpopo	Marico	<i>Barbus motebensis</i>	25	30	0.04	Engelbrecht & Van der Bank (1996, a)
Limpopo	Selons	<i>Barbus neefi</i>	29	36	0.00	Engelbrecht, G. D. (2001)
Limpopo	Dorps	<i>Barbus neefi</i>	29	34	0.05	Engelbrecht, G. D. (2001)
Limpopo	Spekboom	<i>Barbus neefi</i>	29	34	0.04	Engelbrecht, G. D. (2001)
Limpopo	Blyde	<i>Barbus neefi</i>	29	50	0.07	Engelbrecht, G. D. (2001)
Limpopo	Makutswi	<i>Barbus neefi</i>	29	46	0.06	Engelbrecht, G. D. (2001)
Limpopo	Mutamba	<i>Barbus neefi</i>	29	50	0.04	Engelbrecht, G. D. (2001)
Limpopo	Mogalakwena	<i>Barbus brevipinnis</i>	25	21	0.04	Engelbrecht, G. D. (2001)
Limpopo	Palala	<i>Barbus brevipinnis</i>	29	46	0.03	Engelbrecht, G. D. (2001)
Limpopo	Palala	<i>Barbus brevipinnis</i>	25	50	0.03	Engelbrecht, G. D. (2001)
Limpopo	Palala	<i>Barbus bifrenatus</i>	29	46	0.00	Engelbrecht, G. D. (2001)
Limpopo	Grootspuit	<i>Barbus brevipinnis</i>	25	21	0.03	Engelbrecht, G. D. (2001)

CHAPTER 2

Limpopo	Pienaars	<i>Clarius gariepinus</i>	18	27	0.05	Van der Bank <i>et al.</i> (1992)
Limpopo	Pienaars	<i>Chetia flaviventris</i>	40	64	0.02	Van der Bank <i>et al.</i> (1989)
Limpopo	Pienaars	<i>Oreochromis mossambicus</i>	40	91	0.02	Van der Bank <i>et al.</i> (1989)
Limpopo	Mwenezi	<i>Oreochromis mossambicus</i>	38	23	0.05	Feresu-Shohiwa & Horward (1998)
Limpopo	Limpopo	<i>Oreochromis mossambicus</i>	38	24	0.03	Feresu-Shonhiwa & Horward (1998)
Limpopo	Limpopo	<i>Chiloglanus paratus</i>	32	50	0.02	Engelbrecht, G. D., & Mulder (2000)
Limpopo	Limpopo	<i>Tilapia rendalli</i>	38	19	0.00	Feresu-Shonhiwa & Howard (1998)
Limpopo	Limpopo	<i>Tilapia rendalli</i>	10	26	0.07	Lizemore <i>et al.</i> (1989)
Limpopo	Olifants	<i>Mesobola brevianalis</i>	27	60	0.01	Engelbrecht, G. D., & Mulder (1999, b)
Limpopo	Olifants	<i>Labeo rosae</i>	29	50	0.08	Van Vuuren (1989)
Limpopo	Olifants	<i>Labeo cylindricus</i>	29	50	0.10	Van Vuuren (1989)
Limpopo	Olifants	<i>Labeo molybdinus</i>	29	42	0.11	Van Vuuren (1989)
Limpopo	Olifants	<i>Glossogobius callidus</i>	30	57	0.01	Engelbrecht, G. D., & Mulder (1999, a)
Limpopo	Olifants	<i>Hydrocynus vittatus</i>	25	40	0.05	Kotze <i>et al.</i> (1998)
Limpopo	Spekboom	<i>Barbus polylepis</i>	31	20	0.09	Mulder (1989)
Limpopo	Olifants	<i>Barbus mattozi</i>	31	15	0.22	Mulder (1989)
Limpopo	Olifants	<i>Barbus marequensis</i>	31	50	0.11	Mulder (1989)
Limpopo	Origstad	<i>Barbus neefi</i>	25	30	0.03	Engelbrecht, J. S. (1996)
Limpopo	Politsi	<i>Barbus lineomaculatus</i>	29	34	0.02	Engelbrecht, G. D. (2001)
Limpopo	Olifants	<i>Chiloglanis paratus</i>	32	48	0.03	Engelbrecht, G. D., & Mulder (2000)

CHAPTER 2

Limpopo	Olifants	<i>Chiloglanis pretoria</i>	32	46	0.02	Engelbrecht, G. D., & Mulder (2000)
Mbashe	Xuka	<i>Barbus amatolicus</i>	25	10	0.11	Engelbrecht, J.S., & Van der Bank (1996, a)
Mgeni river	Msinduzi	<i>Barbus gurneyi</i>	25	10	0.02	Engelbrecht, G. D. (2001)
Mzimvubu	Inxu	<i>Barbus anoplus</i>	25	10	0.04	Engelbrecht, J.S. (1996)
Nata	Gwavazabuya	<i>Oreochromis mossambicus</i>	38	20	0.07	Feresu-Shonhiwa & Horward (1998)
Nata	Amanzamnyama	<i>Oreochromis mossambicus</i>	38	27	0.04	Feresu-Shonhiwa & Horward (1998)
Nhlabane	Nhlabane	<i>Glossogobius callidus</i>	30	49	0.07	Engelbrecht J. S. & Van der Bank (1996, b)
Nhlabane	Nhlabane	<i>Glossogobius callidus</i>	30	49	0.03	Engelbrecht & Mulder (1999, b)
Olifants (Cape)	Koebee	<i>Barbus serra</i>	31	40	0.11	Mulder (1989)
Olifants (Cape)	Koebee	<i>Barbus capensis</i>	31	42	0.14	Mulder (1989)
Olifants (Cape)	Koebee	<i>Labeo seeberi</i>	29	50	0.02	Van Vuuren (1989)
Orange-Vaal	Mooi	<i>Barbus pallidus</i>	29	33	0.11	Engelbrecht, G. D. (2001)
Orange-Vaal	Bleskopspruit	<i>Barbus anoplus</i>	25	25	0.08	Engelbrecht, J. S. (1996)
Orange-Vaal	Bleskopspruit	<i>Barbus pallidus</i>	29	60	0.10	Engelbrecht, G. D. (2001)
Orange-Vaal	Vaal	<i>Barbus aeneus</i>	31	50	0.13	Mulder (1989)
Orange-Vaal	Vaal	<i>Barbus kimberleyensis</i>	31	40	0.12	Mulder (1989)
Orange-Vaal	Vaal	<i>Labeo capensis</i>	29	50	0.08	Van Vuuren (1989)
Orange-Vaal	Vaal	<i>Labeo umbratus</i>	29	50	0.03	Van Vuuren (1989)
Orange-Vaal	Sands	<i>Barbus pallidus</i>	29	36	0.07	Engelbrecht, G. D. (2001)
Otjikoto	Guinas, sink hole	<i>Tilapia guinasana</i>	26	50	0.07	Oosthuizen <i>et al.</i> (1993)

CHAPTER 2

Otjikoto	Guinas, sink hole	<i>Tilapia guinasana</i>	30	50	0.07	Oosthuizen (1992)
Pongola	Pongola	<i>Barbus polylepis</i>	31	20	0.07	Mulder (1989)
Pongola	Usuthu	<i>Barbus polylepis</i>	31	50	0.11	Mulder (1989)
Pongola	Manzaan	<i>Barbus pallidus</i>	25	10	0.03	Engelbrecht (1996)
Save	Tokwe	<i>Tilapia rendalli</i>	38	21	0.18	Feresu-Shonhiwa & Horward (1998)
Save	Tokwe	<i>Oreochromis mossambicus</i>	38	27	0.04	Feresu-Shonhiwa & Horward (1998)
Save	Runde	<i>Tilapia rendalli</i>	38	13	0.02	Feresu-Shonhiwa & Horward (1998)
Save	Runde	<i>Oreochromis mossambicus</i>	38	12	0.05	Feresu-Shonhiwa & Horward (1998)
Save	Runde	<i>Oreochromis placidus</i>	38	23	0.04	Feresu-Shonhiwa & Horward (1998)
Save	Ruti	<i>Oreochromis mossambicus</i>	38	25	0.09	Feresu-Shonhiwa & Horward (1998)
Save	Runde	<i>Oreochromis mossambicus</i>	38	24	0.03	Feresu-Shonhiwa & Horward (1998)
Swartkops	Swartkops	<i>Barbus pallidus</i>	29	40	0.07	Engelbrecht, G. D. (2001)
Tugela	Spectaclesspruit	<i>Barbus pallidus</i>	29	42	0.09	Engelbrecht, G. D. (2001)
Tugela	Buffels	<i>Barbus anoplus</i>	25	10	0.03	Engelbrecht, G. D. (1996)
Tugela	Bloukrans	<i>Barbus anoplus</i>	25	10	0.07	Engelbrecht G. D. <i>et al.</i> , (1997)
Tugela	Buffels	<i>Barbus pallidus</i>	25	10	0.06	Engelbrecht, J. S., & Van der Bank (1996, b)
Tugela	Buffels	<i>Labeo rubromaculatus</i>	29	50	0.06	Van Vuuren (1989)
Tugela	Buffels	<i>Barbus natalensis</i>	31	50	0.14	Mulder (1989)
Zambezi	Upper Zambezi	<i>Marcosenius marcolapidotus</i>	52	50	0.09	Van der Bank & Van der Bank (1995)
Zambezi	Upper Zambezi	<i>Hydrocynus vittatus</i>	25	35	0.02	Kotze <i>et al.</i> (1998)

CHAPTER 2

Zambezi	Upper Zambezi	<i>Schilbe intermedius</i>	60	60	0.03	Engelbrecht, J. S. <i>et al.</i> (1994, 1997)
Zambezi	Upper Zambezi	<i>Tilapia rendalli</i>	10	52	0.06	Lizemore <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Hemichromis elongatus</i>	40	106	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Oreochromis andersonii</i>	40	75	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Oreochromis andersonii</i>	38	10	0.06	Feresu-Shonhiwa & Horward (1998)
Zambezi	Upper Zambezi	<i>Oreochromis andersonii</i>	38	17	0.03	Feresu-Shonhiwa & Horward (1998)
Zambezi	Upper Zambezi	<i>Oreochromis macrochir</i>	40	103	0.03	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Oreochromis macrochir</i>	38	9	0.07	Feresu-Shonhiwa & Horward (1998)
Zambezi	Upper Zambezi	<i>Oreochromis macrochir</i>	38	34	0.06	Feresu-Shonhiwa & Horward (1998)
Zambezi	Upper Zambezi	<i>Pharyngochromis darlingi</i>	40	5	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis carlottae</i>	40	34	0.03	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis codringtonii</i>	40	24	0.04	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis angusticeps</i>	40	92	0.01	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis giardi</i>	40	24	0.01	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis macrocephalus</i>	40	80	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis robustus jallae</i>	40	15	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Serranochromis thunbergi</i>	40	5	0.02	Van der Bank <i>et al.</i> (1989)
Zambezi	Upper Zambezi	<i>Synodontis leopardinus</i>	51	35	0.09	Van der Bank (1993)
Zambezi	Upper Zambezi	<i>Tilapia sparmanii</i>	38	11	0.04	Feresu-Shonhiwa & Horward (1998)
Zambezi	Mazoe	<i>Oreochromis mossambicus</i>	38	14	0.03	Feresu-Shonhiwa & Horward (1998)

CHAPTER 2

Zambezi	Sinamatella	<i>Oreochromis mortimeri</i>	38	15	0.02	Feresu-Shonhiwa & Horward (1998)
Zambezi	Gwai	<i>Oreochromis mortimeri</i>	38	30	0.06	Feresu-Shonhiwa & Horward (1998)
Zambezi	Mlibizi	<i>Oreochromis mortimeri</i>	38	10	0.06	Feresu-Shonhiwa & Horward (1998)
Zambezi	Mwenda	<i>Oreochromis mortimeri</i>	38	6	0.06	Feresu-Shonhiwa & Horward (1998)
Zambezi	Lake Kariba	<i>Oreochromis mortimeri</i>	38	14	0.03	Feresu-Shonhiwa & Horward (1998)
Zambezi	Deka	<i>Oreochromis mortimeri</i>	38	23	0.03	Feresu-Shonhiwa & Horward (1998)
Zambezi	Hippo	<i>Tilapia rendalli</i>	38	15	0.02	Feresu-Shonhiwa & Horward (1998)
Zambezi	Deka	<i>Tilapia rendalli</i>	38	17	0.01	Feresu-Shonhiwa & Horward (1998)
Zambezi	Middle Zambezi	<i>Tilapia rendalli</i>	38	13	0.04	Feresu-Shonhiwa & Horward (1998)
Zambezi	Middle Zambezi	<i>Oreochromis mortimeri</i>	38	15	0.04	Feresu-Shonhiwa & Horward (1998)
Zambezi	Middle Zambezi	<i>Oreochromis mortimeri</i>	38	24	0.03	Feresu-Shonhiwa & Horward (1998)
Zambezi	Middle Zambezi	<i>Oreochromis mortimeri</i>	38	20	0.03	Feresu-Shonhiwa & Horward (1998)
Zambezi	Middle Zambezi	<i>Tilapia sparmanii</i>	38	19	0.05	Feresu-Shonhiwa & Horward (1998)
Zambezi	Lower Zambezi	<i>Oreochromis macrochir</i>	38	40	0.06	Feresu-Shonhiwa & Horward (1998)

2.5 Results

The total heterozygosity values obtained for this study amounted to 128 records (Table 1). Upon computation of heterozygosity data using SPSS software for statistical analysis, analysis of variance (ANOVA) revealed some significant differences between some populations. Where there was no sign of significant difference between the populations, the data was excluded from further analysis. The results are illustrated in Tables 2 and 3. Heterozygosity was considered significantly different when $p < 0.05$.

The river catchments north of the Orange-Vaal River systems exhibit lower genetic variation than the river catchments southwards. South of the Limpopo River basin, the western and south-western flowing rivers showed very high heterozygosity values. In the Orange-Vaal River systems, the heterozygosity value (H_E) was 0.089 (SD=0.028; SE=0.008). Genetic variation below the Vaal Barrage ridge was averaged to 0.053. In the Vaal Dam $H_E = 0.125$, and in the tributaries flowing into the Vaal River channel far below the barrage ridge, the heterozygosity values were 0.096. Tributaries upstream of the Vaal Dam had $H_E = 0.104$ and an $H_E = 0.118$ when the dam is included.

River catchments north of the Orange-Vaal i.e. the Zambezi, Limpopo, and Incomati River catchments host a heterozygosity range between 0.041 and 0.047. The Limpopo catchment has a H_E of 0.047 (SD=0.047; SE=0.007), the Incomati $H_E = 0.046$ (SD=0.036; SE=0.012), the Zambezi $H_E = 0.041$ (SD=0.027; SE=0.039).

For the Western Cape river catchments, $H_E = 0.091$ (SD=0.0505; SE=0.0223). In the Eastern Cape river catchments, $H_E = 0.069$ (SD=0.069; SE=0.0119) and in the KwaZulu-Natal, $H_E = 0.064$ (SD=0.036; SE=0.010).

Although the Western and the Eastern Cape river catchments have very high heterozygosity values, i.e. $H_E=0.091$ and $H_E=0.069$, respectively, their sample sizes were too small $N=5$ (Table 2). To qualify the results statistically using the Bonferonni test, these populations were excluded and only those with at least 10 individuals per population were further analyzed.

Inter-catchment multiple pairwise comparisons from Bonferroni correction tests exhibited major differences between the Orange-Vaal and the Zambezi River catchments ($p<0.05$; $p=0.020$) and between the Orange-Vaal and the Limpopo River catchments ($p<0.05$; $p=0.023$). This data is illustrated in Table 3.

The p-value describes the significance level of the observations in a study. It is the risk that the researcher is willing to take in rejecting a particular hypothesis. The outcome of a study is not consistent with the null hypothesis, i.e. (there are no genetic variation differences among the populations) whenever the probability of observing certain discrepancy between the observed and the expected values is 5% or less ($p\leq 0.05$), owing to chance alone. If $p< 0.05$, the difference is significant, and it is not chance alone that brought about the discrepancy and thus other factors are involved.

Populations in the Orange-Vaal River basin exhibited higher levels of genetic variability relative to populations from Limpopo and other river systems. One way analysis of variance (ANOVA) confirmed strong and significant impacts of variables of the catchment ($p=0.001$) on the level of genetic variation in the Zambezi River compared to other river catchments.

The Bonferroni test displayed significant differences between the Orange-Vaal and the Zambezi catchments and between the Orange-Vaal and the Limpopo catchments (Table 3).

Table 2. Average expected heterozygosity values of freshwater fish from southern Africa, for all population sizes, grouped according to catchments/river systems.

River systems	Number individuals analysed per population	Mean heterozygosity	Std. deviation	Std. error
Zambezi	52	0.041	0.028	0.039
Limpopo	46	0.047	0.046	0.007
Orange-Vaal	12	0.089	0.028	0.008
Incomati	10	0.046	0.036	0.012
Western Cape	5	0.091	0.051	0.023
Eastern Cape	5	0.069	0.027	0.012
Kwa-Zulu Natal	12	0.064	0.036	0.010
Total	142	0.052	0.041	0.004

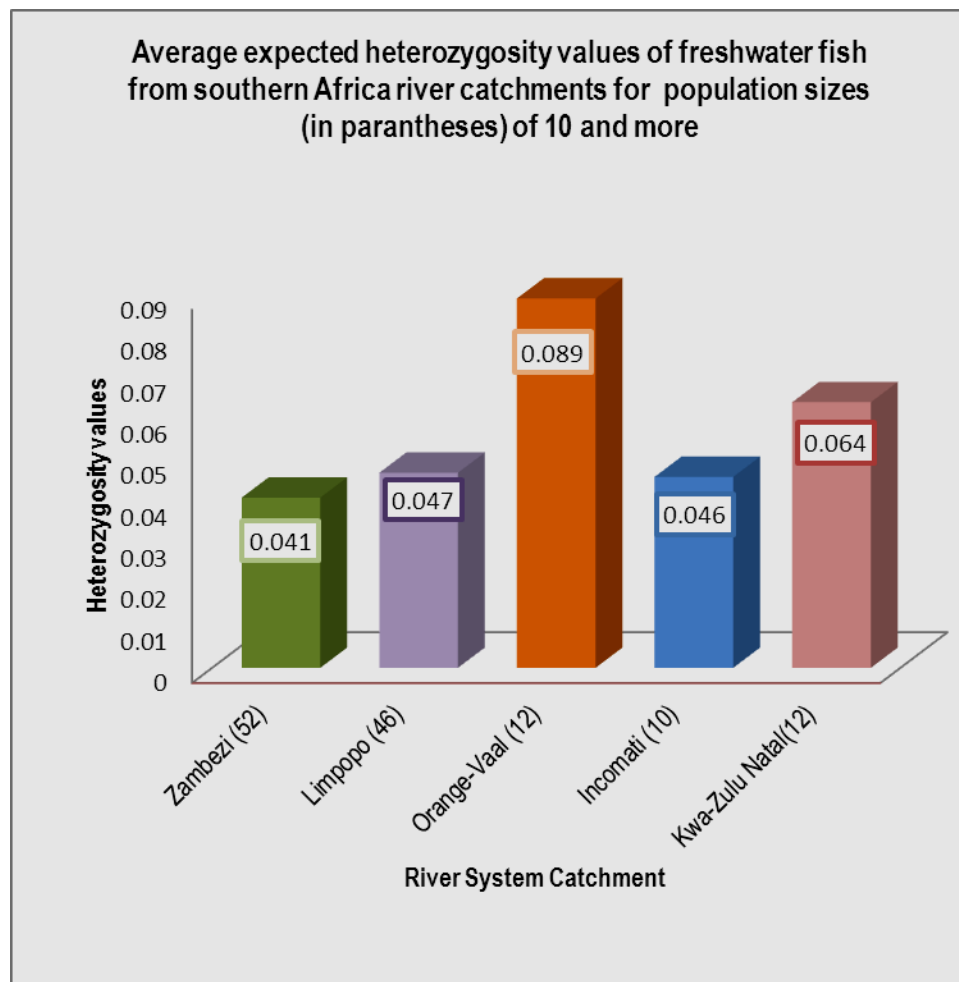


Fig.1. Average expected heterozygosity values of freshwater fish from southern Africa, grouped according to catchments/river systems for population size of 10 or more.

The Bonferroni test displayed significant differences between the Orange-Vaal and the Zambezi catchments and between the Orange-Vaal and the Limpopo catchments (Table 3).

Table 3. Inter-catchments, Bonferroni correction tests for mean differences between the Orange-Vaal-Zambezi and Orange-Vaal-Limpopo catchments areas.

Inter-catchment	Mean Differences	p-value
Orange-Vaal-Zambezi	0.048	0.020
Orange-Vaal-Limpopo	0.040	0.023

2.6 Discussion

Environments have very profound impacts on the diversity of organisms (Templeton, 1980; Kirpichnikov, 1992; Stuart and Gaugler, 1996). The response or adaptation of organisms to environmental disturbances (e.g. climate change, habitat alterations, competition with exotics) depends critically on the genetic structure of the populations. Genetic structures are influenced by environmental variables, and a relationship exists between gene determined physiological responses and the dynamics of that particular gene in a population (Calow and Forbes, 1998). Therefore environments warrant consideration when interpreting any study on organisms (Templeton, 1980; Kirpichnikov, 1992; Stuart and Gaugler, 1996).

The populations in this study were different in terms of the magnitude of genetic variability. This was expected, since diversity in the level of genetic variation is always likely to occur when populations are separated by extrinsic geographical barriers (Templeton, 1980; Stuart and Gaugler, 1996).

The results in this study suggest that genetic variation in the catchment north of the Orange-Vaal was lower than it is in the Orange-Vaal River system and river systems to the south.

A survey of fish data by Gyllensten (1985), estimated the average total heterozygosity in freshwater species to be 0.043, while Kirpichnikov (1992), estimated more than 0.05 for fish populations with the lower margin of the range to be 0.02. Selander and Johnson (1973) estimated 0.05-0.06 for tetrapods. Buth *et al.* (1991) estimated an average heterozygosity of 0.052 in most cyprinid fish populations.

The Zambezi, the Limpopo and the Incomati River catchments conform in terms of heterozygosity levels to what was estimated by the above mentioned authors for fish. The Eastern Cape and the KwaZulu-Natal catchments rated slightly higher. The Orange-Vaal River catchment and the Western Cape catchments rated very high.

One possible explanation for low heterozygosity in the Zambezi River could be mining, industrial activities, landfill and sewage mismanagement along the catchment. Aquatic organisms, due to their sensitivity to changing environments, become highly vulnerable to pollution and habitat change. Since genetic variation within a population is indicative of effective population size and their ability to adapt to change (Spellerberg, 1996), severe reduction in genetic variation threatens the population with ultimate extinction should any major catastrophic incidence (natural or human induced) occur.

Considering droughts, salt intrusion, aquatic weed proliferation, pollution and dam constructions, flood plains and wetlands were subjected to reduced flow (Bourgeois *et al.*, 2003/4). As a result floodplain spawners such as *Labeo* are reduced to minute population sizes. In the Zambezi River, drought interchanges with flooding which leads to soil degradation. Soil degradation itself worsens the drought conditions of the environment (Harden and Mathews, 2002; Bourgeois *et al.*, 2003/4).

The Zambezi River is under pressure from a cocktail of pollutants such as mining, sewage, chemical, fertilizer and textile industrial effluents (Bourgeois *et al.*, 2003/4). Mining is considered a major point source polluter (BBC news, 2004; Bourgeois *et al.*, 2003/4, Earth Crash, Earth Spirit, 2004). It discharges highly toxic metals like arsenic, gold, mercury, copper, cadmium, cobalt and

lead. Mine waste landfills are inadequately designed and consequently mine waste poses a threat to the ecosystem by causing lots of water acidification. Of the non-point pollution sources, agriculture and forestry and the non decaying DDT used to control tsetse flies for the last three decades, threatens the river and the riparian ecosystems. Most aquatic populations have had their populations drastically reduced by DDT toxicity (Bourgeois *et al.*, 2003/4).

Poverty and unemployment make management of this environment to range from difficult, to impossible (Bourgeois *et al.*, 2003/4). Taking over-fishing into consideration, population bottlenecks arise, while agriculture demands more and more water for irrigation. Negative pressures also emanate from mining and industry, with consequences of deterioration and degradation of very sensitive areas, like wetlands in the catchment (Earth Crash, Earth Spirit, 2004).

The SADC has had several civil wars in different countries of the Zambezi River catchment area. Any form of war has detrimental and devastating effects on the aqua infrastructure of the country as a whole. The impacts include destruction of agricultural beds, hydroelectricity and river pollution, to name a few (BBC News, 2004). The fact that most of the waste in the Zambezi River catchment riparian countries is disposed of on land poses a threat to the river basin since the river becomes the recipient of the storm runoff. Land fill leachate and inadequate sewage effluent management led to eutrophication and massive fish kill in the Zambezi (Chenje, 2004. SARDC-IMERCSA, 2004.)

Transport is another polluter through fuel gas emission and fuel spillages. Of the most difficult to control, is the storm water runoff pollution since it originates from multiple non-point sources. The

complexity of this type of pollution emanates from it being a blend of non-organic, organic and biotic waste contaminants (SARDC-IMERCSA, 2004).

Aquatic life is the most vulnerable to pollutants since pollution affects adversely their photosynthesis, respiration, growth, reproduction, strength, entire physiology, behaviour (Gaigher, 1973; Weis *et al.*, 1999; SARDC-IMERCSA, 2004) and genetic expression (Heithaus and Laushman, 1997).

Besides being used as a waste depot by industry, the Zambezi River is over fished to maintain food security. Furthermore the correlation that exists between higher growth rate and high genetic variation in a population predisposes the highly variable genetically populations to fishing pressure and angling (Mitton and Grant, 1984; Beacham, 1991).

For most rivers in mine escarpments a by-product of acid mine drainage (AMD), which is renowned for low pH and high levels of some metals, is released into the environment. It results from oxidation reactions of sulphide containing rocks. The oxidation is activated by the bacteria *Ferrobacillus ferrooxidans*. The product of this oxidation is sulphuric acid which dissolves metal sulphides from the rocks, through the catchment into the river channels. AMD is a serious hazard to the aquatic ecosystems. Among the dissolved metals, copper is renowned for reaching extremely high toxicity levels at very low concentration of 1-10 µg/l. Fish juveniles are particularly sensitive to copper toxicity. Other effects of copper toxicity include reduced immunity, decreased growth rate, impaired swimming ability and decreased stress resistance (Barry *et al.*, 2000).

Other industrial activity by-products include pulp and paper mill effluents. Pulp and paper mill effluents proved to be fatal to aquatic fauna including fish through O₂ depletion, excessive chlorination and turbidity (Kleynhans *et al.*, 1992; Sharples and Evans, 1998). Turbidity itself is notorious for habitat reduction, obscuring migration routes, reducing growth rate and feeding ability of aquatic flora and fauna (Sharples and Evans, 1998).

The Orange-Vaal River catchment seems to be hosting a relatively high genetic variation, $H_E=0.089$ (SD=0.028; SE=0.008). Variation of heterozygosity below the Vaal Barrage ridge was averaged to 0.053. In the Vaal Dam, H_E was 0.125 and in the tributaries which have their insertion points on the main river channel far beyond the barrage ridge, 0.096. The upstream dam tributaries hosted a H_E of 0.104 and with the dam inclusive the H_E was 0.118.

The tributaries forming the Orange-Vaal catchment seem to be responsible for isolation of highly variable gene pools and for maintaining the gene integrity in their populations. The high genetic variation seems to stem from the fact that most of the population samples were collected from the less polluted areas of southern Gauteng. Beyond the Vaal barrage ridge, sampling localities were not on the main channel but on tributaries which form the catchment of the basin.

The Orange-Vaal River system is shared by Botswana, Lesotho, Namibia and South Africa. The largest portion of the river basin is in South Africa and the river basin is intensely developed. Twenty-four dams have been constructed along its length for various purposes, the major of which is irrigation (Assessment Sample, 2004). It is also the source of the LHWP waters. The Vaal River at its waterhead receives on average ± 1800 mm of rainfall per annum, which gets reduced to ± 25 mm per annum at its estuary. Evaporation is low in Lesotho and high in the west.

Due to high impoundment on the system, there is a diminished flow regime with salt intrusion on the lower parts of the river system (Pottinger, 2003). It may be that the relatively high genetic variability recorded for the Orange-Vaal river system was a result of the lack of sampling in the parts closer to the estuary, but further studies would be needed to determine this.

It is striking that while industrial activities are relatively high in the KwaZulu-Natal, KwaZulu-Natal still exhibited high heterozygosity (0.064) in its river systems. Populations which were analyzed were collected from river tributaries of the Tugela and the Pongola which are demarcated along major routes into and out of the province and where accidental oils spillages are often problematic.

The Western Cape and the Eastern Cape catchments exhibited high heterozygosity and the H_E was 0.091 and 0.069 respectively. The two catchments are constituted by smaller tributaries with mostly isolated gene pools. In this manner the genetic integrity of each population is maintained. These two catchments were excluded during further statistical analysis due to the fact that they had only small sample population numbers.

Since genetic variability within a population is an indication of population effective size and the evolutionary potential to adapt to rapid environmental change (Spellerberg, 1996), it may be that most of the species in the Zambezi and the Limpopo River are being threatened with elimination.

Low genetic variation in natural (non-captive) populations is often incidental to severe reduction in population size due to founder effects, historical population bottlenecks and unsustainable exploitation of natural resources to a point beyond recovery of effective population size. Low

genetic variation is common in breeding centres and captivity areas and it is also spatio-temporally related (Smith *et al.*, 1991).

2.7 Conclusion and recommendations

A tributary is part of the river basin and should receive the same status of reverence and development management as the main river channel. The mishap that occurs is that, more often than not, it is treated as not being part of the main basin. The international river basins (IRBs) that a country shares with other countries are of crucial importance in terms of water supply, hydro-politics and management (BBC news, 2004).

There should be joint comprehensive catchment management and development by SADC countries, both spatially (including tributaries in other countries) and temporally. Mining, agricultural, interbasin transfer, domestic infrastructure and industrial activities should be strictly regulated so as to comply with and adhere to the International and National Laws on environmental safety and human health and safety. All riparian countries of shared catchments should be treated coherently. Each riparian country should execute constitutional directives to regulate, monitor, enforce and implement appropriate environmental management practices in all its industrial sectors. There should also be the involvement of all stake holders (educators, legislature, sociologists, health teams, engineers, researchers and communities), in order to create sound environmentally protective technologies and practices.

Every stakeholder in the catchment utilization programmes should have their communities developed to a point of environmental awareness and sustainable development and sustainable exploitation. Prevention of environmental mishaps should be made a priority, while active responses to mishaps and incidences and consequence management should be first on the priority list of the contingency environmental plans according to the 'polluter pays principle'.

These measures ensure that the environment is not degraded to a point of having to close the industry down. This is important because economic dwarfism leads to poverty across the nation where the particular industry was established. Poverty in a nation leads to activities which compound the problems of an already degraded and even non-viable environment. Poverty dictates and disregards the evidence that some species have endangered or vulnerable status attached to their survival.

2.8 Summary

There exists a relationship between natural resource management and economic development within human society. With development, there is a trend in human population growth, energy consumption, global warming, ozone depletion, cropland scarcity, freshwater scarcity, fisheries decline, rural environment degradation and loss of biodiversity (Mathews and Zimmerman, 1990). Environmental degradation this far, has crossed the threshold level of reversibility in most parts of developing countries in Africa. The irreversible ecological damage from anthropogenic degradation may be so permanent as to cause conflict even after the socio-politico-economic factors which caused tension then are alleviated or stabilized (Haber, 1995).

The scarcity of renewable environmental resources, and hence inequities of resource distribution contributes to tension, development of ethnic violence and guerrilla revolutions within countries. Inequities of natural resource distribution also lead to mass displacements/migration of the poor into productive areas but most often into sensitive ecosystems, e.g., overcrowding in informal settlements close to big cities in South Africa frequently occurs around wetlands. The effects of concentration or overcrowding of any species on any part of the landscape are over exploitation

and pollution of natural resources. Water resource is the most vulnerable of natural resources and most vulnerable are the organisms which depend on water for habitat, survival, breathing, reproduction and thriving.

Conservation of biodiversity cannot be addressed politically alone and several approaches are necessary. These include poverty eradication, economic incentives, research, education, *ex situ* and *in situ* remediation of the environment and establishment of protected areas. The *in situ* protection, remediation and rehabilitation become important because aquatic systems for a particular population cannot be easily duplicated elsewhere without adversely influencing the latter environment. To be successful in the *in situ* treatment the integrity of ecosystems and ecological services must be maintained (Lèvéque, 1997). Natural resource scarcity interacts with the politico-economic utilization of the scarce resource and can precipitate social violence, civil strife or ethnic conflicts.

CHAPTER 3. An investigation into the allozyme genetic structure of four populations of *Labeo cylindricus* Peters, 1852 (Pisces: Cyprinidae) from the Okavango, the Upper Zambezi, the Olifants (Mpumalanga Province), and the Incomati River systems.

3.1 Introduction

Labeo is a genus of carp-like primarily freshwater fish in the family Cyprinidae (Reid, 1985 Tshibwabwa *et al.*, 2006). It is one of the most plentiful and widely distributed among African freshwater fishes (Skelton, 1988; Skelton, 1993; Montchowui *et al.*, 2009). Together with *Barbus*, they form the two major genera of the family on the African continent (Soulsby, 1960; Skelton, 1988, Skelton, 1993).

The genus comprises a group of fish with ventrally located sucker-like mouths and fleshy lips bearing sharp, horny edges to facilitate their feeding mechanism i.e., that of scraping-suctioning food substances from the surfaces. The bucco-pharyngeal cavities bear pharyngeal teeth and the fish possesses a very long, coiled intestine. The snout is also fleshy and often bears tubercles (Reid, 1985). Balon, (1977) stated that snout size and body width varies geographically. The author observed different snout sizes in populations of the same species from the same river system, but are separated by certain physical barriers.

Reid (1985) reflects that not only the snout, but even tuberculations, lips and dorsal fins are territorially variable, the fin highly curved at the caudal end for very rapid flowing water habitats. Active tuberculations on the snout seem to be associated with hydrodynamic benefits, like reducing the drag caused by the current velocity and pressure (Skelton, 1993). Tuberculations are more pronounced on forms living in fast flowing water and rapids (Reid, 1985).

The genus has a feeding mode which allows it to scrape the algae off the substrata in its habitat (Pienaar, 1978; Minshull, 1985). It thus cleans both the biotic and abiotic substrata off algae, fungi, invertebrates, microorganisms and detritus (Corbet, 1961; Pienaar, 1978; Reid, 1985; Minshull, 1985; National Parks Board of Trustees of South Africa, 1994). *Labeos* are also commercially very important as forming very marketable fish in most African fisheries (Tomasson *et al.*, 1984; Weyl and Booth, 1999; Montchowui *et al.*, 2009).

Despite the distinct diagnostic features of the genus *Labeo*, there has been great uncertainty and confusion about the taxonomic status and interspecific relationships in the African *Labeo* species based on morphological characters (Bell-Cross and Kaoma, 1971; Reid, 1985; Tshibwabwa and Teugels, 1995). Several revisions of the genus have been advanced so far (Jackson, 1961; Bell-Cross and Kaoma, 1971; Reid, 1985; Robert, 1986; Tshibwabwa and Teugels, 1995), but more disagreements than consensus emerged with each revision. Robert (1986) describes *Labeo* as inadequately understood and very problematic to systematize and questions the validity of some species in the genus. Paugy *et al.* (1990), utilized morphometrics, parasite specificity and genetics in an attempt to characterize some of the *Labeo* species.

Reid (1985) organized the species into groups based on common features they share. The species in this study belong to the group Forskali. Much of the failure to achieve proper identification in this group seems to be centred on *L. cylindricus* (Soulsby, 1960). Bell-Cross and Kaoma (1971), in trying to clear the confusion on the similarity between some species, stated that in the Zambian *Labeo*, all species previously identified as *Labeo annectens* prior to 1971 should read *L. cylindricus* and those that were identified as *L. cylindricus* read *L. molybdinus*. They further suggested that *L. annectens* is a synonym to *L. cylindricus*. Balon (1977), in his findings, insisted that *L. annectens* and *L.*

cylindricus are genotypically isolated. However, this opinion is subject to criticism since the author made this inference without substantiating genetic studies on the species specified. Du Plessis (1963) resorted to stating that *L. parvulus* is synonymous to *L. cylindricus*. Reid (1985) and Skelton (1993) reported closer ecophenotypic characteristics between *L. molybdinus* and *L. cylindricus*, while Balon (1977) described them as sympatric siblings.

The high degree of ecophenotypic similarity among most of the *Labeo* species (Reid, 1985; Skelton, 1993) prompts for studies into their systematic status. *Labeo molybdinus* and *L. cylindricus* differ in habitat preferences and also have ecophenotypic variations. However, there exists some degree of overlap in these features (Reid, 1985, Gaigher, 1973). In the field, identification of these species is often confusing since they are closely similar. The red eye colour of *L. cylindricus* is not a reliable indicator in identifying the species, because it may be absent in certain individuals and the orange annulet around the pupils of *L. molybdinus* (Reid, 1985) may be doubtful. Furthermore it was observed (during on site sampling visit) that morphological separation has shown not to be a very reliable to distinguishing between *L. cylindricus* and *L. molybdinus* in the Sabie River (the Crocodile River system). In the light of the above, therefore, it becomes necessary to investigate the taxonomic status of these species (*L. molybdinus* and *L. cylindricus*) at genetic level in order to validate morphological characteristics (Buth, 1984).

***Labeo cylindricus* (Peters 1852)**

Type locality: Mumbu, Zimbabwe. Nyuridzi, Sabi-Lundi Rivers.

Synonyms: *Labeo parvulus* (*L. parvulus*) Du Plessis (1963).

Labeo darlingi (*L. darlingi*) Jackson (1961).

Labeo annectens (*L. annectens*) Bell-Cross and Kaoma (1971).

Reid (1985) reported that *L. cylindricus* has uncertain taxonomic status and as such there are several synonyms for it. These include *L. darlingi* (Jackson (1961), *L. parvulus* Du Plessis (1963) and *L. annectens* (Bell-Cross and Kaoma, 1971). Several authors have identified *L. cylindricus* and *L. annectens* as different species but Bell-Cross and Kaoma (1971) identified them as one species, therefore suggesting that *L. annectens* should be synonymous to *L. cylindricus*.

Labeo cylindricus is commonly known as the red eyed *Labeo*. As an adaptation for fast currents, *L. cylindricus* has a streamlined body (Barlow, 1961; Reid, 1985; Skelton, 1993) and a characteristic cylindrical shape (Soulsby, 1960; Jubb, 1961), but its growth is geographically variable. Fowler (1936) reported that the Kenyan fully mature specimens of *L. cylindricus* have a length of 4.1- 8.8 cm, but Skelton (1993) observed growths of up to 23 cm in standard length (SL). There are, however, larger specimens and in Lake Malawi, it was noted to reach 1kg and 40 cm (SL), twice the size elsewhere in its distribution range (Skelton *et al.* 1991; Tweddle, 1996).

From the data gathered by Hecht and Mashego (1981), *L. cylindricus* adult fish collected during summer in the tributaries of the Olifants River (Mpumalanga) are of a larger size than those collected in the same localities in winter. Furthermore the cylindrical body can not be used reliably in classification or identification, since in large specimens, the belly becomes distended and the cylindrical shape is lost (Jubb, 1961).

Colouration in live specimens is seasonal, geographical (Balon, 1977) and seasonally variable. The older the fish gets, the darker it becomes (Pienaar 1978; Reid, 1985) and it is also variable depending on the river substratum, water clarity and locality (Jubb, 1961). The iris (eye) is often distinctly red (Reid, 1985; Skelton, 1993), and this is used in species identification (Reid, 1985).

However several species of *Labeo* have red or almost red eye colouration i.e., *L. rosae*, *L. altivelis* (Skelton, 1993) and *L. molybdinus* (orange annulet around the pupil) (Reid, 1985), which reduces the reliability of this feature for identification. Furthermore Fowler (1936) reported a greyish iris, and dark grey inconspicuous axial lateral line in the Kenyan populations of *L. cylindricus*. Furthermore the sympatric occurrence of *L. molybdinus* and *L. cylindricus* (Reid, 1985; Balon, 1977) and their overlapping ecophenetic characters (Pienaar, 1978; Reid, 1985) add to the difficulties in identification and separation on morphological and ecological basis (Pienaar, 1978).

3.2 Relationship between *Labeo* and other organisms

Algae are a basic food source for the genus *Labeo* (Jackson, 1961; Whitton, 1975; Pienaar, 1978; Reid, 1985). However *Labeo* not only cleans substrata of algae, but also off fungi, invertebrates, microorganisms and detritus from other living organisms such as plants, bodies of hippopotami and fish (Corbet, 1961; Pienaar, 1978; Reid, 1985; Minshull, 1985; National Parks Board of Trustees of South Africa, 1994).

Labeo is prey to a number of predators, such as Tigerfish (*Hydrocyon vittatus*) (Jackson, 1961; National Parks Board of Trustees of South Africa, 1994), Silver Catfish (*Schilbe intermedius*) (Bell-Cross, 1973; Groenewald, 1964; Hecht and Mashego, 1981), Largemouth Bass (*Micropterus salmoides*) (Weyl and Booth, 1999), birds (Coulin, 1993), reptiles and amphibians (Pienaar, 1978). They are also subjected to over fishing by humans in some countries in southern Africa (Jackson, 1961; Pienaar, 1978; Jackson *et al.*, 1988; Tweddle, 1996.; Lowe-McConnell, 1997). The species has developed several predator-avoidance and predator-escape mechanisms (Emlen, 1977; Gamberale and Tullberg, 1996) to protect itself and its progeny from predation (Jackson, 1961; Reid,

1985; Bowen, 1988; Skelton, 1993). These mechanisms include being able to cling strongly to cracks on rocks and onto submerged vegetation and having adaptive camouflaging colours (Reid, 1985).

Labeo as a genus and a member of the family cyprinidae is infected by a host of pathogens (Table 4). The pathogens have strict specificity or narrow range specificity to their hosts and pathogens which infect parental species infect the hybrid offsprings as well (Paugy *et al.*, 1990). The presence of particular pathogens on organisms may thus be used as identification markers of hybridization (Smith, 1966; Paugy *et al.*, 1990). Some of the parasites are just opportunistic pathogens on the fish; causing disease only when the organism succumbs to stress (Stewart, 1991). For the genus *Labeo* intestinal pathogens are contracted easily due to their feeding mode, that of scraping the substrata off algae (Jackson, 1961; Pienaar, 1978; Reid, 1985; Oosthuizen and Davies, 1994).

Toxic environments can compromise the fish's immune response, causing the fish to become more predisposed to infections by Trichodinids which thrive very well in polluted water (Lafferty, 1997). Besides the parasitic infestations mentioned above, *L. cylindricus* is also infected by a host of parasites of the genus *Trichodina* (Basson *et al.*, 1983; Van As and Basson, 1992). Furthermore *Tripartiela lechridius* is a new species that is specific to the gills (Van As and Basson, 1992; Lafferty, 1997) of the Limpopo and Olifants Rivers' *L. cylindricus* (Van As and Basson, 1992).

Table 4. Some pathogens infecting *Labeo* and other freshwater fish.

Pathogen	Host	Specified organ	Effect/impact	Authors
Cestode, <i>Ligula intestinalis</i>	Cyprinidae	Intestine	Bleeding and malabsorption of nutrients	Viljoen, 1986; Lauzanne, 1988; Barber <i>et al.</i> , 1995; Barber and Huntingford, 1996).
<i>Dactylogyrus</i> spp. (strict host specificity)	<i>Labeo</i>	Gills	Erosion of gill epithelium and blood loss. Predisposition to secondary infection by fungi and bacteria. An increase mucus production which suffocates the fish.	Schmidt and Roberts, (1989); Paugy <i>et al.</i> , (1990); Gibson <i>et al.</i> , (1996); Timofeeva <i>et al.</i> , (1997).
genera <i>Dactylogyrus</i> and <i>Dogielius</i> (very strict host specificity)	<i>Labeo roseopunctus</i> , <i>Labeo senegalensis</i> and <i>L. coubie</i> (strict host specificity)	Gills	Blood loss, impairment of respiration.	Paugy <i>et al.</i> , (1990).
<i>Dogielius dubicornis</i>	<i>Labeo cylindricus</i>	Gills	Blood loss and secondary infection by other pathogens.	(Guégan <i>et al.</i> , 1989).
The genus <i>Chonopeltis</i> (Crustaceans) <i>Chonopeltis koki</i> n. sp <i>Chonopeltis victori</i> <i>Chonopeltis australis</i>	<i>Labeo cylindricus</i> and most freshwater fish	Fins and the body surface	Skin infection, fluid imbalance and cell lysis, death	Van As, (1992); Avenant-Oldewage <i>et al.</i> , (1994); Viljoen, (1986). Luus-Powell and Avenant-Oldewage, 1996
<i>Chonopeltis australis</i> <i>Lernaea barnimiana</i> (Crustaceae: Copepoda).	<i>Labeo umbratus</i> and <i>Labeo capensis</i>	Fins and the body surface	Skin infection, fluid imbalance and cell lysis, death	Avenant-Oldewage <i>et al.</i> , (1994). Viljoen, (1986).
The trematode <i>Nematobothrium labeonis</i> a genus-specific parasite	Genus <i>Labeo</i>	The eye sockets	Inflammation of the tissues of the eye ball	(Schmidt and Roberts, 1989).
genus <i>Trichodina</i>	<i>Labeo cylindricus</i>	Gills	Respiratory membrane inflammation	Basson <i>et al.</i> , (1983); Van As and Basson, (1992).
<i>Tripartiella lechridius</i>	<i>Labeo cylindricus</i>	Gills	Respiratory membrane inflammation	Van As and Basson, (1992); Lafferty, (1997).

CHAPTER 3

<i>Camallanus</i> species (Nematoda: Camallanidae) (narrow specificity)	<i>Labeo molybdinus</i> <i>Barbus</i> species	Intestinal Tract	Impairment of nutrient absorption	Viljoen, (1986); Lauzanne, (1988), Schmidt and Roberts, (1989); Boomker, (1994).
The leech, <i>Placobdelloides jaegerskioedi</i>	<i>Labeo cylindricus</i>	Intestinal tract	Blood loss	Oosthuizen and Davies, (1994)
<i>Clonorchis sinensis</i> (Chinese liver fluke)	Cyprinids	Skin	bleeding	Schmidt and Roberts, (1989).
cestode <i>Ligula intestinalis</i>	Cyprinids	Intestine	Bleeding and malabsorption of nutrients	Viljoen, 1986; Lauzanne, 1988; Barber <i>et al.</i> , 1995; Barber and Huntingford, 1996).
<i>Dactylogyrus</i> spp. (strict host specificity)	<i>Labeo</i>	Gills	Erosion of gill epithelium and blood loss. Predispose to secondary infection by fungi and bacteria. Increase in mucus production which suffocates the fish.	Schmidt and Roberts, (1989). Paugy <i>et al.</i> , (1990); Gibson <i>et al.</i> , (1996) Paugy <i>et al.</i> , (1990); Timofeeva <i>et al.</i> , (1997).
genera <i>Dactylogyrus</i> and <i>Dogielius</i> (very strict host specificity)	<i>Labeo roseopunctus</i> , <i>L. senegalensis</i> and <i>L. coubie</i> (strict host specificity)	Gills	Blood loss, impairment of respiration.	Paugy <i>et al.</i> , (1990).
East Africa and South East Africa, <i>Dogielius dubicornis</i>	<i>Labeo cylindricus</i>	Gills	Blood loss and secondary infection by other pathogens	(Guégan <i>et al.</i> , 1989).
The genus <i>Chonopeltis</i> (Crustaceans) <i>Chonopeltis koki</i> n. sp <i>Chonopeltis victori</i> <i>Chonopeltis australis</i>	<i>Labeo cylindricus</i> and most freshwater fish	Fins and the body surface	Skin infection, fluid imbalance and cell lysis, death	Van As, (1992); Avenant-Oldewage <i>et al.</i> , (1994); Viljoen, (1986).
<i>Chonopeltis australis</i> <i>Lernaea barnimiana</i> (Crustaceae: Copepoda).	<i>Labeo umbratus</i> and <i>L. capensis</i>	Fins and the body surface	Skin infection, fluid imbalance and cell lysis, death	Avenant-Oldewage <i>et al.</i> , (1994). Viljoen, (1986).
The trematode <i>Nematobothrium labeonis</i> a genus-specific parasite	genus <i>Labeo</i>	The eye sockets	Inflammation of the tissues of the eye ball	(Schmidt and Roberts, 1989).
genus <i>Trichodina</i>	<i>Labeo cylindricus</i>	Gills	Respiratory membrane inflammation	Basson <i>et al.</i> , (1983); Van As and Basson, (1992).

<i>Tripartiela lechridius</i>	<i>Labeo cylindricus</i>	Gills	Respiratory membrane inflammation	Van As and Basson, (1992); Lafferty, (1997).
<i>Camallanus</i> species (Nematoda: Camallanidae) (narrow specificity)	<i>Labeo molybdinus</i> <i>Barbus</i> species	Intestinal tract	Impairment of nutrient absorption	Viljoen, (1986); Lauzanne, (1988), Schmidt and Roberts, (1989); Boomker, (1994).
The leech, <i>Placobdelloides jaegerskioedi</i>	<i>Labeo cylindricus</i>	Intestinal tract	Blood loss	Oosthuizen and Davies, (1994)
<i>Clonorchis sinensis</i> (Chinese liver fluke)	Cyprinids	Skin	bleeding	Schmidt and Roberts, (1989).

Once individuals in a population are infected they can easily infect one another. The risk of cross infection with parasites and other microorganisms among individuals of the school/shoal is higher during migration (Barber *et al.*, 1995). Some of the parasites are just opportunistic pathogens on the fish; causing disease only when the organism succumbs to stress (Stewart, 1991).

3.3 Distribution

The genus *Labeo* has a wide but interrupted distribution on the African continent and South East Asia (Reid, 1985, Skelton, 1993; Tshibwabwa and Teugels, 1995). This pattern of distribution is the result of geographical, ecological and geomorphological discontinuities (Skelton *et al.*, 1995), such as large waterfalls, different water temperatures and pH, rift valleys and ridges. Such factors influence taxonomic differences and isolation can result in specific divergence over time (Bowen, 1988).

In Africa *Labeo* species occur in rivers and lakes (Gaigher and Pott, 1973; Walsh, 1996). In southern Africa the distribution stretches from Afrotropical Africa to the Olifants River in the Western Cape (Allanson, 1979; Gaigher *et al.*, 1980; Skelton *et al.* 1995). Central tropical Africa,

particularly the Congo River, is assumed to be the origin of most freshwater fishes in southern Africa (Allanson, 1979). Dispersal of fish to other river systems results from flooding of fish from one water body into another during heavy rain falls (Allanson, 1979; Skelton *et al.* 1995).

Primary and secondary freshwater fish species appear predominantly in the Zambezi River systems. South Africa may have received primary fresh water fish species through connections between Okavango, Upper and Lower Zambezi River basins (Gaigher and Pott, 1973). This may have resulted in the three river systems which cross the southern Mozambique plain (Pongola, Limpopo and Incomati) having such diverse fish populations (Allanson, 1979). Although Allanson (1979), claimed primary freshwater fish species extend no further south than the Pongola River, Skelton (1993) located *Labeo molybdinus* as far south as the Tugela River.

Labeo cylindricus is widely distributed in the rivers of southern Africa, being more abundant in warmer latitudes (Skelton *et al.*, 1991). In South Africa, *L. cylindricus* occurs in the Limpopo River system. Included in this river system are the Olifants River and its tributaries (Groenewald, 1964; Kleynhans, 1980; Hecht and Mashego, 1981; Skelton, 1989; Merron *et al.*, 1993; Engelbrecht *et al.*, 1994). The species is also found in the Incomati and Pongola River systems (Jubb, 1961; Du Plessis, 1963; Bell-Cross, 1988; Hyslop, 1991). *L. cylindricus* forms a very small percentage (1.5%) of all fish populations in the Piet Gouws Dam in the Olifants River in the Western Cape (Schoonbee *et al.*, 1995). It appears to be absent from the cold highveld streams (Barnard, 1943; Bell-Cross, 1973; Gaigher 1973). Apart from the effects of water temperature, numbers can vary due to factors such as poor oxygenation of the water, lack of submerged vegetation and the presence of predators such as *Schilbe intermedius* (Jackson, 1961; Groenewald, 1964).

Outside the borders of South Africa, *L. cylindricus* is also found in the Zambezi River system (Jubb, 1961; Du Plessis, 1963; Bell-Cross, 1988; Tweddle and Skelton, 1993, Gaigher and Pott, 1973) and northwards through the Cunene and Okavango to the Congo River system in Central Africa (Du Plessis, 1963; Bell-Cross, 1988). Hyslop (1991) reported the presence of *L. cylindricus* in the Mlawula River, Swaziland.

3.4 Habitat

Labeo occupy depths ranging from 0 to 305 metres (Jackson, 1961; Jubb, 1961; Gaigher, 1973). Juveniles prefer shallow water environments to escape predation by larger predators (Jackson, 1961). The species thrives well in the presence of vegetation (Sanyanga *et al.*, 1995). As for temperature preferences, Carey (1967), Pienaar (1978), Bowen (1988), and Schoonbee *et al.* (1995), reported survival of *Labeo* in temperatures between 17°C and 32.5°C. Silt deposition and mineralization from farming and chemical industries result in changes in water temperatures, depth, pH and salinity, thus posing difficulties for fish to occupy their preferred habitats (Gaigher *et al.*, 1980). The *Labeo* species do not seem to prefer confined waters. In the man-made lakes of Tanzania, *Labeo* species formed 5% of the total fish population in 1974 and was not found in 1994 (Bailey, 1996). Merron and Bruton (1993) reported that only twenty *L. cylindricus* were caught the whole year of 1986 in the Pongola Dam, indicating that the dam confinement is not a suitable habitat for the species (Merron and Bruton, 1993).

Although *L. cylindricus* occurs predominantly in fast flowing water (Soulsby, 1960; Jubb, 1961; Gaigher, 1973; Reid, 1985) and is the most abundant *Labeo* of almost every tributary of the Zambezi and Limpopo River systems, some lacustrine populations are also found (Balon, 1977;

Tweddle, 1996). In these lacustrine populations, the majority of the fish gets restricted to the stream entries into the lake but survive there only in less abundant numbers (Carey, 1967; Balon, 1977). Around Lake Malawi, there are both permanent riverine and lacustrine populations (Balon, 1977; Tweddle, 1996). *Labeo cylindricus* also prefers sheltered and shallow water environments of lagoons, which are unfavourable conditions for large predators (Carey, 1967).

The species inhabits fast flowing rocky rapids in many perennial streams, though they may also be found in pools of both perennial and annual streams (Gaigher, 1973; Reid, 1985; Bell-Cross, 1988; Tweddle, 1996). *Labeo cylindricus* may also occur in sluggishly flowing waters and lakes, generally when the water flows over rocks, tree trunks or other hard substrata (Bell-Cross and Kaoma, 1971; Reid, 1985). *Labeo cylindricus* was reported by Hecht and Mashego (1981) to be able to survive in all water types, whether pools or rapids. Gaigher (1973) supported this perspective by indicating that they seem to prefer rapids for feeding, but not fundamentally for existence. Rapids have certain feeding advantages for *Labeo species*. Rapid currents clean debris and deposits, and remove metabolic wastes from the surface of the algal mat upon which the fish feed (Whitton, 1975; National Parks Board of Trustees of South Africa, 1994).

Labeo cylindricus is found in all limnological levels (Jubb, 1961). Gaigher (1973) indicated the preference for limnological altitudes of 0 to 305 m. The largest specimens of *L. cylindricus* (in the Zambezi, the Bua and Shire rivers of Malawi's game reserves) were found in streams of 3-4 m wide and 2 m deep with rather slow flow, overhanging plants, near dead trees with rock and gravel (Tweddle and Willoughby, 1976; Kleynhans, 1980; Mattson and Mutale, 1992).

Carey (1967) reported water temperature preferences of 20° to 28°C for *L. cylindricus*. The species was also found in water temperature of 17°C and pH range of 8.3-8.4 (Schoonbee *et al.*, 1995).

3.5 Pollution

There have been reports, that in some river systems of Mpumalanga Province, agricultural activities have increased the concentration of nitrates and phosphates in rivers (Hyslop, 1991). This opinion is also shared by Walsh (1996) with regards to pesticides and herbicides. The Sabie, the Crocodile and the Olifants Rivers, among others, are subjected to pollution from land use changes, irrigation, mining and industrial toxin mismanagement (Du Preez and Steyn, 1992). Hyslop (1991), however, indicated that *Labeo molybdinus* was abundant in these sampling locations despite all those potential hazards.

The effluent (chlorine based bleaches and other toxins) spillage from the wood, pulp and paper production industry like the Ngodwana in 1989 and minor spillages in the years before caused very high mortality rates of fish in the Incomati River system (Kleynhans *et al.*, 1992). For two years after the spillage most fish species, with *L. cylindricus* inclusive, were not found from several localities in this system (Kleynhans *et al.*, 1992). Lafferty (1997) indicated that pathogens like Trichodinids and a host of others thrive in large numbers in water contaminated by oil, industrial, thermal and pulp mill effluents, adding morbidity and immune-suppression to toxicity (Lafferty, 1997). On populations that are to contaminated environments, pollution poses higher mortality threats compared to populations which are accustomed to the contamination (Heithaus and Laushman, 1997).

3.6 Feeding ecology

The object of feeding is to acquire proteins and energy for survival and reproduction (Bone, 1995). The genus *Labeo* is notable as specialized epilithic, epiphytic and epizoic algae feeders (Corbet, 1961; Reid, 1985; Skelton *et al.*, 1991). They also feed on the aquatic invertebrates that dwell in the algal mat (*aufwuchs*) to augment their protein requirements (Bowen, 1980; Reid, 1985; Jackson *et al.*, 1988; Skelton, 1993; Bone, 1995; Montchowui *et al.*, 2009). Algae substrata include the submerged bodies of organisms like the hippopotami (Corbet, 1961; Pienaar, 1978; National Parks Board of Trustees of South Africa, 1994), submerged vegetation, rocks, gravel and sand (Reid, 1985; Jackson *et al.*, 1988; Skelton, 1993; National Parks Board of Trustees of South Africa, 1994; Engelbrecht *et al.*, 1994).

Upon feeding from the substratum, *Labeo* leave characteristic vermiform post scraping paths on the hard substrata which usually becomes clearly visible when the substratum is dry (Pienaar, 1978; Skelton, 1989; 1993). Feeding preferences include detritus (decaying organisms and the decomposing bacteria in them) (Lauzanne, 1988), fungi growing on other fish (Corbet, 1961; Pienaar, 1978; Minshall, 1985), rotifers (Corbet, 1961; Reid, 1985; Lauzanne, 1988) and microcrustaceans e.g. copepods (Lauzanne, 1988). *Labeo* are therefore generalized feeders (Hyslop, 1991), with algae being the main food source.

The gut of algivorous fishes is faced with breaking down the cell wall. The processes used are mechanical grinding, acid lysis, generalized digestion and digestion by cellulase enzymes derived from microflora in the gut (Power, 1983). *Labeo* have pharyngeal teeth for grinding up the algae

on which they feed (Reid, 1985; Bowen, 1988; Skelton, 1993), thereby increasing the surface area of algae, and the accessibility of digestive enzymes to them (Bowen, 1988).

No stomach has been identified in the genus *Labeo*. Its role seems to have been partly taken by the pharyngeal teeth. The intestine is long and coiled. This suggests that the diet may be quite resistant to enzymatic digestion demanding a longer transit period and a greater surface area for digestion and for absorption (Bowen, 1988; Bone *et al.*, 1995). The most resistant components of the diet are said to be detrital amino acids (Bowen, 1988). Fungi and bacteria act on the external material of the detritus, breaking it up to release and increase nitrogen bio-availability in the food (Yossa and Arango-Lima, 1998).

When found living in the same habitat, *Tilapia rendalli* and *L. cylindricus* live symbiotically. Both feed on algae (Hyslop, 1991; Bailey, 1996). Juveniles of *L. cylindricus* were observed feeding on the fungi that grow on the bodies of *T. rendalli* (Minshall, 1985; Skelton, 1993). *Tilapia rendalli* signals for the procedure by displaying a head down position (Minshall, 1985).

Labeo cylindricus, by inhabiting fast rapids, gains certain advantages from this environment (National Parks Board of Trustees of South Africa, 1994). Fast currents wash loosely attached species and debris from algae and leave only firmly attached algae on rocks. The currents also prevent the coat of depleted water and silt on the surface of algal mats, thus replacing algal waste with fresh material (Whitton, 1975). Consequently, exposed food becomes optimal for the fish (National Parks Board of Trustees of South Africa, 1994), and the grazing behaviour of *Labeo* and other organisms contribute to the growth of some algae species (Whitton, 1975). For fast current habitat exploitation, *L. cylindricus* have streamlined bodies, are fast strong swimmers and

they have suctorial mouths and pectoral fins for clinging purposes (Du Plessis, 1963; Reid, 1985; Bell-Cross, 1988; National Parks Board of Trustees of South Africa, 1994).

3.7 Reproductive ecology

Labeo are seasonal rhythmic breeders. Spawning time is under the influence of light versus dark cycles (Emlen, 1977; Jackson and Coetzee 1982). Increasing ratios of light to dark intervals in spring enhance faster gonadal development, while temperature increments induce gonadal maturation in fish (Imai and Tanaka, 1997). *Labeo*, like all cyprinids respond sexually to rain. Rain induces sexual behaviour and spawning migration, while floods induce spawning. Cold weather, hunger, hypoxia and low water levels inhibit sexual activity (Emlen, 1977).

Spawning in this genus is polyandrous. Species of this genus undergo long spawning migrations to flooded areas in big groups (shoals or schools). Migration is upstream and laterally into flood plains (Jackson and Coetzee, 1982; Bowen, 1988). Seasonal development of tubercles aid in the migration and have also been used as organs of contact in reproduction in the fish (Bone *et al.*, 1995; Reid, 1985). Synchronized grouping or schooling movements have antipredatory benefits (Pitcher and Parish, 1993; Barber and Huntingford, 1996). These benefits are gained through numerous behavioural responses, including dilution of the risk of capture because, usually, the predator is only able to ingest single isolated individuals during attacks. The dividends of schooling are amplified by maximum coordination and similarities in external morphology (Barber and Huntingford, 1996). The ability to school effectively with optimal coordination depends on the optimal functioning of the sensory system and of the visual system (Barber and Huntingford, 1996). A school is also more effective in locating scattered food than single foraging individuals

(Barber *et al.*, 1995). Weyl and Booth (1999), however, observed that *Labeo cylindricus* do not feed just prior and during spawning.

Labeo cylindricus are migratory, flood-dependent and obligatory total spawners, breeding at flood times and spawning on flooded banks or floodplains (Carey, 1967; Jackson, 1984; Tomasson *et al.*, 1984; Reid, 1985; Bowen, 1988). *Labeo* like most fish have very high fecundity (Jackson, 1984; Tomasson *et al.*, 1984; Reid, 1985; Bowen, 1988), the average number of eggs per gonad reported ranges from 11 500 to 82 400 eggs per gonad (Carey, 1967; Tomasson *et al.*, 1984; Skelton *et al.*, 1991). The eggs are small with diameter ranges of 0.76 mm to 1.78 mm (Jackson and Coetzee, 1982; Tomasson *et al.*, 1984; Skelton *et al.*, 1991) and the mass from 127 000 eggs/kg and 450 000 eggs/kg per fish (Bowen, 1988). The eggs are laid among rocks (Tomasson *et al.*, 1984; Skelton *et al.*, 1991) for refuge of the fry (Tomasson *et al.*, 1984; Skelton *et al.*, 1991). Reid (1985) reported that the eggs adhere to inundated vegetation, Tomasson *et al.* (1984), however argues that the eggs are not adhesive but only buoyant after fertilization. The Eggs develop and hatch quickly (Tomasson *et al.*, 1984; Skelton *et al.*, 1991) then larvae swim in large numbers to the river water column and then sink down (Tomasson *et al.*, 1984).

Floodplains offer security from large aquatic predators like the tiger fish (*Hydrocyon vittatus*) and the Silver Catfish (*Schilbe intermedius*) and high nutritional value to the fry, since the shallow water detritus is rich in protein (Bowen, 1988; Merron and Bruton, 1989; Skelton *et al.*, 1991). Apart from the said benefits, floods connect water bodies on lower regions to the river channel making migration and exchange of some nutrients between the river channel and the floodplains easier (Merron and Bruton, 1989; Smith, 1996).

It is worth noting that not all *Labeo* species have single total spawning. Jackson (1973), observed *Labeo capensis* in the Gariep Dam, Orange River, to have two spawning stages occurring three months from each other. The first spawning cycle is triggered by an increase in the water temperature, while the second is stimulated by flooding. Tomasson *et al.* (1984) reported that there can be more than one spawning in a population due to differential gonadal development.

There is upstream migration of the fish for spawning (Van Someren, 1961; Daget *et al.*, 1988; Olivier, 1994). Migration starts at the peak of flooding or shortly thereafter. The apparent breeding season for *L. cylindricus* is between December and April, the peak period being in January (Carey, 1967; Olivier, 1994). Weyl and Booth (1999) reported spawning as early as November in *L. cylindricus* of Mozambique. In November the fish gets golden brown dorsally, golden yellow ventrally and the fins get pinkish-purple. The colouration pattern is geographically dependent (Balon, 1977). The species is dependent on flood onset for spawning (Sanyanga *et al.*, 1995). Despite the fact that floods induce spawning in flood-dependent spawners, food availability and warmer temperatures also play a contributory role (Jackson, 1973; Merron and Bruton, 1993; Olivier, 1994).

Downstream migration at a peak of, or shortly after flood times, occurs in spent adults, which have migrated upstream during early flood (Van Someren, 1961). If juveniles are found downstream at flood times, they have just accidentally been swept down, being the progeny of the previous spawning cycle (Van Someren, 1961; Jackson *et al.*, 1988). Juveniles only move downstream during rain using pectoral fins for climbing weirs and their sucker-like mouths for clinging (Bell-Cross, 1988).

Fish survival in dams is sustained by building large weirs. While this has beneficial effects on fish survival in drought, it impedes migration to a certain extent and therefore inhibits breeding (Kleynhans, 1980; Olivier, 1994). *L. cylindricus*, however, have been seen climbing weirs of up to 2.m high, clinging to the surface with their mouths, pressing and holding with the paired fins, thus all the way up (Du Plessis, 1963).

Variations in the values of gonado-somatic index (GSI) can be used to determine the onset and the termination of ovarian development and thus even the breeding seasons (Van der Merwe, 1987). Experiments performed by Tomasson *et al.* (1984) and Hyslop (1989), indicated that reproductively matured females have a GSI which increases from 3 to 11 during gonodal maturation, whereas it declines during spawning and post spawning periods (Van der Merwe, 1987; Rosenblum *et al.*, 1991).

3.8 Objectives of the study

It is recognised that knowledge of the population genetic structure of a species is a basic requirement for its rational and sustainable exploitation and management, and also for the correct interpretation of ecological investigations. Taking into consideration the heterozygosity status of the assemblage of fish populations, previously studied from river systems and tributaries of the SADC countries (Chapter 2), the allozyme genetic structure of *Labeo cylindricus* from four chosen river systems in southern Africa was investigated. The objectives of this study were: to assess and verify the hypothesis that since river systems network to a larger extent, there may be very little difference in the genetic structure of fish in the sub-Saharan river basins (1) To explore the pattern and the amount of variation in the genetic structures between geographically isolated

populations of *L. cylindricus*. This information could provide a routine and objective basis for discrimination between *L. cylindricus* populations and populations of other species in the genus even in the case of severe crypticism. (2) To determine their phylogenetic relationship; (3) To investigate the extent of genetic differentiation between the populations of *L. cylindricus*; (4) To enhance improved conservation management of these populations in South Africa, and in other regions of their distribution.

3.9 Materials and methods

Specimens of *L. cylindricus* were collected in rapids at locations indicated in Table 5.

Table 5. Sampling localities from which the fish was collected

River system	Sampling Location
Okavango	18°6.5' S, 21°35.6' E
Upper Zambezi	17°29.7' S, 24°16' E
Olifants	25°25.5' S, 29 °22' E
Incomati	25°40' S, 31°46' E

Sampling was undertaken with hand held electrofishing apparatus powered by a 220V AC, portable generator at locations indicated by Table 5 above. Morphometric measurements and meristic counts were determined only as a means to facilitate identification (Blackith and Reyment, 1971). Meristics and morphometrics were taken on the left side of the fish, except for the circumpeduncle meristic counts. After species identification on morphological basis (morphology, morphometrics and meristics), each fish specimen was dissected and tissue samples of the liver, heart and skeletal muscles excised. The tissues samples were placed into respective sample vials and stored in liquid nitrogen (-196°C) temporarily, for onsite storage and transportation purposes. In the laboratory they were transferred to a freezer (-40 °C) until electrophorized.

The equipment used was as outlined in Ferreira (1984). Horizontal starch gel electrophoresis based on the methods of Harris and Hopkinson (1976), was performed. Gel preparation was done ahead of electrophoresis, and it involved pouring heated and degassed (Sigma S-4501) 13% hydrolyzed potato starch (in Tris Citric acid at pH 6.9, Tris Boric acid EDTA at pH 8.6 and Tris Citric acid at pH 8.7 gel buffer solutions) into a gel mold (20 x 18 x 1 cm³) to make slabs of starch gels. The starch slabs were chilled in the refrigerator for a period between 1.5 to 10 hours before use.

The gel slabs were then placed on a chilled stage, the two ends of the gel slabs made to be in contact with the electrode buffers (of a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970), a continuous Tris, boric acid, EDTA buffer system as described by Markert and Faulhaber (1965), a discontinuous Tris, citric acid (gel pH 8.7), Lithium hydroxide, boric acid (electrolyte pH 8.0) buffer system (Ridgway et al., 1970), respectively.

Samples of liver, heart and muscle tissues were homogenized in an equal volume of distilled water and the homogenates centrifuged at 1000 rph for 30 seconds at 2.0 °C. The supernatants were soaked onto 5x15 mm² filter paper strips (wicks) and loaded onto the prepared starch gel slabs.

An electrode sponge-cloth was placed on top of the gel (with the ends dipping into the electrode wells) to insure good flow of current across the gel. The samples were subjected to a constant current of 50 mA.

Proteins which gave the best results appear in Table 6. The proteins were left to migrate for 4-5 hours at a constant temperature of 2.7°C before staining was commenced (Jung *et al.*, 1993). Staining procedures followed Harris and Hopkinson (1976), except for peptidases (Glycine-Leucine) GL which were according to Hillis and Moritz (1990). Terminology and notations for gene loci were based on the recommendations by Shaklee *et al.* (1990). Arabic numerical suffixes for multiple loci (1, 2, 3...) were in order of decreasing anodal mobility. Alleles were identified by their electrophoretic mobilities, relative to the most common allele. The most common allele was designated 100% of the mobility. Being mindful that the success of this technique depends wholly on the reliable and proper identification of zymographs (Quicke, 1993), the interpretation of patterns of enzyme activity followed the guidelines set out by Ferreira (1984).

Ten (10), 9, and 9 enzymes were screened for, in the liver, heart and skeletal muscle tissue samples, respectively. Although many authors (Selander and Johnson, 1973; Gorman and Renzi, 1979; Creech, 1991) maintain that a small number of individuals is not a problem for estimation of heterozygosity if only the number of loci is large enough, Nei (1978) argues that small samples may lead to sampling errors on average heterozygosity and may also affect the gene frequency badly. Nei (1987) advocated for many loci and at least 50 individuals if gene frequency distributions are to be considered.

Statistical analysis of all allozyme data was done using a BIOSYS-2 computer programme for analysis of genetic data by Swofford *et al.*, (1997). Genetic variability was assessed by calculating average heterozygosity (H_E) per locus and percentage polymorphic loci ($P_{0.95}$). The polymorphic genotypes were tested by chi-square (X^2) analysis for fit of allelic frequencies into Hardy-Weinberg proportions. Nei's (1978) genetic distance coefficients and Wright's F-Statistics (F_{ST}) were also calculated to determine the extent of genetic differentiation among the populations (Weir and Cockerham, 1984). F-Statistics (F_{ST}) was based on the methods of Wright's (1978) and represents differentiation among populations (Lewis *et al.*, 2000). A measure of allelic fixation index, F_{ST} , in populations was also used to estimate the effective number of individuals exchanged between populations of each generation.

$F_{ST} = 1/1+4 N_{em}^{\alpha}$ Where $\alpha = n / (n-1)^2$, and n is the number of populations analysed

Table 6. Proteins, Locus Abbreviations and Enzyme Commission (EC) Numbers used in the genetic analysis of *Labeo cylindricus* populations.

ENZYME	LOCUS	EC. NUMBER	BUFFER	TISSUES
Adenylate kinase	AK-1	2.7.4.3	A	Muscle, Heart
Creatine kinase	CK-1, 2	2.7.3.2	B	Muscle
Esterases	EST-1*, 2*, 3*	3.1.1.-	B	Muscle, Heart, Liver
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-1, 2	1.2.1.12	B	Muscle, Heart
Glucose-3-phosphate dehydrogenase	G3PDH-1	1.1.1.8	B	Muscle, Heart, Liver
Glucose-6-phosphate isomerase	GPI-1, 2, 3	5.3.1.9	C	Muscle, Heart
Hexokinase	HK-1	2.7.1.1	C	Liver
Isocitrate dehydrogenase	IDH-1, 2	1.1.1.42	C	Muscle, Liver
Lactate dehydrogenase	LDH-1*, 2, 3	1.1.1.27	C	Muscle, Heart, Liver
Malate dehydrogenase	MDH-1*	1.1.1.37	B	Muscle, Heart
Malic enzyme	ME-1, 2	1.1.1.38	B	Muscle
Mannose-6-phosphate isomerase	MPI-1	5.3.1.8	A	Heart
Peptidases: (Glycine-Leucine)	PEP-A-1*, 2	3.4.-.-	B	Muscle
Phosphogluconate dehydrogenase	PGDH-1	1.1.1.44	B	Liver
Phosphoglucomutase	PGM-1	5.4.2.2	C	Muscle
Superoxide dismutase	SOD	1.15.1.1	B	Muscle, Heart, Liver
<p>A: a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970).</p> <p>B: a continuous Tris, boric acid, EDTA buffer as described by Markert and Faulhaber (1965).</p> <p>C: a discontinuous Tris citric acid (gel pH 8.7), lithium hydroxide, boric acid (electrolyte pH 8.0) buffer system Ridgway <i>et al.</i>, 1970).</p> <p>*Polymorphic loci ($P_{0.95}$)</p>				

3.10 Results

The enzymes systems which were best resolved and their respective buffer systems together with tissues which exhibited best resolution are presented in Table 5. Gene products of thirty two (32) loci were analyzed but only twenty eight (27) enzyme loci had interpretable resolutions. Populations were monomorphic and identical at 23 (82%) of the loci, viz. AK; CK-1, 2; GAP-1, 2; GPD-1; GPI-1, 2, 3; HK-1; IDH-1, 2; LDH-2, 3; ME-1, 2; MPI-1; PEP-2; PGD-1; PGM-1 and SOD. The third locus of creatine kinase (CK- 3), though resolved in *L. cylindricus* of the Incomati and the Olifants River populations, was not consistent in other populations; this locus only appeared in the other populations during repeats with NAD added in the cathode of the electrode buffer. This extra locus appears at almost the same migration level as the AK-1 locus. The GAP-1, 2 loci were uninterpretable initially and only resolved well in the samples that were homogenized overnight and centrifuged. The GPI-3 locus migrated cathodally. The populations were fixed for the same alleles at all monomorphic loci. Enzyme loci which exhibited differences in terms of mobility and/or allele frequencies are represented in Table 6.

Initially the MDH-1 locus would not stain. It only appeared in repeats with NAD added in the cathode electrode buffer. At the MDH-1 locus, Okavango, Zambezi and the Incomati River populations were monomorphic for the same allele. The Olifants River population exhibited some polymorphism but deviated significantly from Hardy-Weinberg proportions.

Table 7. Polymorphic loci, relative mobility (R_M) of alleles, mean number of alleles per locus (N_A) percentage of polymorphic loci using 0.95 criterion ($P_{0.95}$), average expected heterozygosities (H_E) with standard error (S. E.) and X^2 values for the loci which deviated significantly ($p < 0.05$), from expected Hardy-Weinberg proportions, for four populations of *Labeo cylindricus*. OKAV: Okavango, ZAMB: Zambezi, OLIF: Olifants: INCOM: Incomati River system, respectively.

Locus	R_M	Allelic frequencies of different populations			
		OKAV (50)	ZAMB (50)	OLIF (37)	INCOM(28)
EST-1	105	0.48	1.00	1.00	0.02
X^2 (DF)	100	0.52	0.00	0.00	0.98
P value		50.00 (1)			0.01 (1)
		0.00			0.92
EST-2	105	0.02	0.27	1.00	1.00
	100	0.92	0.45	0.00	0.00
X^2 (DF)	95	0.06	0.28	0.00	0.00
P value		0.38 (3)	56.09 (3)		
		0.95	0.000		
EST-3	105	0.12	0.14	0.00	0.00
	100	0.88	0.65	0.96	1.00
X^2 (DF)	95	0.00	0.210	0.04	0.00
P value		50.00 (1)	6.89 (3)	0.07 (1)	
		0.00	0.08	0.79	
LDH-1	105	0.12	1.00	1.00	1.00
	100	0.88	0.00	0.00	0.00
X^2 (DF)					
P value					
MDH-1	105	1.00	1.00	0.82	1
	100	0.00	0.00	0.10	0
X^2 (DF)	95	0.00	0.00	0.08	0
P value				37.49 (3)	
				0.00	
PEP-GL-1	105	1.00	1.00	1.00	0.60
	100	0.00	0.00	0.00	0.40
X^2 (DF)					5.00 (1)
P value					0.025
N_A		1.18	1.14	1.11	1.07
$P_{0.95}$		14.29	7.14	3.57	3.57
H_E (S. E.)		0.039 (0.021)	0.042 (0.029)	0.014 (0.01)	0.020 (0.019)

Three loci were resolved for esterases. They showed complex banding patterns in some loci. The heterozygotes at the EST loci showed double banding, consistent with the monomeric subunit structure of the proteins and the EST-1 locus showed two variants of alleles which presented simple monomeric appearances of the banding zones. EST-2 and EST-3 showed three variants of alleles.

According to Gorman and Renzi (1979), esterases are frequently variable in fishes. Contrary to homozygosity obtained by Van Vuuren (1989) at all EST loci, the Okavango River population was polymorphic at all EST loci, while the Zambezi River population was polymorphic at EST-2 and EST-3 loci. The Olifants River system population was polymorphic at the EST-3 locus and the Incomati River system population polymorphic at EST-2, confirming the observations made by Gorman and Renzi, (1979). All populations displayed heterozygosity deficits of 50% and more at the EST loci in relation to Hardy-Weinberg proportions. At EST-1 locus the Zambezi and the Olifants River populations were monomorphic and fixed for the same allele.

At the LDH-1 locus, except for the Okavango River, all populations were monomorphic, with fixation for the same allele. In the Okavango population, the allelic frequencies for the LDH-1(100) were lower, and those for the LDH-1(95), higher than was obtained by Van Vuuren (1989). The lack of heterozygosity at the LDH locus for the Zambezi and the Olifants River populations was congruent with what was observed by Van Vuuren (1989) for *L. cylindricus* from the Olifants River. Variability for this cold resistant enzyme could not be expected to occur in this study, since the samples were not collected from colder localities (Bell-Cross, 1973; Gai gher, 1973; Kirpichnikov, 1992).

The four populations shared the most common allele on three loci: EST-3, MDH-1 and PEP-A-1. This confirms similarity between the populations. For the EST-1 locus, the Zambezi and the Olifants River populations were fixed for the same allele and for the EST-2 locus the most common allele was shared between the Olifants and the Incomati River populations. On the LDH-1 locus, the Zambezi, the Olifants and the Incomati River populations shared the same allele. This indicates a close relationship between allele frequency distribution and these adjacent river systems.

After allozyme analysis, EST, MDH and GPI were found to be polymorphic with considerable differences in the allozyme patterns. G6PDH, GAP, and G3PDH were polymorphic but difficult to detect, while SOD were all monomorphic and fixed in all populations and unsuitable therefore for use in distinguishing between the between genetic difference. EST, MDH, GPI, renowned for their polymorphic nature, stability and ease of detection in most organisms (Baillie *et al.*, 1998) were polymorphic in most populations sampled. The EST was highly polymorphic possessing five different electrophoretic alleles across all population (Table 7).

In this study, significant heterogeneity in allelic frequencies was found. Statistical significant ($p < 0.05$) deviation of genotypes at polymorphic loci from Hardy-Weinberg proportions was observed at EST-1, 3 and LDH-1 loci in the Okavango, at EST- 2 locus in the Zambezi, at MDH-1 locus in the Olifants and at PEP-A-1 in the Incomati River populations respectively. At all of these loci there were deficiencies in the heterozygotes (Table 7).

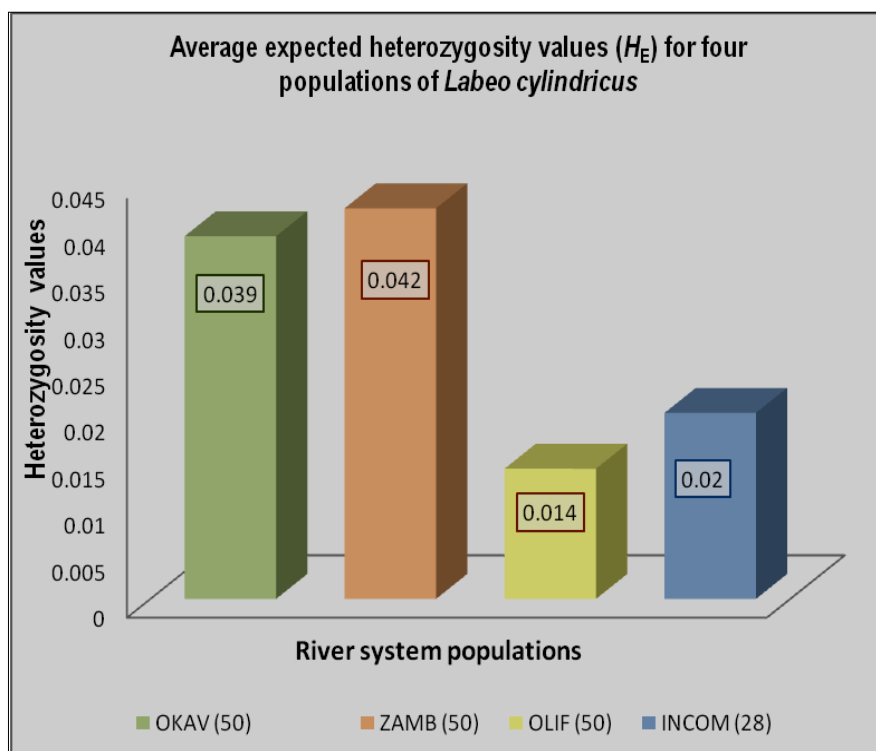


Fig. 2. Average expected heterozygosity values (H_E) for four populations of *Labeo cylindricus*. OKAV: Okavango, ZAMB: Zambezi, OLIF: Olifants: INCOM: Incomati River systems, respectively with their sample numbers in the brackets.

With regards to the degree of divergence between the populations as signified by the genetic distance coefficients between the pairs of populations it was found that the genetic distance coefficients ranged from 0.018 to 0.078. The genetic distance coefficient was the least between the Zambezi and the Olifants River system populations i.e. 0.018. Between the Zambezi and the Okavango River system populations, the genetic distance coefficient was 0.048.

Table 8. Genetic distance coefficients (D) for various taxonomic levels as defined by literature. Nei's theoretical genetic distance of D=1 is equivalent to five (5) million years of divergence (Grant *et al.*, 1988).

RELATIONSHIP	GENETIC DISTANCES (D) AS DEFINED BY DIFFERENT AUTHORS			
	Grant <i>et al.</i> , 1988	Selander and Johnson, 1973	Nei, 1978 a	Avise & Smith 1977
Semispecific		0.03	<0.1	
Conspecific	> 0.05	0.13-0.20	<0.1	
Congeneric	± 0.40			0.16<D<1.39
Confamilial	± 1.0			

The divergence as determined by the genetic distance coefficient between the Zambezi and the Incomati River system populations was 0.060. The largest distance existed between the Okavango and the Incomati River system populations (0.078) Fig. 2 and Table 9.

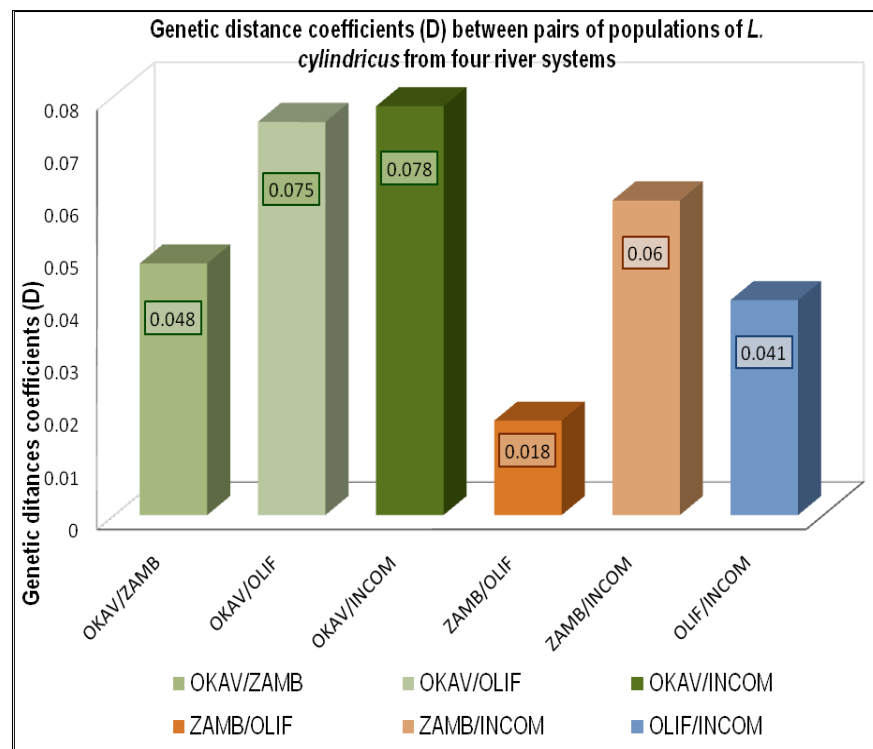


Fig. 3.. Genetic distance coefficients (D) between pairs of populations of *L. cylindricus* from four river systems, according to the methods of Nei (1978). River systems represented are those of the Okavango (OKAV), Zambezi (ZAMB), Oifants (OLIF) and Incomati (INCOM).

Genetic pool differentiation as indicated by the matrix of pair wise allelic fixation index values, F_{ST} , ranged from 0.245 to 0.735 (Table 8). This showed the gene pools of the populations of the Incomati and the Olifants River systems to be highly differentiated. Differentiation was also exhibited between the Incomati and the Zambezi River systems ($F_{ST}=0.613$) and between the Incomati and the Okavango River systems ($F_{ST}=0.670$). While the effective number of individuals exchanged between other river system populations or gene flow (N_{em}) ranged from 0.103 to 0.433, it was the lowest between the Olifants and the Incomati River systems, (0.051), (Table 9).

Table 9. Matrix of pairwise allelic fixation index values (F_{ST}) (above the diagonal) and effective number of individuals exchanged between populations (N_{em}) (below the diagonal) Values for four *L. cylindricus* populations on the basis of polymorphic loci.

Population	1. OKAVANGO	2. ZAMBEZI	3. OLIFANTS	4. INCOMATI
1. OKAVANGO	*****	0.361	0.577	0.670
2. ZAMBEZI	0.249	*****	0.245	0.613
3. OLIFANTS	0.103	0.433	*****	0.735
4. INCOMATI	0.069	0.089	0.051	*****

This showed the gene pools of the Olifants River systems to be highly differentiated. Differentiation was also exhibited between the Incomati and the Zambezi River systems ($F_{ST}=0.613$) and between the Incomati and the Okavango River systems ($F_{ST}=0.670$). While the effective number of individuals exchanged between populations in different river systems or gene flow (N_{em}) ranged from 0.103 to 0.433, (Table 9) it was the lowest between the Olifants and the Incomati River systems ($N_{em}=0.051$).

3.11 Discussion

Environments have profound effects on the biodiversity of organisms (Templeton, 1980; Kirpichnikov, 1992) and therefore warrant consideration when interpreting any study of the organisms (Stuart and Gaugler, 1996). Geographical isolation over wide distribution ranges and population sizes are advantageous for the alteration of genetic integrity and for accumulation and preservation of genetic variability in populations (Templeton, 1980; Mork and Giæver, 1995). The above factors lead to the accumulation of adaptive genetic divergence in populations (Templeton, 1980). Populations in this study were sampled from localities divided by extensive extrinsic barriers. The magnitude of allelic frequencies at polymorphic loci differed in every population, confirming the above statement.

The populations under study were monomorphic at most of the loci examined. This is in accordance with the monomorphic nature of skeletal muscle structural proteins in most fish species (Kirpichnikov, 1973). Interesting though, is that even the liver and heart tissues displayed high levels of monomorphism.

Percentage polymorphic loci and degree of heterozygosity are the most common tools for expressing variability in the genetic structure of populations. Levels of genetic variation differ among organisms (Nei, 1987), and lower than expected observed heterozygosity are common in natural populations (Kirpichnikov, 1992). Selander and Johnson (1973) estimated the degree of heterozygosity to be consistently 0.05-0.06 in tetrapods. Nevo (1978) recorded heterozygosity of 0.051 for the bonefish species and Avise and Aquadro (1982) estimated the average heterozygosity to be 0.054 for fish in general. According to Kirpichnikov (1992) the percentage polymorphic loci may range from 8-20%, with heterozygosity levels between 0.02 and just more

than 0.05 in fish populations. Buth *et al.* (1991) estimated the average heterozygosity to be 0.052 in most cyprinids.

The expected heterozygosity for the Olifants River population (0.014) deviated remarkably from the predictions for panmictic fish populations. The values for the Okavango, Zambezi and the Incomati populations however, almost match the above mentioned predictions for panmictic fish populations and compare favourably with the observations of Van Vuuren (1989) for certain species of *Labeo* species and with the lower margins of heterozygosity (0.02-0.03) referred to by Kirpichnikov (1992).

The genetic variation in the Olifants and Incomati River populations ($P=3.57\%$, $H_E = 0.014-0.020$) was lower compared to the lower margins of all of the above mentioned estimates. The levels of genetic variation in all the populations under study were even lower than values obtained for the Olifants River populations of *L. cylindricus* ($H_E = 0.098$) by Van Vuuren (1989). The heterozygosity results obtained for the Olifants and Incomati River populations (0.014-0.020 respectively) almost compares with the H_E value Van Vuuren (1989) obtained for *L. ruddi* (0.011) from the Limpopo River system. The gene diversity values obtained in this study for all populations (H_E 0.014-0.042) fall within the range obtained for different *Labeo* species (H_E range 0.011-0.108) reported for South Africa (Van Vuuren, 1989).

Variation in the degree of polymorphism between the populations ranging from 3.57% to 14.9% is consistent with values reported by Buth (1984) and Selander and Johnson (1973) who observed that the genetic variability of 4.2% to 16.7% was the norm for geographically and reproductively isolated populations of vertebrates.

The low levels of genetic variability are attributable to deficits in heterozygotes displayed by all populations in the study. Selective agents might have acted against heterozygotes at each geographical locality, each set of selective agents acting against the loci of its choice in each population. Apart from this, selection against heterozygosity is a common occurrence in fish populations (Engelbrecht and Van der Bank, 1997). Heterozygosity values are also strongly influenced by the loci chosen for electrophoresis (Gorman and Renzi, 1979) and selection of staining materials (Grant *et al.*, 1988).

While the present estimates of $P_{0.95}$ and H_E for the Okavango and the Zambezi River populations are not low compared to the lower margins in Kirpichnikov's (1992) estimation for fish species, it is worth noting that they are considerably lower than the corresponding values for *L. cylindricus* from the Olifants River system, based on estimates of ($H_E = 0.098$) by Van Vuuren (1989). The gene diversity distribution obtained for all populations (H_E 0.014-0.042) in this study fall in between the variability range noted for other *Labeo* species (H_E range 0.011-0.108) studied in South Africa by Van Vuuren, (1989).

The heterozygosity value for the Olifants population ($H_E=0.014$) is very similar to the *L. ruddi* ($H_E=0.011$) from the Mogalakwena tributary of the Limpopo River system, suggesting almost similar genetic and aquatic conditions in the two river systems. The heterozygosity found in the Incomati River system compares favourably with the *L. seberi* from the Olifants river system. The Okavango ($H_E=0.039$) and the Zambezi River ($H_E=0.042$) populations' heterozygosity values fall in a range between *L. umbratus* ($H_E=0.033$) from the Vaal River (Gauteng) and *L. rubromaculatus* ($H_E=0.058$) from Buffels River (KwaZulu-Natal).

Van der Bank (1995) studied 35 loci in freshwater mussels from the same locality in the Zambezi River and obtained the average heterozygosity of 0.075. Van der Bank and Van der Bank (1995), estimated $H_E=0.082$ over 51 loci in the momyrids (fish species). Engelbrecht *et al.* (1994), observed an $H_E=0.029$ for *Schilbe* over 60 loci. The value obtained in this study ($H_E=0.042$) falls between the lowest heterozygosity value ($H_E=0.029$) and the highest value ($H_E=0.081$) obtained for this locality. From the Olifants River system, Van Vuuren (1989) determined $H_E=0.098$ for *L. cylindricus* and $H_E=0.108$ for *L. molybdinus*. Engelbrecht and Mulder (1999), studied 30 loci for the River Goby and obtained $H_E=0.025$. The results of this study show that the Olifants River population has a value far lower than those from the Okavango and the Zambezi River systems (Fig. 2), suggesting very critical genetic erosion in this population. The Olifants River is crossing through areas of diverse farming and industrial activities like coal mines and low genetic variability suggests possible pollution from industrial effluents.

The genetic variation results reported in this study, for the Okavango and the Zambezi River systems populations, however low, suggest that the level of genetic variability can still sustain the populations if no major detrimental disturbances occur in those environments.

Apart from the heterozygosity and percentage polymorphic loci, the Hardy-Weinberg equilibrium can also be used to assess alignment of polymorphic loci in a population. It is worth noting, however, that although important as a yardstick and quick reference point in identifying discrepancies in populations, fit to the Hardy-Weinberg proportions is not an absolute criterion for inferring the genetic nature of a population (Althukov, 1981; Grant *et al.*, 1988). Significant departures may occur as a result of natural selection, gene flow, founder effects, genetic drift, small effective population size, assortive mating, sampling error, population bottlenecks, genetic drift, the Wahlund effect and even various anthropogenic activities (Ferreira, 1984; Grant, 1993).

In this study departure from the Hardy-Weinberg proportions was represented by deficits in heterozygotes at 75% of polymorphic loci in the Okavango and at 50% of the polymorphic loci in the Zambezi, Olifants and Incomati River populations. Parasitic infestation reported in the former two river systems (Okavango and Zambezi) is among factors which may be responsible for the discrepancy since parasites may either reduce the population size through death or reduction in reproductive potential (Smith, 1980).

Pollution and low population density coupled by reduced gene flow between the populations of a species are highly correlated with lowered genetic variation (Heithaus and Laushman, 1997; Yokota and Watanabe, 1997). Spillage of paper mill effluent, led to a massive fish kill in the Incomati River system in 1989 (Kleynhans *et al.*, 1992). This devastation also affected *L. cylindricus* and possibly caused severe population bottleneck, since for more than a year after the 1989 paper mill pollution incident, the river was devoid of *L. cylindricus*, among other fish fauna, at several localities monitored during different temporal surveys (Kleynhans *et al.*, 1992). The paper mill effluents carry the risk of morbidity and mortality for fish, from egg stages to adult stages due to toxic stress. Paper mill toxicity also causes chronic damage to the sex organs and massive kill of larvae (Karås *et al.*, 1991). Apart from the paper mill effluent pollution, acid mine drainage from coal mines in the catchment areas of the river system results in metallic acidity, which is known to increase fish mortality (Henry *et al.* 1999).

Exposure to toxicity or pollution is well known for eroding variation at certain loci and thereby changes genotypic frequencies over time in several fish fauna (Heithaus and Laushman, 1997). Loci whose genetic variability is more susceptible to erosion due to toxicity include MDH, GPI, IDH and PGM (Heithaus and Laushman, 1997). Heithaus and Laushman (1997) identified the PGM-2 locus to be a suitable and useful tool in monitoring water quality, and that monomorphism

at this locus suggests poor quality or pollution in water. Even though PGM was previously found to be highly variable in most fish species, (Selander and Johnson 1973), it has been monomorphic in populations of this study, indicative of poor water quality, lack of resistance, and susceptibility to agricultural pesticides and herbicides and industrial toxins (Heithaus and Laushman, 1997). Over several years of further industrial development and considering the population growth rate, this situation could worsen, unless pollution control measures can run ahead of pollution creation.

Direct effects of pollution and the smaller population size of *L. cylindricus* in the Incomati River system have probably led to the reduction in the genetic variation within the system. Severe genetic drift might have consequently occurred. This situation is likely to last for many more years since bottleneck effects are retained for thousands of years in the genetic structure, even after the recovery of the population size (Frankel and Soule, 1981; Doyle *et al.*, Waples, 1991; O'Brien, 1994). Developmental instability and reduced growth rate is also expected (Nei, 1978). In contrast the populations from relatively less polluted water bodies (the Okavango and the Zambezi Rivers) had slightly higher genetic variation.

For LDH, variation may be decreased due to higher temperatures in the sampling localities (more tropical environments). Lack of variation at this locus suggests constraints imposed upon alleles to adaptation to extremely elevated water temperatures (Yoshiyama and Sassaman, 1983; Buth, 1984). The monomorphic nature of this locus in this population does not seem life threatening though. While agriculture may be disturbing the ecosystems in terms of insecticide and herbicide pollution in rivers and water bodies, esterase variants are resistant to these influences particularly to the organophosphates (Owusu *et al.*, 1996; Grafton-Cardwell *et al.*, 1998).

Lack of genetic variation is itself a constraint that violates the population's potential of survival, procreation, disease resistance, growth and adaptation to any change in the environmental conditions (Heithaus and Laushman, 1997).

Distribution ranges of populations correspond with the amount and the distribution of genetic variation (Nevo *et al.*, 1984; Selander and Johnson, 1973; Carvalho, 1993). Genetic variability may also differ between populations if the populations occupy contrasting habitats even within the same water body. Where distribution is interrupted, even dispersal of genetic variation is correspondingly different (Carvalho, 1993). The river barriers act as impediments to migration and gene flow among populations of aquatic species and act as major drivers of diversification (Carvalho, 1993). The difference in allelic frequencies in these populations seems likely to have resulted from lack of gene flow and genetic drift resulting from bottlenecks and founder influences.

Differences in allelic frequencies at polymorphic loci contribute to genetic divergence between conspecific populations (Oosthuizen *et al.*, 1993). One of the methods of quantifying the amount of genetic differentiation within and among populations is through the F-statistics by Wright (1978). F_{ST} values range from 0-1. The values are zero when allelic frequency within the species is the same in all populations. The values approach one when there are fixed differences of all allelic frequencies among the populations. F_{ST} values become larger when populations are constrained by geographical barriers.

The F_{ST} range of 0.670-0.735 between the Incomati River system population and other populations has evolutionary significance. It signifies genetic divergence beyond subspecies level of differentiation (0.20-0.30) according to Templeton, (1999). It also suggests that dispersal,

migration and gene flow between the tributaries of the river systems in the study are completely handicapped. Taking into consideration the geographical barriers dividing freshwater bodies, such differentiation is expected (Carvalho, 1993).

The F_{ST} values ranging from 0.245 to 0.735 could be generated by the N_{em} ranges of 0.051-0.433. An N_{em} value of greater than one (1) suggests sufficient gene flow to counteract the effects of differentiation between the populations. In the present study the levels of gene flow is low since N_{em} is less than one (1). Migration of the populations is prevented by physical-geographical barriers. Such lack of gene flow may result into fixation of the population to particular monomorphic alleles and increased differentiation among the populations of the *L. cylindricus* in the river systems.

An alternative method to F_{ST} for quantifying genetic differentiation among populations is through genetic distances. There are several models of the genetic distance concept and a number of different indices for genetic distances are being used (Ayala, 1982). In this study Nei's (1978) genetic distances were used. Genetic distances defining conspecific species according to Grant *et al.*, (1988) occur at values between 0.05 and 0.40 (Table 8).

Fixation of most monomorphic loci for the same alleles in this study (Oosthuizen *et al.*, 1993), in conjunction with genetic distances values obtained for Nei (1978) (0.018-0.078), suggest that these populations are conspecific. Different authors assign different categories of genetic distances between pairs of organisms. Apart from this factor, the demarcation between the categories is not a sharp or a solid one (Ferreira *et al.*, 1984; Grant *et al.*, 1988). This phenomenon prompts for incorporation of other comparative statistical mechanisms like the F_{ST} .

Genetic distances (Nei, 1978) Fig. 3, together with the F_{ST} results (Wright, 1978) (Table 9), in this study exhibited closer relationships between the Zambezi and the Olifants River systems populations than between the Okavango and the Olifants River systems. There exists a shorter genetic distance between the Zambezi and the Olifants River systems populations. The Okavango river system population stands out as quite distinct from the former two populations. This phenomenon can be ascribed to the distribution patterns of the *L. cylindricus* and is supported by the fact that the primary freshwater fishes were distributed to South Africa through connections between the Okavango-Zambezi River basins (Gaigher and Pott, 1973). It also suggests a more recent divergence or recent isolation of gene pools between the Zambezi and the Olifants River systems populations.

The shorter genetic distance between the Zambezi and the Incomati River populations coupled to the low F_{ST} value (0.245) and moderate magnitude of gene flow ($N_{em}=0.433$), suggest a recent divergence between these two populations.

For populations to maintain integrity at a molecular level while undergoing divergence, gene flow between them needs to be eliminated (Selander and Johnson, 1973). With the integrity of gene pools maintained, the unique protein complement per population is sustained. With such uniqueness in protein complement, identification and systematization of the population or species can be achieved (Selander and Johnson, 1973; Paugy *et al.*, 1990).

3.12 Conclusion and recommendations

The populations in this study are fixed for allele A on all the monomorphic loci tested and the results suggest a less diverse environment and lack of gene flow between *L. cylindricus* populations. Furthermore, the results in this study also suggest more variable and healthy environmental conditions for the Zambezi and the Okavango River system populations than for the Olifants and the Incomati River systems. It is more likely that the Olifants River system and its populations has been affected by pollution and droughts reported in South Africa over recent years (Du Preez and Steyn, 1992).

The replacement of some of the alleles A by alleles B at monomorphic loci could occur through mating of an intermediate AB with the said population or by monozygotes BB. The new allele can be introduced into the population through migration or translocation. Any population thought of for translocation purposes must first be thoroughly surveyed for pathologies and compatibility of the genetic structure with the local population. If breeding becomes successful then the populations can be polymorphic until selection or genetic drift causes a loss or fixation of the new allele or the old allele again (Mabee and Humphries, 1993).

Any situation causing stress in habitat or biota should be avoided or minimised. Toxicity levels of every river should also be evaluated from time to time but at least quarterly. Interpopulational gene flow in the same river system should be maintained or established where possible. There should be strict public educational campaigns to intensify environmental awareness and to enforce compliance with the regulations of the DWA & F (1997), which stipulates that the quality, quantity and reliability of water be reserved for aquatic and associated ecosystems, for both the social and economic benefit of the society.

CHAPTER 4: References

ACACIA AFRICA. 2004.

Southern Africa Zambia Zambezi Victoria Falls Game Wildlife Parks.

http://www.acacia-africa.com/Country_zambia.htm. Date accessed: 21-04-2004.

AGNESE, J. F., TEUGELS, G. G., GALBUSERA, P., GUYOMARD, R., AND VOLCKAERT, F. 1997.

Morphometric and genetic characterization of the sympatric populations of *Clarias ssp.*, from Senegal. *Journal of Fish Biology* **50** (6): 1143-1157.

ALLANSON, B. R. (ED.). 1979.

Lake Sibaya. (*Monographiae Biologicae*, **36**). Dr. W. Junk Publishers, The Hague-Boston, London.

ALLANSON, P., MURDOCH, J., GARROD, G., AND LOWE P, .1995.

"Sustainability and the rural economy: an evolutionary perspective" *Environment and Planning A* **27**(11): 1797 – 1814

ALLENDORF, F. W., AND THORGAARD, G. H. 1984.

Tetraploidy and the evolution of Salmonids fishes. In: Tuneer, B. J. (Ed.). *Evolutionary genetics of fish*. Plenum Press, New York.

ALLENDORF, F. W. 1991.

Ecological and genetic effects of fish introductions: Synthesis and recommendations.
Canadian Journal of Fisheries and Aquatic Sciences **48** (Suppl.1): 178-181.

ALTHUKOV, Y. P. 1981.

The stock concept from the view point of population genetics. *Journal of Fisheries and Aquatic Sciences* **38**: 1523-1538.

ANDREWS, R. H., AND CHILTON, N. B. 1999.

Multilocus enzyme electrophoresis: a valuable technique for providing answers to the problem in parasite systematics. *International Journal for Parasitology* **29** (2): 213-253.

ARIZMENDIC, C., CUEZVA, J. M., AND MEDINA, J. M. 1983.

Lactate dehydrogenases and their correlation with blood oxygen concentration. *Enzymes* **29** (1): 66-70.

ARTHINGTON, A. H. 1991.

Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia.
Canadian Journal of Fisheries and Aquatic Sciences **48** (Suppl.1): 33-43.

ASINAR.NET GLOBAL ENCYCLOPEDIA. 2004.

River. <http://georgia.asinar.net/en/wikipedia/river.html>.

Date accessed: 15-04-2004.

ASSESSMENT SAMPLE. 2004.

“Vulnerability of water resources to environmental change in Southern Africa.”

<http://www.unep.org/dewa/water/vulnerability/Reports/sample-Africa.pdf>.

Date accessed: 18-06-2004.

AVENANT-OLDEWAGE, A., SWANEPOEL, J. H., AND KNIGHT, E. 1994.

Histomorphology of the digestive tract of *Chonopeltis australis* (Crustacea: Brachiura). *South African Journal of Zoology* **29** (1): 74-81.

AVISE, J. C., AND SMITH, M. H. 1977.

Gene frequency comparisons between sunfish (Centrarchidae) populations at various stages of evolutionary divergence. *Systematic Zoology* **26**: 319-335.

AVISE, J. C., AND AQUADRO, C. F. 1982.

A comparative summary of genetic distances in vertebrates. Patterns and correlations. *Evolutionary Biology* **15**: 151-185.

AYALA, F. J., TRACEY, M. L., HEDGEcock, D., AND RICHMOND, R. C. 1974.

Genetic differentiation during the speciation process in *Drosophila*. *Evolution* **28**: 574-592.

AYALA, F. J. 1982.

Population and evolutionary genetics: A Primer. The Benjamin/Cumming Publishing Company. Menlopark, California.

BAILEY, J. 1984.

Principles of Wildlife management. John Wiley and Sons, New York.

BAILEY, R. G. 1996.

Changes in fish and fisheries ecology of a large man-made lake in Tanzania, 1965-94.

Fisheries Management and Ecology **3**: 251-260.

BAILLIE, B. K., MONJE, V., SILVESTRE, V., SISON, M., AND BELDA-BAILLIE, C. A. 1998.

Allozyme Electrophoresis as a tool for distinguishing different zooxanthallae symbiotic with giant clams. *Proceedings of Royal Society of London B.* **265** (1409): 1949-1956.

BALON, E. K. 1977.

The golden mudsuckers (*Labeo spp.*) From above and below the Victoria falls: sibling species along different invasion routes. *Hydrobiologia.* **53**: 3-12.

BARBER, I., HUNTINGFORD, F. A., AND CROMPTON, D. W. T. 1995.

The effect of hunger and cestode parasitism on the shoaling decisions of small fresh water fish. *Journal of Fish Biology* **47** (3): 524-536.

BARBER, I., AND HUNTINGFORD, F. A. 1996.

Parasite infection alters schooling behaviour: deviant positioning of Helminth-infected minnows in conspecific groups. *Proceedings of Royal Society of London B.* **263** (1374): 1095-1102.

BARLOW, G. W. 1961.

Causes and significance of morphological variation in fishes. *Systematic Zoology* **10** (3): 105-117.

BARNARD, K. H. 1943.

Revision of indigenous freshwater fishes of South West Cape region. *Annals of the South African Museum* **36** (1): 101-261.

BARRY, K. L., GROUND, J. A., LEVINGS, NIDLE, B. H., AND PIERCEY, G. E. 2000.

Impacts of acid mine drainage on juvenile salmonids in an estuary near Britannia_Beach in Howe South, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **57** (10): 2032-2043.

BASSON, L., VAN AS, J. G. AND PAPERNA, I., 1983.

Trichodinid ectoparasites of cichlid and cyprinid fishes in South Africa and Israel. *Systematic Parasitology*. **5**: 245-257.

BBC NEWS. 2004.

World water Crisis.

<http://news.bbc.co.uk/hi/english/static/in-depth/world-water-crisis/default.stm>.

Date accessed 15-04-2004.

BEACHAM, T. D. 1991.

Developmental stability, heterozygosity, and genetic analysis of morphological variation in pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Zoology* **69**: 274–278.

BELL-CROSS, G., AND KAOMA, J. 1971.

Addition and amendments to the check list of the fishes of Zambia, no. 3. *Fisheries Research Bulletin of Zambia* **5**: 235-244.

BELL-CROSS, G. 1973.

The fish fauna of the Buzi river system in Rhodesia and Moçambique. *Arnolda, Rhodesia: The National Museums and Monuments of Rhodesia* **6** (8): 1-14.

BELL-CROSS, G., AND MINSHULL, J. L. 1988.

The fishes of Zimbabwe. The trustees of National Museum and Monuments of Zimbabwe. Harare, Zimbabwe.

BILLINGTON, N., BROOKS, R. C., AND HEIDINGER, R. C. 1996.

Use of Cellulose Acetate Electrophoresis to rapidly screen Sauger broodstock *Gauge-Walleye* hybrids. *The Progressive Fish Culturist* **58** (4): 248-253.

BLACKITH, R. E., AND REYMENT, R. A. 1971.

Multivariate Morphometrics. Academic Press, London.

BONE, Q., MARSHAL, N. B., AND BLAXTER, J. H. S. 1995.

Biology of fishes, 2nd ed. Blackie Academic and Professional, London.

BOOMKER, J. 1994.

Parasites of South African freshwater fish, VI in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* **61**: 35-43.

BOURGEOIS, S., KOCHER, T., AND SCHELANDER, P. 2003/4.

Case studies on the Zambezi River Basin.

<http://eawag.ch/research-e/apec/seminars/case%20studies/2003/casestudyZambeziRiverBasin.pdf>. Date accessed 16-10-2004.

BOWEN, S.H. 1980.

Detrital nonprotein amino acids are the key to rapid growth of Tilapia in Lake Valencia, Venezuela. *Science* **207**: 1216– 1218.

BOWEN, S. H. 1988.

Detritivory and herbivory. In: C., Bruton, M. N. and Ssentongo, G. W. (Eds.). *Biology and ecology of African freshwater fishes*. Lévêque, Institut Francais de Recherche Scientifique pour le Developpement en Coopération, Paris.

BRISMAR, A. 2002.

River systems as providers of goods and services: A basis for comparing desired and undesired effects of large dam projects. *Environmental Management* **29** (5): 598-609.

BRUTON, M. N. 1989.

Does animal systematics have a future in South Africa? *South African Journal of Science*

85 (6): 348-350.

BUTH, D. G. 1984.

Allozymes of the Cyprinid Fishes. In: Turner, B. J. (ed.). *Evolutionary genetics of fishes*.

Plenum Press, New York.

BUTH, D. G., DOWLING, T. E. AND GOLD, J. R. 1991.

Molecular and cytological investigations. In: Winfield, I. J., and Nelson, J. S. *Cyprinid*

Fishes: Systematics, biology and exploitation. Chapman and Hall, London, Fish and fisheries

series, 3: 667.

CALOW, P., AND FORBES, V. E. 1998.

How do physiological responses to stress translate into ecological and evolutionary

processes? *Comparative Biochemistry and Physiology Part A*. **120**: 11-16.

CAMPBELL, W. C. 1997.

Parasites and Cancer. *Parasitology today* **13** (5):202.

CAREY, T. G. 1967.

Kafue River and floodplain research: Fish population in the lagoon and riverine environments. *Fisheries Research Bulletin of Zambia* **3**: 9-22.

CARIGNAN, R., D'ARCY, P., SÉBASTAIN, L. 2000.

Comparative impacts of fire and forest harvesting on water quality in Boreal Shield lakes.

Canadian Journal of Fisheries and Aquatic Sciences **57** (Suppl. 2): 105-117.

CARVALHO, G. R. 1993.

Evolutionary aspects of fish distribution: Genetic variability and adaptation. *Journal of Fish*

Biology **43** (supplement A): 53-57.

CECIL, K. 2004.

The Zambezi reservoirs of water: Dams on the river.

<http://cgee.hamline.edu/rivers/MA2001/MA2000/Guests/archive/keen3.html>.

Date accessed 13-04-2004.

CHENJE, M. 2004.

Zambezi wetlands priceless.

<http://www.sardc.net/Imercsa/Zambezi/ZnewsLetter/issue2of2/wetland.htm>. Date accessed

19-04-2004.

CIAT, 2004.

Environmental Indicators.

<http://www.ciat.cgiar.org/indicators/project-description.htm>. Date accessed 26-04-2004.

CLAUSEN, J., KECK, D. D., AND HIESEY, W. M. 1947.

Heredity of geographically and ecologically isolated races. *American Naturalist* **81**: 114-133.

CORBET, P. S. 1961.

The food preferences non-cichlid fishes in the Lake Victoria Basin, with remarks on their evolution and adaptation to lacustrine conditions. *Proceedings of Zoology Society of London* **136**: 1-99.

COULIN, P. 1993.

Pelican catch. *Lammergeyer* **42**: 56.

COUNTRY REPORTS. 2004.

Facts by the country: Zimbabwe.

http://www.classbrain.com/cb_cr/geos/zi.html. Date accessed 15-04-2004.

CREECH, S. 1991.

An electrophoretic investigation of populations of *Antherina boreyi* Risso, 1810 and *A. presbyter* Cuvier, 1829 (Teleostei: Athrenidae): genetic evidence in support of the two species. *Journal of Fish Biology* **39**: 807-816.

CROWE, T. M. 1989.

Vertebrate systematics and biogeography in the Tropics. *South African Journal of Science* **85**: 497.

CROWE, T. M., KEMP, A. C., EARLÉ, R. A., AND GRANT, W. S. 1989.

Systematics is an essential but neglected biological science. *South African Journal of Science* **85**: 418.

CROZIER, W. W. 1997.

Genetic heterozygosity and meristic character variance in a wild Atlantic salmon population and a hatchery strain derived from it. *Aquaculture International* **5**: 407-414.

CUTTER, S. L., AND RENWICK, W. H. 1999.

Exploration, conservation, preservation: A geographical perspective on natural resource use, 3rd ed. John Wiley and Sons, New York.

DAGET, J., GAÏGHER, I. G., AND SSENTONGO, G. W. 1988.

Conservation. In: Lévêque, C., Bruton, M. N. and Ssentongo, G. W. (EDS.). *Biology and ecology of African freshwater fishes*. Institut Francais de Recherche Scientifique pour le Developpement en Coopération, Paris.

DIXON, B., TISCH, B., AND KANGAMBA, G. 2004.

Mining in Zambia: Challenges to Effective Regulation.

www.ott.wrcc.osmre.gov/library/proceed/sudbry2003/sudbry03/36.pdf. Date accessed 19-04-2004.

DOBSON, P. A., BRADSHAW, A. D., AND BAKER, A. J. M. 1997.

Hopes for the future: Restoration ecology and conservation biology. *Science* **277** (5325): 515-522.

DOBZHANSKY, TH. 1937.

Genetic nature of species differences. *American Naturalist* **71**: 404-420.

DOYLE, R. W., SHACKEL, N. L., BASIAO, Z., URAIWAM, S., MATRICIA, T., AND TALBOT, A. J. 1991.

Selective diversification of aquaculture stocks: A proposal for economically sustainable genetic conservation. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (suppl. 1): 148-154.

DU PLESSIS, S. S. 1963.

A revision of the Genus *Labeo* (Pisces: Cyprinidae) in the Transvaal, with a description of new species. *Annals of the Transvaal Museum* **24** (4): 327-337.

DU PREEZ, H. H. AND STEYN G. J. 1992.

A preliminary investigation of the concentration of selected metals in tissues and organs of the tigerfish (*Hydrocyon vittatus*) from the Olifants River, Kruger National Park, South Africa. *Water SA*. **18** (2): 131-136.

DWA & F (DEPARTMENT OF WATER AFFAIRS AND FORESTRY). 1997.

White paper on a National water policy for South Africa. Department of Water Affairs and Forestry, Pretoria.

EARTH CRASH, EARTH SPIRIT. 2004.

<http://www.africafront.com/waterwater-sheds/water-sheds2/water-sheds8.phd>.

Date

accessed 16-10-2003.

EMLLEN, J. M. 1977.

Ecology: an evolutionary approach. Addison-Wesley Publishing Company, Menlo Park,

California.

ENGELBRECHT G. D. 1995.

A Biochemical genetic study of three populations of Schilbe intermedius, Rüppel, 1832, (Pisces, Siluriformes). MSc. Dissertation, RAU, South Africa.

ENGELBRECHT, G. D., VAN DER BANK, F. H., AND MULDER P. F. S. 1997.

Allozyme variation in *Schilbe intermedius* Rüppel, 1832 (Pisces: Siluriformes) from two localities in the Limpopo River system, South Africa. *Comparative Biochemistry and Physiology* **117 B** (2): 179-184.

ENGELBRECHT, G. D., AND MULDER P. F. S. 1999 A.

Extremely high genetic differentiation between two populations of the river goby, *Glossogobius callidus* (Smith, 1937). *Water SA* **25** (1): 85-90.

ENGELBRECHT, G. D., AND MULDER P. F. S. 1999 B.

Allozyme variation in the river sardine *Mesobola brevianalis* (Pisces, Cyprinidae). *Water SA* **25** (3): 293-296.

ENGELBRECHT, G. D., AND MULDER P. F. S. 2000.

Allozyme variation in *Chiloganis paratus* and *C. pretoriae* (Pisces, Mochokidae) from the Limpopo River system. *Water SA* **26** (1): 111-114.

ENGELBRECHT, G. D. 2001.

A biochemical genetic analysis of the Southern African goldie barbs (*Barbus*, Cuvier and Cloquet 1816), with special reference to their biodiversity and conservation. Ph.D., Thesis, University of the North, Turfloop campus, South Africa.

ENGELBRECHT, J. S., AND VAN DER BANK, F. H. 1994.

Isozyme and allozyme differences in four shortfin barb (*Barbus brevipinnis* Jubb, 1966) populations with reference to undescribed species from the Transvaal. *Biochemical Systematics and Ecology* **22** (3): 296-276.

ENGELBRECHT, J. S., ROUX, F. AND HOFFMAN, A. H. 1994.

An assessment of the status of the fish community in the Levubu River (Limpopo system) from Albasini dam to the Limpopo confluence in the Kruger National Park. *Workshop report: Department of Water Affairs.*

ENGELBRECHT, J. S. 1996.

Phylogenetic relationships between morphologically similar Barbus species, with reference to their taxonomy, distribution and conservation. Ph.D. Thesis, Rand Afrikaans University, Johannesburg, South Africa.

ENGELBRECHT, J. S., AND VAN DER BANK, F. H. 1996 A.

Genetic relationships between Marico barb (*Barbus motebensis* Steinachner, 1894), Red tail barb (*B. gurnei* Günther, 1868) and Amatola barb (*Barbus amatolicus* Skelton, 1990). *Animal Genetics* **27**: 77-84.

ENGELBRECHT, J. S., AND VAN DER BANK, F. H. 1996 B.

Genetic relationships between seven species within chubbyhead and goldie barb groups of minnow (Pisces, Cyprinidae). *Journal of African Zoology* **100** (6): 381-396.

ENGELBRECHT, J. S., AND VAN DER BANK, F. H. 1997.

Allozyme differences between populations of chubbyhead barb (*Barbus anoplus* Weber, 1897) and Marico barb (*B. motebensis* Steindacher, 1894). *Water S A*. **23** (2): 175-180.

EVERITT, 1998

The Cambridge dictionary of statistics. Cambridge University press, New York.

FERREIRA, J. T. 1984.

Workshop on the Fish Genetics, 8th & 9th August 1984. Rand Afrikaans University, South Africa.

FERESU-SHONHIWA, F., AND HOWARD, J. H. 1998.

Electrophoretic identification and phylogenetic relationships of indigenous *tilapiine* species of Zimbabwe. *Journal of Fish Biology* **53** (6): 1178-1206.

FORBES, L. V., TROUNG, O., WIENTJIES, F. B., MOS, S. J., AND SEGAL, A. W. 1999.

The multicomponent NADH oxidase, which causes the production of Superoxide (SOD). *Biochemical Journal* **338**: 99-105.

FOWLER, H. W. 1936.

Zoological results of the George Vanderbilt African expedition of 1934. Part III, the fresh water fishes. *Proceedings of Academic and National Science of Philadelphia* **88**: 243-335.

FRANKEL, O. H., AND SOULE, M. E. 1981.

Conservation and evolution. Cambridge University Press, Cambridge.

FREEDMAN, B. 1995.

Environmental Ecology, second edition: The ecological effects of pollution, disturbance and other stresses. Academic Press, California.

GAIGHER, I. G. 1973.

The habitat preferences of the fishes from the Limpopo river system, Transvaal and Mocambique. *Koedoe* **16**: 103-116.

GAIGHER, I. G., AND POTT, R. MC. C. 1973.

Distribution of fishes in Southern Africa. *South African Journal of Science* **69**: 25-27.

GAIGHER, I. G., HAMMAN, K. C. D., AND THORNE, S. C. 1980.

The distribution, conservation status and factors affecting the survival of indigenous freshwater fishes in the Cape Province. *Koedoe* **23**: 57-88.

GAMBERALE, G., AND TULLBERG, B. S. 1996.

Evidence of peak shift in predator generalization among posematic prey. *Proceedings of Royal Society of London B.* **263** (1375): 1329-1334.

GIBSON, D. I., TIMOFEEVA, T. A., AND GERASEV, P. I. 1996.

A catalogue of the nominal species of monogenean genus, *Dactylogyrus*, Dieseng, 1850; and their host genera. *Systematic Parasitology* **35**: (1-3): 2-48.

GOODFELLOW, W. L. JR., HOCUTT, C. H., MORGAN, R. P. II, AND STAUFFER, J. R. JR. 1984.

Biological assessment of the taxonomic status of "*Rhinichthys bowersi*" (Pisces: Cyprinidae). *Copeia* **3**: 617-628.

GORE, J. A. 1989.

Setting priorities for minimum flow assessments in Southern Africa. *South African Journal of Science* **85**: 614.

GORE, J. A., KING, Y. M., AND HAMMAN K. C. D. 1991.

Application of the instream flow increment methodology to South African rivers: Protecting endemic fish of the Olifants River. *Water SA.* **17** (3): 225-236.

GORGENS, A., PEGRAM, G., UYS, M., GROBICKI, A., LOOTS, L., TANNER, A., AND BENGU, R. 1998.

Guidelines for Catchment Management to achieve Integrated water Resources Management in South Africa. *WATER RESEARCH COMMISSION*, Report No. KV 108/98.

GORMAN, G. C., AND RENZI, J. JR. 1979.

Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. *Copeia* (2): 242-249.

GRAFTON-CARDWELL, E. E., OUYANG, Y., AND SALSE, J. 1998.

Insecticide resistance and esterase enzyme variation in the California red scale (Homoptera: Diaspididae). *Journal of Economic Entomology* **91**: 812-819.

GRANT, W. S. 1984.

Biological population genetics of Atlanta Herring, *Clupea harengus*. *Copeia* 2: 357-364.

GRANT, W. S., DEMPESTER, Y. L., AND DA SILVA, M. F. 1988.

Use of electrophoresis in evolutionary systematics. *Transactions of Royal Society of South Africa* **46** (4): 295-311.

GRANT, W. S. 1993.

Population genetics in Southern Africa: What are the questions? *South African Journal of Science* **89** (1): 113-118.

GROENEWALD, A. A. V. J. 1964.

The role of *Eutropius depressirostris* (*Schilbe intermedius*) Peters (Pisces: Schilbeidae) as a predator in the lowveld impoundment in the Transvaal. *Hydrobiologia* **23** (1-2): 267-273.

GUÉGAN, J. F., LAMBERT, A., AND EUZET, L. 1989.

É tude des Monogènes des Cyprinidae du genre *Labeo* en Afrique de l' Ouest. II Genre *Dogielius*, (Bykhoski, 1936). *Review Hydrobiology of Tropics* **22** (1): 35-48.

GYLLENSTEN, U. 1985.

Genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine and freshwater species. *Journal of Fish Biology* **26**: 691-699.

HABER, W. 1995.

System ecological concepts for environmental planning. In: Klijn, F. (ed.).1995. *Ecosystem classification for Environment management*. Kluwer Academic Publishers, London.

HANCOCK, P. J. 2002.

Human impacts on the stream-ground water exchange zone. *Environmental Management* **29** (6): 763-781.

HARDEN, P. C., AND MATHEWS, L. 2002.

Rainfall response of degraded soil following reforestation in the Copper Basin, Tennessee USA. *Environmental Management* **26** (2): 163-174.

HARRIS, H., AND HOPKINSON, D. A. 1976.

A handbook of enzyme electrophoresis in Human genetics. Amsterdam-North,Holland.

HECHT, T. AND MASHEGO, S. N. 1981.

The fishes of the Limpopo and Olifants river tributaries (Limpopo drainage basin, South Africa). Part II. Annotated checklist of the fishes of the Mohlapiitse tributary of the Olifants River. Series A 26. University of the North. Republic of South Africa.

HENRY, T. B., IRWING, E. R., GRIZZLE, J. M., WILDHABER, M. L., AND BRUMBAUGH, W. G. 1999.

Acute toxicity of an acid mine drainage mixing zone to juveniles blue gill and largemouth bass. *Transaction of American Fisheries Society* **28**: 919-928.

HEITHAUS, M. S., AND LAUSHMAN, R. H. 1997.

Genetic variation and conservation of stream fishes: influence of ecology life history and water quality. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 1822-1836.

HILLIS, D. M., AND MORRITZ, C. 1990.

Molecular Systematics. Sinauer Associates Publishers, Sunderland.

HITEMAN, M. L., AND EKSTROM, B. 1994.

An investigation of the factor controlling staining and destaining of electrophoretic gels. *Electrophoresis: An International Journal* **15** (2): 200-208.

HOFFMAN, A. A., AND PARSONS, P. A. 1997.

Extreme Environmental Change and Evolution. Cambridge University Press, New York.

HUBBY, J. L., AND THROCKMORTON, L. H. 1967.

Protein differences in *Drosophila*. IV. A study of sibling species. *American Naturalist* **102**: 193-205.

HYSLOP, E. J. 1991.

Notes and Records: Ichthyofauna of Mlawula Nature Reserve, Swaziland. *African Journal of Ecology* **29**: 261-274.

IMAI, C., AND TANAKA, S. 1997.

Effect of sea water temperature on the variability of batch fecundity in Japanese Anchovy from the coastal waters around Miura Peninsula. *Fisheries Science* **63** (4): 489-495.

INTERNATIONAL RIVERS NETWORK. 2005.

A brief history of Africa's largest water projects.

<http://www.internationalrivers.org/africa/brief-history-africa-s-largest-water-project#env>.

Date accessed: 17-10-2010.

JACKSON, P. B. N. 1961.

The impact of predation, especially by Tigerfish (*Hydrocyon vittatus*, Castelnau) on the African freshwater fishes. *Proceedings of the Zoological Society of London* **136**: 603-622.

JACKSON, P. B. N., AND COETZEE, P. W. 1982.

Spawning behaviour of *Labeo umbratus* (Smith) (Pisces: Cyprinidae). *South African Journal of Science* **78**: 293-295.

JACKSON, P. B. N., MARSHALL, B. E. AND PAUGY, D. 1988.

Fish communities in man-made lakes. In: *Biology and ecology of African freshwater fishes*. Lévêque, C., Bruton, M. N., and Ssentongo, G. W. (eds). Institut Francais de Recherche Scientifique pour le Developpement en Coopération. Paris.

JAIN, S. 1983.

Genetic characteristics of populations. In: *Disturbances and ecosystems components of response*. Mooney, H. A., and Godron, M. Springer-Verlag.

JAMESON, D. L. (ED.). 1977.

Genetics of speciation. Dowden, Hutchinson and Ross, Inc. Stroudsburg.

JAWOROSKI, Z. 2006.

All Chernobyl's victims: A realistic assessment of Chernobyl's health effects.

www.ncr.gov/reading-rm/doc-collections/commissions/secy8/2002-0089.

Date accessed: 19-07-2006.

JENERETE, G. D., LEE, J., WALLER, D. W., AND CARLSON, R. E. 2002.

Multivariate analysis of the ecoregion delineation for aquatic systems. *Environmental Management* **29** (1): 67-75.

JUBB, R. A. 1961.

An illustrated guide to the freshwater fishes of Zambezi River, Lake Kariba, Phungwe, Sabi-Lundi and Limpopo rivers. Stuart Manning, Bulawayo.

JUNG, K., KÜHLER, S., KLOTZET, S., BECKER, S., AND WOLFGANG, H. 1993.

The effect of storage temperature on the activity of SOD, catalase, glutathione peroxidase, reductase and S -transferase in rat liver and kidney homogenates. *Enzymes and Proteins* **47** (3): 149-155.

KAMBOLE, M. S. 2004.

Managing the water quality of the Kafue River.

www.waternetonline.org/aboutWN/pdf/Kambole.pdf-93k.

Date accessed: 14-06-2004.

KARÅS, P., NEUMAN, E., AND SANDSTRÖM. 1991.

Effect of Pulp Mill effluent on population dynamics of Perch, *Perca fluviatilis*. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (1): 28-34.

KEENLEYSIDE, M. H. A. (ED.). 1991.

Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman and Hall, London.

KING, E. R., AND STANFIELD, W. D. 1997.

A Dictionary of Genetics, 5th ed. Oxford University Press, New York.

KIRBY, A. 2004.

Dawn of the thirsty country.

<http://news.bbc.co.uk/1/hi/sci/tech755497.stm>. Date accessed: 15-04-2004.

KIRPICHNIKOV, V. S. 1973.

Biochemical polymorphism and Microevolution Processes in fish. In: Schröder, J. H. (ed.).
Genetics and mutagenetics of fish. Springer-Verlag, Berlin.

KIRPICHNIKOV, V. S. 1992.

Adaptive nature of intrapopulational biochemical polymorphism in fish. *Journal of Fish
Biology* **40**: 1-16.

KLEYNHANS, C. J. 1980.

A checklist of the fish species of the Crocodile and Matlabas rivers (Limpopo system) of the
Transvaal. *Journal of Limnological Society of Southern Africa* **6** (1): 33-34.

KLEYNHANS, C. J., SCHULZ, G. W., ENGELBRECHT, J. S., AND ROUSSEAU, F. J. 1992.

The impact of paper mill effluent spill on the fish populations of the Elands and the
Crocodile rivers (Inkomati river system, Transvaal). *Water S A*. **18** (2): 73-80.

KLIJN, F. (ED). 1995.

Ecosystem classification for Environment Management. Kluwer Academic Publishers,
London.

KOEHN, F. K. 1978.

Physiology and biochemistry of enzyme variation: The interface of ecology and population
genetics. In: Brussard P. F. (ed.). *Proceedings in Life Sciences, Ecological Genetics: the
Interface*. Springer-Verlag, New York.

KOOPMAN, K. F. 1950.

Natural selection for reproductive isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution* **4**: 135-148.

KOTZE, A., VAN DER BANK, F. H., STEYN G. J. 1998.

Allozyme variation in two populations of *Hydrocynus vittatus* (Pisces Characidae). *South African Journal of Animal Science* **28** (3/4): 153-160.

KRAMER, D.L., AND BRYANT, M. J. 1995.

Intestine length in the fishes of a tropical stream: 1. Ontogenetic allometry. *Environmental Biology of Fishes* **42** (2): 115-127.

KYLE, C. J., AND BOULDING, E. G. 1998.

Molecular genetic evidence for parallel evolution in Marine Gastropod, *Littorina subrotundata*. *Proceedings of Royal Society of London B*. **265**: 303-308.

LAFFERTY, K. D. 1997.

Environmental parasitology: What can parasites tell us about human impact on the environment? *Parasitology Today* **13** (7): 251-255.

LANDAU, S. I. (ED.). 1986.

International Dictionary of Medicine and Biology, Volume 11. A Wiley Medical publication, John Wiley and Sons, New York.

LAUZANNE, L. 1988.

Les habitudes alimentaires des poissons d'eau douce Africains. In: Lévêque, C., Bruton, M. N. and Ssentongo, G. W. (eds.). *Biology and Ecology of African Freshwater Fishes*. Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris.

LAVIN, P. A., AND MCPHAIL, J. D. 1993.

Parapatric lake and stream sticklebacks on Northern Vancouver Island: disjunct distribution or parallel evolution? *Canadian Journal of Zoology* **71** (1): 11-17.

LEE, M. S. Y. 1996.

Stability in meaning and content of taxon names: an evaluation of Crowd-clade definitions. *Proceedings of Royal Society of London B*. **263**: 1103-1109.

LÉVÊQUE, C. AND QUENSIÈRE. 1988.

Les peuplements ichtyologiques des lacs peu profonds. In: Lévêque, C., Bruton, M. N. and Ssentongo, G. W. (eds.). *Biology and ecology of African freshwater fishes*. Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris.

LÉVÊQUE, C. 1997.

Biodiversity Dynamics and Conservation: the Freshwater Fish of Tropical Africa. Cambridge University Press, UK.

LEWIS, K. M., FEDER, J. L., AND LAMBERTI, G. A. 2000.

Population genetics of the Zebra mussel, *Dreissena polymorpha*, (Pallas): Local allozyme differentiation within Midwestern lakes and streams. *Canadian Journal of Fisheries and Aquatic Sciences* **57** (3): 637-643.

LINCOLN, R. J., BOXSHALL, G. A., AND CLARK, P. F. 1984

A Dictionary of Ecology, Evolution and Systematics. Cambridge University Press, Cambridge.

LIZEMORE, E., FERREIRA J. T., AND VAN DER BANK, F. H. 1989.

An electrophoretic and morphometric evaluation of two subspecies of *Tilapia rendalli* from Southern Africa. *Madaqua* **16** (1): 35-39.

LOVE, M. S., AND CAILLIET, G. M. 1979.

Readings in Ichthyology. Goodyear Publishing Company, Santa Monica.

LOWE-McCONNELL, R. H. 1988.

Broad characteristics of the ichthyofauna. In: Lévêque, C., Bruton, M. N. and Ssentongo, G. W. (eds.). *Biology and ecology of African freshwater fishes*. Institut Francais de Recherche Scientifique pour le Developpement en Coopération. Paris.

LOWE-McCONNELL, R. H. 1997.

EAFRO and After: A guide to key events affecting fish communities in Lake Victoria. *South African Journal of Science* **193** (11/12): 570-574.

LUUS-POWELL, W. J., AND AVENANT-OLDEWAGE, A. 1996.

Surface morphology of the fish parasite *Chonopeltis victori*, Avenant-Oldewage, 1991 and other aspects of histomorphology. *Koedoe* **31** (1): 55-70.

MABEE, P. M. 1993.

Coding polymorphic loci: Examples from allozymes and ontogeny. *Systematic Biology* **42** (2): 166-181.

MARRIOTT, F. H. C. 1991.

A dictionary of statistical terms, 5th edition. Longman Scientific, Technical, New York.

MARKERT, C. L., AND FAULHABER, I. 1965.

Lactate dehydrogenase isozymes in fish. *Journal of Experimental Zoology* **159**: 319-332.

MATTSON, N. S., AND MUTALE, J. C. 1992.

Multi-mesh gillnets to estimate species composition and catch per unit effort of a small water body in Zambia. *Journal of Fish Biology* **41**: 897-908.

MATHEWS, W. J., AND ZIMMERMAN, E. G. 1990.

Potential effects of global warming on the native fishes of the Southern Great Plains and Southwest. *Fisheries* **15**: 26-32.

MAY, B., AND MARSDEN, J. E. 1992.

Genetic identification and implications of another invasive species of Dreissenid mussel in the great lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **49** (7-9): 1501-1506.

MAYR, E. 1959.

Isolation as an evolutionary factor. *Proceedings of American Philosophical Society* **103**: 221-230.

MCCULLY, P. 2003.

Silenced Rivers: The Ecology and Politics of large dams. *The Professional Geographer*, **55** (2): 285-287.

MCKEOWN, B. A. 1984.

Fish Migration. Croom Helm, London.

MEISSNER, R. 2004.

Conflict Trends - No. 2/2000. The case of the Orange River.

<http://www.accord.org.za/web.nsf/0/f4486bc239918e4225>. Date accessed: 13-04-2004.

MENEZES, M. R., AND NAIKS, M. M. 1993.

Genetic characterization in four Sciaenid Species from the Arabian Sea. *Journal of Fish Biology* **43**: 61-67.

MERRON, G. S., AND BRUTON, M. N. 1989.

Recent fisheries research in the Okavango Delta. *South African Journal of Science* **85** (7): 416-417.

MERRON, G. S., AND BRUTON, M. N. 1993.

A survey of the fishes in and around Sun City with particular reference to Mankwe Lake (Pilanesberg National Park) and suggestions for stocking fish into the waters of the Sun City Resort Complex. *J. L. B. Institute of Ichthyology, Investigational Report no.44*: 1-22.

MERRON, G. S., BRUTON, M. N., AND LA HAUSSE DE LALOUVIERE, P. 1993.

Implications of water release from the Pongola poort dam for the fish and fishery of the Phongolo floodplain, Zululand. *South African Journal of Aquatic Science* **19** (1/2): 34-49.

MINSHULL, J. L. 1985.

Cleaning behaviour between the Cichlid fish *Tilapia rendalli rendalli* and the Cyprinid, *Labeo cylindricus* Peters, 1852. *Journal of Limnological Society of Southern Africa* **11** (1): 20-21.

MITTON, J. B., AND GRANT, M. C. 1984.

Associations among protein heterozygosity, growth and developmental homeostasis. *Annual Review of Ecology and Systematics* **15**: 479-499.

MOHAMMED, A. E. 2003.

Cooperation and joint development in International water resources: The case of Limpopo and Orange River basin in Southern Africa.

<http://www.somwat.com/unupaper.html>. Date accessed: 07-08-2003.

MONCEL, C. 2004.

More precious than gold: water at the heart of the conflicts.

<http://www.iucn.org/reuters/1999/article/winningwestafrica.htm>. Date accessed: 13 -04-2004.

MONNA, S. C. 2004.

A framework for international cooperation for the management of the Okavango Basin and the Delta. Ramsar COP7DOC.20.5.

http://www.ramsar.org/cop7_doc_20.5_e.htm. Date accessed: 13-04-2004.

MONTCHOWUI, E., LALEYE, P., MOREAU, J., PHILIPPART, J-C., AND PONCIN, P. 2009.

Population parameters of African carp: *Labeo parvus* Boulenger, 1902 (Pisces: Cyprinidae) in the Ouémé River in Bénin (West Africa), North-Western Journal of Zoology **5** (1): 26-33.

MORK, J., AND GIÆVER M. 1995.

Genetic variation at isozyme loci in blue whiting from the north-east Atlantic. *Journal of Fish Biology* **46**:462-468.

MORITZ, C., AND HILLIS, D. M. 1990.

Molecular systematics: Context and Controversies. pp 1-10. In: Hillis, D. M., and Moritz, C. (eds.). *Molecular Systematics*. Sinauer Associates Publishers, Sunderland.

MOSS, B. 2000.

Biodiversity in freshwater - an issue of species preservation or system functioning. *Environmental Conservation* **27** (1): 1-4.

MOYLE, P. B., AND CECHE, J. J. JR. 1996.

Fishes: An introduction to ichthyology. Prentice Hall, New Jersey.

MOYLE, P. B., AND CECHE, JR., J. J. 2000.

An Introduction to Ichthyology, 4th ed. Prentice-Hall International, London.

MPANDE, R. L., AND TAWANDA, M. 2004.

Population dynamics and the emerging competition for the water use in the Zambezi River basin. <http://www.aaas.org/international/ehn/waterpop/southaf.html>. Date accessed: 19-04-2004.

MULDER, P. F. S. 1989.

Die ontwikkeling van 'n genetiese databank vir die ekonomies belangrike Barbus spesies in Suid Afrika. (Ph.D. Thesis). RAU., South Africa.

MURPHY, R. W., SITE, C. W. JR., BUTH, D. G., AND HAUFLER, C. H. 1990.

Protein 1: Allozyme electrophoresis. In: Hillis, D. M., and Moritz, C. (eds.). *Molecular systematics*. Sinauer Associates, USA. (pp. 45-126).

NATIONAL PARKS BOARD OF TRUSTEES OF SOUTH AFRICA. 1994.

Fish for Africa. Pretoria, South Africa.

NEI, M. 1972.

Genetic distance between populations. *American Naturalist* **106**: 283-292.

NEI, M. 1978

Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics* **89**: 580-590.

NEI, M. 1987.

Molecular Evolutionary Genetics. Columbia University Press, New York.

NEVO, E. 1978.

Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology* **13**:121-77.

NEVO, E., BEILES, E., AND BEN-SHLOMO, R. 1984.

The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. Evolutionary dynamics of genetic diversity. *Lecture Notes Biomath.* **53**: 13-

213.

O'BRIEN, S. J. 1994.

A role for molecular genetics in biological conservation. *Proceedings of National Academy of Science* **91** (13): 5748-5755.

OLIVIER, L. P. 1994.

'n Studie om die effektiwiteit van die Kanniedood dam visleer in die Shingwezirivier, Nasionale Krugerwildtuin, te bepaal. Technikon Pretoria.

OOSTHUIZEN, J. S. 1992.

'n Biochemiese en morfologiese ondersoek van *Tilapia guinasana*, *T. rendalli* en *T. sparrmanii*. MSc. RAU. South Africa.

OOSTHUIZEN, S., VAN DER BANK, F. H., AND FERREIRA, J. T. 1993.

Genetic distances between and evolutionary relationships for *Tilapia guinasana*, *T. rendalli* and *T. Sparrmanii*. *Biochemical Systematics and Ecology* **21** (3): 351-362.

OOSTHUIZEN, J. H., AND DAVIES, R. W. 1994.

The biology and adaptation of the hippopotamus leech *Placobdelloides jaegerskioedi* (Glossophonidae) to its host. *Canadian Journal of Zoology* **72** (3): 418-422.

OWUSU, E. O.; HORIIKE, M., AND HIRANO, C. 1996.

Polyacrylamide gel electrophoretic assessments of esterases in cotton aphid (Homoptera: Aphididae) resistance to dichlorvos, *Journal Economic Entomology* **89**: 302–306.

PADHI, B. K., GHOSH, S. K., AND MANDAL, R. K. 1998.

Characterization of Mbo II satellite in *Cirrhana mrigalla* and *Clarias batrichus* (Pisces). *Genome* **41**: 34-39.

PAPERNA, I. 1973.

New species of Monogenea (Vermes) from African freshwater fish. A Preliminary Report. *Revue de Zoologie et de Botanique Africaines* **87**: 505–518.

PAUGY, D., GUÉGAN, J. F., AND AGNÉSE, J. F. 1990.

Three simultaneous and independent approaches to characterization of a new species of *Labeo* (Teleostei, Cyprinidae) from West Africa. *Canadian Journal of Zoology* **68** (6): 1124- 1131.

PIENAAR, U. DE V. 1978.

The freshwater fishes of the Kruger National park. Trustee of the National Parks Board of The Republic of South Africa.

PITCHER, T. J., AND PARISH, J. K. 1993.

Functions of the shoaling behaviour in teleosts. pp 363-439. In: Pitcher, T. J. (ed.). *Behaviour of Teleost Fishes*, 2nd edition. Chapman and Hall, London.

POTTINGER, L. 2003.

International Rivers Network. IRN's Lesotho Campaign.

<http://www.irn./index.asp?id=/programs/lesotho/background.html>. Date accessed: 05-06-2003.

POWER, M. E. 1983.

Grazing responses of tropical freshwater fishes to the different scales of variation in their food. *Environmental Biology of Fishes* 9 (2): 103-115.

QUICKE, D. L. J. 1993.

Principles and techniques of contemporary taxonomy. Blackie Academic and Professional, London.

RAMSHAW, J. A. M., COYNE, J. A., AND LEWONTIN, R. C. 1979.

The sensitivity of electrophoresis as a detector of genetic variation. *Genetics* **93** (4): 1019-1038.

REID, G. MCG. 1985.

A revision of African species of *Labeo* (Pisces: Cyprinidae) and a re-definition of the Genus. *Theses zoologicae*. Vol 6: J. Cramer, Braunschweig, Germany.

RIBBINK, A. J., AND ECCLES, D. H. 1988.

Fish communities in East African great lakes. In: Lévêque, C., Bruton, M. N. and Ssentongo, G. W. (eds.). *Biology and Ecology of African Freshwater Fishes*. Institut Francais de Recherche Scientifique pour le Developpement en Coopération. Paris.

RIDGWAY, G. J., SHELBURNE, S. W., AND LEWIS, R. D. 1970.

Polymorphism in the esterases of Atlantic herring. *Transaction of American Fisheries Society* **99**: 147-151.

ROBERT, T. R. 1986.

A revision of the Labeo G. M. Reid, 1985. Copeia **3**: 847-849.

ROSENBLUM, P. M., HORNE, H., CHATTERJEE, N., AND BRANDT, T. M. 1991.

Influence of the diet on ovarian growth and steroidogenesis in the Largemouth bass. In: Pott, A. P., Sumpter, J. P., Kime, D. E., and Rolfe, M. S. *Proceedings of the fourth International Symposium on the Reproductive Physiology of Fish*. Fish Symposium 91, Sheffield, United Kingdom.

SABEH, F., WRIGHT, T., AND NORTON, S. J. 1996.

Isoenzymes of SOD from aloe vera. *Enzymes and proteins* **49** (4): 212-221.

SADC COUNTRIES DATABASE (SADC/FSTAU & FAO/GIEWS). 2004.

Database on food and agriculture in SADC Countries.

<http://www.sadc-fanr.org.zw/sadcbase.htm>. Date accessed: 04-05-2004.

SADC FACTS. 2004.

SADC facts. <http://www.sadc-sqam.org/sadc/sadcfacts.html>. Date accessed: 04-05-2004.

SADC SECTORAL RESPONSIBILITIES. 2004.

SADC Sectoral responsibility table. <http://www.sadc-sqam.org/sadc/sadcresp.html>.

Date accessed: 04-05-2004.

SANYANGA, R. A., MACHENA, C., AND KAUTSKY, N. 1995.

Abundance and distribution of inshore fish in fished and protected areas in Lake Kariba

Hydrobiologia: The Hague **306** (1): 67-78.

SARDC-IMERCSA. 2004.

Zambezi Factsheet 11: Pollution.

<http://www.sardc.net/Imercsa/zambezi/zfsheet/zfsheet11.html>. Date accessed: 21-04-2004.

SCALLY, R. 2011.

Severe acute respiratory syndrome (SARS) Update: Tears, Fears and Hope, NurseZone.

http://www.nursezone.com/nursing-news-events/more-news/SARS-Update-Tears-Fears-and-Hope_28666.aspx

Date accessed: 12-03-2011

SCHEIMER, F. 2000.

Fish as indicators for the assessment of the ecological integrity of large rivers.

Hydrobiologia (422 /423): 274-278.

SCHMIDT, J. 1918.

Racial studies in fishes: II. Experimental investigations with *Lebistes reticulatus* (Peters)

Regan. *Genetics* 7: 147-153.

SCHMIDT, G. D., AND ROBERTS, L. S. 1989.

Foundations of Parasitology, 4th edition. Times L mirror/ Mosby College Publishing.
St Louis.

SCHOONBEE, H. J., HECHT, J., SAAYMAN, J. E., AND POLLING L. 1995.

Fish population assessment in a temperate lowveld impoundment of the Transvaal, South Africa. *Water SA*. 21 (2): 147-152.

SCIENCE DAILY. 2006 A.

NASA unveils new "Natural Hazards" web site.

www.sciencedaily.com/releases/2002/01/020128081112.htm. Date accessed: 22-08-2006

SCIENCE DAILY. 2006 B.

NASA's Terra satellite: Transglobal pollution.

www.sciencedaily.com/releases/2004/05/040519064909.htm. Date accessed 22-08-2006

SCOBIE, A. E., AND MACKIE, I. M. 1995.

The use of Sodium Docecyl Sulphate Polyacrimide gel electrophoresis to confirm the presence of Salmon (*Salmo salar*) eggs in an illegal fish bait. *Electrophoresis: An International Journal* **16**: 306-307.

SCHOONBEE, H. J., HECHT, J., SAAYMAN, J. E., AND POLLING L. 1995.

Fish population assessment in a temperate lowveld impoundment of the Transvaal, South Africa. *Water SA*. **21** (2): 147-152.

SELANDER, R. K., AND JOHNSON, W. E. 1973.

Genetic variation among vertebrate species. *Annual Review of Ecology and Systematics* **4**: 75-91.

SERVEISS, V. B. 2002.

Applying ecological risk principles to watershed assessment and management. *Environmental Management* **29** (2): 145-154.

SHAKLEE, J. B., AND TAMARU, C.S. 1981.

Biochemical and morphological evolution of Hawaiian Bonefishes (*Albula*). *Systematic Zoology* **30** (1): 125-146.

SHAKLEE, J. B., ALLENDORF, F. W., MORIZOT, D. C., AND WHITT, G. S. 1990.

Gene nomenclature for protein coding loci in fish. *Transactions of the American Fisheries Society* **119**: 2-15.

SHARPLES, A. D., AND EVANS C. W. 1998.

Impacts of pulp and paper mill effluents on water quality and fauna in a New Zealand hydroelectric lake. *New Zealand Journal of Marine and Freshwater research* **32** (1): 31-53.

SHOKO, D. S. M. 2004.

Small-scale mining and alluvial panning within the Zambezi Basin: an ecological time bomb and tinderbox for future conflicts among riparian states.

<http://dlc.dlib.indiana.edu/archive/00000921/>. Date accessed: 15-04-2004.

SHUGART, H. H. 1998.

Terrestrial Ecosystems in Changing Environment. Cambridge University Press, New York.

SINGH, N., BHATTACHARYA, P., JACKS, G., AND GUSTAFSSON, J. 2003.

Women and water: a policy assessment.

LWR.kth.se/personal/personer/bhattacharya-prosun/WaterPolicy-2003.pdf-92k.

Date accessed: 07-08-2003.

SKELTON, P.H. 1988.

A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa.

Annals of the Cape Provincial Museum 16(10): 201-307.

SKELTON, P. H. 1989.

A preliminary survey of fishes in the Kransberg National Park. *J. L. B. Smith Institute of Ichthyology: Investigational Report* 32: 1-11.

SKELTON, P. H., TWEDDLE, D., AND JACKSON, P. B. N. 1991.

Cyprinids of Africa. In: Winfield, I. J. and Nelson, J. S. (eds.). *Cyprinid fishes: Systematics, biology and exploitation*. Fish and fisheries series 3, Chapman and Hall, London.

SKELTON, P. H. 1993.

A complete guide to the fresh water fishes of Southern Africa. Southern Book Publishers, Halfway House, South Africa.

SKELTON, P. H., CAMBRAY, J. A., LOMBARD A., AND G. A. BENN. 1995.

Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology* 30(3): 711-781.

SMITH, M. J. 1966.

Sympatric speciation. *American Naturalist* **100**: 637-650.

SMITH, I. 1976.

Chromatography and Electrophoretic Techniques, vol. 2. Heineman, London.

SMITH, R. L. 1980.

Ecology and Field Biology, 3rd ed. Harper and Row Publishers, New York.

SMITH, P. J., FRANCIS, R. I. C. C., AND MCVEAGH, M. 1991.

Loss of genetic diversity due to fishing pressure. *Fisheries Research* **10** (3-4): 309-316.

SMITH, G. R. 1992.

Introgression in fishes: significance for palaeontology, cladistics and evolutionary rates.

Systematic Biology **41** (1): 41-57.

SMITH, R. L. 1996.

Ecology and Field Biology, 5th ed. Harper and Collins College Publishers, New York.

SNADDON, C. D., DAVIES, B. R., AND WISHART, M. J. 1999.

A global overview of inter-basin water transfer schemes, with an appraisal of their ecological, socio-economic and socio-political implications for their management. *Water Research Commission*, TT120/00.

SOULÉ, M. E. 1979.

Heterozygosity and developmental stability. *Evolution* **33**: 306-401.

SOULSBY, J. J. 1960.

Some Congo Basin fishes of Northern Rhodesia. *Northern Rhodesia Journal* **4** (4): 319-334.

SPEITH, H. T. 1949.

Sexual behaviour and isolation in *Drosophila*. II. The interspecific mating of species of the *willistoni* group. *Evolution* **3**: 67-81.

SPELLERBERG, I. F. 1996.

Conservation Biology. Longman Group, England.

STEWART, J. E. 1991.

Introduction as a factor in diseases of fish and aquatic vertebrates. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (suppl. 1): 110-117.

STUART, R. J., AND GAUGLER, R. 1996.

Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematode *Steinernema glaseri*. *Canadian Journal of Zoology* **74**: 164-170.

SWANSON, T. 1997.

Global Action for Biodiversity. Earthscan Publications, London.

SWOFFORD, D.L, BLACK, W.C., AND SELANDER R. B. 1997.

BIOSYS-2. Computer software package for allelic frequencies. Department of Microbiology, Colorado State University, USA.

TED CASE STUDIES. 2002.

Lesotho Water Exports, case number 196.

<http://www1.american.edu/TED/LESOTHO.htm>. Date accessed: 15-10-2003.

TEMPLETON, A. R. 1980.

Modes of speciation and inferences based on genetic distances. *Evolution* **34** (4): 719-729.

TEMPLETON, A. R. 1999.

Human races: A genetic and evolutionary perspective. *American Anthropologist* **100** (3): 632-650.

THOMPSON, J. N. 1999.

The evolution of species interactions. *Science* **284** (54): 2116-2118.

TIMOFEEVA T. A. GERASEV P. I., AND GIBSON D. I. 1997

A catalogue of the nominal species of the monogenean family Dactylogyridae Bychowsky, 1933 (excluding *Dactylogyrus* Diesing, 1850). *Systematic Parasitology* **38**: 153–158

TOMASSON, T., CAMBRAY, J. A. AND JACKSON, P. B. N. 1984.

Reproductive biology of four large riverine fishes (Cyprinidae) in a man-made lake, Orange River, South Africa. *Hydrobiologia* **112**: 179-195.

TSHIBWABWA, S. M., AND TEUGELS, G. G. 1995.

Contribution to the systemic revision of the African cyprinid fish genus *Labeo*: species from the lower Zaire River systems. *Journal of Natural History* **29**: 1543-1579.

TSHIBWABWA, S.M., M.L.J. STIASSNY AND R.C. SCHELLY. 2006.

Description of a new species of *Labeo* (Teleostei: Cyprinidae) from the lower Congo River. *Zootaxa* 1224:33-44.

TVE'S EARTH REPORT: 2004.

Boiling Point. <http://www.tve.org/earthreport/archive/doc.cfm?aid=903>.

Date accessed: 13-04-2004.

TWEDDLE, D. AND WILLOUGHBY, N. G. 1976.

Further notes on the fishes of Malawi's Game Reserves. *Nyala* **3** (2): 4-10.

TWEDDLE, D., AND SKELTON, P. H. 1993.

River fishes of the Lake Malawi Catchment area. Part II: First record of the Blotched Catfish, *Clarias stappersii*, Boulenger, in Malawi. *Nyala* **16**: 73-76.

TWEDDLE, D. 1996.

Fish survey of Nkotakota wildlife reserve: a report to the Japanese International Cooperation Agency on behalf of the Wildlife of Malawi. Investigational Report no. 53: Research Associate, J. L. B. Smith Institute of Ichthyology, Grahamstown, RSA.

TWINCH, A. J., AND GROBLER, D. C. 1986.

Pre-impoundment as an eutrophication management option: A simulation study at Haartebeesdam. *Water SA* **12** (1): 19-26.

UDO DE HAES, H. A., AND KLIJN, F. 1995.

Environmental policy and ecosystem classification. In: Klijn, F. (ed). 1995. *Ecosystem classification for environment management*. Kluwer Academic Publishers, London.

OWUSU, E. O., HORIIKE, M., AND HIRANO, C. 1996.

Polyacrylamide gel electrophoretic assessments of esterase in cotton aphid (Homoptera: Aphididae) resistance to dichlorvos. *Journal of Economic Entomology* **89**: 302-306.

VAN AS, J. G. 1986.

A new species of *Chonopeltis* (Crustacea: Branchiura) from the Limpopo river system, South Africa. *South African Journal of Zoology* **21**: 348-351.

VAN AS, J. G. 1992.

A new species of *Chonopeltis* (Crustacea: Branchiura) from the Zambezi river system. *Systematic Parasitology* **22** (3):221-229.

VAN AS, J. G. AND BASSON, L. 1992.

Trichodinid ectoparasites (Ciliophora: Peritrichida) of freshwater fishes of the Zambezi River system with a reappraisal of host specificity. *Systematic Parasitology* **22** (2): 81-109.

VAN DER BANK, F. H., GRANT, W. S., AND FERREIRA, J. T. 1989.

Electrophoretically detectable genetic data for fifteen Southern African cichlids. *Journal of Fish Biology* **34**: 465-483.

VAN DER BANK, F. H., GROBLER, J. P., AND DU PREEZ, H. H. 1992.

A comparative biochemical genetic study of three populations of domesticated and wild African catfish (*Clarius gariepinus*). *Comparative Biochemistry and Physiology* **101B**: 385-390.

VAN DER BANK, F. H. 1993.

Allozyme variation in *Synodontis leopardinus* Pisces, Siluriformes). *Comparative Biochemistry and Physiology* **105B**: 333-336.

VAN DER BANK, F.H. 1995.

Allozyme variation in a freshwater mussel population (*Coelatura kunenensis*, Mousson, 1887) from southern Africa. *Water SA*. **21** (2): 153-157.

VAN DER BANK, F. H., AND VAN DER BANK, M. 1995.

An estimate of genetic variation in a population of the Bulldog *Marcosenius marcolepidotus* Mormyridae). *Water SA*. **21** (3): 265-268.

VAN DER MERWE, W., VAN VUUREN, J. H. J., AND VERMAAK, J. F. 1987.

Cyclic changes of the steroid production activity in the ovary and the estradiol levels of the mud fish, *Labeo capensis*. *South African Journal of Zoology* **22** (3): 213-217.

VAN SOMEREN, V. D. 1961.

Appendix E: River fish migration. *East African Freshwater Fisheries Research Organisation (Annual report)*: 36-39.

VAN VUUREN, N. G. 1989.

·n Ondersoek na die genetiese verskille en die populasie genetica van nege Labeo-spesies in Suid-Afrika. (Ph.D Thesis). Randse Afrikaanse Universiteit. South Africa.

VAN VUUREN, N. G., MULDER, P. F. S., FERREIRA, J. T., AND VAN DER BANK. 1990.

Morphometric and electrophoretic analysis of *Labeo capensis* and *Labeo umbratus* from two localities in Southern Africa. *Water S A*. **16** (3): 135-146.

VAN WYK, E., VAN DER BANK, F. H., AND VERDOORN, G. H. 1992.

A biochemical genetic study of allozyme polymorphism in two natural populations of the Cape Griffon vulture (*Gyps coprotheres*) and in individuals in captivity. *Comparative Biochemistry and Physiology* **103 B**: 481-493.

VILJOEN, B. C. S. 1986.

A seasonal investigation of the genus *Lernaea* (Crustaceae: Copepoda) on Cyprinid fish in Loskop Dam, Transvaal, South Africa. *South African Journal of Wildlife Research* **16** (1): 26-31.

WALSH, M. T. 1996.

Fish and fishing the rivers and wetland of Usangu. *East African Natural History Society* **26** (3/4): 42-47.

WAPLES, R. S. 1991.

Genetic interactions between hatchery and wild Salmonids: Lessons from the pacific North West. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (suppl.1):124-133.

WARHEIT, K. I. 1992.

The role of morphometrics and cladistics in the Taxonomy of fossils: A paleontological example. *Systematic Biology* **41** (3): 345-369.

WEIR, B.S. AND COCKERHAM, C.C. 1984.

Estimating F-statistics for the analysis of population structure. *Evolution* **38**: (6) 1358-1370.

WEIS, J. S., SMITH, G. M., AND ZHOU, T. 1999.

Altered predator/prey behaviour in the polluted environments: implications for fish conservation. *Environmental Biology of Fishes* **55** (1-2):43-51.

WEYL, O. L. F., AND BOOTH, A. 1999.

On the life history of a Cyprinid fish, *Labeo cylindricus*. *Environmental Biology of Fishes* **55** (3): 215-225.

WHITE, M. J. D. 1968.

Models of speciation. *Science* **159**: 1065-1070.

WHITT G. S. 1970.

Developmental genetics of lactate dehydrogenase of fish. *Journal of Experimental Zoology* **175**:1-35.

WHITT, G. S., MILLER, E. T., AND SHAKLEE, J. B. 1973.

Developmental and biochemical genetics of lactate dehydrogenase isoenzymes in fishes. In: Schröder, J. H. (ed.). *Genetics and Mutagenesis of Fish*. Springer-Verlag. Berlin.

WHITTON, B. A. (ED.). 1975.

River Ecology. Blackwell Scientific Publications, Oxford, London.

WILDNET AFRICA NEWS ARCHIVE. 2004.

Ministerial probe into SA's polluted rivers (February 09, 2001).

<http://wildnetafrica.com/wildlifeneeds/2002/02/1097.html>.

Date accessed: 19-04-2004.

WILSON, A. C., MAXSON, L. R., AND SARICH, V. M. 1974.

Two types of molecular evolution. Evidence from studies of interspecific hybridization.

Proceedings of National Academy of Science **71**: 2843-2847.

WORLD HEALTH ORGANIZATION. 2002.

National cancer control programmes: Policies and managerial guidelines. World Health Organization. Geneva. Switzerland.

WORLD HEALTH ORGANIZATION. 2006 A.

Global environmental change. <http://www.who.int/globalchange/en>.

Date accessed: 2006-07-19.

WORLD HEALTH ORGANIZATION. 2006 B.

Qualifying environmental impacts.

<http://www.who.int/qualifyingenvironmentalimpact/en>.

Date accessed: 19-07- 2006.

WRIGHT, S. 1940.

Breeding structure of populations in relation to speciation. *American naturalist* **74**: 232-248.

WRIGHT, S. 1978.

Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

YOKOGAWA, K. 1997.

Morphological and genetic differences between Japanese and Chinese Red Ark Shell, *Scapharca broughtonii*. *Fisheries Science* **63** (3): 332-337.

YOKOTA, M., AND WATANABE, S. 1997.

One way gene flow by stocking and its effects on fish population. *Fisheries Science* **63** (4): 539-542.

YOSHIYAMA, R. M., AND SASSAMAN, C. 1983.

Morphological and allozyme variation in the Stichaeid fish, *Anoplarchus purperescens*. *Systematic Zoology* **32** (1): 52-71.

YOSSA, M. I., AND ARANJO-LIMA, C. A. R. M. 1998.

Detrivory in two Amazonian fish species. *Journal of Fish Biology* **52** (6): 1141-1153.

YOUNG, K. A. 2002.

Riparian zone management in Pacific North West: Who is cutting what?

Environmental Management **26** (2): 131-144.

ZUMDAHL, S. S. (primary contributor). 1997.

Water. *Encyclopædia Britannica*. Benton, W., and Benton, H. H. Publishers, Chicago.