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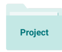

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ASPECTS OF THE ECOLOGY AND POPULATION MANAGEMENT OF THE BUSHVELD SMALLSCALE YELLOWFISH (*LABEOBARBUS POLYLEPIS*)

Report to the

Water Research Commission

by

Gordon O'Brien

(Editor)

Centre for Aquatic Research, Department of Zoology,

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ASPECTS OF THE ECOLOGY AND POPULATION MANAGEMENT OF THE BUSHVELD SMALLSCALE YELLOWFISH (*LABEOBARBUS POLYLEPIS*).

Editor: Gordon O'Brien[1]

EXECUTIVE SUMMARY

Yellowfishes (*Labeobarbus* spp.) are of the most easily related to and are amongst the most widely distributed indigenous fishes of South Africa. These fishes are actively targeted and utilised by various angling and subsistence fishing communities throughout South Africa. They are also used as indicator species by resource managers and conservationists to facilitate with the management of river ecosystems which give them a high ecological, economical and social value to South Africans. Although valuable, very little is known about these fishes and before we have the chance to fully understand the biology of these species, we are losing them.

The Bushveld smallscale yellowfish is a large, small-scaled yellowfish that occurs in the upper reaches of the Limpopo, Inkomati and Phongolo River systems in Southern Africa. Throughout this distribution many fragmented populations of this species occur. Apart from two recent assessments of this species, very little is known and to date no formal conservation initiatives have been established to address the conservation requirements of any potentially unique populations of this species. One population of the Bushveld smallscale yellowfish that historically occurred in the Letaba catchment (Limpopo Province of South Africa) is now locally extinct, potentially due to the unsustainable use of the goods and services of this system by people.

This study has been established to address the conservation and/or management implications associated with the potential determination of any unique populations of the Bushveld smallscale yellowfish from five isolated populations of this species from the greater Inkomati and Phongolo River catchments in Mpumalanga. In particular, this study considers potential differences in the biology and ecology of these populations by undertaking selected assessments that are concerned with the genetic and morphological differences between these populations, the occurrence of metals in the liver and muscle tissues within these populations and the feeding

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biology of these populations. This study has been undertaken on behalf of the Water Research Commission by the Centre for Aquatic Research, Zoology Department of the University of Johannesburg in collaboration with the Department of Genetics, School of Biological Sciences, University of Pretoria.

The Bushveld smallscale yellowfish populations used in this study were obtained from the Inkomati River Catchment including populations from the Elands River, Ngodwana Dam and the Komati River, and two populations from the Phongolo River Catchment including populations from the Assegaai River and the Phongolo River. An out-group population of the KwaZulu-Natal yellowfish, obtained from the Umvoti River in KwaZulu-Natal was included in some of the analyses to facilitate with the assessments.

Findings from the morphological and genetic assessment indicate that consistent morphological and genetic differences do exist between the five populations of Bushveld smallscale yellowfish considered in this study. Based on the genetic assessment of these five populations, findings indicate that three groups, consisting of the Phongolo/Assegaai populations (group 1), individuals from the Komati and selected individuals from the Elands and Ngodwana populations (group 2) and most of the individuals from the Elands and Ngodwana populations (group 3), should be considered as separate conservation units. An extreme case of genetic variation was obtained in this study in the discovery of a group of individuals from the Elands River and Assegaai River that shows a clear unique genetic divergence not only from the remaining populations of Bushveld smallscale yellowfish but also from all of the other small-scaled yellowfishes considered in South Africa to date.

Following the morphological assessment, outcomes indicate that although all of the individuals from the populations considered in this study are very similar, consistent differences in the morphology of the populations do exist. Findings suggest that the Elands River and Ngodwana Dam populations of the Bushveld smallscale yellowfish are unique and that they are the only populations that can with certainty be separated morphologically from the other Bushveld smallscale yellowfish populations. Interestingly, this study showed that although the Elands River and Ngodwana Dam individuals of the Bushveld smallscale yellowfish could be separated from the remaining populations considered, no other populations, including the KwaZulu-Natal yellowfish, which is a different species, could be separated with confidence in this study.

The metal assessment was used as an indication of the extent of metal exposure and uptake in the five different Bushveld smallscale yellowfish populations. The highest concentrations for the selected metals were found in the liver samples for all the sampled populations with the exception of one population which showed the highest Ni concentration in the muscle. However, this was not consistent within all five populations as some populations showed higher bioaccumulation patterns for certain metals in the muscle samples. The metal concentrations found in this study were relatively low and at most, very similar in concentration when compared to other studies completed on other indigenous South African fish species.

From the feeding biology assessment undertaken in this study, results suggest that the Bushveld smallscale yellowfish seems to be an opportunistic omnivore that preys predominantly on aquatic macro-invertebrates and also feeds on detritus. This species is well adapted to forage in substrates to capture their prey as well as in the water column and from the water surface. This ability makes this species a successful predator which can adapt to changing ecosystem types and take advantage of various ecosystem niches. This study suggests that different ecosystem types drive the feeding biology of this species of yellowfish and that they may be able to adapt to moderate changes in ecosystem structure and function.

This study reveals that not only are there genetically based differences between the populations that warrant conservation action, but that there are also morphological differences that can successfully be used to separate at least two of the populations from the rest of the group. Furthermore, this study has revealed that additional experimentation should be undertaken to address the potential genetic differences within this species in order to ascertain if the indication of a unique group of individuals obtained in this study warrants evolutionary significant unit status which would result in it being established as a new species of smallscaled yellowfish. Of the five populations considered in this study, three groups of populations were determined to be sufficiently different from one another to warrant conservation significant unit status at this time. Very little concerning the other remaining isolated populations of this species throughout South Africa has been considered.

Finally, following the outcomes of this study, the current approach to conserve the Bushveld smallscale yellowfish as one species is considered to be erroneous and it is suggested that the isolated populations of the Bushveld smallscale yellowfish that

are determined to be unique should be awarded with an individual conservation status and conserved and/or managed accordingly.

Following the outcomes, it is recommended that the approach adopted in this study should be expanded to consider the genetic, morphology, biology and general ecology of the remaining populations of Bushveld smallscale yellowfish in South Africa. In addition, the following recommendations should be considered by ecosystem users, conservators, regulators and managers in accordance with the outcomes of this study:

- This study has shown that the isolated population of the Bushveld smallscale yellowfish in the Elands River and associated Ngodwana Dam is unique and as such is of great ecological importance. The conservation status of this isolated population should be addressed with urgency as this population has historically been impacted on by chemical spillages and possibly by genetic contamination through individuals from the Komati River, that have been released into this system.
- More comprehensive geographic sampling of the Bushveld smallscale yellowfish individuals from the systems included in the study as well as nuclear DNA markers, to confirm the past and current gene flow between the separate rivers, is required.
- Further research is required to validate the findings of the metal assessment and to possibly establish causes for the levels obtained in this study.
- Additional assessments of the gut length and/or nutrient uptake potential of the gut of Bushveld smallscale yellowfish should be undertaken to contribute in addressing the uncertainty obtained in this study. In addition, due to the unavailability of seasonal data in this study we recommend that additional feeding biology assessments of this species be carried out during the spring/summer periods. Finally, some stomach morphological assessments should be undertaken which would address the uncertainty of the uptake of detritus matter by this species and similar assessments to address differences within and between the feeding biology of other isolated populations of *L. polylepis* in South Africa.

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1 General Introduction

Yellowfishes (*Labeobarbus* spp.) are of the most easily related to and are amongst the most widely distributed indigenous fishes of South Africa (Skelton, 2001; Skelton, 2007). In addition yellowfish in South Africa are considered to be among the most valuable of fishes in South Africa (Jackson and Coetzee, 1982). Yellowfish are actively targeted and utilised by various angling and subsistence fishing communities throughout South Africa and are used as indicator species by resource managers and conservationists to facilitate with the management of river ecosystems (Gaiger, 1976; Jackson and Coetzee, 1982; Wolhuter and Impson, 2007). As such, yellowfish have a high ecological, economical and social value to South Africans (Gaiger, 1976; Jackson and Coetzee, 1982; Wolhuter and Impson, 2007; Skelton and Bills, 2008). Although valuable, very little is known about these useful species, and unfortunately, before we have the chance to fully understand the biology of these species, we are facing the looming dilemma of losing them. Currently, at least one of the six species of yellowfish occurring in South Africa are listed as endangered on the IUCN Red Data List (Wolhuter and Impson, 2007; IUCN, 2008). In the recently released State of the Yellowfish in South Africa Report (Wolhuter and Impson, 2007), the plight of the yellowfishes in South Africa has further been highlighted due to the excessive use of the river systems in which they occur. Wolhuter and Impson (2007) stressed that as a result of excessive resource utilisation and widespread pollution that is impacting many river systems in South Africa, yellowfish populations are being adversely affected in that the distribution and abundance of these populations are diminishing.

The Bushveld smallscale yellowfish (*Labeobarbus polylepis,* Boulenger, 1907) is a large, small-scaled yellowfish that occurs in the upper reaches of the Limpopo, Inkomati and Phongolo River systems in Southern Africa (Skelton, 2001; Roux, 2008). Figure 1 presents the distribution of *L. polylepis*, per quaternary catchment within South Africa. Although *L. polylepis* is widely distributed across north eastern South Africa, many fragmented populations of this species occur due to historical changes in river connectivity, temperature barriers (the species prefers cool waters above 600 m altitude), natural and recently due to artificial barriers (Roux, 2007b).

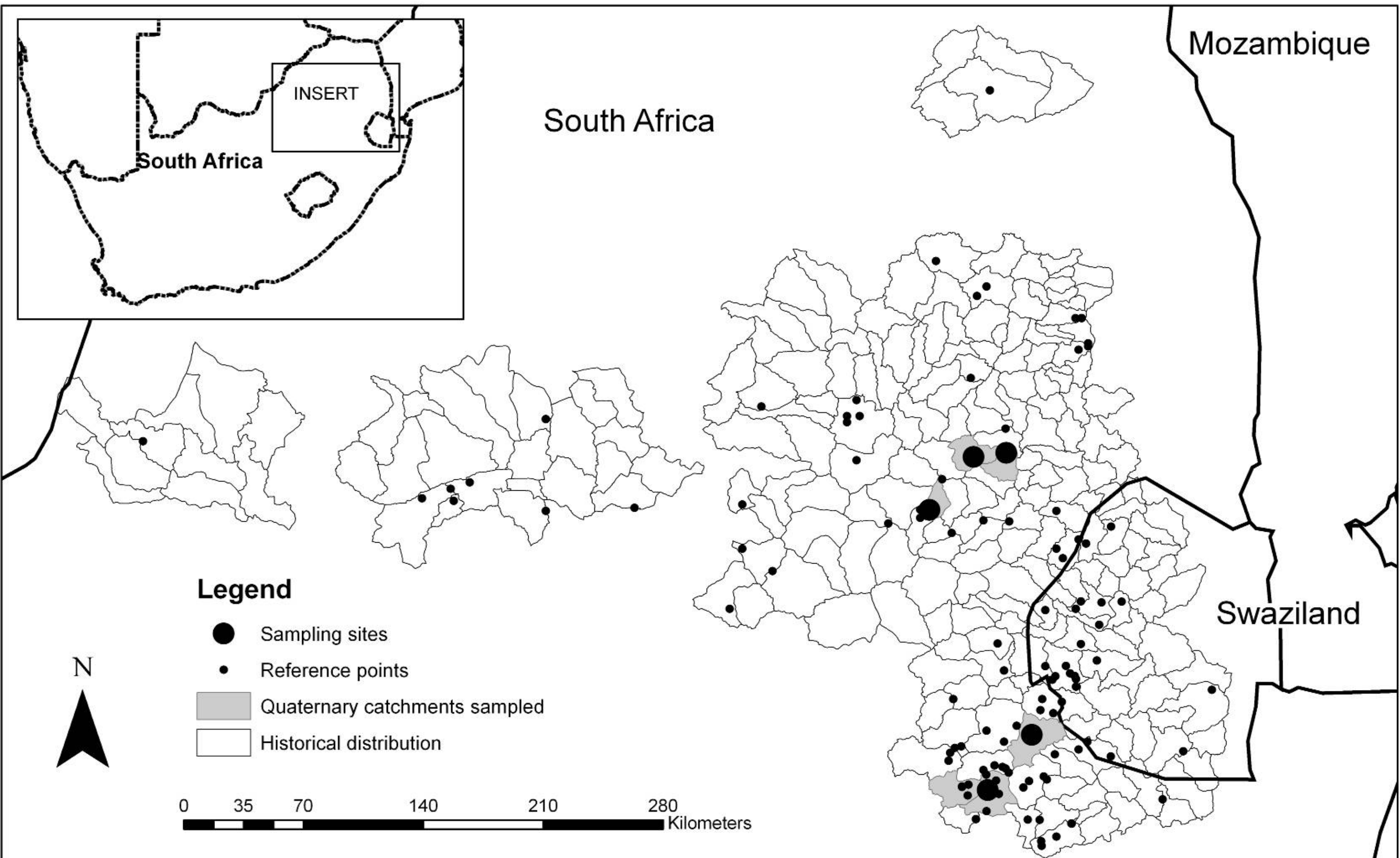

Figure 1: Quaternary catchment-based historical distribution of *Labeobarbus polylepis*. Reference points indicate locations where museum specimens were collected. Shaded areas represent the quaternary catchment sampling sites of populations.

In comparison to international trends, very little is known concerning the biology of the Bushveld smallscale yellowfish. However, two detailed assessments of this species have recently been undertaken; the assessment of the state of the Bushveld smallscale yellowfish populations in South Africa and a comprehensive assessment of the breeding biology of this species (Roux, 2007a, Roux, 2007b; Roux, 2008). *Labeobarbus polylepis* is classified as a true yellowfish in that it has a hexaploid karyotype of approximately 150 chromosomes and parallel striated scales (Oellermann and Skelton, 1990). Following the assessment of the state of the *L. polylepis* communities by Roux (2008), findings indicated that as of 2007 the populations in the Limpopo, Inkomati and Phongolo drainage basins remained widely distributed and abundant. As such, *L. polylepis* populations were generally deemed to be in a fair to good state. However, populations in the upper Olifants catchment and in Gauteng rivers were deemed to be in a poor state and that population size and abundance continued to decline (Roux, 2008). One population that historically occurred in the Letaba catchment is now locally extinct. This system has recently been restocked with *L. polylepis* from the Crocodile catchment (Elands River) (Pers. comm[2]., Wynand Vlok).

The existing populations of Bushveld smallscale yellowfish are widely used by conservators or ecosystem managers ,who are responsible for the management of aquatic ecosystems where these species occur, primarily as an indicator species that has a specific preference for ecological flows and spawning requirements relating to the timing and durations of flows alone (Roux, 2008). Although *L. polylepis* is considered to be very useful and contributes towards the establishment of management plans for the aquatic ecosystems in which they occur (Roux, 2008), the potential importance of conserving individual isolated populations of this species has to date not been addressed. In the recent study that aimed to determine the conservation value of land in Mpumalanga (Emery et al. 2002), various ecologically important species and ecosystem units where considered and utilised to establish a conservation and or management plan for the province. In this assessment eleven species of fishes that are endemic, near endemic, highly sensitive and/or that contain limited distributions in Mpumalanga, were selected for the modelling activities undertaken in this study. The Bushveld Smallscale yellowfish, although mentioned to be useful in determining flows for systems, was not considered in this modelling

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[2] Wynand Vlok, June 2006, Former Researcher, Zoology Department, University of the North. Now Environmental consultant, EcoAssets.

exercise, potentially due to the extensive distribution of the species which extends into Limpopo, Gauteng and North-west provinces of South Africa. In accordance with the National Environmental Management: Biodiversity Act (no 10 of 2004) of South Africa, which states that not only species diversity but also genetic diversity should be considered within the management and conservation of biodiversity, should any uniqueness in any of the isolated *L. polylepis* populations be determined that these populations should be conserved as unique populations contributing towards the biodiversity of the country. As such, within Mpumalanga, should any isolated populations of *L. polylepis* that are endemic, near endemic, highly sensitive and/or that contain limited distributions in Mpumalanga be established, these populations should be used in future conservation and or management activities of the province.

In this study various aspects pertaining to the management of *L. polylepis* populations have been considered along with independent assessments of aspects of the biology of five populations from the Inkomati and Phongolo catchments in South Africa. As an out-group, a population of the KwaZulu-Natal yellowfish (*L. natalensis*), from the Umvoti River, was included in selected assessments within this study.

1.1 Study area

The *L. polylepis* populations used in this study included individuals from two catchments of South Africa namely the Komati River and the Phongolo River. Within the Komati River Catchment three isolated populations including the Elands River, Ngodwana Dam and Komati River populations were used. In the Phongolo River Catchment the Assegaai River and the Phongolo River populations were used.

The Elands River population of *L. polylepis* was included in this study as it was considered to be the only population of *L. polylepis* that exhibited a high frequency of rubber lip forms observed from as early as 1969 (Gaiger, 1969). The Ngodwana Dam population was included in this study due to the close proximity of this population to the Elands River population, the ease of sampling in the Ngodwana Dam, the non-characteristic habitat in which this population occurs and the need to assess the possible genetic contamination of this species by the release of individual *L. polylepis* from the Komati River that were released into this system by

Mpumalanga Parks in the late 1990's (Pers Comm[3], Johan Engelbrecht). The Ngodwana Dam population is separated from the Elands River population by the Ngodwana Dam wall an artificial barrier constructed by Sappi to provide water to the Sappi Ngodwana pulp and paper mill that was commissioned in 1967 (Hocking, 1987). Additional sampling sites for *L. polylepis* populations from the Komati, Assegaai and Phongolo rivers were selected and included in this study according to local expert knowledge of the locations of large abundances of *L. polylepis* in these systems (Pers Comm[4], Johan Engelbrecht and Horst Filter). The Komati River population represented a population of *L. polylepis* that is well known and relatively well documented; this population is additionally the source of individuals that were relocated into the Ngodwana Dam by Mpumalanga Parks Board (Mulder *et al.*, 2004). The two sites selected in the Phongolo River Catchment namely the Assegaai and Phongolo river sites where included to provide the assessment with variation as these sites contain the two most southern distributed populations of *L. polylepis*. For selected assessments in this study where an out group was required a population of *L. natalensis* from the Umvoti River in KwaZulu-Natal was included in this assessment. The locations of the sampling sites and an overview of the sites are presented in Figure 2.

---

[3] Dr. Johan Engelbrecht, June 2004, Aquatic Scientist, Mpumalanga Parks Board.

[4] Dr. Johan Engelbrecht, June 2004, Aquatic Scientist, Mpumalanga Parks Board and Mr. Horst Filter, Professional Bushveld smallscale yellowfish angling guide, River Hunter Safaris.

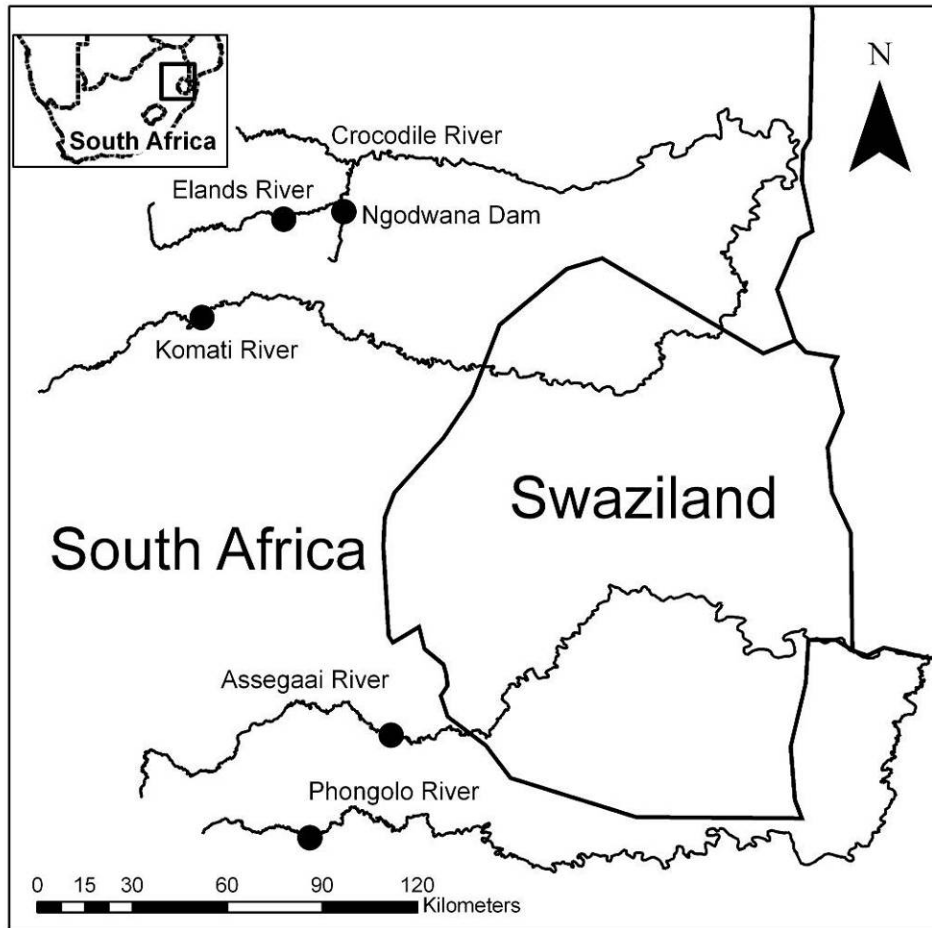

Figure 2: Graphical representation of the location of the sampling sites of the five *Labeobarbus polylepis* used in this study.

1.1.1 Collection

A minimum of thirty *L. polylepis* individuals were collected from each sampling location in the Ngodwana Dam and the Elands, Komati, Assegaai and Phongolo rivers between May and July of 2006 (Table 1). A sample of 22 individual *L. natalensis* were collected in the Umvoti River and used in this study. Individuals where captured using an array of sampling techniques including seine nets, cast nets, electro-shocking, gill nets (mesh size 45 mm-95 mm) and fly fishing techniques. In order to optimise the value of this bio-prospecting endeavour, as much of the *L. polylepis* individuals as possible were used in this study and portions of the remaining specimens, with genetic samples, will be sent to the South African Institute of Aquatic Biodiversity to be lodged in the fish collection.

Table 1: Locations and a brief description of the sites used to collect individual *Labeobarbus polylepis* in this study.

River	Site co-ordinates	Site description
Elands River	25°36'56.09"S 30°30'55.29"E	This site is located between two geographical barriers of this small clear river in Mpumalanga. This system contains a wide diversity of fast and slow, deep and shallow habitats with adequate substrate and cover in the form of cobble and boulder beds, undercut banks and root wads and sufficient deep areas. Pools and backwater areas are common and dominated by cobbles and fine sediments. Within the lower portion of the Elands River, the system widens and slows down before reaching the lower geographical barrier, the Lindenau Waterfall. *Labeobarbus polylepis* is the only large cyprinid that occurs within this reach.
Ngodwana Dam	25°35'36.50"S 30°41'12.62"E	This Ngodwana Dam is located on the lower section of the Ngodwana River, a small tributary of the Elands River, Mpumalanga. This large dam is diverse with extensive deep and shallow areas (in excess of 10m) and contains areas that are extensively vegetated. The dam, constructed in the late 1960's, contains a large population of many exotic, non-endemic and endemic fish species. A barrier (weir) prevents the migration of exotic fish above the dam into the upper Ngodwana River. Non-endemic and exotic fish originating from the dam have frequently been observed in the Elands River. L. polylepis and the exotic Common Carp (*Cyprinus carpio*) are the only two large cyprinids that occurs within this dam.
Komati River	25°53'40.94"S 30°17'1.19"E	This site is located within the upper reaches of this medium sized clear river in Mpumalanga, between the Nooitgedacht and Vygeboom dams. This reach of the river is divided by dams and is now affected by modified flow released from the Nooitgedacht Dam. The existing communities of *L. polylepis* are isolated between these dams that act as barriers. This site is located within a reach of the river that passes a cliff wall, creating an extensive, deep (over 3m) pool. This pool is dominated by boulders and bedrock and the area contains numerous riffle and rapid complexes. *L. marequensis* occurs with and is more common than *L. polylepis* in this reach.
Assegaai River	27° 4'48.22"S 30°49'15.09"E	This site is located within the middle reach of this medium sized clear river, below the Heyshope Dam in southern Mpumalanga. The site characterised by a wide diversity of fast and slow, deep and shallow habitats with adequate substrate in the form of cobble and boulder beds with bed-rock banks. Pools and backwater areas are common and offer adequate cover for the large cyprinids including *L. marequensis* and *Varicorhinus nelspruitensis* which are common in the system.
Phongolo River	27°22'17.93"S 30°35'24.50"E	This site is located within the upper reach of this medium sized clear river in Southern Mpumalanga close to the KwaZulu-Natal border. The site is characterised by a wide diversity of fast and slow, deep and shallow habitats with adequate substrate in the form of cobble and boulder beds with bed-rock banks. Pools and backwater areas are common and offer cover for a large abundance of cyprinids including *L. marequensis* and *V. nelspruitensis*.
Umvoti River	29°14'12"S 31°00'12"E	This site is located within the middle reach of the relatively small clear river in central KwaZulu-Natal. The site characterised by a wide diversity of fast and slow, deep and shallow habitats with adequate substrate in the form of cobble and boulder beds with bed-rock banks. Pools and backwater areas are uncommon and are dominated by sandy bottoms. L. natalensis is the only large cyprinid that occurs within this system.

In this study, the genetic and morphological differences between five isolated populations of *L. polylepis* have been considered. In addition, notes on the feeding biology, and the metal bioaccumulation in the muscle and liver tissue of the five populations were considered.

This study has been divided into four sections in accordance with the aim of this study. These sections include:

- Section 1: Genetic and morphological differences between five populations of the Bushveld smallscale yellowfish, *Labeobarbus polylepis* in South Africa.
- Section 2: Metal bioaccumulation in muscle and liver tissue of five *Labeobarbus polylepis* populations from Mpumalanga, South Africa
- Section 3: Notes on the feeding biology of five selected populations of *Labeobarbus polylepis* in South Africa.

2 Section 1: Genetic and morphological differences between five populations of the Bushveld smallscale yellowfish, *Labeobarbus polylepis* in South Africa.

Carel Oosthuizen[5], Amanda Austin[6], Gordon O'Brien[7] and Paulette Bloomer[8].

2.1 Introduction

Within South Africa, many isolated populations of Bushveld smallscale yellowfish (*Labeobarbus polylepis,* Boulenger, 1907) exist, specifically within the upper reaches (above 600 m) of rivers in the Limpopo, Inkomati and Phongolo catchments (Mulder *et al.*, 2004; Roux, 2008). Due to the preference that this species has for upper reaches of rivers, no fewer than eleven isolated populations of *L. polylepis* occur (Roux, 2008). Although very little of the biology and ecology of *L. polylepis* is known, some morphological and genetic variation between isolated populations have been observed in the past (Gaiger, 1969; Kleynhans *et al*., 1992; Mulder *et al.*, 2004). From as early as 1969, consistent morphological differences between populations of *L. polylepis* have been observed. This morphological difference relates primarily to the occurrence of a single population that exhibited a high percentage of individuals with the rubber-lip form, a rare occurrence in *L. polylepis* (Gaiger, 1969). Following this initial account of morphological differences between *L. polylepis* populations, similar observations have been noted by Kleynhans *et al.* (1992) and Mulder *et al.* (2004). No formal assessment of the possible morphological differences between *L. polylepis* populations has been undertaken. More recently, with the development of methods to characterise genetic variation within and between populations, consistent differences between three *L. polylepis* communities occurring in the Phongolo,

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Komati and Spekboom rivers were discovered (Mulder *et al*., 2004). Some of the genetic variation observed between *L. polylepis* populations from the Phongolo, Komati and Spekboom rivers were attributed to genetic contamination of specifically the Spekboom River population due to the potential hybridisation of *L. polylepis* with *L. aeneus* in this system (Mulder *et al.*, 2004). These findings suggested that although the possibility of genetic differences between isolated populations exists, there is still insufficient data to warrant a change in the currently adopted management and conservation strategies for this species towards conserving isolated populations that may be unique and where the survival of these populations is threatened.

The outcomes from a recently released assessment concerning the state of the identified populations of *L. polylepis* are concerning in that at least five of the eleven populations considered in this assessment are now believed to be rare, threatened or declining in numbers (Roux, 2008). Seven populations were considered to be negatively affected by hybridisation with other *Labeobarbus spp.*, water quality and quantity, river connectivity, river habitat destruction, competition with alien fishes and excessive harvesting usually during spawning activities (Roux, 2008). One population of *L. polylepis* that originally occurred in the Letaba River is now considered to be extinct (Pers. Comm[9] Wynand Vlok). Within South Africa, national legislation makes provision for the conservation of biological diversity between and within species (NEMBA, Act 10 of 2004). Although the possibility of ongoing differentiation between isolated populations of *L. polylepis* exists, no specific management or conservation plans have, as yet, been implemented to conserve any unique populations that may be facing some kind of threat (Emery *et al*., 2002; Roux, 2008).

In this study the genotypic and morphometric differences between the *L. polylepis* populations were considered. In addition, any potential morphological adaptations of the populations in response to different environmental variables were considered. The aim of this portion of the study is to characterise the morphological and genetic differences of the five isolated populations of *L. polylepis* in Mpumalanga. In order to reach this aim the following two objectives have been established. Initially the use of a genetic marker approach using mitochondrial DNA control region was adopted to assess the genetic diversity within the populations. Thereafter the morphometric

[9] Wynand Vlok, June 2006, Researcher, Zoology Department, University of the North.

assessment involved the use of multivariate statistical methods to assess the potential differences of 159 morphological measurements taken

Based on the findings of numerous studies published on freshwater fish species, the mitochondrial DNA (mtDNA) control region was selected as genetic marker for this pilot study of genetic differentiation within *L. polylepis*. Mitochondrial DNA is inherited independently of nuclear DNA and only passed on from the female parent in most animal species (Moritz *et al*., 1987). There is therefore no mixing of maternal and paternal alleles of particular genes. The mtDNA molecule contains 37 genes and a control region (Harrison, 1989). The latter region does not code for a specific molecular product but contains several very important signals for the normal functioning of the mtDNA molecule. Compared to the 37 genes, however, the control region evolves quite rapidly and it allows one to record the pattern of changes within and between different species (Harrison, 1989, Avise, 2000). It can even resolve differences between different populations within the same species, depending on the dynamics of the past and present connections between them. Mitochondrial DNA is not without limitations: As all the genes are linked on the circular molecule, it represents a single locus and thus a single view of the species history (Moritz *et al*., 1987). Due to the maternal inheritance, the mtDNA genealogy is also not always representative of the species' history. In addition, mtDNA has a smaller effective population size than nuclear DNA and this will over many generations of inheritance affect the pattern of variation. Specific alleles will become fixed much faster than nuclear DNA alleles and many alleles will go extinct. For comprehensive reviews on mtDNA and it's utility consult Moritz *et al*., (1987), Avise, (2000) and Zhang and Hewitt, (2003).

The potential expressions of phenotypic differences between the populations were considered in a morphometric assessment of the individuals from the five populations. In order to potentially provide ecosystem stakeholders and users with the ability to distinguish between the populations of yellowfishes assessed in this study, it is important to address the external morphological differences of these populations in an attempt to establish any key measurements that these stakeholders could use. Although the relationship between the yellowfishes (*Labeobarbus spp.*) is unclear, the group contains a broad range of morphological variation within species and between species (Skelton, 2001). In particular, the small-scaled group of yellowfishes namely; *L. aeneus*, *L. kimberleyensis*, *L. natalensis* and *L. capensis* show a large, similar range of morphological characteristics which makes the

identification of these species very difficult. Without prior knowledge concerning the historical distribution of a small-scaled yellowfish population it is very difficult to clearly distinguish between these species using morphological characteristics. In order to provide *L. polylepis* stakeholders with the information required to accurately identify and possibly distinguish between the *L. polylepis* populations, a detailed morphological assessment of all of the individuals used in this study has been undertaken.

This section of the study details the methodologies implemented, the findings and outcomes of the genetic and morphological assessment of five *L. polylepis* populations and one *L. natalensis* population.

2.2 Materials and methods

2.2.1 Sample collection

In total 164 *L. polylepis* specimens were collected from five populations within the Inkomati and Phongolo river systems (Figure 2) and 22 individual of *L. natalensis* were collected from the Umvoti River in KwaZulu-Natal. During June in 2006, 32 specimens of *L. polylepis* were collected from the Phongolo River and 30 specimens from the Assegaai River. During October in 2006, 34 specimens of *L. polylepis* were collected from the Elands River and 38 specimens were collected from the Ngodwana Dam. Finally, during January in 2007, 30 *L. polylepis* specimens were collected from the Komati River. An additional 22 specimens of *L. natalensis* were collected during October in 2006 from the Umvoti River, KwaZulu-Natal, and were included in this study as an out-group.

Fish were collected using an array of standardised fish sampling techniques. These techniques included the use of gill nets (37 mm, 45 mm and 57 mm mesh sizes), small fyke nets, small and medium seine nets, electro-fishing and fly-fishing techniques. Once the fish were sampled they were kept alive either in nylon keep-nets within the river or dam where they were sampled or in a plastic holding tank before they were dissected. The approach followed by Bloomer *et al.* (2007) in an assessment of the morphological differences between *L. aeneus* and *L. kimberleyensis* was followed for this assessment. This approach made use of 57

pre-selected morphological measurements of which 18 were recorded in the field before specimens were dissected and an additional 39 measurements (Figure 3) were taken in the laboratory of the University of Johannesburg. Muscle, heart and liver samples were collected from fish dissected in the field and frozen using liquid nitrogen. A subsample of muscle from each specimen was taken in the lab, preserved in 96% ethanol and later used for the genetic assessment.

2.2.2 DNA extraction, PCR and DNA sequencing

Total genomic DNA was isolated from muscle samples using Chelex resin following the protocol of Estoup *et al*. (1996). A short variable region of the control region was amplified using Polymerase Chain Reaction (PCR) with *Labeobarbus* specific primers designed in earlier research on *L. aeneus* and *L. kimberleyensis* (Bloomer and Naran, 2006). Polymerase Chain Reaction and cycle sequencing were performed in a Geneamp® PCR System 9700 (Applied Biosystems). Amplification reactions were performed in 25 µl volumes, each containing 1 x buffer, 2.5 mM $MgCl_2$, 0.2 mM of each of the four nucleotides (Promega), 12.5 pmol of each primer, 1.5 units of SuperTherm DNA polymerase (Southern Cross Biotechnology) and approximately 100 ng template DNA. Cycling conditions for PCR consisted of an initial denaturation of 5 min at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 51°C and 30 seconds at 72°C, with a final extension of 7 min at 72°C. PCR products were precipitated using sodium acetate and 100% EtOH, followed by elution in Sabax water (Adcock Ingram). Cycle sequencing was performed in 10 µl volumes with the reaction mix containing 100 ng of purified PCR template, 3.2 pmol of one of the above-mentioned primers and 2 µl of ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit V3.1 (Applied Biosystems). Cycle sequencing and precipitation of the products followed the manufacturer's instructions. Nucleotide sequences were determined through electrophoresis on an ABI3130 automated sequencer by Macrogen (Rockville, MD, USA). Consensus sequences were obtained from the forward and reverse sequences through alignment and inspection in Vector NTI (Invitrogen). All consensus sequences were aligned using Clustal X (Thompson *et al.*, 1997) and checked manually. The sequences of unique alleles will be deposited in GenBank.

2.2.3 Analysis of DNA sequence variation

Aligned sequences from Clustal X were analysed using statistical parsimony in TCS (Clement *et al.*, 2000) to identify all unique alleles and their frequencies. DNASP version 4.0 (Rozas *et al*., 2003) was used to test for neutral evolution of the control region analysed using Tajima's D test statistic (Tajima, 1989) and to calculate diversity indices such as allelic (Nei and Tajima, 1981) and nucleotide diversity (Nei, 1987). Arlequin 2.0 (Schneider *et al*., 2000) was used to plot a mismatch distribution of pairwise differences between all samples. The observed distribution was compared to the expected distribution under a population growth and decline model (Harpending, 1994, Rogers, 1995). An Analysis of Molecular Variance (AMOVA, Excoffier *et al*., 1992) was also conducted in Arlequin 2.0. The analysis partitions the overall variation into two or three components such as: between pre-defined groups, among populations within these groups and within populations. The amount of variation within populations relative to the total variation gives an indication of population structure (F_{ST}, Wright, 1951). The significance of the variance components were evaluated using 10 000 permutations.

2.2.4 Phylogenetic and allele based analyses

An allele network was constructed using statistical parsimony in TCS (Clement *et al.*, 2000) which only joins alleles that can be connected with 95% confidence. An unrooted distance based phylogenetic analysis of the sequences was done based on the neighbour-joining algorithm (Saitou and Nei, 1987) as implemented in PAUP (Swofford, 2003). Confidence in inferred relationships was determined based on 1000 bootstrap replicates (Felsenstein, 1985).

2.2.5 Morphological analysis

The morphological assessment of the study involved the initial assessment of the scale counts and the fin ray counts of all *L. polylepis* and *L. natalensis* individuals (refer to Appendix A). This assessment was undertaken by carrying out a Principle Component Assessment (PCA) of the data using Primer version 6, multivariate statistical package (PRIMER-E Ltd, Plymouth Marine Laboratory, United Kingdom). Following this assessment an intra- and inter-species assessment of 54

morphological measurements was undertaken similarly by PCA using the Primer version 6 multivariate statistical packages. Following the PCA assessments, key measurements were determined using the Eigenvectors or coefficients in the linear combinations of variables making up the principle components of the PCA assessment (Clarke and Gorley, 2006). These key measurements where used to discuss any differences within the populations assessed. Finally, standard length and mass of the individuals where considered in order to address the condition of the individuals by dividing the mass (g) by the length (cm).

In order to carry out the intra- and inter-species PCA assessments the approach followed was to convert the initial measurements into a ratio value using the fork length (measurement/fork length) for each individual. Similar approaches have been widely used to assess the morphology of fishes in this manner in order to address the impact of change in shape due to increasing body size, termed allometry (Groenewald, 1958; Stewart, 1977; Kramer *et al.*, 2007).

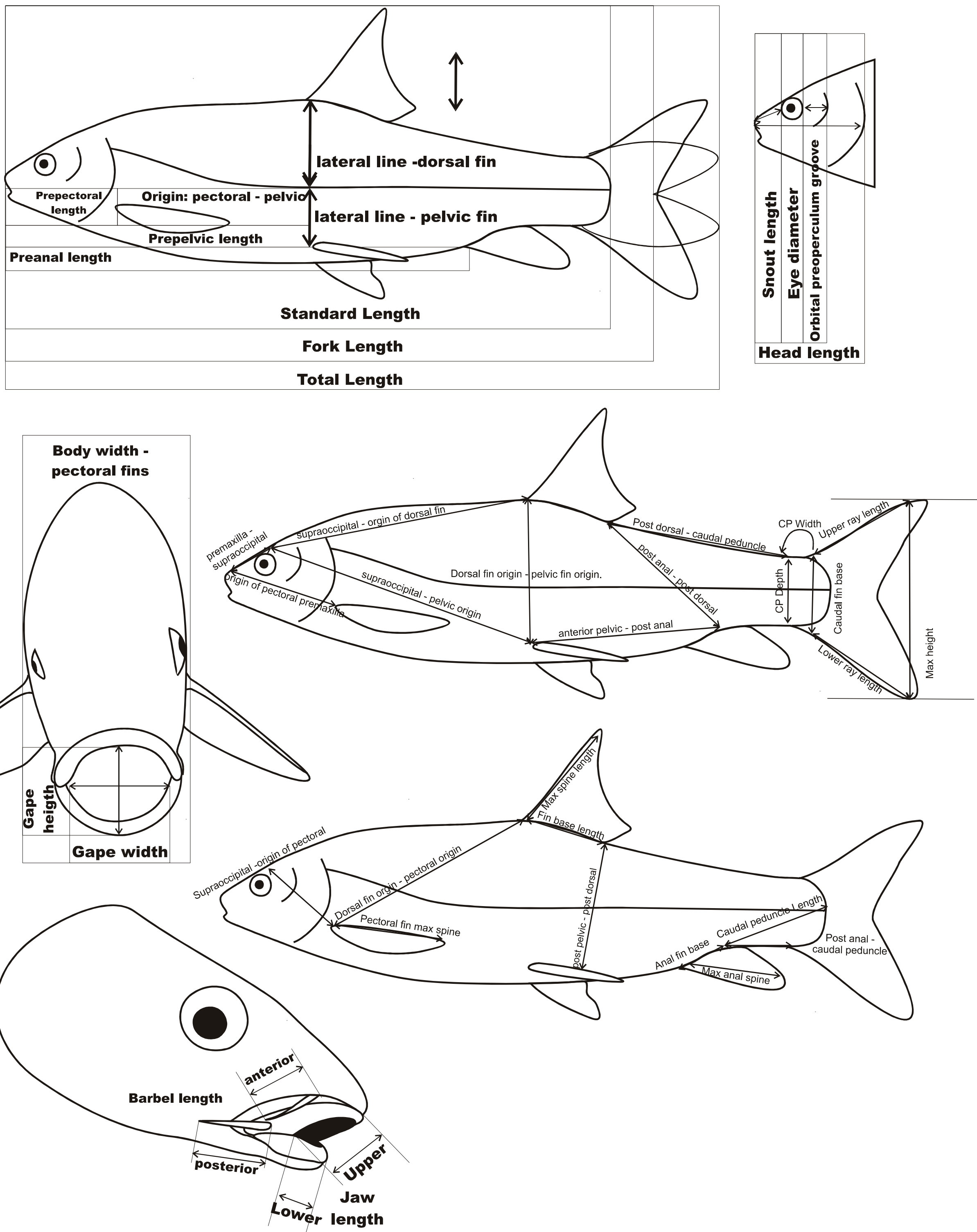

Figure 3: Graphical representation of morphological measurements taken for each yellowfish individual used in this study.

2.3 Results and discussion

The findings of the study are presented in two sections namely the genetic assessment and the morphological assessment.

2.3.1 Genetic assessment findings

Mitochondrial DNA control region sequences were generated for 147 *L. polylepis* individuals from the five localities. Following identification of unique alleles, the analysis was done in the following stages: (Analyses 1) Allele based analysis using statistical parsimony. This analysis only connects the most closely related alleles that can be connected with a 95% confidence limit; (Analyses 2) Alleles not connected in the first analysis were then included in an allele tree that can also connect more distantly related alleles; (Analyses 3) Long branches (i.e. very divergent alleles) reduce resolution and thus a third analysis was conducted, including other *Labeobarbus spp.* outgroups, to determine the placement of the most divergent lineages.

2.3.1.1 Analysis 1: Population structuring and diversity within *L. polylepis*:

The statistical parsimony analysis could only connect 15 alleles (N=109) from the five populations and several Elands River, Ngodwana Dam and one Assegaai River alleles could not be connected with confidence. The fifteen unique alleles were identified based on variation at 22 sites within a 427 base pair fragment of the 5' variable segment of the mtDNA control region (Table 2). The relationships among the alleles are summarized in the allele network (Figure 4). Several of the alleles (Figure 4) are shared (6 of the 15 alleles were found in more than one locality), with a number of high frequency alleles (such as allele 10 that was recorded from 22 individuals). Within our sample, a high number of alleles (9 out of the 15 alleles identified) were only recorded from a single locality. The analysis indicates the distinction of the Assegaai and Phongolo rivers populations from the northern populations.

Table 2: Unique mtDNA control region alleles identified among 109 *L. polylepis* individuals. The unique sequence for each allele is given with reference to allele 1. Dots indicate variable nucleotide positions in the control region sequence where a particular allele has the same base as the reference allele; differences at particular positions are indicated by showing the altered bases (G, A, T or C) relative to the base in the reference sequence. 'N' indicates the number of individuals with a particular allele.

		Nucleotide position																					
Allele	N	010	027	055	056	058	098	099	122	129	200	210	211	216	251	264	300	319	341	369	388	401	419
1	21	A	T	T	A	A	G	A	A	T	T	A	G	T	G	A	T	T	C	C	A	C	G
2	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
3	12	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
4	1	.	C	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
5	10	.	C	.	.	G	.	.	.	C	.	G	.	.	.	.	.	.	.	.	.	.	.
6	2	.	.	.	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A
7	1	.	.	C	G	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	.	T	A
8	7	.	.	C	G	.	.	.	.	.	.	G	A	.	.	C	.	C	T	.	.	T	A
9	8	.	.	C	G	.	.	.	G	.	.	G	A	.	.	C	C	C	T	.	.	T	A
10	21	.	.	C	G	.	.	.	G	.	.	G	A	.	.	C	C	C	T	T	.	T	A
11	1	G	.	C	G	.	.	.	G	.	.	G	A	.	.	C	C	C	T	.	.	T	A
12	1	.	.	C	G	.	.	.	G	.	.	G	A	.	.	C	C	C	T	.	-	T	A
13	4	.	.	C	G	.	.	.	G	.	.	G	A	.	.	C	C	C	T	.	-	.	A
14	5	.	.	C	G	.	.	.	G	.	C	G	A	.	.	C	C	C	T	.	.	T	A
15	9	.	.	C	G	.	.	G	G	.	.	G	A	.	A	C	.	C	T	.	.	T	A

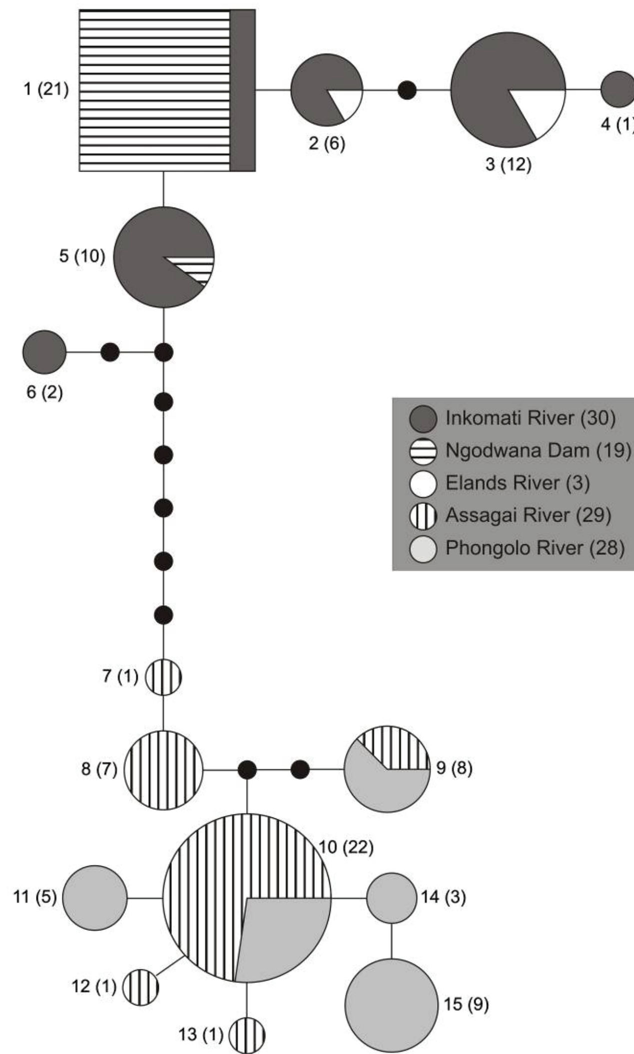

Figure 4: Allele network, based on a statistical parsimony analysis conducted in TCS, depicting the relationships between 15 unique maternal alleles identified among 109 *L. polylepis* individuals, based on the analysis of 427 base pairs of the 5' variable segment of the mitochondrial DNA control region. Each circle/square represents a unique maternal allele defined by a unique set of DNA bases (Table 5). The sizes are drawn relative to the frequency of each of the alleles. Allele 1 was identified as the ancestral allele. The TCS analysis could join alleles with 95% confidence if they were connected with eight or fewer mutational changes. Each line represents a single mutational change and small dark circles indicate missing alleles (alleles not sampled in the present study or extinct alleles).

A moderate level of allele diversity (0.876) and a relatively low level of nucleotide diversity (1.5%) were recorded. These summary statistics not only allow comparison across different freshwater fish species but also can reveal information about the

population/species history of the species under investigation. Allele diversity gives an indication of the number and frequencies of alleles irrespective of the actual sequence differences between them; when randomly drawing any two individuals from the population, it reflects the probability of the two individuals having different alleles. Allele diversity ranges from 0 to 1, with 0 indicating that all individuals are identical whereas a value of 1 would be obtained if each individual had a unique allele. The allele diversity estimated among the 109 samples analysed here thus indicates a reasonable degree of differentiation among individuals and compares well with that found within other freshwater fish in South Africa, for example in redfins (*Pseudobarbus spp.*), where lineages within single species are often isolated in different river systems, values higher than 0.8 are typically recorded (see for example Bloomer and Impson, 2000; Swartz, 2005).

Nucleotide diversity shows the extent of sequence difference among alleles. The estimate is influenced by the frequencies of different alleles but not by the number of different alleles. On average, the alleles in the present study differed from each other at 22 sites within the 427 bp region, i.e. less than 2% divergence; this is reasonably low and expected for within-species variation. The number of pairwise differences however ranged from 0-12.

The estimate of Tajima's D statistic (1.95) was non-significant ($P > 0.98$) indicating that the control region, studied here, is evolving in a neutral fashion (unaffected by selection) and is thus appropriate for studying population/species history.

An Analysis of Molecular Variance was used to test several independently defined groupings. When considering all individuals as a single lineage, most of the variation was recorded between (77.69%) rather than within the populations (22.31%) and the overall population structure ($F_{ST} = 0.78$) was significant. When defining two groups, Ngodwana/Elands/Komati versus the Assegaai/Phongola, 80.05% of the variance could be accounted for by the two groups, 3.75% of the variation was found among populations within these two groups and 16.21% within populations. This confirms the need to investigate the relationship between these rivers in greater detail. We need more comprehensive geographic sampling and nuclear DNA markers to confirm the past and current gene flow between the separate rivers.

The mismatch distribution analysis, comparing the trend of observed pairwise sequence differences among the 109 Bushveld smallscaled yellowfish, showed a significant fit to the trend expected under a population growth model (Figure 5).

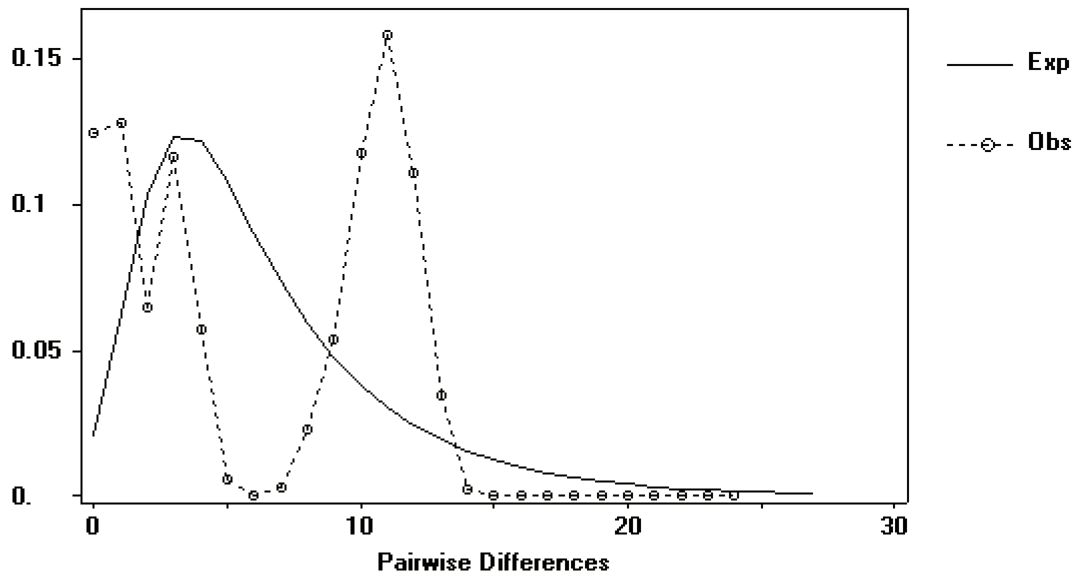

Figure 5: Pairwise comparison of nucleotide differences between 109 Bushveld smallscaled yellowfish from five localities based on 427 base pairs of the 5' end of the mtDNA control region. The observed data show a significant fit to the trend expected under a population growth/decline model [Sum of Squared deviation: 0.036; P(Sim. Ssd >= Obs. Ssd): 0.053; Harpending's Raggedness index: 0.05, P(Sim. Rag. >= Obs. Rag.): 0.081].

2.3.1.2 Analysis two: Allele tree for *Labeobarbus polylepis*

The alleles that could not be connected with confidence in Figure 3 were included in the allele tree (Figure 6). We had to prune a divergent branch connecting two alleles, one from the Assegaai and one from the Elands (n=11). The tree confirms the distinction between the Assegaai/Phongolo population and the northern populations. There is also significant separation between most individuals from the Elands population versus the Inkomati, Assegaai and Phongolo populations. The Ngodwana Dam and Elands River individuals share some alleles with the Komati River population; this could reflect shared history of these populations in the past or may reflect the result of a previous translocation of individual *L. polylepis* from the Komati

River into the Elands River and Ngodwana Dam (Pers. Comm[10] Francious Roux). We recommend that as a precautionary principle, these three groupings (Figure 6) should be treated as separate conservation units, pending more in-depth analysis based on nuclear genes and wider sampling.

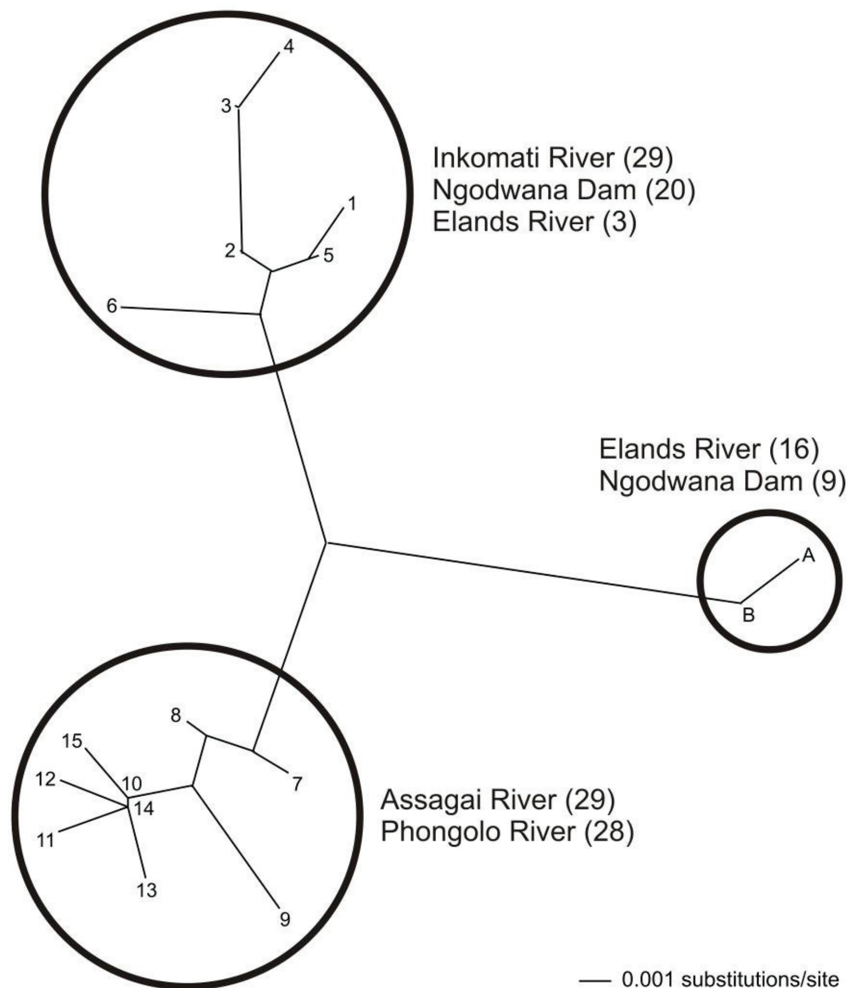

Figure 6: Unrooted phylogram based on a neighbour-joining analysis of the 15 unique control region alleles as well as alleles A and B that represents a unique lineage from the Elands River and Ngodwana Dam, identified among 134 Bushveld smallscaled yellowfish. The branches are drawn relative to the number of mutational changes in the 427 base pair segment of the control region. Notice the longer branches connecting alleles 1-6 and 7-15, compared with the close relationship between alleles 1-5 for example.

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[10] Francious Roux, 2005, Aquatic specialist, Mpumalanga Parks Board.

2.3.1.3 Analyses 3: Broader level phylogenetic relationships

A representative of each of the lineages from the above analyses as well as the two divergent Assegaai and Elands population lineages were compared in terms of the sequence divergence between them. To put the latter into perspective we also compared some of our unpublished data for *L. polylepis* from Swaziland and the other four species of smallscaled yellowfishes (Table 3) and determined the phylogenetic relationships of the divergent *L. polylepis* alleles to these outgroups based on the genetic distance estimates. The sequence divergences show that three *L. polylepis* individuals sampled from the Assegaai River and eleven individuals from Elands Rivers were highly divergent, with the estimates even exceeding those between the isolated *L. capensis* and all other taxa. The unrooted neighbour-joining phylogram (Figure 7) summarize these relationships. Clearly there is highly significant differentiation within *L. polylepis* and we have this far only considered five of at least 11 populations identified by Roux (2008).

Table 3: Sequence divergence (as percentage) between the most divergent sequences after identification of unique alleles for *L. polylepis* and four other species of smallscaled yellowfish species. The light-grey shaded area represents *L. polylepis* alleles identified and the dark-grey shaded area represents the two alleles identified that might not be *L. polylepis.*

No	Species and locality	1	2	3	4	5	6	7	8	9	10	11	12
1	*L. polylepis* Inkomati River/Ngodwana Dam	-											
2	*L. polylepis* Assegaai River/Phongolo River	1.9	-										
3	*L. polylepis* Usuthu system, Swaziland	2.7	1.1	-									
4	*L. polylepis* Elands River/Ngodwana Dam	2.8	2.3	2.6	-								
5	*L. polylepis* ? Elands River	6.6	7.3	5.3	8.2	-							
6	*L. polylepis* ? Assegaai River	6.1	6.8	5.1	7.7	0.5	-						
7	*L. aeneus* Lower Orange	2.8	3.3	2.9	4.2	6.5	6.1	-					
8	*L. aeneus* / *L. kimberleyensis* Orange/Vaal	4	4.5	4	5.4	7.5	7	2.6	-				
9	*L. natalensis* Mtamvuna River	4.2	4.7	4.5	4.7	6.8	6.8	3.5	4	-			
10	*L. natalensis* Mkuze River	4.2	4.2	3.7	4.2	7.5	7	2.8	4	3.5	-		
11	*L. natalensis* Tugela River	4.4	4.9	3.4	4.9	7.7	7.2	2.6	3.8	3.7	2.6	-	
12	*L. capensis* Clanwilliam Olifants	5.9	6.3	4.8	6.3	8	8	5.2	5.6	5.4	5.4	4.2	-

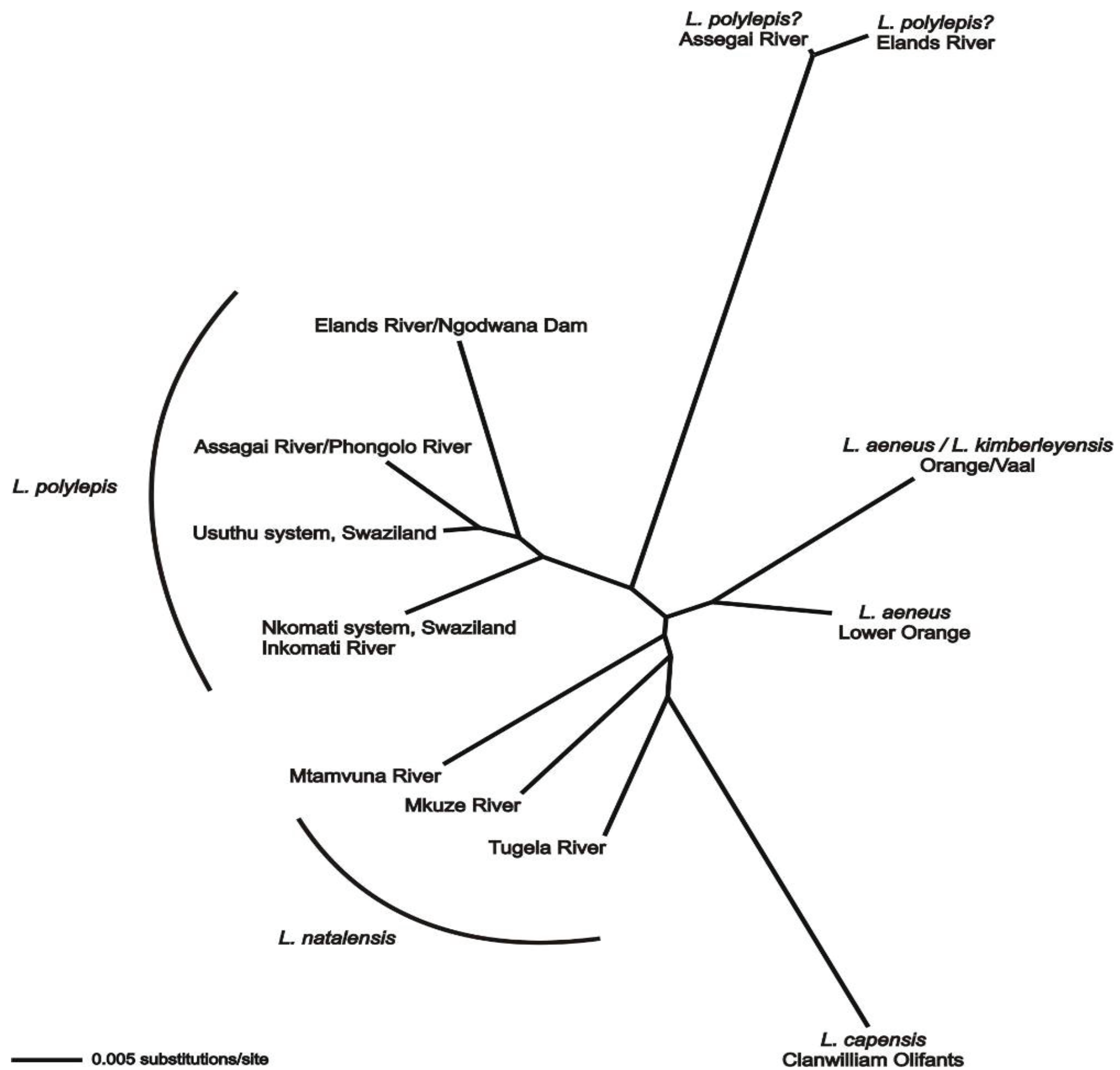


Figure 7: Unrooted phylogram based on a neighbour-joining analysis of the unique control region alleles identified for *L. polylepis* and four other smallscaled yellowfish species. The branches are drawn relative to the number of mutational changes in the 427 base pair segment of the control region. Notice the longer branch connecting the two alleles identified for *L. polylepis* from the Assegaai and Elands rivers.

2.3.2 Morphological assessment

The morphological assessment was undertaken by carrying out a multivariate statistical analyses approach using the Principle Components Analyses (PCA) to identify any driving measurements that may result in the unique grouping of any given measurements or morphological variable.

Initially an assessment of the scale counts and amount of fin rays were undertaken to test for any consistent differences between the populations of *L. polylepis* and the *L. natalensis* out-group collected from the Umvoti River (KwaZulu-Natal).

The initial assessment considered the amount of rays for all fins, and scale counts from the lateral line to the origin of the dorsal fin, lateral line scales, predorsal scales and caudal peduncle scales (Refer to Appendix A). Finding of the initial scale and fin ray PCA assessment are presented in Figure 8. Findings indicate that an overlap of all populations exists and that no individual or combination of scale and or fin ray counts can be useful in identifying any of the individual populations.

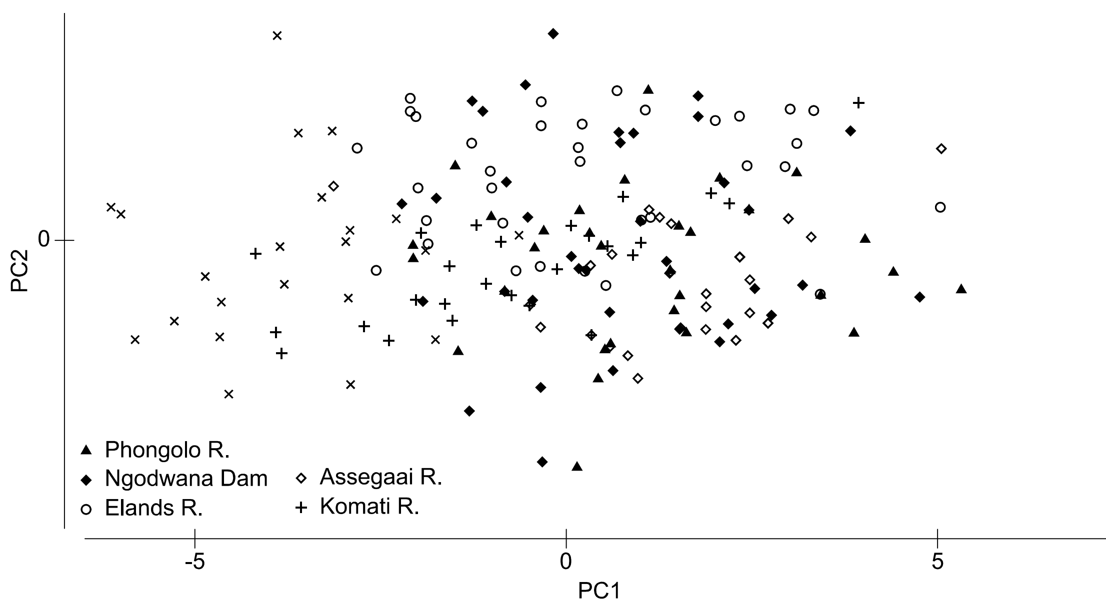


Figure 8: Principle Component Analysis based on the fin ray counts and the scale counts of the five *Labeobarbus polylepis* populations and the one *Labeobarbus natalensis* population considered in this study.

Following the scale and fin ray assessment, consideration of the morphological measurements of the intraspecies differences between the *L. polylepis* populations has been carried out using a PCA (Figure 9) (Refer to Appendix B). Findings suggest that based on morphology, the populations can be separated into three groups: one group consisting of specimens from the Phongolo and Assegaai River, a second group of specimens from the Komati River only, and the third group consisting of specimens sampled at Elands River and Ngodwana Dam. The second group that contained specimens from the Komati River partially overlapped with the Phongolo and Assegaai rivers group. Findings revealed no overlap between the Elands River and Ngodwana Dam groups, with any of the other two groups.

The grouping of the Elands River and Ngodwana Dam populations confirms that these two populations, although occurring in vastly different habitats, are similar. If the effects of different habitat variables were a main driver of differing morphology within this species the Ngodwana Dam sample, which occurs in a lentic ecosystem, should have separated from the remaining populations which were all collected in lotic systems. As such these findings suggest that the morphological differences, although slight amongst these populations, may be arising as a result of genotypic influences rather than phenotypic influences.

The groupings of the individuals from the Phongolo and the Assegaai rivers suggest that although these populations currently seem to be isolated from one another, these populations may still be connected and/or may have only recently been isolated. Considering these results, the morphological assessment for the *L. polylepis* populations correspond well to the geographical distribution of these populations. The Elands River population (most northerly population) is morphologically very similar to the Ngodwana Dam population which occurs in close proximity to this population. Thereafter the Komati River population is more similar to the Elands River and Ngodwana Dam populations of which all three occur within the greater Inkomati River catchment. The Assegaai River, and lastly the Phongolo River populations, that occur within the greater Phongolo River catchment, link onto the initial grouping of the Inkomati River populations. Important to highlight is that the Elands River and Ngodwana Dam populations do not overlap with any other population whilst the remaining Komati River, Assegaai River and Phongolo River populations do overlap (Figure 9), suggesting the uniqueness of this population.

An inter species assessment (Refer to Appendix B) was undertaken by the addition of a KwaZulu-Natal yellowfish *L. natalensis* sample, obtained from the upper Umvoti River (Figure 10). . Morphologically, the new sample of *L. natalensis* overlapped the *L. polylepis* individuals from the Komati, Assegaai and Phongolo rivers. This suggests that the *L. natalensis* population included were morphologically similar to the *L. polylepis* communities from the Komati, Assegaai and the Phongolo rivers. This also indicated that there is no overlap between the *L. natalensis* population with the Elands River and Ngodwana Dam *L. polylepis* populations.

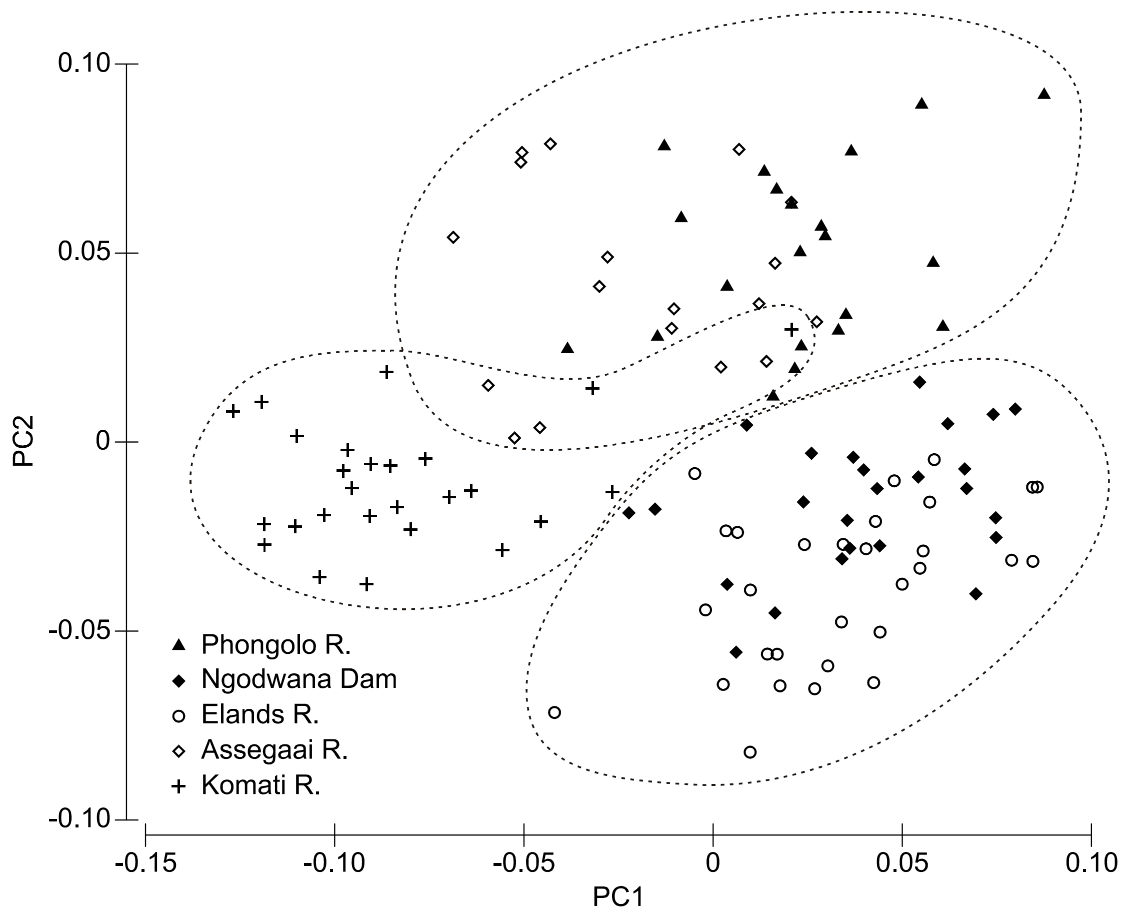


Figure 9: Principle Component Analysis based on morphological measurements from five *Labeobarbus polylepis* populations.

Previous assessments (Groenewald, 1958; Gaiger, 1969; Bloomer *et al.*, 2007) concluded that large morphological variation exists, specifically within the Small-scale yellowfish group. This variation is evident when comparing the morphology of the *L. natalensis* population from the Umvoti River with the three southern *L. polylepis* populations. These findings suggest that a gradient of morphological differences may exist across some of the isolated populations of *L. polylepis* and *L. natalensis*. There were no overlap in the findings from the morphological assessment of the Elands River and Ngodwana Dam populations of *L. polylepis* and any other population, this may originate from an extended period of isolation that has rendered this population morphologically different from the other *L. polylepis* and the *L. natalensis* populations.

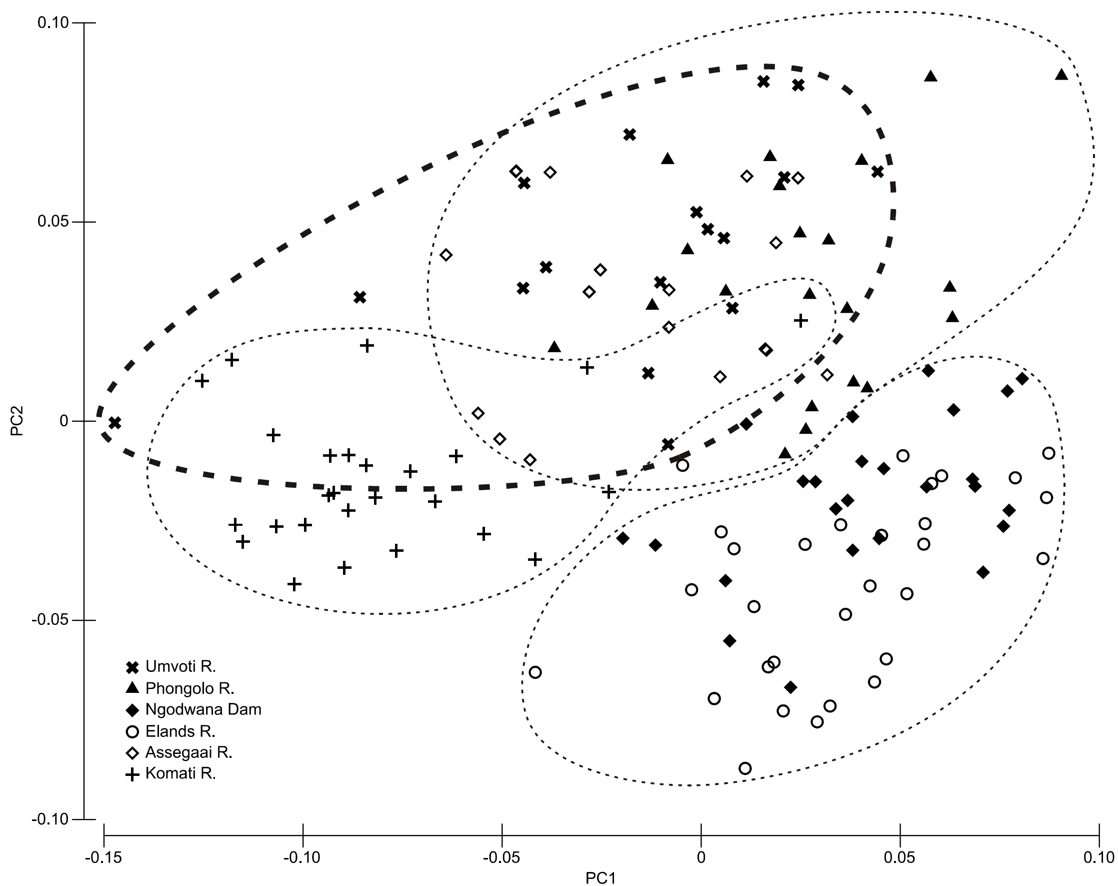


Figure 10: Principle Component Analysis based on morphological measurements from five *Labeobarbus polylepis* populations and the *Labeobarbus natalensis* population considered in this study.

In order to carry out an assessment of the individual measurements that contributed towards the establishment of the groupings presented in the interspecies assessment (Figure 9), the measurements that act as key coefficients in the PCA where considered. Key coefficients in the PCA were those measurements that were considered to have the most influence in the formation of the PCA, these measurements were selected as those that had coefficient values of $\geq$ 0.1 and those that are $\leq$ -0.1.

Table 4) Key coefficient measures are considered to be those that contributed significantly to the groupings observed in the PCA graph (Figure 9) that was drawn using the five *L. polylepis* populations. Results indicated that there were ten key measurements that contributed to the variation explained by PC1 and ten measurements (eight key measurements similar to those indicated in PC1) that contributed to the variation observed in PC2. Standardised key measurement values (measurement/fork length) are presented in Table 5, and, using Microsoft

Excel box and whisker plots, presenting the minimum and maximum value, upper and lower quartile and the median of the key measurements are presented in Figure 11, Figure 12, Figure 13 and Figure 14.

Key measurement assessment results indicated that the morphological differences between populations were slight but consistent. Key measurements included the median sizes of the fins, differences in the median lengths of the heads, mouth measurements, trunk lengths (pre-pelvic length, pre-pectoral length and pre-anal length measurements) and median differences in height (lateral-line to pelvic and post pelvic to post dorsal) and in width (before dorsal fin) of individuals between the different populations.

Graphical representation, Figures 11, 12, 13 and 14) of key measurements allow for the comparison between measurements from the *L. polylepis* populations. In this assessment the *L. natalensis* population data has been included to allow for a holistic review of the morphological.

Table 4: Key coefficients, those above 0.1 and below -0.1 (based on variable measurements), in linear combinations making up PC1 and PC2.

Variable	PC1	PC2
Caudal fin max height	-0.768	-0.355
Lower ray length	-0.204	-
Head length	-0.200	0.277
Prepectoral length	-0.193	0.265
Prepelvic length	-0.192	0.189
Origin of pectoral - premaxilla	-0.176	0.214
Upper ray length	-0.173	-0.118
Premaxilla - supraoccipital	-0.138	0.154
Pre anal length	-0.131	0.338
Pectoral fin length	-0.103	-
Dorsal fin origin - pelvic origin	-	-0.236
Post pelvic - post dorsal	-	-0.203
Max dorsal spine length	-	-0.171
Anterior pelvic - origin of pectoral	-	-0.159
Lateral line - dorsal fin	-	-0.139
Lateral line - pelvic fin	-	-0.133
Body width (before dorsal fin)	-	-0.100
Origin of pectoral - supraoccipital	-	0.119
Lower jaw length	-	0.160
Gape height	-	0.176

Table 5: Standardised key measurement median values (measurement/fork length) of *Labeobarbus* spp. assessed in the study.

	Umvoti R.	Phongolo R.	Assegaai R.	Komati R.	Ngodwana Dam	Elands R.
Caudal fin max height	0.32	0.32	0.36	0.42	0.32	0.34
Upper ray length	0.25	0.22	0.22	0.24	0.22	0.22
Lower ray length	0.25	0.22	0.22	0.24	0.22	0.22
Head length	0.23	0.23	0.23	0.23	0.21	0.20
Origin of pectoral - premaxilla	0.22	0.23	0.23	0.23	0.21	0.21
Premaxilla - supraoccipital	0.12	0.12	0.11	0.12	0.10	0.09
Origin of pectoral - supraoccipital	0.18	0.18	0.18	0.18	0.17	0.17
Gape height	0.07	0.09	0.09	0.08	0.07	0.07
Lower jaw length	0.06	0.07	0.07	0.06	0.05	0.05
Anterior pelvic - origin of pectoral	0.26	0.26	0.26	0.26	0.26	0.27
Prepectoral length	0.23	0.23	0.23	0.24	0.21	0.20
Prepelvic length	0.49	0.49	0.49	0.49	0.47	0.47
Pectoral fin length	0.19	0.17	0.19	0.18	0.17	0.17
Max dorsal spine length	0.19	0.15	0.16	0.18	0.17	0.17
Pre anal length	0.71	0.70	0.71	0.70	0.68	0.68
Lateral line - dorsal fin	0.17	0.15	0.16	0.17	0.16	0.16
Lateral line - pelvic fin	0.07	0.07	0.07	0.08	0.08	0.09
Dorsal fin origin - pelvic origin	0.23	0.19	0.22	0.23	0.22	0.23
Post pelvic - post dorsal	0.22	0.20	0.20	0.21	0.21	0.21
Body width (before dorsal fin)	0.12	0.13	0.13	0.13	0.14	0.14

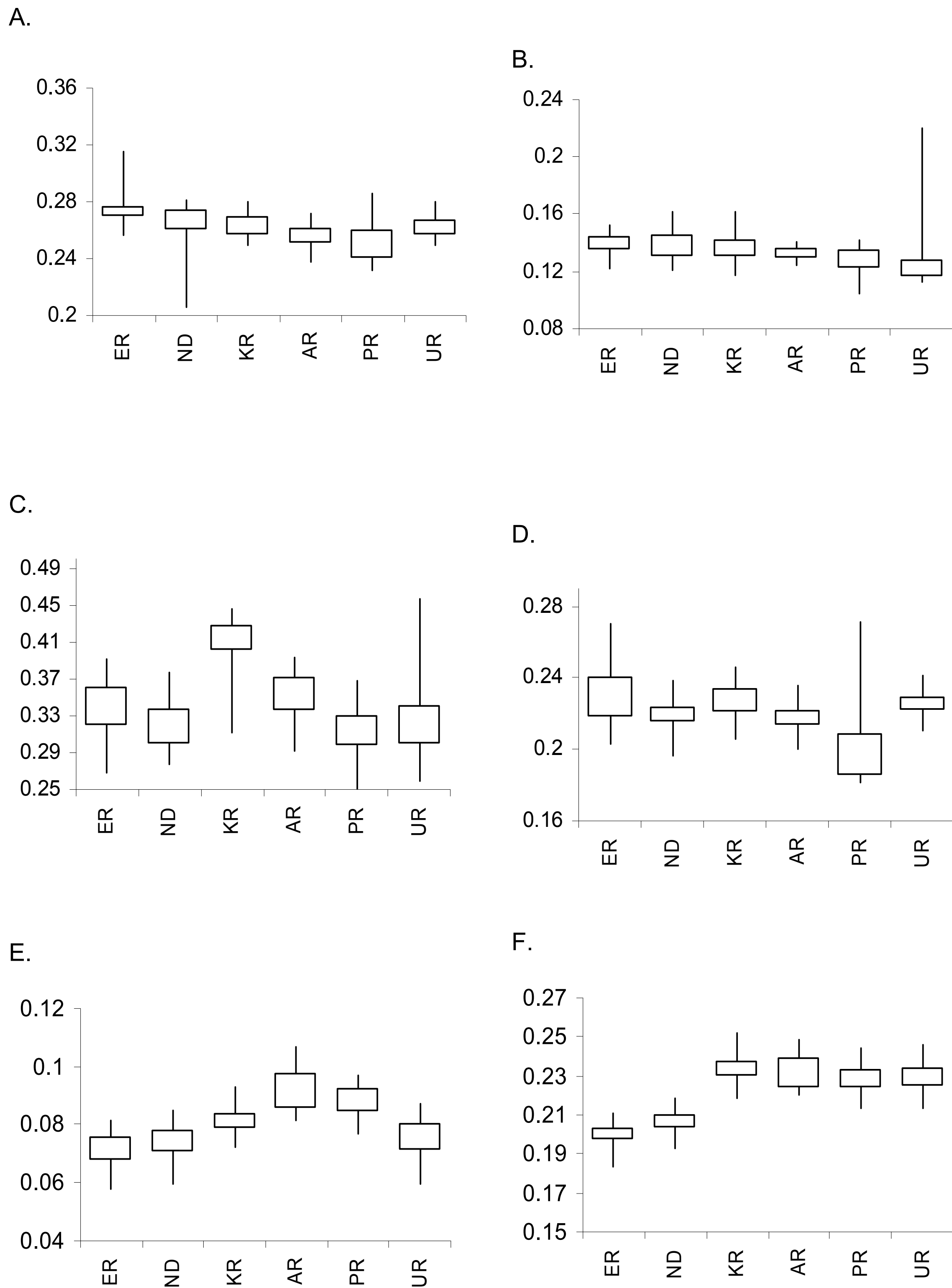


Figure 11: Graphical representation of the key measurements contributing to morphological differences obtained in the study. Measurements include: (A) anterior pelvic – origin of pectoral, (B) body width (before dorsal fin), (C)caudal fin max height, (D) dorsal fin origin – pelvic origin, (E) gape height and (F) head length.

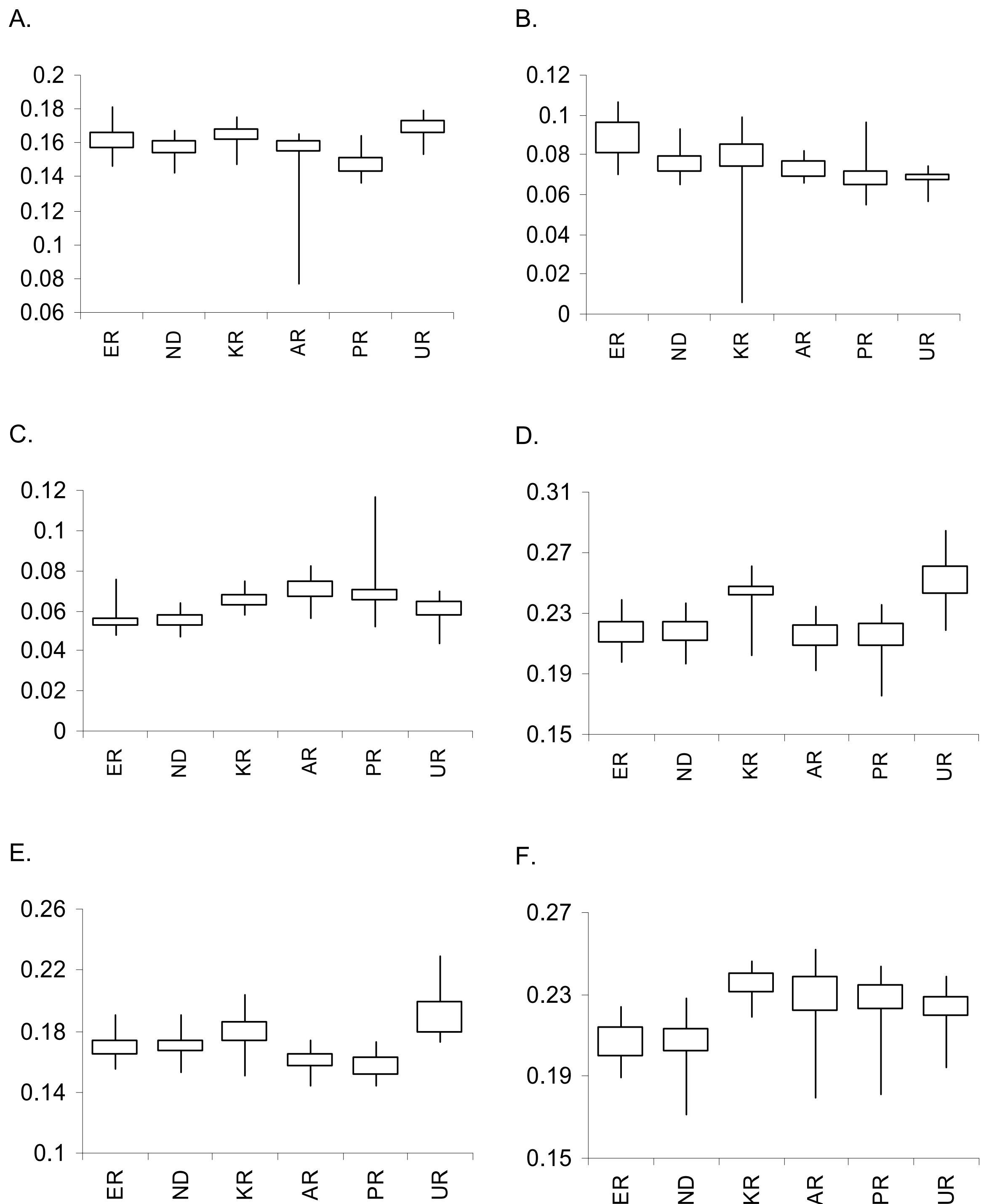


Figure 12: Graphical representation of the key measurements contributing to morphological differences obtained in the study. Measurements include; (A) lateral line – dorsal fin, (B) lateral line – pelvic fin, (C) lower jaw length, (D) lower ray length, (E) max dorsal spine length and (F) origin of pectoral – premaxilla.

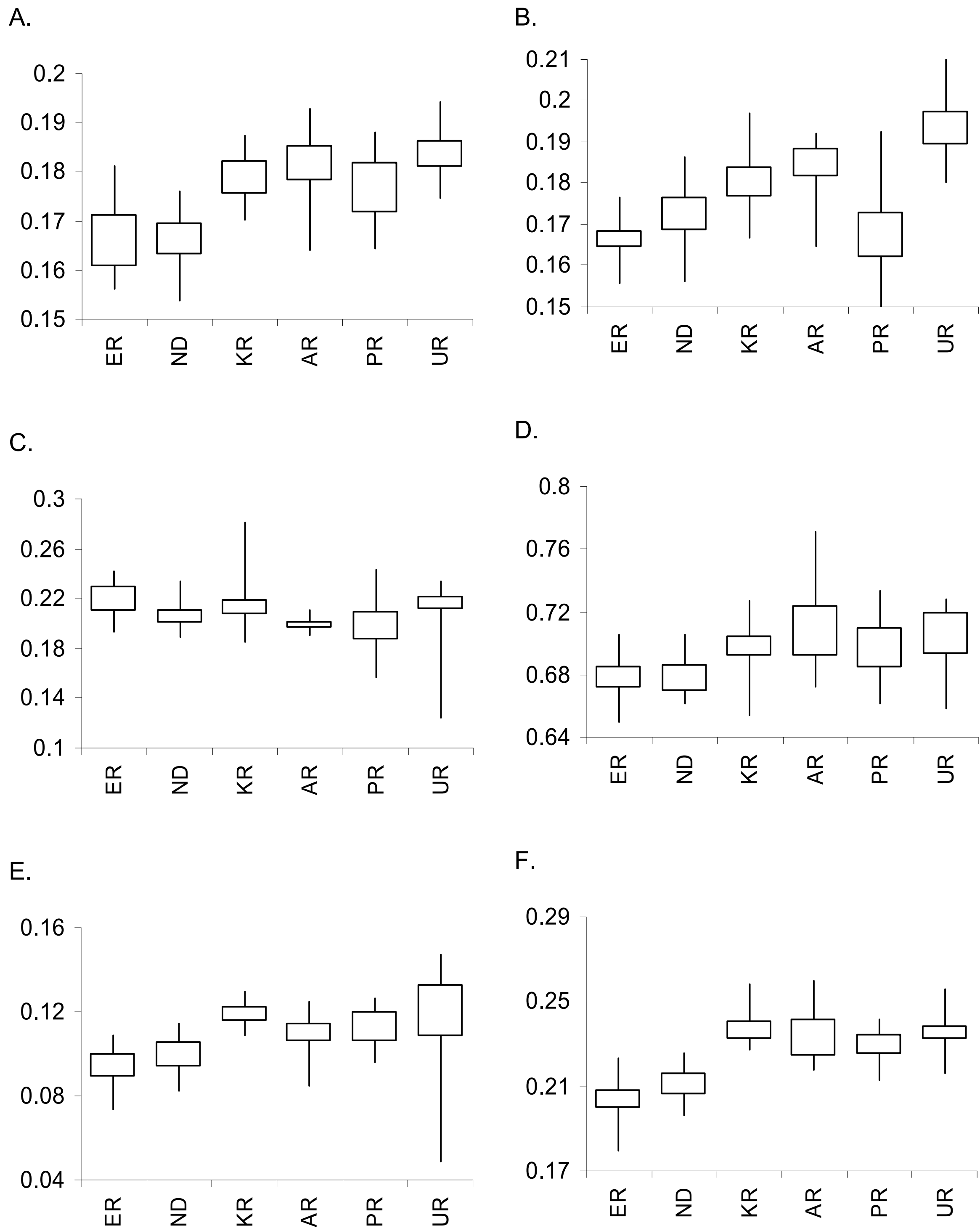
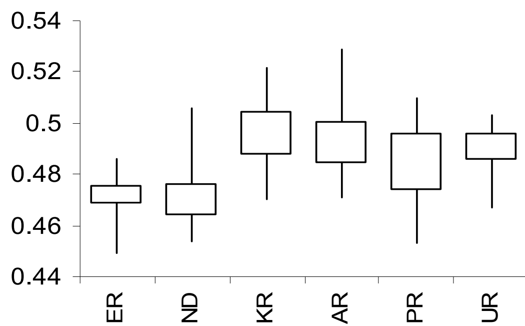


Figure 13: Graphical representation of the key measurements contributing to morphological differences obtained in the study. Measurements include; (A) origin of pectoral – supraoccipital, (B) Pectoral fin length, (C) post pelvic – post dorsal, (D) pre anal length, (E) premaxilla – supraoccipital and (F) prepectoral length.

A.



B.

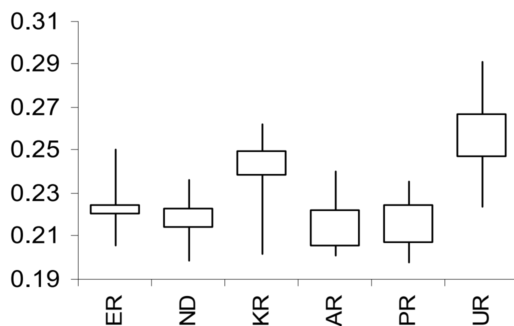


Figure 14: Graphical representation of the key measurements contributing to morphological differences obtained in the study. Measurements include; (A) prepelvic length and (B) upper ray length.

Findings indicate that a large amount of variation and overlap exists in the key morphological measurements of the populations. This does not allow for easy separation of the *L. polylepis* populations or for the *L. natalensis* population. This study confirms that a large amount of variation and overlap exists at least amongst the *L. polylepis* and *L. natalensis*, small-scaled yellowfishes of South Africa. In particular the *L. natalensis* population collected from the Umvoti River in KwaZulu-Natal showed the highest amount of variation particularly due to the outliers of the measurements including body width (before dorsal fin) (Figure 11 (B)), caudal max fin height (Figure 11 (C)), dorsal fin origin to pelvic origin (Figure 11 (D)), premaxilla to supraoccipital (Figure 13 (E)) and Upper ray length of the caudal fin (Figure 14 (B)). Interestingly, in the species comparison of the body width, the *L. natalensis* population reflected a larger variation in body width between individuals, while all of the *L. polylepis* showed uniform widths, particularly in the Assegaai and Elands populations.

Within the *L. polylepis* populations a large amount of variation was observed in the following measurements:

- (Figure 11 (A)) Anterior pelvic to origin of pectoral for the Ngodwana population.
- (Figure 11 (C)) Caudal fin max height predominantly for the Komati River population.
- (Figure 11 (D)) Dorsal fin origin to pelvic fin origin for the Elands River and the Phongolo River populations.
- Figure 12 (A)) Lateral line to dorsal fin origin for the Assegaai River population.
- Figure 12 (B)) Lateral line to pelvic fin for the Komati River population.
- Figure 12 (C)) Lower jaw length for the Phongolo River population.
- Figure 12 (F)) Origin of pectoral to premaxilla for the Ngodwana, Assegaai and Phongolo populations.
- (Figure 13 (A)) Origin of pectoral to supraoccipital for all of the *L. polylepis* populations.
- (Figure 13 (B)) Pectoral fin length for all of the *L. polylepis* populations.
- (Figure 13 (C)) Post pelvic to post dorsal for the Komati River and Phongolo River populations.
- Pre-anal length (13 (D)) predominantly for the Komati, Assegaai and Phongolo populations.
- (Figure 14 (A)) Pre-pelvic length for all of the *L. polylepis* populations.
- (Figure 14 (B)) Upper ray length predominantly for the Komati River population.

This large amount of variation within the *L. polylepis* populations makes the clear separation between populations difficult. The assessment, presented in Figure 9, does however indicate that separations are possible between the grouping of the Elands River and Ngodwana Dam populations, with the rest of the *L. polylepis* populations.

Initially, by considering the box and whisker plots (Figures 11-14), the probability is high, although not certain, that an individual with a measurement equal to the measurement represented in the box would belong to the population represented by that the box, if no overlap of any given measurement occurs between populations. This is possible as 75% of the data points, or those data that occur between the

upper and lower quartile of the measurements of each population, are represented by the boxes. To be certain of the distinctions, only those measurements that occur within a data range of any populations that occur above or below the minimum and maximum values for all other populations can be used. As such the morphological measurements that can be used with certainty to separate the Elands River and Ngodwana Dam populations from the rest of the *L. polylepis* populations, considered in the assessment, include the gape height (Figure 11 (E)), the head length (Figure 11 (F)), the origin of pectoral to supraoccipital (Figure 13 (A)) and the prepectoral length (Figure 13 (F)). In particular, if any individual *L. polylepis* individual originating from any of the populations included in this study are obtained with a gape height smaller than 0.72%, a head length smaller that 0.21%, an origin of pectoral to supraoccipital less than 0.164% and a prepectoral length smaller than 0.213% of the fork length of the individual the individual belongs to the Elands River or Ngodwana Dam populations.

Based on the key measurements of the study, the measurements that can be used with a high probability and those that can be used with certainty to identify the *L. polylepis* populations considered are presented in Table 6. In accordance with the PCA assessment of the *L. polylepis* populations (Figure 9) the Elands River and Ngodwana populations are similar and contain seven measurements that can be used with a high probability to identify individuals from these populations.

Of the seven measurements, five can be used with certainty to identify these individuals. Following the Elands River and Ngodwana Dam populations, four measurements can be used with a high probability to identify individuals from the Komati River, three measurements can be used with certainty. For the Assegaai River population only two measurements are available to identify populations with certainty and for the Phongolo River population only one measurements can be used with a high probability and with certainty should the measurement fall within the range of the certainty measurement. One additional measurement (Max dorsal spine length) can be used with a high probability to identify individuals from the Assegaai River and Phongolo River populations.

Table 6: Key measurements that can be used with a high probability and with certainty to identify the *L. polylepis* populations considered in this study.

Population	Measurements	High probability			With certainty
Elands River and Ngodwana Dam	Gape height	0.07	&	0.08	<0.007
	Head length	0.2	&	0.21	<0.21
	Lower jaw length	0.05	&	0.06	<0.052
	Origin of pectoral - premaxilla	0.2	&	0.21	-
	Origin of pectoral - premaxilla	0.16	&	0.17	<0.179
	Premaxilla - supraoccipital	0.09	&	0.11	-
	Prepectoral length	0.2	&	0.22	<0.21
Komati River	Caudal fin max height	0.40	&	0.43	>0.39
	Lateral line - pelvic fin	-	-	-	<0.055
	Lower ray length	0.24	&	0.25	>0.239
	Max dorsal spine length	0.17	&	0.19	-
	Upper ray length	0.24	&	0.25	-
Assegai River	Gape height	-	-	-	>0.097
	Lateral line - dorsal fin	-	-	-	<0.136
Phongolo River	Dorsal fin origin - pelvic origin	0.19	&	0.21	<0.197
Assegai and Phongolo rivers	Max dorsal spine length	0.15	&	0.17	-

Results indicate that the Ngodwana Dam and Elands River populations have noticeably smaller heads compared to the other populations. Smaller heads may be attributed to the slightly different ecological niche of this species as they are the only two populations of *L. polylepis* (in this study) that do not occur with any other large cyprinids such as *L. marequensis* and/or *Varicorhinus nelspruitensis*. In relation to head size, populations from the Elands River and the Ngodwana Dam had relatively smaller mouth sizes. The Assegaai and Phongolo rivers populations had the largest mouths of the populations assessed.

In addition, a relatively high prevalence of the rubber-lip form amongst *L. polylepis* individuals from the Elands River and from the Ngodwana Dam was observed. The relationship between head size, gape size and lip form are unknown. Occurrence of the rubber-lip form, considered not to exist in *L. polylepis* (Mulder, 1989), within the populations from the Phongolo and Komati rivers was also observed and has not been noted before. It is currently believed that the rubber-lip formation within the smallscaled *Labeobarbus spp.* is due to their feeding behaviour as a result of grubbing between pebbles, cobbles and loose rocky substrates (Skelton, 2001).

Historical assessments of these mouth formations within *L. aeneus* indicated that the rubber-lip form can revert to the varicorhinus lip form if the feeding behaviour of the individual changes (Groenewald, 1958).

Results further indicate that although the heads of the Elands River and Ngodwana Dam communities are noticeably smaller than the remaining populations, the trunk lengths of these populations were the greatest. The Komati River population has the largest head lengths, in relation, which are similar to the Umvoti River population of *L. natalensis*. Finally in consideration of the trunk lengths of the remaining populations, the Phongolo River population had the smallest trunk lengths followed by the Umvoti and Assegaai rivers populations and finally the Komati River population which were all smaller than the Ngodwana Dam and Elands River populations.

In consideration of the fin lengths, although the *L. natalensis* population had relatively longer fins compared to the *L. polylepis* populations, only the Phongolo and Assegaai rivers populations have pectoral fins that were longer than their dorsal fins. In all of the remaining populations the lengths of the dorsal and pectoral fins were equal. These differences in fin length of *L. polylepis* and *L. natalensis* populations are considered to possibly occur as a result of the influence of different habitats on the populations.

In consideration of the height of individuals from all of the populations, results showed that all the populations were similar in height. Interestingly all the populations (excluding the Phongolo River population) had a dorsal fin origin to pelvic fin origin measurement that was slightly longer than the post pelvic to post dorsal length. In the Phongolo River population the post pelvic to post dorsal length was slightly longer than the dorsal fin origin to pelvic fin origin.

In consideration of the width from all of the populations, this assessment revealed that the width of the *L. polylepis* populations was relatively greater than the width of the *L. natalensis* population. Findings showed that the width of the *L. polylepis* populations from the Elands River and the Ngodwana Dam were greater than for any of the other *L. polylepis* populations. The width of the Komati and Assegaai rivers populations were similar whilst the width of the Phongolo River population was slightly less than that of the Assegaai River population, but greater than that of the *L. natalensis* population.

Finally this study allowed for an assessment of the condition of populations that were obtained from the different systems.

Findings of the condition assessment are presented in Figure 15 and reveal that based on the mass (in g) per cm, the condition of individuals assessed in the study ranged from 0.35g per centimetre to 43.3g/cm. Both the minimum and maximum condition values were obtained from the Phongolo River population. A better refection of the overall condition of the populations is provided by considering the box of the box and whisker plots. These data points include 75% of the data from the populations. By considering this the Assegaai River and the Ngodwana Dam populations had the highest condition, followed by the Elands River population, the Komati River population and the Umvoti River population respectively (Figure 15). Interestingly the amount of variation of the condition of the *L. polylepis* populations where considerably greater than the *L. natalensis* population from the Umvoti River. This assessment indicates that differences in the condition of the populations assessed in this study exist. These differences may have occurred as a result of differing habitats that are available to these individuals and that the availability of food for populations in these systems may be different.

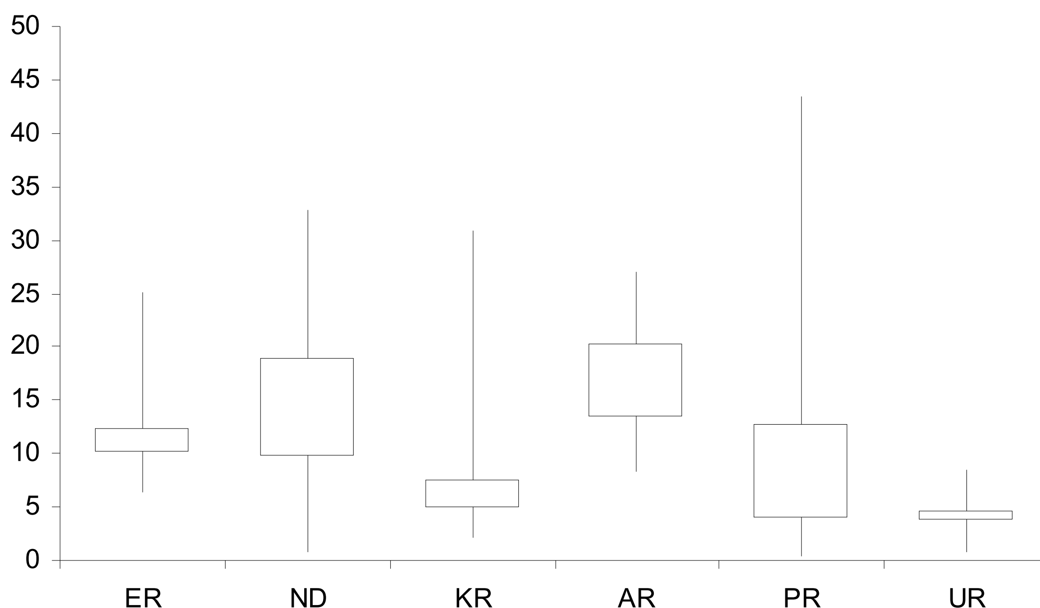


Figure 15: Graphical presentation of the condition index outcomes (grams per cm of Standard length) of the populations assessed in this study.

2.4 Conclusions

In conclusion, results from the genetic and morphological assessment of the isolated populations of *L. polylepis* showed that consistent morphological and genetic differences do exist between the five populations of *L. polylepis* considered. Based on the genetic assessment of the populations, outcomes indicate that a large range of genetic variation exist within *L. polylepis*. An extreme case of genetic variation is represented by a group of individuals from the Elands River and Assegaai River that show a clear unique divergence not only from the remaining populations of *L. polylepis* but also from all of the other small-scaled *Labeobarbus spp* in South Africa. Findings further suggest that three groups, the Phongolo/Assegaai populations (group 1), the Komati and selected individuals from the Elands and Ngodwana populations (group 2) and the Elands and Ngodwana populations (group 3), should be considered as separate conservation units pending further investigation within the species and/or the small-scaled group whereby this status may be elevated to management units or even evolutionary units. Due to individuals from the Elands River and the Ngodwana Dam containing alleles from two of the proposed conservation units, this study may indicate that remnants from a stocking exercise of *L. polylepis into* these systems from the Komati River may still remain or alternatively that these two conservation units may have a shared history.

Following the morphological assessment, outcomes indicate that although very similar, consistent differences in the morphology of the populations considered do exist. These outcomes suggests that the Elands River and Ngodwana Dam populations of *L. polylepis* are unique and that they are the only populations that can with certainty be separated morphologically from the other *L. polylepis* populations and the *L. natalensis* populations from the Umvoti system.

In conclusion, the use of a genetic marker such as the mitochondrial DNA (mtDNA) control region, is extremely useful in identifying populations of yellowfish that are sufficiently different from other populations to warrant specific conservation and management. Although the morphological and genetic assessment identified the uniqueness of the Elands River and the Ngodwana Dam populations of *L. polylepis,* without the genetic assessment the uniqueness of the isolated Assegaai and Phongolo river Bushveld smallscale yellowfish in comparison to the northern populations considered in this study would not have been established.

3 Section 2: Metal bioaccumulation in muscle and liver tissue of five *Labeobarbus polylepis* populations from Mpumalanga, South Africa

Victor Wepener[11] and Andrew Husted[12]

3.1 Introduction

Metal pollution of rivers is a world-wide phenomenon and this can be attributed to the growth in mining, industrial and agricultural activities, as well as a proliferating human population (He and Morrison, 2001). According to Abel (1989) the most important metals in water pollution management are cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn). Some of these studied metals are essential trace elements to living organisms (i.e. Cu and Zn), while other metals (i.e. Cd and Pb) are non-essential and have no known biological function (Connel et al., 1999). At elevated levels, all metals are toxic to aquatic organisms. This toxicity may cause direct or indirect effects such as histological damage or a reduction in the survival, growth and reproduction of species (Heath, 1987). Environmental factors such as temperature, pH and water hardness may have an influence on the toxicity of metals. According to Abel (1989) these conditions help to determine the chemical speciation of metals and as a result influence the bioavailability of the metals to aquatic organisms. Interactions between pollutants, the developmental stage of the organism and interspecific variations in susceptibility to metals are other factors which may influence metal toxicity (Hellawell, 1986).

The need to monitor river systems which may be impacted either directly or indirectly by industrial and mining activities is extremely important when viewed in the light of the consequences of metal pollution in aquatic ecosystems. These determined metal concentrations can then be compared to the set metal concentrations published in the existing water quality guidelines for these systems (Wepener et al., 2000). The state of the system to which the aquatic organisms is exposed can then be assessed.

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According to Abel (1989), biological monitoring is very important in order to obtain a reliable and general assessment of the metal pollution of the impacted system.

According to Hellawell (1986) the aquatic organisms which accumulate pollutants from their environment and/or food, sequestering them in their bodies, so that an indirect estimate of prevailing environmental concentrations of these substances can be made once the tissues are analysed. Van der Oost et al. (2003) suggest that the concept associated with the term “biological indicator” is that of an organism, which accumulates substances in it’s tissues in a way so as to reflect the environmental levels of these substances or the extent to which the organism has been exposed to them. Organisms such as these are “bio-accumulators” of these substances, and as they are able to concentrate very low environmental levels of substances they are very useful, as they facilitate with detection and analysis (Hellawell, 1986).

According to Dallinger et al. (1987) many fish species are considered to be top consumers in an aquatic ecosystem. As a result, fish are most likely to accumulate pollutants and pose a potential risk not only to themselves but also piscivorous birds and mammals, including humans (Grimanis et al., 1978; Adams et al., 1992). The uptake of metals by fish through the diet can be as important as waterborne metal uptake and the relative importance of the different uptake routes is variable (Dallinger et al., 1987; Kraal et al., 1995; Langevoord et al., 1995). Little information is available on the relationship between internal tissue levels of metals and condition of fish under natural exposure conditions (Bervoets & Blust, 2003).

In an aquatic ecosystem, organisms which are near the top of the food chain such as fish are generally considered to be reliable indicators of the health of the overall system. The use of fish for this study as a biological monitoring organism is based on the fact that living organisms can provide useful information on the chemical quality of the water as they have experienced it throughout their lives, whereas a chemical analysis (purely physical and chemical analysis of the water) can only indicate the conditions of the system at the time of the sampling (Abel, 1989). A number of reasons are available as to why fish are good organisms to use for biological monitoring. According to Hellawell (1986) fish are known to accumulate metals in their organs and tissues. In addition to this, fish are easily identified in comparison to other aquatic organisms, they are sampled with relative ease and they have a wide distribution. According to Van der Oost et al. (2003) fish have an economic

importance as a resource which provides fish with an added feature of great importance.

Many factors influence the uptake of metals by fish and their use in environmental assessment programmes (Van der Oost et al., 2003). Such factors are morphometry, pH, alkalinity, modes of metal uptake and release, dissolved organic matter, trophic relationships of fish, differences among species, and fish weight within populations (Johnson, 1987, Saiki and May, 1988, Wren and MacCrimmon, 1986).

The uptake routes of pollutants can vary greatly and bioaccumulation can only occur if the rate of uptake by the organism exceeds the rate of elimination (Spacie and Hamelink, 1983). In fish, a control mechanism for the uptake of metals is found, and as a result, elimination rates may be more dependent upon uptake rates (Bryan, 1964, 1967) than is probably the case for non-essential metals such as lead. The oral route is the most significant uptake route for metals by fish, through ingested food (Manahan, 1989, Berg et al., 1995), ingested non-food particles such as sediment, drinking water, the gills or the skin (Du Preez, 1990). According to Mason (1991) contaminants accumulate faster in fish with higher metabolic rates and, because a higher metabolism is a result of feeding, a greater uptake of contaminants across the gills may occur in feeding as opposed to starved fish. It is for this reason that gills should be assessed for metal accumulation, which was excluded for this study. According to Klaassen (1976) the liver is known as a storage and detoxification organ and as a result the liver as considered for the study as the amount of metal accumulated therein might reflect the severity of the pollutant. According to Du Preez et al. (1997) the muscle is the tissue generally consumed by humans and the metal accumulation content is important for the presumed effect on human health, for this reason muscle was considered for this study.

The *Labeobarbus* genus is generally considered to be a cosmopolitan species as they are distributed all over South Africa and for this reason *L. polylepis* was selected for this study. This distribution will assist in acquiring information about the relevant and respective systems sampled through the *L. polylepis* distribution. In addition to this, little information on yellowfish is published. The lack of research with regard to *L. polylepis,* as well as the status of this species is a concern that needs to receive urgent attention. An assessment of the bioaccumulation of *L. polylepis* will help to determine the state of the systems sampled for this study as well generate information for this species.

The objectives of this component of the study were to determine the extent of metal bioaccumulation in the organs and tissues of *L. polylepis,* to determine the preferred order of bioaccumulation of the 9 selected metals in the different organs and tissues of *L. polylepis* and to determine if there were any temporal differences in metal bioaccumulation between the selected sampling localities.

3.2 Materials and methods

3.2.1 Study area

The Bushveld smallscale yellowfish, *L. polylepis* is widely distributed, occurring in the southern tributaries of the Limpopo, Incomati and the Phongolo river systems in South Africa (Skelton, 2001). Fish populations assessed in this study were collected from three separate catchments in Mpumalanga (Figure 2), from the Assegaai River (Usutu Catchment), the Phongola River (Phongola Catchment) and the Elands and Komati Rivers and the Ngodwana Dam (all three from the Komati Catchment).

3.2.2 Field sampling

Twenty individual *L. polylepis* were sampled from the five different rivers between May 2006 and July 2006 using array of sampling techniques which included seine nets, cast nets, electro-shocking, gill nets (mesh size 45 mm-95 mm) and fly fishing techniques. The sampled fish were processed in the field where the following data was recorded from each fish according to the process adopted by Coetzee *et al*. (1996).

The captured fish were (i) individually weighed and their total length measured. The sampled fish were (ii) dissected on a polyethylene work-surface, using stainless steel work instruments (Heit and Klusek, 1982) whilst wearing surgical gloves. The following tissues were removed for metal analysis: muscle and liver. All the samples were then frozen, until they could be subjected to metal concentration analysis in the laboratory.

3.2.3 Laboratory procedures

In the laboratory distilled water was used to thaw and rinse the tissues to remove the excess mucus coating and/or other foreign particles that could have absorbed metals (Nussey, 1998). An inductive coupled plasma mass spectrometry (ICP-MS) was used for metal screening for prepared whole body tissues. According to the procedures used in Nussey (1998), the samples were weighed in pre-weighed polypropylene falcon tubes, the tissues were then dried in an oven at 60°C for a period of 48 hours, and in order to determine the moisture content of the tissues, both the wet and dry weights of the samples were recorded. The samples were then digested by adding 5 ml nitric acid (65%) and 200 µl hydrogen peroxide (50%) to each sample. These samples were then left to stand for a period of 12-24 hours. A 1000 watt microwave oven was used for the digestion of the samples. Samples were place in the microwave oven for 15 minutes at 10-40% full power until the solutions appeared clear (fully digested) (Blust et al., 1988). After digestion, each of the samples as made up with 9.5 ml ultrapure water produced by a Milli-Q Academic system and was ready to be analysed. The concentration of the following metals: Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were measured using an ICP-MS. These metals were selected based on the results of the ICP-MS scan of the water sample. The metal concentrations of each sample were calculated as follows:

$$\text{Metal concentration } (\mu g/g) = \frac{\text{ICP-MS reading } \mu g/l}{\text{Sample dry mass (g)}} \times \text{Sample volume (10 ml)}$$

3.2.4 Statistical analyses

In accordance with Zar (1984) the statistical analysis of the data was performed by using standard ANOVA tests using Tukey's multiple comparison-tests in order to be able to measure significant differences. The $P < 0.05$ level was where significance was tested.

The differences in metal concentrations were tested by one-way analysis of variance (ANOVA), considering sites as variables. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively (Zar, 1984). When the ANOVA revealed significant differences, post-hoc multiple comparisons between sites were made using the appropriate Scheffe (parametric) or

Dunnette-T3 (non-parametric) test to determine which means differed significantly. The significance of results was ascertained at $P<0.05$.

3.3 Results

The findings of the metal bioaccumulation experiment are presented here. The metal concentrations (aluminium – Al, Cd, Cr, Cu, iron – Fe, manganese – Mn, Ni, Pb and Zn) found in the tissues (muscle and liver) of *L. polylepis,* were analysed to obtain site specific bioaccumulation data. The mean and standard error of heavy metal concentrations (µg/g dry mass) of the 9 selected metals found in the muscle and liver samples of the five *L. polylepis* populations are presented in Figure 16, Figure 17, Figure 18 and Figure 19. The summary statistics for all the bioaccumulation data are presented in Appendix H and Appendix I.

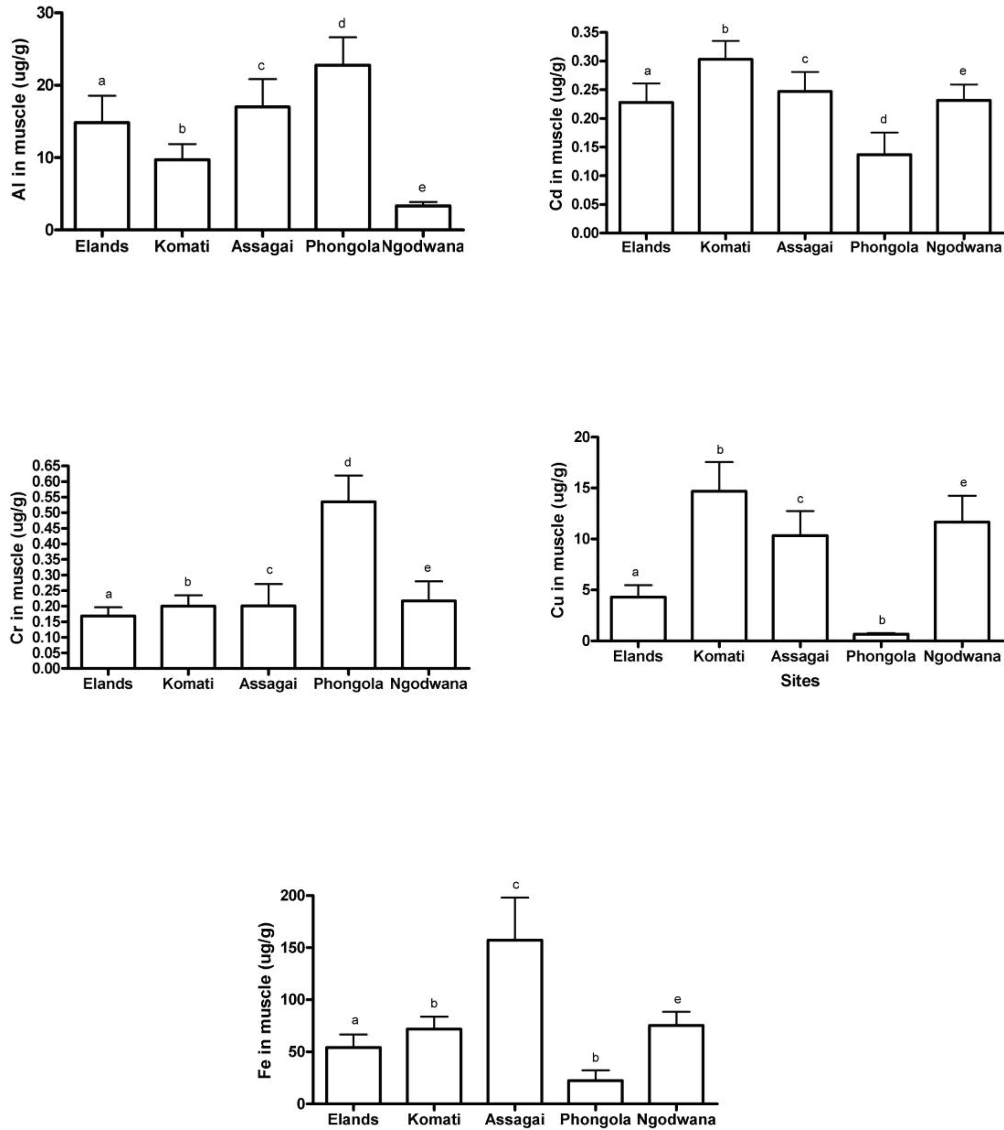

Figure 16: The mean metal concentration in the muscle from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant differences (P< 0.05).

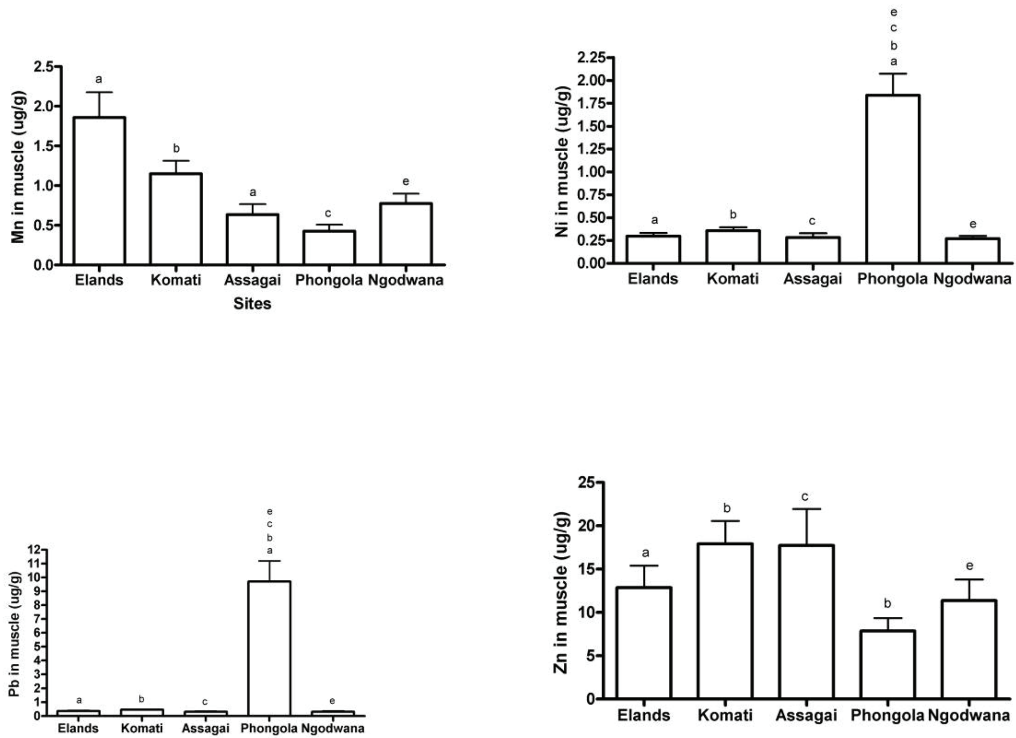

Figure 17: The mean metal concentration in the muscle from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant differences (P< 0.05).

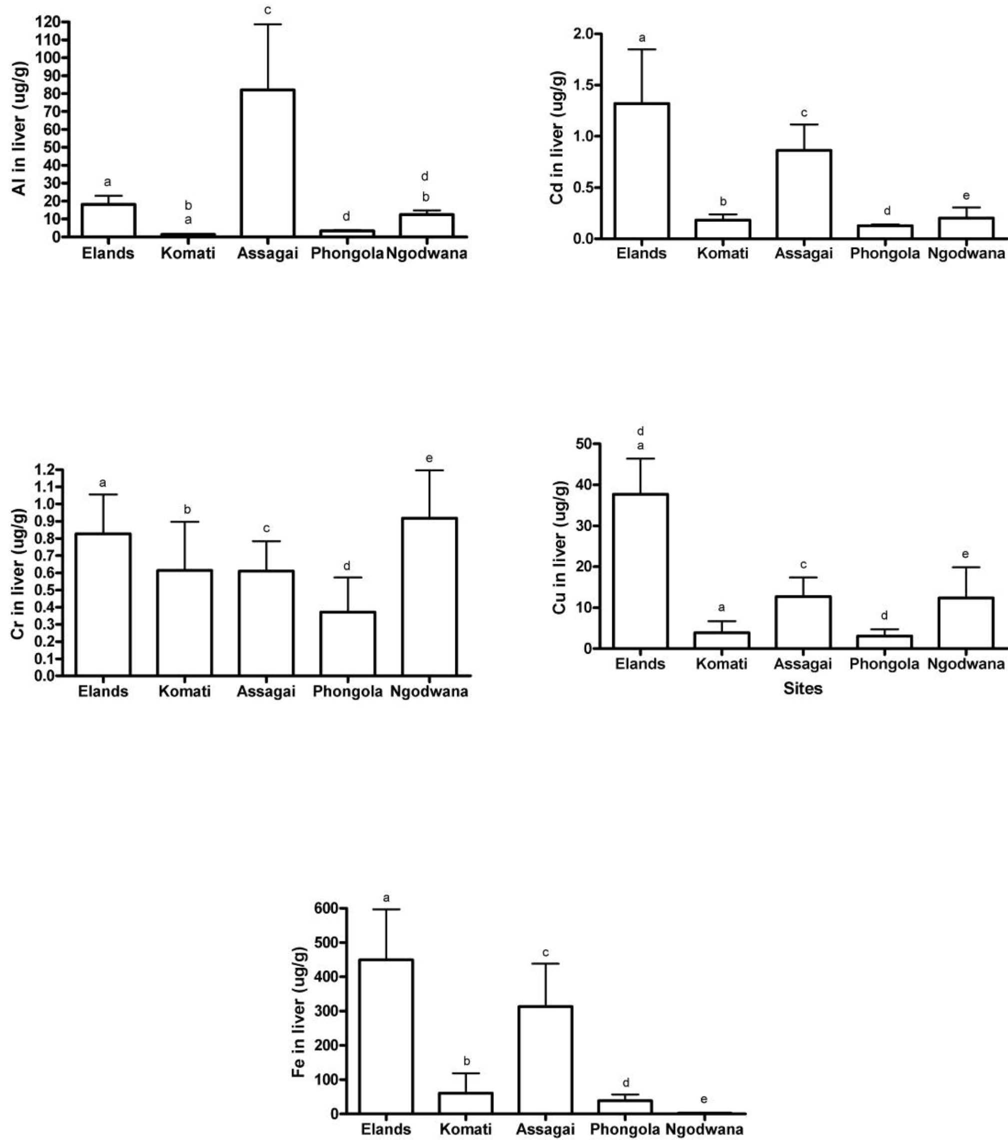

Figure 18: The mean metal concentration in the liver from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant difference (P< 0.05)

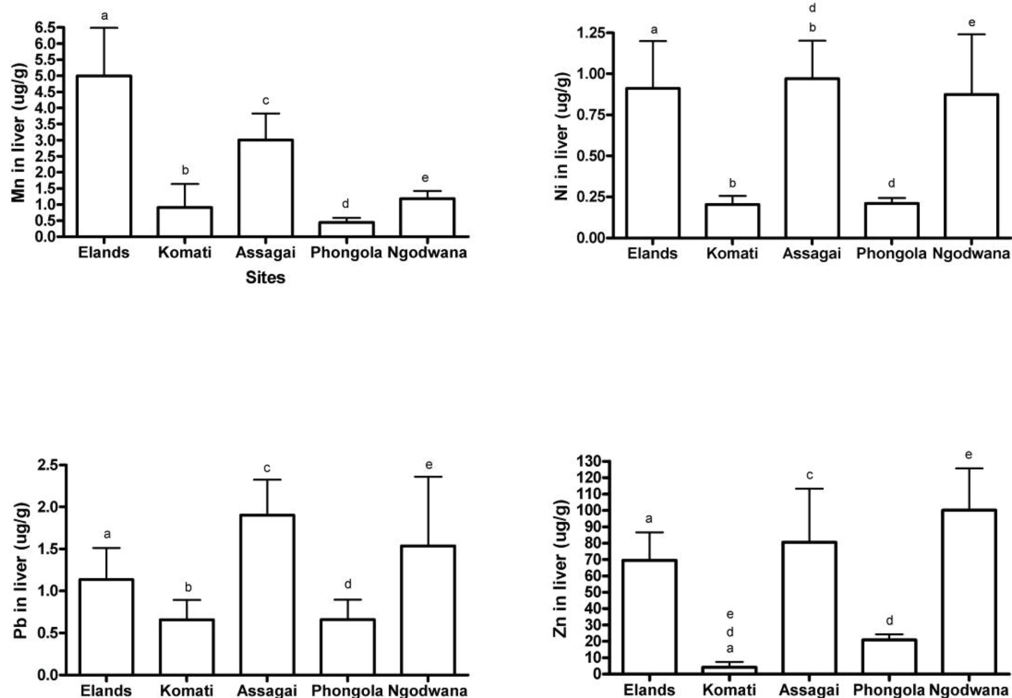

Figure 19: The mean metal concentration in the liver from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant difference (P< 0.05)

3.3.1 Aluminium

The order of bioaccumulation for Al in *L. polylepis* was the highest in the liver for the Elands River, Assegaai River and the Ngodwana Dam populations (Figure 18). Significant differences (P < 0.05) were found between the liver samples of the Elands River and Komati River populations, The Komati River and Ngodwana Dam populations as well as the Phongola River and Ngodwana Dam Populations. The Al concentrations of *L. polylepis* showed high variations in both the liver and muscle samples. The highest Al concentrations were found in the liver samples of the Assegaai River population with the lowest Al concentration being found in the liver samples of the Komati River population. Variations in the Al concentrations found in the muscle samples from the sampled populations are not significant.

The tissue which showed the highest Al concentration (82 µg/g) was the liver samples taken from the Assegaai River. The remaining Al concentrations from both

tissue samples taken from the remaining four systems were significantly lower than the Assegaai River liver sample.

3.3.2 Cadmium

Cadmium showed the highest bioaccumulation in the liver of *L. polylepis* in the Elands River and Assegaai River populations (Figure 18). No significant differences ($P < 0.05$) were found between any of the populations of *L. polylepis.* High variations in the Cd concentrations of the liver samples were evident between all five populations with the Elands River populations showing the highest concentration and the Phongola River population showing the lowest concentration. Cadmium concentrations in the muscle samples of all five populations were consistently low (0.137-0.3025 µg/g) (Figure 16). The Assegaai River showed the second highest Cd concentration which was found in the liver sample (0.9 µg/g). The remaining Cd concentrations found in the liver and muscle samples from all five systems are similar in concentration.

3.3.3 Chromium

The order of bioaccumulation for Cr in *L. polylepis* was the highest in the liver samples for all the sampled populations except for the Phongola River population which showed higher Cr concentrations in the muscle sample (Figure 6.1) No significant differences ($P < 0.05$) were found between any of the populations of *L. polylepis.* Variations in the Cr concentrations of the liver samples was very little (0.3-0.9 µg/g) with the Phongola River population showing the lowest bioaccumulation. In contrast, the Phongola River population showed the highest Cr concentration in the muscle samples with the other four populations showing very little variation in concentrations (0.16-0.2 µg/g) (Figure 16).

The highest Cr concentration found in the muscle samples taken from the five sampled *L. polylepis* populations was found in the Phongola River population (0.5 µg/g). In addition to this, the Cr concentrations found in the muscle samples from the other *L. polylepis* populations were similar in concentration to the Phongola River population. This concentration is significantly lower than the chromium concentrations found in muscle samples from other fish species from four other

systems (Table 7). The highest Cr concentration found in the liver samples from this project was found in the Ngodwana Dam population (0.9 µg/g) (Figure 18) and again all Cr concentrations from liver samples for the *L. polylepis* were similar. This concentration is also significantly lower than the Cr concentrations found in the liver samples from other fish species from other systems (Table 7). This is an indication that all sampled systems for this project have low chromium concentration levels.

3.3.4 Copper

The Cu bioaccumulation order in *L. polylepis* was highest in the liver samples of all populations except for the Komati River population (Figure 18), with significant differences ($P < 0.05$) between the liver samples between the Elands River and Phongola River populations as well as between the Komati River and Phongola River populations. *L. polylepis* showed the highest Cu concentrations in the liver samples in the Elands River population, with the Phongola River populations showing the lowest Cu concentrations. Similar concentrations of Cu were found in the muscle samples between all populations excluding the Phongola River population which showed lowest Cu concentration (Figure 16).

The highest Cu concentration found in the muscle samples from the five *L. polylepis* populations was found in the Komati River populations with a concentration of14.7 µg/g. In comparison with the copper concentrations found in the fish species in Table 7 we are able to deduce that the Cu concentrations for the muscle samples identified in this project are reasonably low as well as normal when compared to other the fish species found in other systems.

3.3.5 Iron

Iron showed the highest bioaccumulation in the liver of *L. polylepis* in the Elands, Assegaai and Phongola rivers populations (Figure 18). A significant difference ($P < 0.05$) was found between the muscle samples of the Komati River and Phongola River populations. The highest Fe concentrations were found in the Elands River liver populations, and with the lowest concentrations being found in the liver samples of the Ngodwana Dam population. The iron concentrations showed little variation between all the sampled populations except for the Assegaai River population which

was relatively higher in Fe concentration. A large variation in Fe concentrations was evident in the liver samples.

3.3.6 Manganese

The Mn bioaccumulation order in *L. polylepis* was highest in the liver sample taken from the Elands River population (Figure 19). The Mn concentrations were highest in the liver samples for all the populations except for the Komati River population where the muscle sampled showed a higher Mn concentration (Figure 17). A significant difference ($P < 0.05$) was found in the muscle sample between the Elands River and Assegaai River populations. The highest Mn concentration was found in the liver sample taken from the Elands River population, and the lowest Mn concentration was taken from the muscle sample from the Phongola River population. Variations in the Mn concentrations of both the muscle and liver samples taken from the five populations were found.

3.3.7 Nickel

Nickel showed the highest bioaccumulation in the muscle of *L. polylepis* in the Phongola River population (Figure 17). The liver samples taken from the Elands and Assegaai Rivers, as well as the Ngodwana Dam showed higher Ni concentrations than the muscle samples (Figure 19). A significant difference ($P < 0.05$) was found in the muscle samples between the Elands and Phongola River populations, the Komati and Phongola River populations, the Assegaai and Phongola River populations as well as between the Ngodwana Dam and Phongola River populations. In addition, a significant difference ($P < 0.05$) was found in the liver samples between the Komati and Assegaai River populations as well as between the Assegaai and Phongola River populations. The Ni concentrations from the liver samples were very similar between the Komati and Phongola river populations, as well as between the Elands and Assegaai rivers and the Ngodwana Dam. Little variation in the Ni concentration was evident with the muscle samples except for the Phongola River population which showed the highest overall Ni concentration.

The highest Ni concentration for this project was found in the muscle samples from the Phongola River (1.8 µg/g). The Ni concentrations found in the four remaining

populations were all relatively similar in concentration to the Phongola River sample. The Assegaai and Elands Rivers' populations as well as the Ngodwana Dam population had very similar Ni concentrations found in the liver samples with 0.97 µg/g being the highest.

3.3.8 Lead

The order of bioaccumulation for Pb in *L. polylepis* was the highest in the liver of all the sampled populations except for the Phongola River population which showed the highest Pb concentration in the muscle of all populations (Figure 17). A significant difference ($P < 0.05$) was found in the muscle samples between the Elands and Phongola River populations, the Komati and Phongola River populations, the Assegaai and Phongola River populations and lastly between the Ngodwana Dam and Phongola River populations (Figure 17). The highest Pb concentration was found in the muscle samples taken from the Phongola River population. Variations in the Pb concentrations found in the muscle samples were found to be limited, with the exclusion of the Phongola River population which showed a significantly higher Pb concentration in the muscle sample. A limited variation in Pb concentrations was evident in the liver samples taken from the five populations.

The highest Pb concentration measured was in the muscle samples taken from the Phongola River population (9.7 µg/g). The remaining Pb concentrations for the four remaining *L. polylepis* populations were significantly lower and similar in concentration to one another.

3.3.9 Zinc

The order of bioaccumulation for Zn in *L. polylepis* was the highest in the liver samples taken from the Ngodwana Dam (Figure 19). The Zn concentrations were highest in all the liver samples except for the Komati River populations which showed higher Zn concentrations in the muscle samples (Figure 17). A significant difference ($P < 0.05$) was found in the muscle samples between the Komati and Phongola River populations. A significant difference ($P < 0.05$) was also found in the liver samples between the Elands and Komati River populations, the Komati and Phongola River populations and also between the Komati River and Ngodwana Dam populations.

The variations in the Zn concentrations taken from the liver samples were greater as well as more significant (4.1-100.1 μg/g) than variations amongst the muscle samples. Variations in the Zn concentrations taken from the muscle samples were found to be relatively small (7.8-17.9 μg/g).

The highest Zn concentration found in the muscle samples of *L. polylepis* was 17.9 μg/g found in the Komati River population. The Ngodwana Dam population had the highest Zn concentration in muscle tissue (100.1 μg/g) with the lowest Zn concentrations being recorded for the Komati River population (4.1 μg/g).

3.4 Discussion

Bioaccumulation results of other bioaccumulation studies on indigenous South African fish species are presented in Table 7. From this table comparisons can be made with the metal concentrations found in *L. polylepis during* this project.

Aluminium is not considered an essential nutrient in organisms but it is one of the more toxic metals (Dallas and Day, 1993). In spite of free Al ions being scarce, in an aqueous solution, aluminium can form a diversity of complexes with water, fluoride, hydroxide, silicate and sulphate (Freeman and Everhart, 1971). The toxicity of Al is dependant on the chemicals involved, and it's solubility is very dependant on the pH. With a pH less that 6 (acidic), Al is present as a soluble, available and toxic hexahydrate (aqua) species. Aluminium is partially soluble and probably occurs as a polyhydroxo- and hydroxo-complexes with an intermediate pH. With a pH above 8 (alkaline), Al is present as soluble but biologically unavailable hydroxide complexes or as colloids and flocculants (Dallas and Day, 1993; DWAF, 1996). Although Al has been described as a non-critical metal, there is increased concern over the effects that elevated concentrations of Al may have on the aquatic environment. This is particular for areas where it has been mobilised as a result of acid precipitation and acid mine drainages (DWAF, 1996). The toxicity of Al is dependant on the biological species exposed, life stages of the organism, pH and temperature of the water as well as the calcium concentration in the water (Neville, 1985).

The highest Al concentrations were found in the liver samples with exception to the Phongola and Komati Rivers populations which showed the highest Al concentrations in the muscle tissues. The high Al concentration found in the liver samples from the

Assegaai River may suggest a higher presence of Al in this system when compared to the other systems. Further research would need to be conducted on the Assegaai River to verify these findings. The relatively high Al concentrations found in the muscle samples of the Phongola and Komati Rivers population would require further research to validate this finding and to possibly establish a reason for this. The comparisons made with two other fish species (Table 7) indicate that the Al concentrations found in this study were lower and at the most, similar to those concentrations found in *L. capensis* and *L. umbratus.* The lowest Al concentrations found in this study were found in the liver samples from the Komati and Phongola Rivers as well as from the muscle samples from the Ngodwana Dam. These concentrations were similar to concentrations found by Groenewald (2000).

Cadmium requires added attention due to it's potential hazards to aquatic biota (Mayer et al., 1991; Barber and Sharma, 1998) as well added potential hazards to human beings (Groten and Van Bladeron, 1994; Vanderpool and Reeves, 2001). Cadmium is the type of heavy metal which is biologically non-essential, persistent and non-biodegradable and it's compounds are known to have high toxic potentials (Panchanathan and Vattapparumbil, 2006)**.** According to Panchanathan and Vattapparumbil (2006) a gross biological impact resulting from continuous, low level exposure may be comparable to that of recurring exposures at much greater intensity. The uptake of Cd in fish has three primary routes, namely the gills, the skin and then also from food via the intestinal wall (Karlsson-Norrgran and Runn, 1985). The retention capacity of Cd by the fish is dependant on the metal assimilation and excretion capacities of the fish concerned (Rao and Patnaik, 1999). Cadmium is a common aquatic pollutant and is known to be very toxic to most organisms and holds true even at low concentrations in natural waters (Lovert et al., 1972)

The liver samples taken from the Elands and Assegaai Rivers showed the highest Cd concentrations, with the highest Cd concentration being found in the liver of *L. polylepis*, whilst the muscle accumulated the lowest Cd concentration. The Cd concentrations in all the tissues suggest no serious Cd exposures in the study areas, in spite of the significant difference in Cd concentrations between the Elands and Assegaai Rivers populations and the remaining populations. The Cd concentrations found in this study were relatively low when compared to *L. capensis* and *L. umbratus* (Groenewald, 2000).

Chromium is a relatively scarce metal and thus the occurrence of concentrations found in aquatic ecosystems is generally very low (0.001-0.002 mg/l – Moore & Ramamoorthy, 1984; DWAF, 1996). In spite of the naturally low concentration of Cr in the aquatic ecosystems, natural water can receive Cr from anthropogenic sources such as, effluent from industry, resulting from the production of corrosion inhibitors and pigment (Galvin, 1996), thus resulting in a pollutant to the aquatic ecosystem being harmful to aquatic ecosystems (Srivastava et al., 1979). Aspects such as species, body size and life stage of the organism, pH of the water and to a lesser extent, hardness, salinity and temperature all affect the degree of toxicity of Cr to the organism (Holdway, 1988; Wepener et al., 1992a). Fish are generally more resistant to Cr than other aquatic organisms, but they may be affected sublethally when exposed to concentrations ranging from 0.013 to 50 mg/l (Olson and Foster, 1956; Van der Putte, 1982), lethal concentrations range from 3.5-280 mg/l Cr (Moore and Ramamoorthy, 1984; Van der Putte et al., 1981a; 1981b). These variations in exposure concentrations can be attributed to different species response and a difference in water chemistry (Wepener et al., 1992a).

The highest Cr concentrations were found in the liver samples with exception to the Phongola River population which showed the highest Cr concentration in the muscle samples. The detected concentrations found in the fish tissues suggested no serious Cr contamination in the study areas. These concentrations are lower than the Cr concentrations found in muscle samples from other fish species from four other systems (Table 7). The concentrations found in the liver were also lower than the Cr concentrations found in the liver of fish species from other systems (Table 7).

Copper is one of the world’s most widely used metals (DWAF, 1996). Copper is essential for the formation of bone and thus appears as a micronutrient in animals. It also aids in maintenance of myelin within the nervous system, synthesis of haemoglobin, a component of key metalloenzymes and forms an important part of cytochrome oxidase and various other enzymes involved in redox reactions in the cells (Sorensen, 1991; Dallas and Day, 1993). In spite of Cu occurring naturally in most waters, it is regard as being potentially hazardous (USEPA, 1986). Anthropogenic sources such as industrial, mining and plating operations, the use of Cu salts to control aquatic vegetation or influxes of Cu containing fertilizers result in Cu reaching the natural waters (Felts and Heath, 1984; El-Domiaty, 1987). With a high pH (alkaline), Cu precipitates and is thus not toxic, whilst at a low pH (acidic) Cu is mobile, soluble and toxic. A reduction in water dissolved oxygen, hardness,

temperature, pH, chelating agents such as NTA and EDTA amino acids and suspended solids increases the toxicity of Cu (II) (EIFAC, 1978, Hellawell, 1986).

The liver accumulated the highest Cu concentrations, with exception to the Komati River population which showed the highest Cu concentrations in the muscle. The high Cu concentration found in the muscle samples of the Komati River population would require further research to validate this finding and to possibly establish a reason for this. The highest Cu concentration found in the liver samples was 37.7 µg/g and when this is compared to the Cu concentrations found in other fish species from other systems (Table 7) are lower with the exception of the concentrations found in L. marequensis by Seymore *et al*. (1995). The Cu concentrations found in the liver samples were very similar to those found by Seymore *et al.* (1995).

Iron is present in many types of soils, in particular clay soils, it may also be present in natural waters in varying quantities depending on the geology of the specific area and other chemical properties of the water body (Train, 1979). In addition to leaching and weathering of sulphide ores as well as igneous metamorphic and sedimentary rocks into the aquatic environment, Fe concentrations can also be elevated in the aquatic environment through anthropogenic sources such as industrial and mine drainage waste, sewage and burning of coal (Nussey, 1998). In the aquatic environments the form in which Fe is present is determined by the pH and redox potential (Environment Canada, 1987). Various forms of Fe can be found but the two forms of common concern in water, are the ferrous of bivalent (Fe (II)) and the ferric or trivalent (Fe (III)) states (DWAF, 1996). According to Dallas and Day (1993) Fe is an important nutrient in all organisms, in fish microcytic anaemia is a result of Fe deficiency and elevated Fe concentrations can be lethal.

The highest Fe concentrations were found in the liver samples with the exception of the Komati River and the Ngodwana Dam populations which showed higher Fe concentrations in the muscle tissues. The Fe concentrations found in the muscle for this study were higher than concentrations found by Groenewald (2000) in *L. capensis* and *L. umbratus.* This may give an indication of slightly higher Fe concentrations being available to *L. polylepis* populations in the Assegaai River. The Fe concentrations found in this study indicate that when compared to previous studies (Table 7) they are relatively low.

According to Dallas and Day (1993), Mn is an essential micronutrient, which does not occur naturally as a metal in aquatic ecosystems but does occur in various minerals and salts (<1.0 mg/l – Hellawell, 1986). Manganese may be available in the soluble manganous Mn (II) form but it can be effortlessly oxidized to the insoluble manganic (Mn (IV)) form (WHO, 1986; DWAF, 1996). Although as a pollutant Mn has little significance (Hellawell, 1986), it is one of the first metals to show increased concentrations levels in acidic waters (Bendell-Young and Harvey, 1986). Manganese can be moderately toxic to aquatic organisms (Kempster *et al.*, 1982). The toxicity of Mn can be affected by the pH of water (Wepener et al., 1992b). The haematology and carbohydrate metabolism of freshwater fish can be impacted by sublethal Mn concentrations (2584 mg/l – Nath and Kumar, 1987; 4.43 mg/l – Wepener et al., 1992b; 172 259 and 345 mg/l – Barnhoorn, 1996).

L. polylepis bioaccumulated the highest Mn concentrations in the liver tissue samples, with the exception to the Komati River population which showed the highest Mn concentrations in the muscle samples. The Mn concentrations found in the muscle samples from previous projects on three different fish species (Table 7) are all higher than the highest Mn concentration recorded in this study, which was found in the Elands River population. In addition to this, the highest Mn concentration found amongst the liver samples was also found to be in the Elands River population. In spite of this, the concentrations found in this population (4.9 µg/g) were lower than most of the concentrations found in the three previous projects (Table 7).

According to Birge and Black (1980), Ni constitutes approximately 0.008% of the earth's crust. Nickel is a natural ever-present element of the earth and earth's water (0.001-0.003 mg/l – Snodgrass, 1980). Nickel is discharged into the water and air through increased industrial activities such as mining, electroplating and steel plant operations (Galvin, 1996). Nickel ions form insoluble Ni hydroxides at a pH above 6.7 and otherwise tend to be soluble ions at a pH below 6.5 (Dallas and Day, 1993). Dissolved Ni concentrations in aquatic ecosystems are generally between 0.005 and 0.010 mg/l (Galvin, 1996). The toxicity of Ni to aquatic organisms is dependant on the organism species, pH, water hardness amongst others (Doudorff and Katz, 1953; McKee and Wolf, 1963; Pickering and Henderson, 1966; Birge and Black, 1980). According to Khangarot and Ray (1990) the toxicity of Ni is generally low, but sublethal effects of Ni are possible at increased concentrations. The range for sublethal Ni concentrations is 0.04-6.0 mg/l (Baylock and Frank, 1979; Dave and Xiu, 1991).

In this study, the liver tissue accumulated the highest Ni concentrations with exceptions to the Komati and Phongola River populations that showed the highest Ni concentrations in the muscle samples. In addition to this, the highest over Ni concentration was found in the muscle sample from the Phongola River. With reference to Table 7 it is noted that Seymore (1994) had a similar uptake pattern for *L. marequensis.* The Ni concentrations found in the muscle and liver tissue for this project are also lower than the Ni concentrations found in the muscle samples from three different fish species (Table 7).

Lead is available in several oxidation states (0, I, II and IV) of which all are environmentally important (Nussey, 1998). According to DWAF (1996), the divalent form, Pb (II), is the stable ionic species present in the environment and is thought to be the form in which most Pb is bioaccumulated by aquatic organisms. The physiological importance of Pb to living organisms is considered to be non-essential and is defined as being potentially hazardous to most forms of life by the USEPA (1986). According to DWAF (1996) Pb is relatively accessible to aquatic organisms and considered to be toxic. Lead is used in industry for the production of pesticides, paints, fuels and batteries, and as a result of erosion and leaching from the soil, Pb-dust fallout, municipal and industrial waste discharges, runoff of fallout deposits from streets and other surfaces as well as precipitation it enters the aquatic environment (Pagenkopf and Newman, 1974). Lead is known to accumulate in the organs and tissues of fish, which consists mainly of the bone, gills, kidneys, liver and scales. The uptake of aqueous Pb (II) across the gills is the primary mode of uptake in freshwater fish (Coetzee, 1996). Variables such as the life stage of fish, pH and hardness of the water as well as the presence of organic materials all influence the toxicity of Pb (Pickering and Henderson, 1966).

The highest Pb concentrations were found in the liver samples with exception to the Phongola River population which showed the highest Pb concentrations in the muscle tissues. The detected Pb concentrations found in the fish tissues suggests no serious Pb pollution problems in the study areas. The significantly higher Pb concentration found in the muscle samples of the Phongola River population would require further research to validate this finding and to possibly establish a reason for this. The comparisons made with the Pb concentrations found in the muscle samples of three different fish species (Table 7) indicates that the Pb concentrations found in the muscle of *L. polylepis* is low when compared to *L. marequensis* (Seymore, 1994).

Zinc forms the active sites in various metallo-enzymes, including DNA and RNA polymerases and is thus an important micronutrient for organisms (Dallas and Day, 1993; DWAF, 1996). In spite of Zn being a metallic element, it is relatively scarce in nature and it occurs in combination with many minerals (Moore and Ramamoorthy, 1984). According to Hellawell (1986) Zn is a common pollutant of surface waters in many industrial areas, since it is a constituent of industrial and mining effluent. Liquid effluent discharge, atmosphere deposition, the leaching of domestic sewage and metal bearing minerals can also cause elevated concentrations of Zn in the aquatic environment (Van Loon and Beamish, 1977; Weatherly et al., 1980). According to DWAF (1996) Zn occurs in two oxidation states in the aquatic ecosystems, namely Zn (II) and the metal (Zn), and in the aquatic environment the Zn (II) is toxic to aquatic organisms and fish at relatively low concentrations (0.02 mg/l – Sellers et al., 1975). The toxicity of Zn to fish is dependent on dissolved oxygen concentrations, hardness, pH and temperature of the water (Skidmore, 1964; Buthelezi et al., 2002)

The liver of *L. polylepis* accumulated the highest Zn concentrations, whilst the muscle accumulated the lowest. The Zn concentrations in all the tissues suggest no serious Zn exposure problem in the study areas, although the Zn levels detected in the liver samples from the Ngodwana Dam population might indicate chronic Zn exposure of the fish, causing possible sub-lethal effects. In comparison to work carried out on three different fish species (Table 7), the Zn concentrations found in the muscle samples of this project appear to be relatively low. A significant variation in the Zn concentrations found in the liver samples of *L. polylepis* was evident for this project. When compared to the Zn concentrations found in the liver of three different fish species (Table 7), the concentrations found in *L. polylepis* do not appear out of ordinary, with the concentrations for the Komati and Phongola River populations appearing relatively low.

Table 7: Historical data assessment of the levels of metals in fish found in South Africa (Nussey 1998, Groenewald 2000, Nussey et al., 2000, Robinson & Avenant-Oldewage 1997, Seymore 1994, Kotze et al.,1999, Wepener 1997). Concentrations are expressed in µg/g. BDL represents below detection limits.

Metal	Species	System	Reference	Muscle	Liver
Al	*Labeo umbratus*	Olifants River	Nussey, 1998	21.7-41	21.6-224.9
	Labeo capensis	Vaal River & Dam	Groenewald, 2000	13.1-672.8	33.4-451.9
Cd	*Labeo capensis*	Vaal River & Dam	Groenewald, 2000	BDL-4.7	0.1-4.8
Cr	*Labeo umbratus*	Witbank Dam	Nussey *et al*., 2000	12.4-60.3	10.1-66.2
	Oreochromis mossambicus	Olifants River	Robinson & Avenant-Oldewage, 1997	11.6-21.1	25.4-224.8
	Clarias gariepinus	Olifants River	Robinson & Avenant-Oldewage, 1997	10.3-69.6	16.1-67.1
	Labeobarbus marequensis	Olifants River	Seymore, 1994	7.6-39.1	11.6-33.3
Cu	*Oreochromis mossambicus*	Olifants River	Robinson & Avenant-Oldewage, 1997	1.6-8.1	69.1-305.3
	Clarias gariepinus	Olifants River	Robinson & Avenant-Oldewage, 1997	1.5-12.5	42.7-152.8
	Oreochromis mossambicus	Olifants River	Kotze *et al.,* 1999	1-4.2	48-466
	Labeobarbus marequensis	Olifants River	Seymore, 1994	4.4-8.7	11.1-16.7
Fe	*Labeobarbus marequensis*	Olifants River	Seymore, 1994	132.6-273.9	240.7-624.1
	Labeo umbratus	Vaal River & Dam	Groenewald, 2000	2-480.1	103.5-6615.3
Mn	*Labeo umbratus*	Witbank Dam	Nussey *et al.,* 2000	3.1-9.3	5.6-55.7
	Oreochromis mossambicus	Olifants River	Robinson & Avenant-Oldewage - 1997	2-16.8	5.6-35.3
	Labeobarbus marequensis	Olifants River	Seymore, 1994	4.5-10.4	4.8-14.3
Ni	*Labeo umbratus*	Witbank Dam	Nussey *et al.,* 2000	10-35.8	9.6-38.3
	Labeobarbus marequensis	Olifants River	Seymore, 1994	6.5-17.4	7.4-22.5
	Labeo rosae	Olifants River	Wepener, 1997	1.8-9.2	5.8-13.3
Pb	*Labeo umbratus*	Witbank Dam	Nussey *et. al.,* 2000	4.02-10	3.5-9.6
	Labeobarbus marequensis	Olifants River	Seymore, 1994	26.1-47.8	18.5-51.9
Pb	*Labeo capensis*	Vaal River & Dam	Groenewald, 2000	3.6-60.3	7.9-86.5
Zn	*Labeo capensis*	Vaal River & Dam	Groenewald, 2000	25.7-53.3	77.1-138.3
	Labeo umbratus	Vaal River & Dam	Groenewald, 2000	25.6-46.1	96.8-151.7
	Oreochromis mossambicus	Olifants River	Wepener, 1997	15-43.6	17.9-64.6

3.5 Conclusion and Recommendations

This section reported on the extent of the bioaccumulation of Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in two different tissues of *L. polylepis* from five localities within Mpumalanga, South Africa. In this study the bioaccumulation of metals in fish tissue were used as an indication of the extent of metal exposure and uptake in the five different *L. polylepis* populations. The highest concentrations for the selected metals were found in the liver samples for all the sampled populations with the exception of one population which showed the highest Ni concentration in the muscle. However, this was not consistent within all five populations as some populations showed higher bioaccumulation patterns for certain metals in the muscle samples. The metal concentrations found in this study were relatively low and at the most, very similar in concentration when compared to other studies completed on other indigenous South African fish species. It is suggested that further research be conducted on these systems in order to verify these findings. Monitoring programmes and further research would also need to be conducted on the other systems with an aim to expand the research by including other fish species, water and sediment as well as other tissues.

The accumulated metals (Al, Cd, Cr, Cu, Mn, Ni, Fe, Pb, Zn) found in the liver and muscle samples taken from the five different *L. polylepis* populations provided a good indication of the metal levels to which these fish were exposed. The extent of metal exposure is considerably lower when compared to the metal bioaccumulation in fish from metal contaminated systems such as the Vaal Barrage and the Olifants River, Mpumalanga.

The use of fish as biological indicators provides valuable information for effective water resource management. Management of the water resources is critical to ensure a healthy system as well as to secure a future for these resources. The management of these water resources will only be effective if the information gathering process is appropriate. Thus the correct information needs to be collected, processed analysed and presented in a way that allows the success or failure of a particular action or decision to be evaluated objectively (Heath, 2000). Through these monitoring programmes, current conditions can then be compared to these critical guideline values.

4 Section 3: Notes on the feeding biology of five selected populations of *Labeobarbus polylepis* in South Africa.

Gordon O'Brien[13] and Andrew Husted[14].

4.1 Introduction

Yellowfish are a generally cosmopolitan species and are distributed all over South Africa (Wolhuter and Impson, 2007). *L. polylepis* is a good indicator species as it occurs throughout the Mpumalanga area, in the Usutu Catchment (Assegaai River) the Phongola Catchment (Phongola River) and the Komati Catchment (Komati River, Elands River and the Ngodwana Dam). It features in the catch of both the subsistence and recreational fisheries. The conservation initiative associated with *L. polylepis* has not only an influential role on science, but also on the general public who are now able to associate environmental impacts with the Smallscale yellowish.

Of all of the yellowfishes that occur in South Africa very little, relating to the biology of the Bushveld Smallscale Yellowfish (*Labeobarbus polylepis*), is known. Apart from a recently completed, comprehensive assessment of the breeding biology of this species (Roux, 2007a) no specific assessments have been carried out to characterise any additional biological aspects of this species. The Bushveld Smallscale Yellowfish is considered to be a cool water species, as the distribution range of this species does not extend below an altitude of 600 m (Skelton, 2001). This species is known to select a range of habitats depending on the time of year, including deep pools and flowing waters of permanent rivers and this species readily establishes in dams although it is not clear if the species can successfully breed in still waters (Skelton, 2001; Roux, 2007b). Due to the limited distribution of this species, above an altitude of 600 m, many isolated populations of *L. polylepis* occur within many of the upper river reaches and tributaries of the Phongolo, Inkomati and Limpopo catchments (Scott et al., 2006). Currently this species is managed as one population and to date no research assessments have been undertaken to determine if any differences between the isolated populations exist. This study forms a part of a research programme that has been established to study selected biological aspects

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of five isolated populations of *L. polylepis* in Mpumalanga, South Africa. In this chapter the any potential differences in the feeding biology of these populations have been considered.

Although very little regarding the feeding biology of *L. polylepis* is known, there is a considerable amount of speculation surrounding this topic. According to Le Roux and Steyn (1968) *L. polylepis* is a bottom feeder that selectively feeds on algae and detritus covering the substrates and similar surfaces. Gaigher (1969) considered *L. polylepis* to be an opportunistic feeder that is capable of accepting any food types depending on the availability of the food type. In addition, Gaiger (1969) described *L. polylepis* in quiet, deep, still waters to feed predominantly on algae during the winter and spring months. During the high flow period throughout the summer and autumn months this species is considered to change it's dietary requirements to an insectivorous diet due to a reduction in the availability of algae. Gaigher (1969) further proposes that detritus, in the form of decomposing roots stems and leaves, is accidentally consumed in greater portions during the high flow season while the species targets aquatic macro-invertebrates. Skelton (2001) proposed that *L. polylepis* feeds primarily on algae and is an opportunistic aquatic macro-invertebrate predator.

What can be assured if that as member of the cyprinid family *L. polylepis* does not have a real stomach (Eccles, 1985). The *Labeobarbus* spp. has an alimentary canal which is made up of a pseudogaster, varying lengths of a mid gut and a simple hind gut (Eccles, 1985). The length of the gastro-intestinal tract within the *Labeobarbus* genus is variable and considered to be dependent on the feeding biology of the species. Some *Labeobarbus spp* such as *L. kimberleyensis* has a simple relatively short alimentary canal whilst other species such as *L. aeneus* has a relatively long, convoluted alimentary canal (Eccles, 1985). The relatively short length of the *L. kimberleyensis* alimentary canal is indicative of the carnivorous feeding biology of this species while the extended length of the alimentary canal of *L. aeneus* is indicative of the omnivorous feeding biology of this species (Eccles, 1985). Today *Labeobarbus polylepis* is considered to be an omnivore which feeds on filamentous algae and detritus during autumn and winter and on invertebrates during the rest of the year (Roux, 2007b). The mouth of this species is sub-terminal, with simple, generally un-fleshy lips although some authors have reported observing numerous rubber-lip forms, specifically in the Elands River, Mpumalanga (Gaiger, 1969; Skelton, 2001; Roux, 2007b).

The potential uniqueness of the Elands River population of *L. polylepis* has received a considerable amount of attention in recent years in that from as early as 1969 this population was considered to be only population of *L. polylepis* that exhibited a high frequency of a rare mouth formation termed the rubber lip formations (Gaiger, 1969). Due to the historical account of the potential morphological uniqueness of *L. polylepis* in the Elands River, this study was initiated in this area. The additional populations considered include the populations from the Ngodwana Dam, the Komati, Assegaai and Phongolo rivers. The habitat and food availability of the systems in which the populations occur is potentially different and should be considered. In addition, the Ngodwana Dam represents a population occurring within a still water (lentic) reservoir while to the remaining populations which were collected from lotic, river ecosystems.

The aim of this chapter is to characterise the feeding biology of the *L. polylepis* individuals obtained in this study to allow for an inter-population and intra-population comparisons. As such this chapter aims to present the general feeding biology of five *L. polylepis* populations within South Africa, thereby contributing towards the knowledge base on the biology of this species.

4.2 Materials and methods

4.2.1 Study area

The *L. polylepis* populations used in this study included individuals from the Elands River and Ngodwana Dam (Crocodile River Catchment), and the Komati, Assegaai and Phongolo Rivers (Figure 2).

4.2.2 Collection of specimens

Twenty *L. polylepis* individuals were collected from each sampling locality between May and July of 2006. The individuals were captured using array of sampling techniques including seine nets, cast nets, electro-shocking, gill nets (mesh size 45 mm-95 mm) and fly fishing techniques. Following the methodology prescribed by Coetzee (1996) the captured individuals were individually weighed and the total and fork length of each individual was measured. The individuals were then dissected on

a cleaned polythene work-surface, using cleaned stainless steel work instruments. The entire alimentary canal was removed according to the method adopted by Mandima (1999), and preserved in a 10% neutral buffered formalin solution prior to laboratory analysis.

4.2.3 Stomach content analysis

In the laboratories of the University of Johannesburg, the stomach contents were removed from each stomach and preserved in an 80% ethanol solution, in preparation for later identification. A dissection microscope was initially used to analyse the stomach contents, and where a higher magnification the contents were required for identification a high power Nikon inverted compound microscope was used. The food items were identified to the lowest taxonomic level possible. The stomach contents of the *L. polylepis* individuals were analysed using the approach prescribed by Lima-Junior and Goitein (2001). Following this method the total wet weight of the stomach contents were determined and then the frequency of occurrence of each food item, the Volumetric Analyses Index and the Food Item Importance Index were determined. The different methodologies adapted from Lima-Junior and Goitein (2001) and used in this study are presented below:

1. Frequency of occurrence:
    a. This assessment is based on the following formula:
       $F_i = 100n_i/n$
       Where:
       F_i: frequency of occurrence of the i food item in the sample;
       n_i: number of stomachs in which item i is found;
       n: total amount of stomachs with food in the sample.
2. Volumetric Analyses Index:
    a. Determine the stomach contents standard weight (SW) or the arithmetic mean of stomach contents weight of all specimens captured per community assessed. The SW of each community is used as a constant to analyse the differences between individuals within each community and the differences between populations.
    b. Following the establishment of the SW for each community, using an integer point scoring system, a score was assigned to each of the

identified stomach contents of each community in relation to the SW of each community.

c. The points ascribed to each food item are then transformed into an mean abundance for each food item using the following equation:

$M_i = \sum_i / n$

Where:

M_i: mean of the ascribed points for food item i;

$\sum_i$: sum of the ascribed points of for the food item i;

n: total number of stomachs with food in the sample.

d. In order to communicate the outcome of the Volumetric Analyses Index the mean (M_i) was transformed into a percentage as follows:

$V_i = 25. M_i$

Where:

: Volumetric Analyses Index if the i food item in the sample;

25: multiplication constant to obtain a percentage;

M_i: mean of the ascribed points for food item i.

3. Importance Index:

a. The relative importance of each food item per community was determined using the following formula:

$AI_i = F_i . V_i$

Where:

AI_i: Importance index if the food item in the sample;

F_i: Occurrence of frequency of the item;

V_i: Volumetric analyses Index of the item.

4.2.4 Statistical analysis

Finally, to delineate the possible spatial differences in distribution of *L. polylepis* populations based on diet through the stomach contents, multivariate statistical techniques were applied to the findings. Non metric multi-dimensional scaling (NMDS) based on Bray-Curtis similarity coefficients and group averaged sorting was performed on both the percentage contribution of taxa making up the stomach content at each site and the Volumetric Analyses Index (%) data using the PRIMER (Plymouth Routines in Marine Environmental Research) program v6.1, (Plymouth Marine Laboratory).

4.3 Results

Of the 100 stomachs examined in this study, none were empty. Table 8 presents the findings of the Occurrence of Frequency, mean ascribed points, Volumetric Analyses Index and Importance Index of food types consumed by the *L. polylepis* populations assessed from the five locations included in this study. Results revealed that a relatively high diversity of food types (minimum of five types) were obtained in the stomach contents of all populations of *L. polylepis*. The Frequency of Occurrence findings (Table 1) indicate that the food types which appear to have incidentally been consumed (F $\leq$15) were limited. This included the Philopotamids in the Phongolo River community where only one individual *L. polylepis* from this sample contained this food type in it's stomach and the Gomphids in the Elands River population. The mean of ascribed points (M) were consistently low in all populations showing that there was no clear single dominant food type that were targeted by *L. polylepis* within the study. The only food type with an M score that was consistently above a value of 1 was the Baetidis. The percentage volumetric analyses (V%) results have further been presented graphically in Figure 20. Findings indicate that food types consumed per population vary considerably and that there does not seem to be any clear relationship between the populations apart from the V% results of the Baetid content which were observed to be between 30% and 33% in the populations collected in the rivers and only 15% in the population collected from the Ngodwana Dam. All populations had a V% value of between 18% and 24% for detritus.

Finally when considering the Importance Index values of the food types which are presented in Table 1 and Figure 21, findings indicated that as a species *L. polylepis* may be selecting Baetids and detrital matter, while individual populations may be selecting selected additional food types. The results further indicate that of the population which had the highest preference for selected food types, the Phongolo River population seemed to select in the order of importance; Baetids, Gomphids and detritus while the other populations such as the Komati River population targeted fish. In addition to the Frequency of Occurrence findings which reveal that the occurrence of Philopotamids in the Phongolo River population and the Gomphids in the Elands River population may be incidental, the Importance Index findings indicate that the Corbiculids and the Lebellulids do not appear to be targeted by any population included in the study

Table 8: Findings of the Occurrence of Frequency, mean ascribed points, Volumetric Analyses Index and Importance Index of food types consumed by *L. polylepis* in the five locations assessed in this study.

	Food items	Baetidae	Chironomidae	Corbiculidae	Detritus	Gomphidae	Libellulidae	Philopotamidae	Fish	Unidentified
Phongolo	Frequency of Occurrence (F)	95	70	0	85	80	20	5	0	60
	Mean of ascribed points (M)	1.225	0.55	0	0.75	0.85	0.15	0.025	0	0.45
	Volumetric Analysis Index (V%)	30.625	13.75	0	18.75	21.25	3.75	0.625	0	11.25
	Importance Index (AI)	2909.4	962.5	0	1593.8	1700	75	3.1	0	675
Assegaai	Frequency of Occurrence (F)	90	50	0	100	40	0	50	0	50
	Mean of ascribed points (M)	1.275	0.375	0	0.95	0.35	0	0.675	0	0.4
	Volumetric Analysis Index (V%)	31.25	9.375	0	23.75	8.75	0	16.875	0	10
	Importance Index (AI)	2812.5	468.8	0	2375	350	0	843.8	0	500
Komati	Frequency of Occurrence (F)	85	50	0	85	0	0	45	50	50
	Mean of ascribed points (M)	1.325	0.35	0	0.75	0	0	0.325	0.925	0.35
	Volumetric Analysis Index (V%)	33.125	8.75	0	18.75	0	0	8.125	23.125	8.125
	Importance Index (AI)	2815.6	437.5	0	1593.8	0	0	365.6	1156.3	406.3
Elands	Frequency of Occurrence (F)	90	35	35	90	15	35	40	25	45
	Mean of ascribed points (M)	1.375	0.225	0.225	0.7	0.15	0.25	0.325	0.4	0.325
	Volumetric Analysis Index (V%)	34.375	5.625	5.625	17.5	3.75	6.25	8.125	10.625	8.125
	Importance Index (AI)	3093.8	196.9	196.9	1575	56.3	218.8	325	265.6	365.6
Ngodwana	Frequency of Occurrence (F)	55	60	0	95	0	0	60	30	50
	Mean of ascribed points (M)	0.6	0.7	0	0.925	0	0	0.675	0.825	0.325
	Volumetric Analysis Index (V%)	15	17.5	0	22.125	0	0	16.625	20.625	8.125
	Importance Index (AI)	825	1050	0	2101.9	0	0	997.5	618.8	406.3

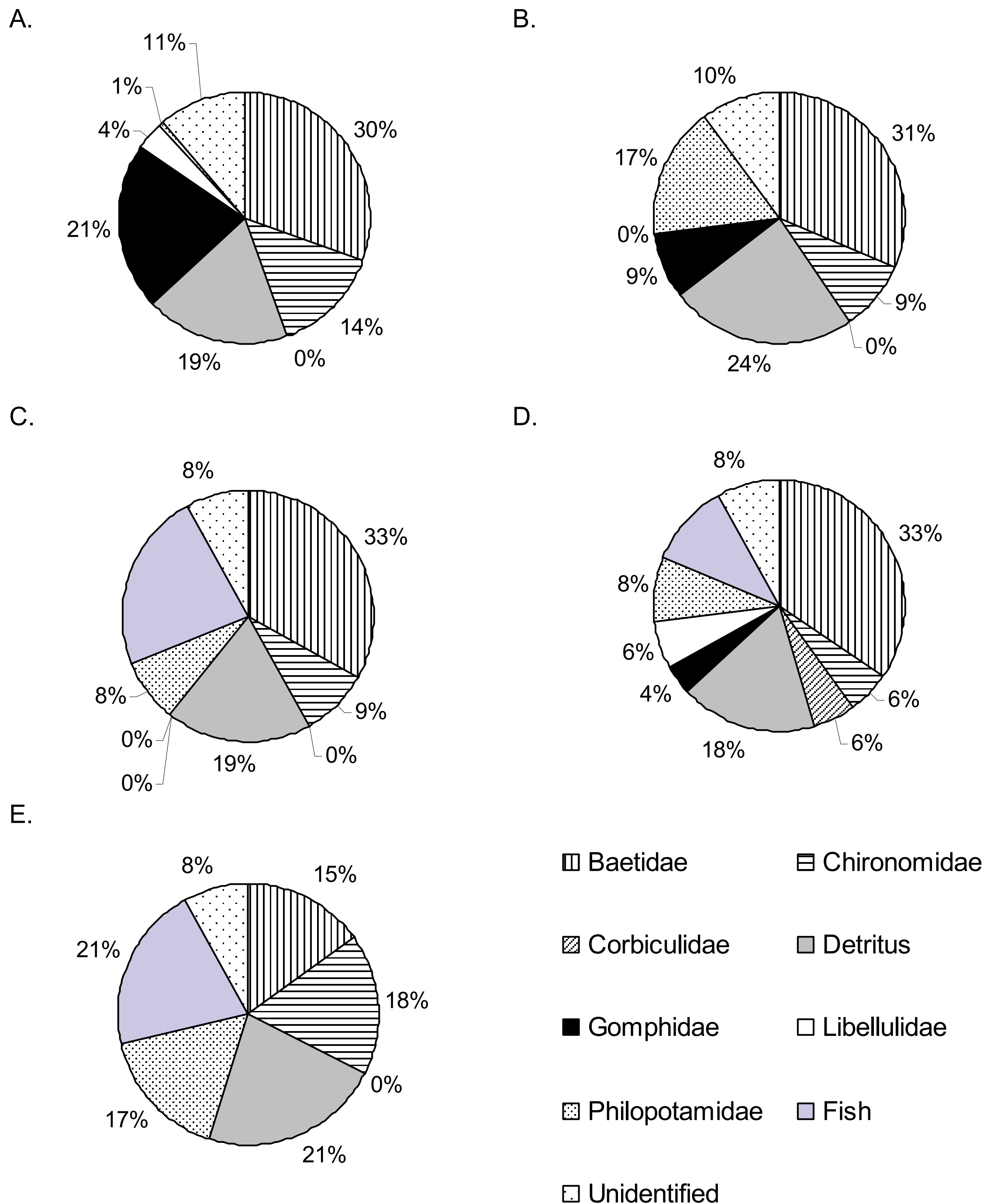

Figure 20: Graphical representations of the overall percentage of stomach contents of the *L. polylepis* populations sampled in the study (legend in the figure). Graphs represent the Elands (A), Komati (B), Phongolo (C) and Assegaai (D) rivers as well as the Ngodwana Dam (E).

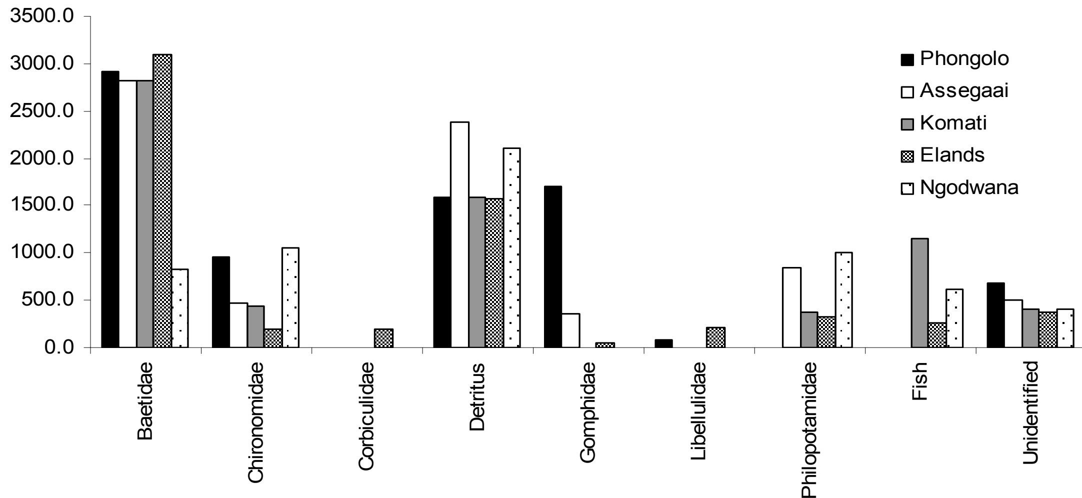

Figure 21: Graphical overview of the Importance Index results for each community of *L. polylepis* surveyed in this study.

Results of the multivariate statistical assessments (Figure 22 and Figure 23) reveal that using untransformed data three significantly different groups emerge which reduce to two groups if the data is square root transformed. Untransformed findings indicate that the Elands River and Komati River populations are distinctly different from the Assegaai River and Phongolo River individual. The findings reveal that the Ngodwana Dam community's feeding biology seems to be isolated when using untransformed data but is included with the Elands River and Komati River populations when a square root transformation is applied to the data.

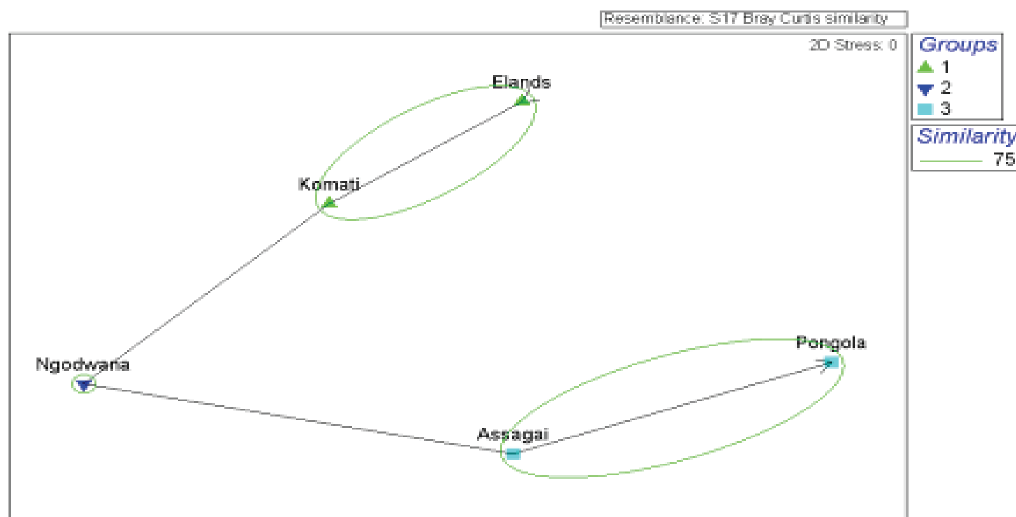

Figure 22: Bi-plots representing the NMDS ordination of the stomach content based on A. percentage contribution and B. Importance Index values (%) of the populations of *L. polylepis* assessed in this study. MDS of the raw data represented at a similarity cut off of 75%. Differences between group 1, 2 and 3 represented spatially in the graph.

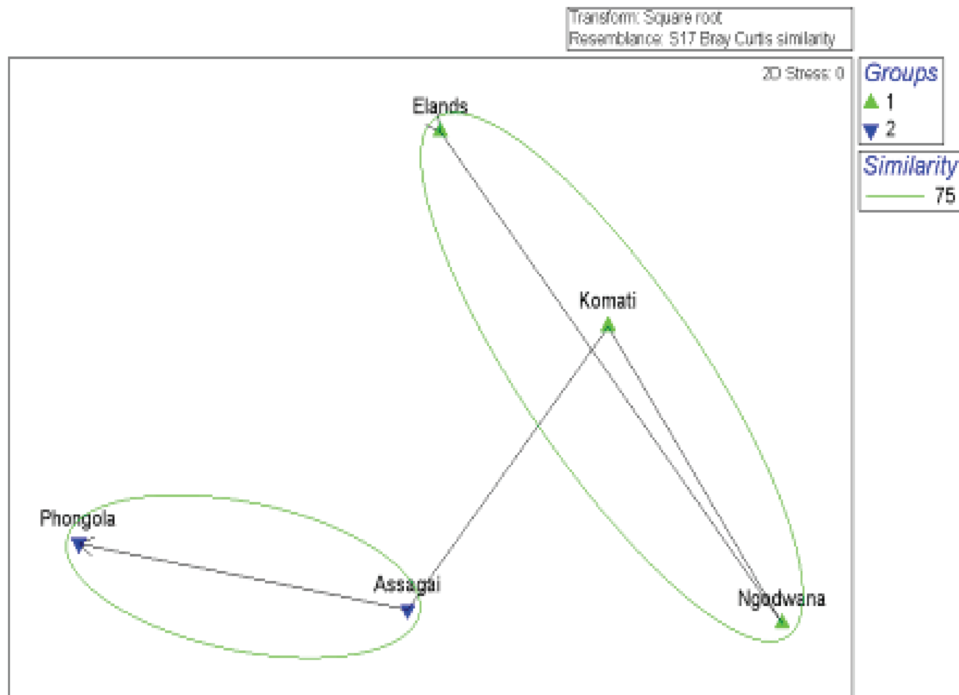

Figure 23: Multi-disciplinary Scaling (MDS) of the stomach content of the populations of L. polylepis assessed in this study. MDS of the square root transformed data represented at a similarity cut off of 75%. Differences between group 1 and 2 represented spatially in the graph.

4.4 Discussion

Outcomes of this study indicate that *L. polylepis* is an omnivore, feeding as an insectivore, piscivore and as a herbivore. With the ability to uncover small organisms in sandy substrate, *L. polylepis* can be classified as diggers of localized excavations (Sazima, 1986) and this type of feeding biology is specifically evident in the Phongolo and Assegaai rivers where numerous small excavations are evident in the softer finer sediments revealing the locations where L. *polylepis* individuals forage for embedded aquatic macro-invertebrates. This species undoubtedly makes use of their sensory barbels to locate prey mainly through touch which would be required during the warmer months when turbidity levels may potentially increase in the systems (Moyle and Cech Jr., 1982).

Additionally, as a successful predator of aquatic macro-invertebrates and fish, findings indicate that a possible reliance on vision to find prey exists especially during the cooler autumn/winter months when the clarity of the rivers in which these species

occurs improves. In addition, the upper reaches of river systems do not generally become as turbid as the lower reaches and this may be a factor for the selection of reaches of rivers above an altitude of 600 m by this species. This possible requirement by this species should be considered in the management of river ecosystems where these species occur in that as a result of anthropogenic activities these reaches may be become excessively turbid impacting on the potential of this species to feed successfully. Although the dominance of detritus within the gut of the *L. polylepis* populations suggests that this species targets this food type sufficient uncertainty exists suggesting that the intake of this food type may be accidental (Gaiger, 1969). This species appears to frequently forage in embedded substrates for aquatic macro-invertebrates, suggesting that the occurrence of the high percentages of detritus observed in the stomach contents may be elevated and that this species may primarily be more carnivorous than and herbivorous. Additional assessments of the gut length and or nutrient uptake potential of the gut of *L. polylepis* should be able to contribute to addressing this uncertainty. Detritus did however contribute towards a noticeable portion of the diet of all populations assessed and at this point in time, cannot be ignored and as such the possibility that this species is omnivorous remains.

This study was undertaken in the cooler autumn/winter months of 2006, a period when the *L. polylepis* populations are not expected to be breeding or conditioning themselves for breeding (Roux, 2007). Based on the available literature *L. polylepis* should switch feeding modes from a predominantly predatory mode to a herbivorous mode where individuals would rely on filamentous algae and detritus to maintain them through the winter months. Findings in this study however suggest that not only does *L. polylepis* continue dedicate a considerable amount of time to foraging for food but that this species actively targets aquatic macro-invertebrates throughout the cooler winter months. The findings of this study further suggest that this species is an opportunist predator during the cooler autumn/winter months and will predate on high protein food types by foraging, targeting Corbiculids and Odonates or by preying on other fish and invertebrates within the water column.

The variation in the size of prey items consumed by *L. polylepis* individuals observed in this study may be an indication of a shift in targeted prey items by larger individuals, which develop the ability to target relatively large prey items such as Barbs, large odonates, amphibians and even small mammals (fur was collected in

one individual). This is in line with the feeding biology in other *Labeobarbus spp.* (Mulder, 1973; Skelton, 2001; Wolhuter and Impson, 2007).

The multivariate statistical analysis of the stomach content data revealed specific groupings of populations based on the percentage food type contributions and resulted in the distinct groupings of fish from Elands and Komati Rivers (both part of the greater Inkomati River Catchment) and the Phongolo and Assegaai Rivers (part of the greater Phongolo/Usuthu River Catchment). The feeding biology of the Ngodwana Dam community of *L. polylepis* appears to be unique which is possibly attributed to the unique (amongst the populations included in this study) ecosystem in which this community occurs. When the data are analysed in the form of the Importance Index values, the Ngodwana population's stomach contents group with the other two populations in the greater Inkomati Catchment. These findings suggest that the feeding biology of the populations are driven by the unique invertebrate structure of the particular catchment rather than the particular habitat type, *viz*. *L. polylepis* feeding biology differing between lotic and lentic habitats.

During this study only a few individual *L. polylepis* individuals were observed to have the "rubber-lips" formation. Individuals with this mouth-form were collected in the Elands River as well as in the Phongolo River. Of the 100 individuals used in this assessment only three exhibited the "rubber-lips" formation while the remaining 97 individuals contained the simple non-fleshy, varicorhinus lip formation. Although considered to be absent from *L. polylepis* the "rubber-lips" form has been observed on occasion (Crass, 1964; Gaiger 1969; Skelton, 2001). Although there is speculation concerning the origin of the "rubber-lips" form within the larger *Labeobarbus spp.* Group, the individuals which contained this mouth form (Elands River and Phongolo River) contained Corbiculids or Gomphids which can only be obtained by aggressive, deep foraging within the sediment/substrate. This would suggest that there is a relationship between the mouth form and the ability of *L. polylepis* to feed on benthic invertebrates or that this mouth form develops as a result of the individual foraging in deep sediments/substrates. This possibility needs to be further explored as this relationship is only based on three individuals and as such cannot be considered to be a confident outcome.

4.5 Conclusion and Recommendations

Labeobarbus polylepis seems to be an opportunistic omnivore that preys predominantly on aquatic macro-invertebrates and detritus. This species is well adapted to forage in substrates to capture their prey as well in the water column and from the water surface. This ability makes *L. polylepis* a successful predator which can adapt to changing ecosystem types and take advantage of various ecosystem niches. This study suggests that different ecosystem types drive the feeding biology of this species of yellowfish and that they may somewhat be able to adapt to moderate changes in ecosystem structure and function. From a feeding biology perspective, as a single species it appears that L. polylepis has the potential to adapt to different ecosystem types that does not warrant conservation actions for individual populations.

Due to the unavailability of seasonal data in this study we recommend that additional feeding biology assessments of this species be carried out during the spring/summer periods. In addition some stomach morphological assessments should be undertaken which would address the uncertainty of the uptake of detritus matter by this species. Similar assessments should be undertaken to address and differences within and between other isolated populations of *L. polylepis* in South Africa.

5 General conclusion

Within South Africa it is of the utmost importance that the conservators and the managers of the biodiversity in the country are provided with the information and or technology needed to facilitate, prioritise and direct their efforts. These stakeholders of biodiversity rely heavily on the conservation status of species within the area that they are mandated to conserve and or manage. Without the scientific evidence initially required to characterise the biodiversity of these areas and then the information needed to facilitate this conservation and or management their efforts will often be misguided and possibly ineffective.

In this study, selected biological and ecological differences of five populations of the Bushveld smallscale yellowfish in Mpumalanga have been considered. Prior to this study no specific conservation or management actions have been put in place to conserve any of the at least eleven isolated populations of this species, presumably

due to the lack of any scientific proof that these isolated populations warranted any action.

The outcomes of this study reveal that not only are there genetically based differences between the populations that warrant conservation action, but that there are morphological differences that can successfully be used to separate at least two of the populations from the rest of the group. Furthermore this study has revealed that additional experimentation should be undertaken to address the potential genetic differences within this species in order to ascertain if the indication of a unique group of individuals obtained in this study warrants evolutionary significant unit status which would result in it being established as a new species of smallscaled yellowfish. Of the five populations considered in this study three groups of populations were determined to be sufficiently different from one another to warrant conservation significant unit status at this time. Very little concerning the other remaining isolated populations of this species throughout South Africa has been considered.

This study reveals that differences in the biology and ecology of these populations exist in that it presents the influences that different habitat availability within each of the systems has on the morphology and the feeding biology of the populations. In addition, this study illustrates that the unique geology of these systems results in unique metal composition of these systems that is accumulated into the individuals of these systems resulting in different chemical constituents within these populations.

Finally, following the outcomes of this study, the current approach to conserve the Bushveld smallscale yellowfish as one species is considered to be erroneous. The authors suggest that isolated populations that are determined to be unique should be awarded with an individual conservation status and conserved and or managed accordingly.

6 Recommendations

Initially, following the outcomes of this study, it is recommended that the approach adopted in this study should be expanded to consider the genetic, morphology, biology and general ecology of the remaining populations of *L. polylepis* in South Africa. This study has the potential to contribute towards the future conservation of ecologically important populations of this species that are currently not being

considered as unique ecologically important species and prevent the possible loss of this biodiversity within South Africa similar to the *L. polylepis* population that has become locally extinct in the Letaba River system. In addition, within South Africa should any additional isolated populations of *L. polylepis* that are endemic, near endemic, highly sensitive and/or that contain limited distributions be established, these populations can be used the establishment of future conservation and or management activities for the country.

In addition the following recommendations should be considered by ecosystem users, conservators, regulators and managers in accordance with the outcomes of this study:

- This study has shown that the isolated population of *L. polylepis* in the Elands River and associated Ngodwana Dam is unique and as such is of great ecological importance. The conservation status of this isolated population should be addressed with urgency as this population has historically been impacted on by chemical spillages and possibly by genetic contamination of *L. polylepis* individuals from the Komati River that have been released into this system.
- The outcomes of the genetic assessment component of this study resulted in the establishment of three separate population groups of *L. polylepis* that are of ecological importance (conservation units) and should be conserved as such, pending more in-depth analysis based on nuclear genes and wider sampling. More comprehensive geographic sampling of *L. polylepis* individuals from these systems and nuclear DNA markers to confirm the past and current gene flow between the separate rivers is required.
- Following the metal accumulation assessment, interesting outcomes in the assessment of the cadmium, copper, Iron, nickel, lead and zinc and manganese concentrations in the livers and muscles of the populations were obtained that requires further research to validate these findings and to possibly establish causes for the levels obtained in this study. It is suggested that further research be conducted on these systems in order to verify these findings. Monitoring programmes and further research would also need to be conducted on the other systems with an aim to expand the research by including other fish species, water and sediment as well as other tissues.

- Following the outcomes of the feeding biology assessment, additional assessments of the gut length and or nutrient uptake potential of the gut of *L. polylepis* should be undertaken to contribute to addressing the uncertainty obtained in this study concerning the feeding status of this species. In addition, due to the unavailability of seasonal data in this study we recommend that additional feeding biology assessments of this species be carried out during the spring/summer periods. In addition some stomach morphological assessments should be undertaken which would address the uncertainty of the uptake of detritus matter by this species. Similar assessments should be undertaken to address and differences within and between the feeding biology of other isolated populations of L. polylepis in South Africa.

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Appendix A: Table of the fin ray counts and the scale counts of the *L. polylepis* and *L. natalensis* populations used in this study.

Umvoti River (*Labeobarbus natalensis*)	Anal fin rays	5	5	5	5	6	6	5	6	5	6	6	5
	Caudal fin rays	19	17	18	21	18	19	18	18	18	17	17	18
	Caudal peduncle scales	13	16	15	15	16	16	15	15	15	16	15	16
	Dorsal fin rays	8	8	8	8	8	8	8	8	8	8	8	8
	Lateral line - dorsal fin (scales)	6	6	6	6	6	6	6	6	6	6	6	6
	Lateral line scales	34	35	37	37	36	36	35	38	38	35	37	38
	Pectoral fin rays	14	14	14	14	14	14	14	14	16	15	16	14
	Pelvic fin rays	7	8	7	8	8	8	8	8	8	8	8	8
	Predorsal scales	13	13	13	13	13	13	13	12	13	13	12	13
Phongolo River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	5	6	6	6	6	6	6	6	6
	Caudal fin rays	18	18	18	17	18	16	18	18	18	18	18	18
	Caudal peduncle scales	16	15	15	16	15	15	16	14	15	15	15	15
	Dorsal fin rays	9	9	10	9	8	8	9	9	8	8	8	8
	Lateral line - dorsal fin (scales)	6	7	6	7	7	6	6	7	6	7	7	7
	Lateral line scales	40	44	38	40	39	39	39	40	41	41	40	42
	Pectoral fin rays	17	16	15	14	14	12	14	15	14	12	14	14
	Pelvic fin rays	8	8	8	8	8	8	8	8	8	7	8	8
	Predorsal scales	13	13	12	11	13	12	15	15	13	15	14	14
Ngodwana Dam (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	6	6	6	6	6	6	6	6
	Caudal fin rays	18	18	18	18	18	19	18	18	17	18	17	18
	Caudal peduncle scales	15	16	16	15	16	16	16	17	16	15	16	16
	Dorsal fin rays	8	9	8	9	9	8	9	8	8	9	9	8
	Lateral line - dorsal fin (scales)	7	7	7	7	6	6	7	7	7	6	6	6
	Lateral line scales	40	39	38	40	41	41	42	40	42	42	39	39
	Pectoral fin rays	15	15	15	17	15	15	16	15	13	16	16	15
	Pelvic fin rays	8	9	8	8	8	8	8	8	8	8	8	8
	Predorsal scales	13	14	12	14	14	13	15	14	14	15	14	13
Elands River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	5	6	5	6	6	5	5	6
	Caudal fin rays	18	18	18	18	19	20	19	18	19	20	19	20
	Caudal peduncle scales	16	16	16	16	16	16	16	16	16	16	16	16
	Dorsal fin rays	9	9	9	10	8	10	8	9	9	9	7	9
	Lateral line - dorsal fin (scales)	6	7	7	6	6	6	7	6	7	6	6	6
	Lateral line scales	38	40	43	43	41	42	43	37	41	39	39	40
	Pectoral fin rays	14	15	14	13	15	14	14	14	15	14	15	14
	Pelvic fin rays	8	8	8	8	8	8	7	8	8	9	8	8
	Predorsal scales	13	13	13	14	13	15	14	14	13	12	14	12
Assegaai River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	5	6	6	6	5	5	5	5
	Caudal fin rays	18	18	18	18	18	18	18	18	18	18	18	18
	Caudal peduncle scales	16	16	17	17	16	16	16	16	17	16	12	16
	Dorsal fin rays	8	9	8	8	8	8	8	8	8	8	8	8
	Lateral line - dorsal fin (scales)	7	6	7	7	7	7	7	7	7	8	6	7
	Lateral line scales	42	43	42	40	39	41	42	40	39	41	37	42
	Pectoral fin rays	15	15	15	16	15	15	16	16	15	14	14	14
	Pelvic fin rays	8	8	8	8	8	8	8	8	8	8	8	8
	Predorsal scales	14	13	14	15	14	14	14	13	14	13	14	15
Komati River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	6	6	6	6	6	5	6	6
	Caudal fin rays	18	18	18	18	18	18	18	18	18	18	18	18
	Caudal peduncle scales	16	16	16	16	16	16	16	16	16	16	15	16
	Dorsal fin rays	8	8	8	8	8	8	8	8	8	8	8	8
	Lateral line - dorsal fin (scales)	7	7	7	7	7	6	6	6	6	7	7	7
	Lateral line scales	42	41	40	42	37	38	36	39	38	39	41	40
	Pectoral fin rays	14	15	14	14	15	15	15	15	15	14	15	14
	Pelvic fin rays	9	9	9	9	9	9	9	9	9	8	9	9
	Predorsal scales	13	12	14	14	14	14	12	12	12	16	13	15

Umvoti River (*Labeobarbus natalensis*)	Anal fin rays	6	5	6	6	7	6	6	6	6	6	6	6
	Caudal fin rays	17	17	18	22	21	21	19	20	20	19	19	18
	Caudal peduncle scales	15	16	16	14	12	14	16	12	14	15	12	15
	Dorsal fin rays	7	8	8	8	8	8	8	8	9	8	8	7
	Lateral line - dorsal fin (scales)	6	7	7	6	6	6	6	6	5	6	7	6
	Lateral line scales	34	37	35	36	37	34	37	37	39	37	37	35
	Pectoral fin rays	14	14	14	12	12	15	14	13	16	14	14	15
	Pelvic fin rays	8	8	7	7	8	8	8	8	8	8	8	8
	Predorsal scales	13	12	13	12	13	13	13	12	15	12	12	11
Phongolo River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	5	6	6	6	6	6	5	5	6
	Caudal fin rays	18	18	18	18	16	18	18	17	18	18	18	16
	Caudal peduncle scales	16	16	16	16	17	16	17	16	16	16	16	17
	Dorsal fin rays	8	8	10	8	8	9	9	9	8	8	8	10
	Lateral line - dorsal fin (scales)	6	7	7	7	7	7	7	6	7	6	7	7
	Lateral line scales	39	40	38	42	39	44	40	45	41	41	43	43
	Pectoral fin rays	15	14	16	14	16	16	16	16	15	14	16	15
	Pelvic fin rays	8	8	8	8	7	8	8	8	7	8	8	8
	Predorsal scales	13	14	13	15	15	14	13	14	14	16	14	15
Ngodwana Dam (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	6	5	6	6	5	5	6	5
	Caudal fin rays	18	18	18	18	18	18	18	19	18	17	19	18
	Caudal peduncle scales	16	16	15	15	15	16	16	15	16	16	15	16
	Dorsal fin rays	8	9	8	8	9	9	8	9	8	9	10	9
	Lateral line - dorsal fin (scales)	7	7	7	6	6	7	6	6	7	7	7	6
	Lateral line scales	41	41	38	39	42	40	44	40	43	44	41	40
	Pectoral fin rays	16	16	17	12	17	15	16	13	16	13	14	15
	Pelvic fin rays	8	8	8	8	8	8	8	8	8	8	9	9
	Predorsal scales	14	16	14	14	13	13	16	12	13	13	13	13
Elands River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	5	6	6	6	5	6	6	6
	Caudal fin rays	18	20	18	19	20	19	19	19	19	19	18	19
	Caudal peduncle scales	16	16	16	16	16	16	15	16	15	16	15	18
	Dorsal fin rays	9	9	8	10	8	9	8	10	8	9	8	10
	Lateral line - dorsal fin (scales)	6	7	7	7	6	7	7	6	6	6	6	6
	Lateral line scales	38	38	43	40	42	42	41	40	40	39	39	37
	Pectoral fin rays	13	13	16	13	14	14	13	16	13	15	14	12
	Pelvic fin rays	8	8	7	8	8	8	8	7	8	8	8	8
	Predorsal scales	13	13	14	14	14	15	13	14	13	15	14	13
Assegaai River (*Labeobarbus polylepis*)	Anal fin rays	5	6	6	6	5	5	6	6	5	5	5	6
	Caudal fin rays	18	18	18	18	18	18	18	18	18	19	18	18
	Caudal peduncle scales	16	16	16	16	16	16	16	16	16	16	16	16
	Dorsal fin rays	8	8	8	8	8	8	8	8	8	8	8	8
	Lateral line - dorsal fin (scales)	7	7	6	7	7	7	7	7	7	6	7	7
	Lateral line scales	41	41	41	42	43	41	42	41	40	41	45	40
	Pectoral fin rays	15	15	14	16	15	16	16	14	16	16	14	16
	Pelvic fin rays	8	8	8	8	7	8	8	8	9	8	7	8
	Predorsal scales	14	16	15	15	14	14	15	14	14	16	14	15
Komati River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	6	6	6	6	6	6	6	6	6
	Caudal fin rays	19	18	16	18	18	18	18	18	18	18	18	18
	Caudal peduncle scales	15	15	16	16	16	16	16	16	16	16	16	16
	Dorsal fin rays	9	8	8	8	8	8	8	8	8	8	8	9
	Lateral line - dorsal fin (scales)	7	7	7	7	7	7	6	7	7	7	7	7
	Lateral line scales	44	38	36	40	40	39	37	39	40	39	41	38
	Pectoral fin rays	14	14	14	14	14	14	15	15	16	15	14	15
	Pelvic fin rays	9	8	8	9	9	9	9	8	8	8	8	8
	Predorsal scales	14	13	12	14	13	13	13	13	13	14	12	13

Umvoti River (*Labeobarbus natalensis*)	Anal fin rays	-	-	-	-	-	-	-	-	-	-	-
	Caudal fin rays	-	-	-	-	-	-	-	-	-	-	-
	Caudal peduncle scales	-	-	-	-	-	-	-	-	-	-	-
	Dorsal fin rays	-	-	-	-	-	-	-	-	-	-	-
	Lateral line - dorsal fin (scales)	-	-	-	-	-	-	-	-	-	-	-
	Lateral line scales	-	-	-	-	-	-	-	-	-	-	-
	Pectoral fin rays	-	-	-	-	-	-	-	-	-	-	-
	Pelvic fin rays	-	-	-	-	-	-	-	-	-	-	-
	Predorsal scales	-	-	-	-	-	-	-	-	-	-	-
Phongolo River (*Labeobarbus polylepis*)	Anal fin rays	5	6	5	6	6	6	6	-	-	-	-
	Caudal fin rays	18	17	18	18	18	18	18	-	-	-	-
	Caudal peduncle scales	16	16	16	16	17	16	16	-	-	-	-
	Dorsal fin rays	8	8	8	9	8	8	8	-	-	-	-
	Lateral line - dorsal fin (scales)	7	7	7	6	7	8	7	-	-	-	-
	Lateral line scales	40	41	41	43	41	41	38	-	-	-	-
	Pectoral fin rays	16	15	14	14	15	16	14	-	-	-	-
	Pelvic fin rays	8	8	8	8	8	8	8	-	-	-	-
	Predorsal scales	14	14	15	14	14	14	12	-	-	-	-
Ngodwana Dam (*Labeobarbus polylepis*)	Anal fin rays	6	6	5	5	6	6	6	5	6	6	5
	Caudal fin rays	19	19	19	22	19	19	18	18	19	18	19
	Caudal peduncle scales	15	15	16	14	15	14	16	16	16	16	16
	Dorsal fin rays	10	9	8	8	8	8	9	8	8	9	8
	Lateral line - dorsal fin (scales)	8	8	6	7	6	6	6	7	6	6	7
	Lateral line scales	39	41	39	40	41	42	38	39	38	39	42
	Pectoral fin rays	13	14	14	14	14	14	13	18	14	13	13
	Pelvic fin rays	8	8	8	7	8	9	8	8	8	8	8
	Predorsal scales	12	12	15	14	13	14	14	13	12	14	13
Elands River (*Labeobarbus polylepis*)	Anal fin rays	5	6	6	6	5	6	5	5	6	5	-
	Caudal fin rays	19	19	19	19	20	19	20	20	19	20	-
	Caudal peduncle scales	15	18	16	15	16	16	16	15	13	16	-
	Dorsal fin rays	9	8	8	9	9	9	9	9	9	9	-
	Lateral line - dorsal fin (scales)	7	7	7	7	6	7	6	7	6	7	-
	Lateral line scales	40	44	38	39	38	38	40	41	39	43	-
	Pectoral fin rays	14	14	14	14	13	12	14	14	15	14	-
	Pelvic fin rays	7	8	9	8	8	8	9	8	8	8	-
	Predorsal scales	14	17	13	13	13	13	14	14	14	15	-
Assegaai River (*Labeobarbus polylepis*)	Anal fin rays	6	6	5	5	6	-	-	-	-	-	-
	Caudal fin rays	17	18	18	18	18	-	-	-	-	-	-
	Caudal peduncle scales	16	16	15	16	15	-	-	-	-	-	-
	Dorsal fin rays	8	8	8	7	8	-	-	-	-	-	-
	Lateral line - dorsal fin (scales)	7	7	7	8	7	-	-	-	-	-	-
	Lateral line scales	42	42	40	40	41	-	-	-	-	-	-
	Pectoral fin rays	16	15	15	14	16	-	-	-	-	-	-
	Pelvic fin rays	8	8	8	8	9	-	-	-	-	-	-
	Predorsal scales	13	14	14	15	16	-	-	-	-	-	-
Komati River (*Labeobarbus polylepis*)	Anal fin rays	6	6	6	-	-	-	-	-	-	-	-
	Caudal fin rays	18	18	18	-	-	-	-	-	-	-	-
	Caudal peduncle scales	16	15	16	-	-	-	-	-	-	-	-
	Dorsal fin rays	8	8	8	-	-	-	-	-	-	-	-
	Lateral line - dorsal fin (scales)	6	6	7	-	-	-	-	-	-	-	-
	Lateral line scales	39	36	38	-	-	-	-	-	-	-	-
	Pectoral fin rays	14	14	14	-	-	-	-	-	-	-	-
	Pelvic fin rays	8	9	8	-	-	-	-	-	-	-	-
	Predorsal scales	12	12	14	-	-	-	-	-	-	-	-

Appendix B: Table of the morphological measurements obtained from the *L. polylepis* individuals from the Elands River (ND=No data).

Measurements	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	E23	E24	E25	E26	E27	E28	E29	E30	E31	E32	E33	E34	E35	E36	E37	E38	E39
Fork length	25.76	39.25	30.60	25.20	32.50	26.60	26.50	22.40	26.30	24.40	24.00	26.40	27.10	23.70	24.80	25.40	25.80	25.30	20.00	25.70	21.50	25.40	25.80	25.10	29.60	29.00	21.00	25.90	24.90	22.30	25.20	26.30	24.10	28.40
Lateral line - dorsal fin	4.11	6.16	4.47	4.27	5.06	4.65	4.02	3.79	4.34	4.06	3.67	4.79	4.49	4.03	4.45	4.04	4.09	4.15	3.18	3.87	3.47	4.05	3.85	4.03	4.54	4.66	3.47	4.04	4.15	3.41	3.86	4.14	3.67	4.49
Lateral line - pelvic fin	1.91	3.00	2.35	2.48	3.06	2.59	2.33	2.24	2.62	2.34	2.41	2.40	2.61	2.27	2.64	2.30	2.41	2.26	1.63	2.04	2.13	2.31	2.09	2.09	2.42	2.26	1.69	1.86	2.13	1.62	2.02	2.02	1.69	2.06
Body width (before pectoral fins)	3.59	5.27	3.98	3.10	3.94	3.23	3.44	3.10	3.66	3.31	3.33	3.50	3.46	3.02	3.42	3.42	3.66	3.35	2.16	3.10	2.81	3.16	3.33	3.12	3.47	3.89	2.56	3.08	3.26	2.72	3.09	3.37	2.87	3.47
Eye diameter	0.93	1.13	1.03	0.93	1.03	0.96	0.95	0.83	0.90	0.88	0.87	0.92	0.96	0.81	0.94	0.94	0.87	0.89	0.85	0.90	0.83	1.03	0.80	0.89	1.05	1.09	0.89	0.92	0.91	0.83	0.92	0.92	0.90	0.20
Snout length	1.41	2.26	1.65	1.34	1.61	1.61	1.98	1.25	1.45	1.44	1.31	1.65	1.42	1.23	1.42	1.42	1.54	1.52	1.02	1.35	1.12	1.06	1.09	1.57	1.38	2.43	1.14	1.21	1.29	1.21	1.46	1.54	1.33	1.42
Orbital preoperculum groove	1.01	1.86	1.35	1.01	1.26	0.98	1.09	0.73	1.02	0.82	0.81	0.78	0.96	0.82	1.04	0.86	1.01	0.97	0.68	1.03	0.78	0.94	0.94	1.04	1.15	1.29	0.83	0.90	0.91	0.76	1.04	1.09	0.90	0.97
Gape width	1.86	2.70	2.32	2.00	2.21	1.66	1.87	1.83	2.10	1.91	1.67	1.78	2.02	1.70	1.83	1.74	1.83	1.91	1.48	1.83	1.59	2.04	1.77	2.06	2.10	2.22	1.58	1.75	1.85	1.53	1.81	1.76	1.78	2.01
Gape height	2.10	2.82	2.26	2.03	2.22	2.12	1.85	1.81	1.82	1.82	1.54	1.73	1.84	1.59	1.97	1.64	1.79	1.84	1.42	1.72	1.53	1.92	1.53	1.99	2.06	2.57	1.54	1.65	1.43	1.38	1.86	1.66	1.82	1.89
Upper jaw length	2.26	3.35	2.80	1.96	2.62	2.20	1.88	2.05	2.14	1.94	1.54	1.88	2.11	1.80	2.10	1.93	2.00	2.06	1.51	1.83	1.62	2.26	1.87	2.14	2.27	2.72	1.56	1.93	1.99	1.80	2.00	2.02	2.02	2.40
Lower jaw length	1.45	2.19	1.70	1.43	1.63	1.45	1.99	1.29	1.33	1.42	1.39	1.37	1.41	1.14	1.38	1.36	1.45	1.49	1.03	1.26	1.19	1.34	1.21	1.39	1.54	1.72	1.08	1.26	1.53	1.09	1.33	1.40	1.38	1.53
Anterior barbel length	1.05	1.56	0.99	1.03	1.42	1.06	1.20	0.88	1.01	1.13	0.87	0.87	1.11	0.98	1.01	0.76	0.99	1.04	0.77	0.92	0.82	1.05	0.91	0.95	1.24	1.16	1.86	0.77	0.90	0.88	0.95	1.02	0.91	1.09
Posterior barbel length	1.06	1.81	1.40	1.15	1.47	1.16	1.20	1.07	1.12	1.02	1.00	1.03	0.96	1.03	1.14	0.93	1.25	1.95	0.84	1.12	0.93	1.22	0.86	0.99	1.45	1.33	0.86	1.04	0.80	0.88	1.02	1.16	1.17	1.27
Premaxilla - supraoccipital	2.22	3.78	3.08	2.34	2.53	2.16	2.78	1.96	2.34	2.39	2.30	2.67	2.36	2.21	2.69	2.26	2.65	2.58	1.88	2.59	1.91	2.31	2.40	2.55	2.17	2.33	2.06	2.48	2.21	1.96	2.42	2.32	2.11	2.64
Origin of pectoral - premaxilla	5.78	8.75	6.60	5.22	6.49	5.53	5.36	4.91	5.85	4.96	4.82	5.50	5.39	4.59	5.49	4.95	5.30	5.53	3.98	4.87	4.46	5.04	5.02	5.38	5.99	6.54	4.22	5.05	5.19	4.61	5.25	5.61	4.99	5.87
Max dorsal spine length	4.16	6.72	5.08	4.13	5.43	4.37	4.47	3.76	4.38	4.19	4.24	4.76	4.72	4.26	4.72	3.95	4.09	4.52	3.31	4.01	3.95	4.20	4.13	4.00	4.94	4.35	3.68	4.51	4.07	3.82	4.23	4.77	4.00	4.46
Dorsal fin base length	2.89	4.49	3.39	2.82	3.63	2.93	2.68	2.40	2.92	2.75	2.54	2.76	2.84	2.67	2.90	2.90	2.89	2.76	2.13	2.95	2.28	2.97	2.87	2.62	3.23	3.05	2.34	2.85	2.68	2.28	2.72	2.82	2.69	3.20
Anal fin base length	1.65	2.84	2.55	1.67	2.54	1.84	1.73	1.38	1.82	1.63	1.48	1.60	1.91	1.51	1.77	1.95	1.53	1.62	1.21	1.81	1.34	1.62	1.60	1.64	2.09	1.68	1.19	1.63	1.66	1.42	1.63	1.70	1.40	1.96
Max anal spine length	3.89	6.17	5.43	3.73	4.24	3.76	4.27	3.16	3.53	3.33	3.37	4.46	4.15	3.52	3.72	3.20	3.57	3.92	2.79	3.58	3.06	3.65	3.41	3.57	3.84	3.69	2.95	3.57	3.33	3.09	3.73	3.79	3.51	3.59
Pectoral fin length	4.33	6.77	5.16	4.14	5.43	4.26	4.28	3.83	4.46	3.87	3.78	4.60	4.52	3.93	4.38	4.09	4.34	4.26	3.36	4.00	3.54	4.08	4.08	4.17	4.93	4.42	3.47	4.36	4.09	3.61	4.28	4.58	3.96	4.65
Prepectoral length	5.75	7.06	6.62	5.03	6.47	5.39	5.38	4.75	5.50	4.91	4.79	5.40	5.26	4.54	5.14	5.10	5.32	5.46	3.99	4.79	4.67	5.10	5.02	5.24	6.03	6.71	4.23	5.07	5.09	4.59	5.19	5.48	4.97	5.80
Prepelvic length	12.45	18.70	14.87	11.98	15.28	12.40	11.91	10.86	12.61	11.60	10.79	12.37	12.48	11.16	11.76	11.86	12.22	12.01	9.31	11.93	10.18	11.91	11.64	11.94	14.25	14.71	9.88	11.86	12.01	10.45	11.81	12.47	11.26	13.25
Preanal length	17.49	26.83	21.60	17.03	22.02	17.80	17.79	15.14	18.37	16.72	15.79	18.25	18.27	15.86	16.78	16.51	17.52	17.16	13.29	16.99	14.68	16.76	17.36	17.28	20.36	20.31	14.15	17.35	17.28	14.81	17.09	18.02	16.30	19.37
Anterior pelvic - origin of pectoral	8.11	10.80	8.48	7.33	8.90	7.41	6.79	6.14	7.43	6.68	6.42	7.14	7.16	6.64	6.78	6.61	7.11	6.62	5.41	7.10	5.90	6.87	6.64	6.73	7.97	7.74	5.67	6.77	6.89	5.83	6.79	6.96	6.27	7.42
Origin of pectoral - supraoccipital	4.47	6.96	5.02	4.15	5.43	4.58	4.30	4.01	4.76	3.92	3.78	4.39	4.57	3.77	4.26	4.06	4.09	4.31	3.20	4.09	3.55	4.22	4.23	4.25	5.12	5.03	3.55	4.16	4.27	3.78	4.17	4.11	4.03	4.57
Supraoccipital - origin of dorsal fin	9.89	14.69	11.24	8.77	11.98	9.52	9.72	7.77	9.45	8.73	8.56	9.44	9.56	8.63	8.24	9.25	10.08	8.20	7.08	9.19	8.09	9.31	9.03	9.28	10.77	11.89	7.71	9.43	9.38	8.29	9.88	9.87	9.02	10.28
Dorsal fin origin - pelvic origin	5.63	8.95	6.44	6.05	7.41	6.76	5.71	5.44	6.26	5.79	5.74	6.72	6.59	5.89	6.41	5.65	5.86	5.89	4.45	5.54	5.15	5.74	5.24	5.55	6.24	6.34	5.67	5.53	5.79	4.58	5.46	5.59	4.90	6.21
Dorsal fin origin - pectoral origin	8.21	12.20	9.27	7.75	9.88	7.95	8.03	6.60	8.05	7.25	6.95	7.72	8.04	5.43	7.89	7.62	8.22	7.79	5.93	7.68	6.75	8.08	7.56	7.43	6.58	8.85	6.27	7.59	8.00	6.50	7.94	8.27	7.07	8.43
Supraoccipital - pelvic origin	10.96	16.85	12.42	10.76	13.90	11.12	10.19	9.25	11.16	9.89	9.64	10.77	10.86	9.75	10.27	9.98	10.42	10.13	8.13	10.18	8.73	9.70	10.38	10.16	12.83	12.82	8.57	10.18	10.27	8.87	10.29	10.43	9.44	11.36
Anterior pelvic - post anal	6.77	11.24	8.87	6.35	8.78	7.72	7.74	6.27	7.68	7.11	6.42	7.73	7.99	6.70	7.09	6.85	7.07	7.17	5.64	7.04	6.40	7.16	7.78	6.98	8.41	7.66	6.05	7.41	7.12	5.95	7.08	7.17	6.69	8.25
Post anal - post dorsal	5.94	8.75	6.42	5.47	7.70	6.34	6.17	5.40	6.38	6.15	5.35	6.64	6.41	5.65	5.68	5.38	5.93	5.83	4.65	5.64	5.07	5.70	6.23	6.06	6.11	6.06	5.02	6.04	5.98	5.35	5.71	5.75	5.35	6.52
Pelvic fin base length	1.02	1.53	1.14	0.93	1.25	0.93	0.89	0.89	1.02	0.87	0.83	0.94	0.45	0.91	0.96	0.84	0.94	0.84	0.68	0.96	0.71	0.90	0.87	1.03	1.02	0.96	0.76	0.84	0.77	0.75	0.76	0.94	0.77	1.08
Post pelvic - post dorsal	5.36	8.26	6.20	6.08	7.03	6.19	5.68	5.07	6.07	5.69	5.40	6.15	6.22	5.50	5.95	5.45	5.90	5.54	4.30	5.22	4.90	5.59	5.28	5.31	5.77	5.60	4.51	5.07	5.35	4.18	5.14	5.39	4.66	5.90
Body width (before dorsal fin)	3.40	5.43	4.14	3.78	4.71	4.03	3.75	3.31	3.90	3.46	3.84	4.01	3.92	3.36	3.77	3.56	3.62	3.59	2.67	3.43	3.08	3.42	3.44	3.57	4.07	3.67	2.83	3.45	3.69	2.75	3.49	3.37	2.95	3.69
Caudal peduncle length	5.05	7.66	5.29	4.67	6.01	5.35	5.36	4.39	4.63	4.50	4.67	4.89	5.09	4.73	4.77	4.94	5.21	4.63	3.71	5.07	4.41	4.79	5.12	4.63	6.07	5.44	4.28	5.13	4.71	4.20	4.67	5.26	4.66	5.61
Caudal peduncle dept	2.33	3.73	2.51	2.22	2.79	2.36	2.39	1.83	2.34	2.19	2.09	2.59	2.14	2.10	2.23	2.12	2.20	2.12	1.57	2.09	1.90	2.04	2.21	2.00	2.41	2.44	1.68	1.90	2.17	1.79	2.05	2.28	1.87	2.25
Caudal peducle width	1.00	1.95	1.28	0.85	1.34	1.19	1.46	0.91	1.28	1.17	1.14	1.09	1.10	1.04	1.26	1.01	1.08	0.98	0.78	1.02	1.03	1.02	1.12	0.95	1.28	1.18	0.88	1.14	1.30	0.94	1.19	1.08	0.88	1.31
Caudal base length	2.72	4.23	3.01	2.65	3.21	2.71	2.83	2.25	2.72	2.38	2.38	2.78	2.71	2.35	2.62	2.62	2.59	2.60	1.94	2.62	2.08	2.53	2.43	2.32	2.78	2.95	1.95	2.46	2.51	2.18	2.47	2.63	2.23	2.82
Upper ray length	5.66	8.11	6.87	5.27	6.69	5.64	5.96	4.73	5.84	5.46	5.14	5.92	5.97	5.31	6.21	5.76	5.77	5.83	4.75	5.67	4.77	5.59	5.41	5.85	6.62	6.29	4.78	5.65	5.58	5.14	5.60	6.22	5.42	6.01
Lower ray length	5.31	8.20	6.92	5.37	6.63	5.31	5.23	4.98	5.92	5.52	5.25	5.92	5.75	5.59	5.93	5.67	5.49	5.74	4.69	5.39	4.49	5.39	5.46	5.48	6.65	5.71	4.54	5.47	5.21	ND	5.46	5.97	5.26	5.98
Caudal fin max height	9.03	13.46	11.08	8.85	10.95	9.70	8.87	7.89	9.48	8.79	ND	7.21	9.26	8.84	9.70	9.11	6.93	7.44	7.51	9.29	6.64	8.18	7.20	9.05	9.69	9.39	6.90	7.60	7.71	ND	7.73	8.54	7.38	8.15
Post dorsal - caudal peduncle	5.44	10.57	7.50	6.34	8.89	7.52	7.47	6.39	5.43	7.29	6.27	8.10	7.77	6.75	6.14	6.94	7.76	7.66	5.48	6.72	6.29	7.13	7.95	6.99	7.59	7.55	6.23	7.48	7.17	6.66	6.99	7.30	6.86	7.96
Post anal - caudal peduncle	2.80	3.96	2.56	2.26	3.53	2.83	2.93	2.35	2.42	2.48	1.96	2.51	2.59	2.65	2.45	2.96	2.55	2.73	1.98	2.76	2.43	2.56	2.84	2.67	2.83	2.51	2.38	2.62	1.99	1.99	2.60	2.61	2.53	2.81
Head length	5.39	8.01	6.12	5.00	6.42	5.33	5.34	4.62	5.31	4.48	4.68	5.21	5.14	4.59	5.00	4.99	5.09	5.34	4.07	4.85	4.40	5.12	4.85	5.12	5.84	6.42	4.14	4.96	4.89	4.49	4.99	5.34	4.97	5.58
Eye diameter	0.93	1.13	1.03	0.93	1.03	0.96	0.95	0.83	0.90	0.88	0.87	0.92	0.96	0.81	0.94	0.94	0.87	0.89	0.85	0.90	0.83	1.03	0.80	0.89	1.05	1.09	0.89	0.92	0.91	0.83	0.92	0.92	0.90	0.20
Snout length	1.41	2.26	1.65	1.34	1.61	1.61	1.98	1.25	1.45	1.44	1.31	1.65	1.42	1.23	1.42	1.42	1.54	1.52	1.02	1.35	1.12	1.06	1.09	1.57	1.38	2.43	1.14	1.21	1.29	1.21	1.46	1.54	1.33	1.42
Orbital preoperculum groove	1.01	1.86	1.35	1.01	1.26	0.98	1.09	0.73	1.02	0.82	0.81	0.78	0.96	0.82	1.04	0.86	1.01	0.97	0.68	1.03	0.78	0.94	0.94	1.04	1.15	1.29	0.83	0.90	0.91	0.76	1.04	1.09	0.90	0.97
Gape width	1.86	2.70	2.32	2.00	2.21	1.66	1.87	1.83	2.10	1.91	1.67	1.78	2.02	1.70	1.83	1.74	1.83	1.91	1.48	1.83	1.59	2.04	1.77	2.06	2.10	2.22	1.58	1.75	1.85	1.53	1.81	1.76	1.78	2.01
Gape height	2.10	2.82	2.26	2.03	2.22	2.12	1.85	1.81	1.82	1.82	1.54	1.73	1.84	1.59	1.97	1.64	1.79	1.84	1.42	1.72	1.53	1.92	1.53	1.99	2.06	2.57	1.54	1.65	1.43	1.38	1.86	1.66	1.82	1.89
Upper jaw length	2.26	3.35	2.80	1.96	2.62	2.20	1.88	2.05	2.14	1.94	1.54	1.88	2.11	1.80	2.10	1.93	2.00	2.06	1.51	1.83	1.62	2.26	1.87	2.14	2.27	2.72	1.56	1.93	1.99	1.80	2.00	2.02	2.02	2.40
Lower jaw length	1.45	2.19	1.70	1.43	1.63	1.45	1.99	1.29	1.33	1.42	1.39	1.37	1.41	1.14	1.38	1.36	1.45	1.49	1.03	1.26	1.19	1.34	1.21	1.39	1.54	1.72	1.08	1.26	1.53	1.09	1.33	1.40	1.38	1.53
Anterior barbel length	1.05	1.56	0.99	1.03	1.42	1.06	1.20	0.88	1.01	1.13	0.87	0.87	1.11	0.98	1.01	0.76	0.99	1.04	0.77	0.92	0.82	1.05	0.91	0.95	1.24	1.16	1.86	0.77	0.90	0.88	0.95	1.02	0.91	1.09
Posterior barbel length	1.06	1.81	1.40	1.15	1.47	1.16	1.20	1.07	1.12	1.02	1.00	1.03	0.96	1.03	1.14	0.93	1.25	1.95	0.84	1.12	0.93	1.22	0.86	0.99	1.45	1.33	0.86	1.04	0.80	0.88	1.02	1.16	1.17	1.27
Premaxilla - supraoccipital	2.22	3.78	3.08	2.34	2.53	2.16	2.78	1.96	2.34	2.39	2.30	2.67	2.36	2.21	2.69	2.26	2.65	2.58	1.88	2.59	1.91	2.31	2.40	2.55	2.17	2.33	2.06	2.48	2.21	1.96	2.42	2.32	2.11	2.64

Appendix C (continued): Table of the morphological measurements from the *L. polylepis* individuals from the Ngodwana Dam (ND=No data).

Measurements	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	N18	N19	N20	N21	N22	N23	N24	N25	N26	N27	N28	N29	N30	N31	N32	N33	N34	N35
Fork length	34.92	29.38	29.43	31.45	27.53	30.61	31.38	29.60	29.12	25.36	29.59	27.34	33.65	42.80	41.73	15.52	42.37	38.87	41.84	30.72	30.67	30.09	27.25	38.53	26.94	28.78	22.00	20.70	20.40	20.60	20.60	20.40	19.60	19.30	30.27
Lateral line - dorsal fin	5.77	4.51	4.91	5.12	4.56	4.74	5.26	4.69	4.69	4.15	4.66	4.32	5.23	6.27	6.39	2.49	6.29	5.99	6.37	4.38	4.73	4.47	4.21	6.15	4.35	4.47	3.41	3.35	3.24	3.29	3.08	3.10	3.00	3.07	4.37
Lateral line - pelvic fin	2.66	2.09	2.46	2.77	2.34	2.19	2.51	2.34	2.20	1.80	2.38	2.03	2.61	3.19	3.87	1.09	3.06	3.28	3.41	2.38	2.34	1.93	2.22	2.73	2.22	2.18	1.42	1.51	1.45	1.61	1.46	1.55	1.56	1.35	1.98
Body width (before pectoral fins)	4.57	3.54	3.72	4.21	3.41	3.73	4.38	3.74	4.09	3.01	4.08	3.59	4.82	5.65	6.03	1.49	5.62	5.04	5.66	3.39	3.77	3.08	3.10	5.07	3.29	3.43	2.33	2.60	2.53	2.82	2.37	2.28	2.43	2.47	3.31
Eye diameter	1.23	1.07	1.04	0.99	1.01	1.06	1.09	1.13	1.08	0.93	0.95	1.00	1.10	1.43	1.30	0.65	1.25	1.12	1.20	1.06	0.96	0.94	0.84	1.28	0.97	0.99	0.93	0.96	0.93	0.88	0.84	0.87	0.79	0.85	0.99
Snout length	2.21	1.58	1.27	1.76	1.23	1.97	1.96	1.47	1.37	1.21	1.63	1.63	2.11	2.76	2.48	0.75	2.64	2.36	2.83	2.03	1.56	2.08	1.76	2.54	1.89	1.96	0.92	1.35	1.28	1.11	1.18	1.14	1.04	1.00	2.12
Orbital preoperculum groove	1.56	1.06	1.30	1.29	1.19	1.43	1.37	1.41	1.25	1.07	1.41	1.26	1.39	1.97	2.16	0.61	1.98	2.16	2.04	1.27	1.33	1.17	1.11	1.89	1.38	1.25	0.79	0.81	0.85	0.78	0.74	0.79	0.76	0.78	1.33
Gape width	2.85	2.15	2.02	2.34	2.01	2.29	2.28	2.00	2.36	1.75	2.20	2.02	2.17	3.31	3.28	1.22	3.42	3.15	3.31	1.93	2.43	2.00	1.66	3.14	0.84	2.05	1.78	1.54	1.46	1.66	1.62	1.58	1.67	1.55	1.83
Gape height	2.90	2.08	2.14	2.47	2.10	2.11	2.45	2.35	2.14	1.97	2.40	2.05	2.36	3.48	3.16	1.34	3.44	3.31	3.12	1.85	2.22	2.03	1.70	3.14	1.82	1.97	1.82	1.50	1.50	1.48	1.52	1.59	1.61	1.46	1.80
Upper jaw length	2.92	2.14	2.10	2.59	2.27	2.43	2.77	2.51	2.45	1.99	2.48	2.17	2.98	3.47	3.55	1.21	3.89	3.28	3.81	2.19	2.53	2.34	1.97	3.41	2.11	2.38	1.82	1.55	1.54	1.59	1.71	1.75	1.79	1.71	2.35
Lower jaw length	2.16	1.40	1.54	1.93	1.38	1.85	2.01	1.59	1.58	1.32	1.67	1.54	2.10	2.70	2.39	0.80	2.50	2.25	2.61	1.60	1.78	1.68	1.42	2.46	1.38	1.72	1.33	1.04	1.00	0.96	1.08	1.09	1.12	1.02	1.76
Anterior barbel length	1.42	1.17	1.26	1.47	1.21	1.41	1.09	1.19	1.33	0.88	1.16	1.03	1.39	1.26	1.54	0.53	1.29	1.64	1.74	1.08	1.01	0.96	ND	1.31	0.87	0.85	1.08	0.84	0.63	0.73	1.03	0.91	0.75	1.08	0.95
Posterior barbel length	1.50	1.36	1.32	1.46	1.40	1.69	1.36	1.38	1.42	0.99	1.67	1.06	1.71	1.83	1.42	0.73	1.31	1.89	1.96	1.22	1.48	1.31	0.92	1.57	1.25	1.17	1.08	0.97	0.94	1.05	0.91	1.07	0.89	1.01	1.03
Premaxilla - supraoccipital	3.78	2.74	2.77	3.44	2.84	2.59	3.22	3.07	2.94	2.47	3.07	2.83	2.77	4.46	4.41	1.47	4.50	4.44	4.60	3.23	3.25	2.83	2.44	4.30	2.98	2.65	2.37	1.73	1.89	2.22	1.75	2.08	1.88	2.03	2.93
Origin of pectoral - premaxilla	5.50	5.91	6.01	6.55	5.28	6.18	6.64	6.13	6.21	5.15	6.15	5.76	6.77	9.33	8.85	3.22	8.71	8.39	8.70	6.66	5.98	5.97	5.55	8.05	4.85	6.04	4.64	4.39	3.49	4.39	4.46	4.33	4.39	4.41	6.12
Max dorsal spine length	5.34	5.16	5.21	5.19	4.86	5.28	5.70	5.13	4.83	4.82	5.30	4.76	6.02	6.77	6.40	2.87	6.62	5.97	7.42	5.12	4.68	4.84	4.14	6.32	4.67	4.56	3.70	3.55	3.50	3.50	3.49	3.42	3.47	3.30	4.77
Dorsal fin base length	4.09	3.06	3.32	3.76	3.07	3.36	3.81	3.28	3.55	2.82	3.48	3.37	3.76	5.10	4.79	1.66	5.06	4.89	4.86	3.71	3.58	3.34	3.30	4.36	3.50	3.29	2.23	2.17	2.39	2.27	2.12	2.20	2.29	2.25	3.52
Anal fin base length	2.28	1.87	1.93	2.22	1.86	2.04	2.47	2.10	2.06	1.70	2.14	1.99	2.12	3.45	2.75	1.01	3.28	3.00	3.35	2.14	2.13	2.01	2.03	2.92	1.80	2.02	1.32	1.27	1.27	1.25	1.33	1.27	1.32	1.24	2.22
Max anal spine length	5.44	4.10	4.34	5.20	4.42	5.00	4.91	4.61	4.24	3.87	4.18	3.96	5.62	7.98	5.98	2.20	7.35	6.26	7.05	4.40	4.28	3.90	3.83	6.65	3.94	4.09	2.97	2.83	3.06	2.78	2.90	2.79	2.75	2.62	4.28
Pectoral fin length	4.94	5.17	5.01	5.38	4.81	5.30	5.28	5.33	5.28	4.49	5.22	5.05	5.73	7.18	7.45	2.55	6.97	6.83	7.57	5.39	5.24	4.91	5.07	6.67	5.02	4.85	3.48	3.23	3.44	3.61	3.29	3.44	3.46	3.34	5.25
Prepectoral length	7.41	5.77	5.81	6.41	5.67	6.34	6.68	6.18	6.12	5.14	6.25	5.91	6.81	9.24	8.96	3.16	8.84	8.63	8.95	6.79	6.21	5.97	5.58	8.14	5.80	6.14	4.77	4.29	4.27	4.31	4.25	4.48	4.37	4.35	6.22
Prepelvic length	17.27	13.43	13.36	15.01	12.88	14.57	14.65	13.86	13.87	11.75	13.81	12.85	15.50	20.09	19.55	6.91	20.77	18.72	19.19	14.24	14.49	13.46	12.24	18.29	12.45	13.37	10.44	9.82	9.88	9.49	9.59	9.96	9.91	9.08	14.11
Preanal length	24.70	20.26	19.87	22.18	18.66	21.21	21.50	19.81	19.72	17.11	19.73	18.25	22.90	31.07	28.94	10.49	30.41	26.57	29.45	20.57	21.05	19.87	18.38	26.79	17.98	19.78	14.70	14.14	13.70	13.84	13.69	14.10	12.97	13.03	20.56
Anterior pelvic - origin of pectoral	10.09	8.16	7.76	8.66	7.47	8.46	8.60	7.95	8.18	7.03	7.85	7.17	8.90	11.68	11.30	6.11	12.46	10.78	11.01	6.33	8.11	7.60	6.82	10.21	6.84	7.54	5.57	5.50	5.31	5.13	5.42	5.45	4.97	5.02	8.02
Origin of pectoral - supraoccipital	5.67	4.78	4.89	5.21	4.36	5.28	5.26	4.71	4.82	4.14	4.75	4.61	5.60	7.25	6.86	2.52	7.04	6.65	7.01	5.18	4.96	4.59	4.39	6.46	4.55	4.92	3.73	3.51	3.23	3.28	3.46	3.39	3.45	3.27	4.65
Supraoccipital - origin of dorsal fin	12.58	10.61	10.34	11.22	10.37	11.72	11.65	10.60	10.32	9.13	10.57	9.86	12.56	16.12	15.52	5.91	15.37	14.48	15.51	11.45	11.48	11.07	9.76	13.33	10.77	11.08	8.19	7.70	7.62	7.45	5.42	7.44	7.18	7.04	10.47
Dorsal fin origin - pelvic origin	7.90	6.33	6.82	7.11	6.36	6.59	7.42	6.50	6.69	5.62	6.69	6.12	7.51	9.00	9.97	3.71	8.70	8.40	9.18	6.30	6.69	6.01	5.84	8.06	6.21	6.22	4.50	4.48	4.39	4.52	4.37	4.28	4.23	4.14	5.95
Dorsal fin origin - pectoral origin	11.08	8.14	9.16	11.62	8.79	9.28	9.97	9.11	8.86	7.78	8.89	7.97	10.34	13.18	13.61	4.88	12.39	12.05	12.97	9.84	9.73	9.06	8.39	11.38	8.96	8.65	6.29	6.35	6.02	6.21	5.83	5.89	5.57	5.59	9.15
Supraoccipital - pelvic origin	14.73	11.96	11.69	12.89	11.09	13.08	12.64	11.93	11.99	10.29	11.77	11.10	13.73	17.28	16.91	6.05	17.83	16.23	16.91	12.25	12.44	11.57	10.30	15.39	10.79	11.97	8.80	8.64	8.45	8.29	8.12	8.60	7.83	7.78	11.84
Anterior pelvic - post anal	9.72	9.05	8.77	9.54	7.64	8.99	9.37	8.38	8.10	6.98	8.10	7.53	9.49	14.28	11.52	4.71	12.19	11.11	12.84	8.85	8.74	8.59	8.14	11.63	7.82	8.48	6.22	6.00	5.56	6.07	5.52	5.56	5.40	5.41	9.31
Post anal - post dorsal	8.19	6.84	7.19	7.30	6.71	7.03	7.33	7.51	7.02	6.12	6.98	6.56	8.15	9.82	9.98	3.71	10.30	9.78	9.72	6.68	7.66	7.24	6.22	9.18	6.44	6.85	4.95	4.80	4.81	6.16	4.82	5.06	4.67	4.78	6.87
Pelvic fin base length	1.22	1.02	0.94	1.08	1.06	1.10	1.14	1.16	1.06	0.93	1.04	1.02	1.26	1.50	1.40	0.60	1.62	1.50	1.58	1.24	1.16	1.03	1.11	1.46	1.10	1.09	0.79	0.75	0.75	0.75	0.82	0.77	0.78	0.85	1.16
Post pelvic - post dorsal	7.53	6.09	6.52	6.28	6.16	5.77	6.63	6.12	6.28	5.44	6.36	5.76	7.85	8.37	8.66	3.42	7.81	8.20	8.64	6.04	6.21	5.72	5.28	7.62	5.92	5.69	4.25	4.26	4.12	4.34	4.32	4.13	3.93	3.79	5.88
Body width (before dorsal fin)	5.16	4.27	4.40	4.79	4.03	4.44	4.31	3.77	4.38	3.85	4.15	3.88	4.84	5.45	6.75	1.66	5.68	5.65	5.80	4.01	4.00	3.83	3.75	5.52	3.70	3.97	2.66	2.79	2.66	2.80	2.52	2.58	2.59	2.56	3.76
Caudal peduncle length	6.89	5.97	5.78	5.89	5.75	5.70	6.64	5.76	6.00	5.08	5.65	5.76	6.96	6.77	7.86	3.00	8.29	7.16	7.70	5.80	6.38	5.95	5.26	7.16	5.42	5.47	4.34	4.00	4.04	4.09	3.98	3.85	3.73	3.84	5.53
Caudal peduncle dept	3.25	2.60	2.65	2.72	2.65	2.61	2.87	2.80	2.64	2.35	2.79	2.58	3.07	3.49	3.70	1.25	3.73	3.73	3.84	2.49	2.77	2.41	2.41	3.08	2.28	2.69	1.79	1.59	2.01	1.57	1.73	1.74	1.62	1.67	2.47
Caudal peducle width	1.51	1.61	1.45	1.47	1.18	1.38	1.50	1.28	1.23	1.22	1.10	1.27	1.75	1.88	1.97	0.62	1.65	1.87	2.03	1.45	1.34	1.39	1.35	1.69	1.17	1.46	0.86	1.06	0.77	0.99	1.15	0.85	1.03	0.78	1.24
Caudal base length	3.42	2.80	2.84	3.03	2.92	2.98	3.25	2.97	3.13	2.64	3.07	2.91	3.27	3.85	3.90	1.54	4.21	4.11	4.04	3.05	3.37	2.86	2.88	5.67	2.91	3.25	2.27	1.82	2.00	1.90	2.01	1.99	1.83	1.97	2.94
Upper ray length	8.09	6.49	6.47	6.69	ND	6.55	6.85	6.84	6.58	5.88	6.42	5.98	7.04	8.74	8.60	3.62	8.18	8.11	8.63	6.48	6.10	6.03	6.14	7.98	6.02	6.43	4.81	4.42	4.54	4.60	4.39	4.62	4.63	4.30	6.60
Lower ray length	7.85	6.40	6.49	6.67	ND	6.34	6.53	6.63	6.70	5.96	6.28	6.18	6.77	9.41	8.44	3.60	8.18	8.70	8.81	6.50	6.54	6.15	6.03	ND	5.95	5.92	5.09	4.37	4.48	4.50	4.57	4.59	4.63	4.27	5.96
Caudal fin max height	ND	8.74	8.82	9.96	ND	8.48	10.48	9.51	10.34	9.23	9.45	8.42	9.87	12.21	11.61	5.28	13.37	13.41	11.60	9.18	9.78	8.61	8.43	ND	8.77	9.45	7.31	6.16	6.59	6.93	7.39	5.83	7.38	7.26	9.44
Post dorsal - caudal peduncle	9.78	8.58	8.73	8.45	8.62	8.70	8.60	8.76	8.33	7.80	8.39	7.96	10.24	10.95	11.97	4.59	12.24	11.26	11.34	7.87	9.29	9.44	7.45	11.41	7.62	7.80	6.42	6.00	5.69	6.35	5.83	6.27	5.77	5.79	8.21
Post anal - caudal peduncle	2.96	3.38	3.28	3.10	2.68	3.04	3.66	2.81	2.97	2.62	2.86	2.77	3.90	2.92	3.93	1.42	3.95	3.80	3.79	2.85	2.89	2.90	2.68	3.80	2.77	2.51	2.39	2.04	2.23	2.28	2.27	2.14	2.02	2.31	2.83
Head length	7.28	5.67	5.78	6.46	5.57	6.21	6.63	6.14	6.00	5.17	6.17	5.73	6.79	9.11	8.77	3.19	8.57	8.42	9.13	5.99	6.03	5.90	5.38	8.25	5.64	5.84	4.54	4.21	4.19	4.33	4.18	4.45	4.23	4.13	5.98
Eye diameter	1.23	1.07	1.04	0.99	1.01	1.06	1.09	1.13	1.08	0.93	0.95	1.00	1.10	1.43	1.30	0.65	1.25	1.12	1.20	1.06	0.96	0.94	0.84	1.28	0.97	0.99	0.93	0.96	0.93	0.88	0.84	0.87	0.79	0.85	0.99
Snout length	2.21	1.58	1.27	1.76	1.23	1.97	1.96	1.47	1.37	1.21	1.63	1.63	2.11	2.76	2.48	0.75	2.64	2.36	2.83	2.03	1.56	2.08	1.76	2.54	1.89	1.96	0.92	1.35	1.28	1.11	1.18	1.14	1.04	1.00	2.12
Orbital preoperculum groove	1.56	1.06	1.30	1.29	1.19	1.43	1.37	1.41	1.25	1.07	1.41	1.26	1.39	1.97	2.16	0.61	1.98	2.16	2.04	1.27	1.33	1.17	1.11	1.89	1.38	1.25	0.79	0.81	0.85	0.78	0.74	0.79	0.76	0.78	1.33
Gape width	2.85	2.15	2.02	2.34	2.01	2.29	2.28	2.00	2.36	1.75	2.20	2.02	2.17	3.31	3.28	1.22	3.42	3.15	3.31	1.93	2.43	2.00	1.66	3.14	0.84	2.05	1.78	1.54	1.46	1.66	1.62	1.58	1.67	1.55	1.83
Gape height	2.90	2.08	2.14	2.47	2.10	2.11	2.45	2.35	2.14	1.97	2.40	2.05	2.36	3.48	3.16	1.34	3.44	3.31	3.12	1.85	2.22	2.03	1.70	3.14	1.82	1.97	1.82	1.50	1.50	1.48	1.52	1.59	1.61	1.46	1.80
Upper jaw length	2.92	2.14	2.10	2.59	2.27	2.43	2.77	2.51	2.45	1.99	2.48	2.17	2.98	3.47	3.55	1.21	3.89	3.28	3.81	2.19	2.53	2.34	1.97	3.41	2.11	2.38	1.82	1.55	1.54	1.59	1.71	1.75	1.79	1.71	2.35
Lower jaw length	2.16	1.40	1.54	1.93	1.38	1.85	2.01	1.59	1.58	1.32	1.67	1.54	2.10	2.70	2.39	0.80	2.50	2.25	2.61	1.60	1.78	1.68	1.42	2.46	1.38	1.72	1.33	1.04	1.00	0.96	1.08	1.09	1.12	1.02	1.76
Anterior barbel length	1.42	1.17	1.26	1.47	1.21	1.41	1.09	1.19	1.33	0.88	1.16	1.03	1.39	1.26	1.54	0.53	1.29	1.64	1.74	1.08	1.01	0.96	ND	1.31	0.87	0.85	1.08	0.84	0.63	0.73	1.03	0.91	0.75	1.08	0.95
Posterior barbel length	1.50	1.36	1.32	1.46	1.40	1.69	1.36	1.38	1.42	0.99	1.67	1.06	1.71	1.83	1.42	0.73	1.31	1.89	1.96	1.22	1.48	1.31	0.92	1.57	1.25	1.17	1.08	0.97	0.94	1.05	0.91	1.07	0.89	1.01	1.03
Premaxilla - supraoccipital	3.78	2.74	2.77	3.44	2.84	2.59	3.22	3.07	2.94	2.47	3.07	2.83	2.77	4.46	4.41	1.47	4.50	4.44	4.60	3.23	3.25	2.83	2.44	4.30	2.98	2.65	2.37	1.73	1.89	2.22	1.75	2.08	1.88	2.03	2.93

Appendix D (continued): Table of the morphological measurements from the *L. polylepis* individuals from the Komati River (ND=No data).

Measurements	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	K27	K28
Fork length	42.35	22.04	16.91	18.38	15.60	19.09	22.16	17.21	22.44	17.38	49.58	43.99	18.45	18.75	19.72	11.15	20.32	16.71	16.83	16.76	18.05	17.16	40.94	16.96	16.95	12.90	13.28	15.09
Lateral line - dorsal fin	6.77	3.70	2.83	2.80	2.41	3.17	3.61	3.02	3.78	2.74	7.49	6.61	3.03	2.76	2.96	1.79	3.35	2.83	2.89	2.73	3.02	2.85	6.87	2.86	2.83	2.10	2.31	2.51
Lateral line - pelvic fin	4.10	1.91	1.56	1.34	1.12	1.52	1.91	1.36	2.23	1.29	3.69	3.04	1.45	1.25	1.51	0.83	1.51	1.46	1.24	1.27	1.44	1.40	3.82	1.17	1.05	0.08	1.04	1.04
Body width (before pectoral fins)	5.88	3.01	2.26	2.20	1.90	2.28	3.03	1.97	3.03	2.09	6.67	5.75	2.21	2.12	2.51	1.36	2.86	2.05	2.11	2.05	2.29	2.00	5.76	2.06	2.01	1.59	1.61	1.85
Eye diameter	1.32	0.93	0.85	0.86	0.79	0.87	1.02	0.95	1.09	0.87	1.45	1.43	0.96	0.88	0.92	0.78	0.97	0.93	0.93	0.89	0.96	1.01	1.35	0.92	0.84	0.78	0.78	0.87
Snout length	2.20	1.53	0.92	1.21	0.90	0.98	1.33	0.97	1.50	0.96	3.69	3.12	1.10	0.91	1.07	0.60	1.34	0.97	1.07	0.85	0.96	0.96	2.70	0.85	0.87	0.69	0.71	0.75
Orbital preoperculum groove	2.26	1.05	0.71	0.83	0.71	0.82	1.16	0.64	1.16	0.75	2.78	2.50	0.78	0.75	0.91	0.51	0.99	0.79	0.88	0.72	0.75	0.78	2.37	0.87	0.71	0.59	0.51	0.66
Gape width	2.98	1.50	1.15	1.34	1.04	1.16	1.48	0.97	1.45	1.00	3.63	3.21	1.13	1.12	1.12	0.79	1.13	1.08	1.11	0.96	1.16	1.09	2.88	1.02	1.07	0.79	0.81	0.88
Gape height	3.38	1.84	1.43	1.53	1.27	1.46	1.99	1.26	1.87	1.39	4.43	3.57	1.48	1.46	1.54	1.04	1.71	1.47	1.35	1.43	1.48	1.35	3.21	1.32	1.42	1.05	0.96	1.20
Upper jaw length	3.97	2.13	1.56	1.81	1.44	1.66	2.13	1.50	2.34	1.49	5.03	4.11	1.68	1.69	1.84	0.94	2.07	1.61	1.65	1.45	1.64	1.60	4.00	1.52	1.62	1.24	1.17	1.40
Lower jaw length	2.90	1.54	1.06	1.17	0.95	1.15	1.52	1.06	1.54	1.11	3.93	3.14	1.19	1.09	1.23	0.70	1.33	1.20	1.25	1.04	1.17	1.13	2.91	1.07	1.07	0.84	0.82	0.98
Anterior barbel length	1.46	0.91	0.83	1.08	0.81	0.72	0.97	0.69	1.09	0.81	1.29	2.33	0.93	0.89	1.02	0.60	0.87	1.02	0.82	0.82	0.88	0.83	1.75	0.73	0.76	0.73	0.59	0.69
Posterior barbel length	1.81	0.95	0.81	0.85	0.92	0.70	1.07	0.96	1.15	0.99	1.74	2.23	0.94	0.97	1.02	0.68	0.95	0.93	0.97	0.92	0.85	1.06	1.74	0.65	0.84	0.60	0.79	0.64
Premaxilla - supraoccipital	5.00	2.41	2.09	2.16	1.81	2.13	2.84	2.01	2.85	1.98	6.25	5.02	2.11	2.15	2.44	1.28	2.45	1.99	2.06	1.96	1.98	2.23	4.79	1.98	1.97	1.66	1.60	1.76
Origin of pectoral - premaxilla	9.76	5.38	3.90	4.52	3.66	4.26	5.33	3.84	5.40	4.01	11.81	10.07	4.28	4.10	4.71	2.58	4.72	4.02	4.08	3.89	4.19	4.04	9.15	3.88	3.92	3.10	3.11	3.65
Max dorsal spine length	6.37	3.68	3.22	3.43	3.04	3.36	3.99	3.18	3.89	3.29	7.50	7.06	3.28	3.24	3.59	2.16	3.48	2.99	2.87	3.13	3.22	3.04	6.87	3.08	2.90	2.33	2.71	2.66
Dorsal fin base length	5.00	2.40	2.01	2.15	1.88	2.25	2.66	2.27	2.61	2.02	5.63	4.93	2.08	2.12	2.19	1.26	2.39	1.98	2.09	2.23	2.10	1.92	4.90	2.03	1.83	1.51	1.55	1.79
Anal fin base length	3.43	1.46	1.17	1.26	1.08	1.31	1.62	1.32	1.57	1.17	4.22	3.56	1.11	1.25	1.33	0.73	1.43	1.17	1.08	1.16	1.25	1.14	3.27	1.17	1.15	0.89	0.92	1.02
Max anal spine length	6.73	3.12	2.57	2.59	2.28	2.76	3.53	2.33	3.34	2.67	7.56	6.26	2.50	2.45	2.98	1.45	2.95	2.33	2.53	2.36	2.54	2.39	6.88	2.53	2.35	1.70	1.77	2.12
Pectoral fin length	7.64	3.85	3.25	3.37	2.86	3.46	4.37	3.27	4.31	3.11	8.44	7.85	3.33	3.27	3.48	1.95	3.65	2.96	3.02	2.79	3.32	3.08	7.04	2.95	3.12	2.36	2.54	2.69
Prepectoral length	9.88	5.30	3.95	4.45	3.67	4.37	5.40	3.91	5.80	4.03	11.93	10.28	4.34	4.26	4.63	2.56	4.88	4.02	4.18	3.90	4.19	4.15	9.50	3.89	4.05	3.20	3.20	3.63
Prepelvic length	20.58	10.81	8.34	9.26	7.73	9.24	11.55	8.69	11.56	8.38	23.59	21.02	9.09	8.82	9.87	5.48	10.28	8.12	8.45	8.19	9.19	8.78	20.00	8.48	8.26	6.27	6.84	7.57
Preanal length	29.82	15.46	11.79	13.07	11.14	13.44	16.10	12.12	16.16	11.77	35.30	30.08	12.65	12.65	13.63	7.75	14.19	11.63	11.93	11.57	12.69	12.07	26.79	11.87	11.75	8.75	9.52	10.43
Anterior pelvic - origin of pectoral	11.32	5.60	4.42	4.81	4.12	5.05	6.19	4.64	5.81	4.40	12.41	11.59	4.75	4.93	5.43	2.80	5.30	4.17	4.32	4.31	4.91	4.63	11.14	4.47	4.32	3.29	3.67	3.93
Origin of pectoral - supraoccipital	7.45	4.01	3.04	3.22	2.80	3.35	3.99	3.00	4.15	3.14	8.89	7.89	3.30	3.22	3.58	1.94	3.60	2.98	3.15	2.87	3.34	3.21	6.97	3.01	3.02	2.37	2.43	2.75
Supraoccipital - origin of dorsal fin	14.92	7.61	6.07	7.02	5.55	7.09	8.09	5.80	8.50	6.18	17.40	16.31	6.69	6.79	7.23	3.95	7.22	5.92	6.10	5.78	6.58	6.08	15.30	6.31	6.07	4.59	4.68	5.52
Dorsal fin origin - pelvic origin	9.76	5.33	4.04	4.03	3.49	4.38	5.02	4.23	5.50	3.75	10.16	9.17	4.23	3.87	4.37	2.44	4.64	4.03	3.81	3.69	4.17	4.00	9.78	3.80	3.66	2.93	3.11	3.29
Dorsal fin origin - pectoral origin	12.72	6.72	5.17	5.56	4.67	5.88	6.65	5.25	7.12	5.06	14.41	13.36	5.72	5.52	6.09	3.31	6.04	4.97	5.17	5.12	5.60	5.29	12.70	5.32	5.03	3.91	3.90	4.70
Supraoccipital - pelvic origin	17.52	9.34	7.11	7.73	6.50	7.94	9.57	7.21	9.28	7.05	19.24	17.62	7.46	7.26	8.19	4.47	8.31	7.13	7.20	6.86	7.53	7.23	17.10	6.84	6.87	5.03	5.61	6.22
Anterior pelvic - post anal	12.16	6.46	4.61	4.55	4.25	5.41	6.29	4.73	6.61	4.63	14.57	11.96	5.01	4.98	5.32	3.21	5.67	4.76	4.84	4.70	4.95	4.41	11.96	4.83	4.83	3.32	3.49	4.08
Post anal - post dorsal	9.75	5.07	4.03	3.84	3.54	4.37	4.87	4.15	4.81	3.90	10.87	9.87	4.10	4.25	4.05	2.54	4.67	3.79	3.77	3.62	4.02	4.03	9.75	3.82	3.96	2.86	3.25	3.30
Pelvic fin base length	1.75	0.87	0.70	0.75	0.65	0.81	0.98	0.75	0.89	0.68	2.04	1.69	0.70	0.70	0.77	0.46	0.80	0.66	0.70	0.64	0.70	0.65	1.51	0.65	0.71	0.51	0.59	0.62
Post pelvic - post dorsal	9.32	4.76	3.77	3.93	3.18	4.32	4.56	3.58	5.14	3.48	9.31	8.25	3.89	3.59	4.10	2.06	4.53	3.61	3.63	3.52	3.89	4.82	9.13	3.61	3.40	2.76	2.85	2.94
Body width (before dorsal fin)	6.16	3.23	2.50	2.40	1.95	2.63	3.14	2.28	3.62	2.31	6.70	6.14	2.40	2.20	2.61	1.38	2.71	2.33	2.38	2.40	2.45	2.28	6.07	2.31	2.14	1.64	1.71	1.98
Caudal peduncle length	8.49	4.40	3.47	3.72	3.08	3.84	4.51	3.47	4.52	3.57	9.16	8.24	3.59	4.08	4.27	2.08	4.05	3.49	3.34	3.30	3.63	3.50	7.80	3.46	3.52	2.60	2.45	3.17
Caudal peduncle dept	3.91	2.10	1.59	1.62	1.48	1.75	2.16	1.62	2.04	1.46	4.10	4.00	1.72	1.41	1.79	0.99	1.93	1.13	1.58	1.46	1.73	1.69	4.18	1.56	1.58	1.17	1.32	1.40
Caudal peducle width	2.17	1.08	0.65	0.72	0.56	0.76	0.96	0.71	0.96	0.73	1.91	1.97	0.69	0.71	0.83	0.40	0.93	0.65	0.83	0.71	0.77	0.75	1.84	0.61	0.66	0.52	0.51	0.73
Caudal base length	4.71	2.58	1.84	1.98	1.70	1.96	2.47	1.91	2.46	1.79	5.34	4.62	1.96	1.97	2.01	1.20	2.12	1.77	1.72	1.82	1.90	1.88	4.70	1.79	1.73	1.35	1.49	1.62
Upper ray length	8.55	5.01	4.04	4.59	3.89	4.53	5.61	4.16	5.67	4.56	9.94	ND	4.41	4.58	4.81	2.87	4.66	4.19	4.17	3.87	4.40	4.15	8.43	4.01	4.09	3.17	3.31	3.74
Lower ray length	8.55	5.01	4.04	4.69	3.83	4.60	5.68	4.16	5.41	4.53	ND	9.17	4.44	4.58	4.81	2.85	4.70	4.19	4.17	3.91	4.37	4.15	8.45	4.14	4.20	3.17	3.31	3.74
Caudal fin max height	13.17	7.80	6.50	7.81	6.47	7.62	9.63	7.33	9.49	7.66	ND	ND	7.42	7.44	8.53	4.59	8.77	7.45	6.64	7.02	7.57	7.33	15.51	7.09	6.96	5.36	5.84	6.37
Post dorsal - caudal peduncle	10.92	5.73	4.70	4.71	4.32	5.33	5.74	4.79	5.41	4.53	13.03	11.66	5.17	5.76	5.20	3.12	5.63	4.70	4.65	4.40	4.91	5.01	11.56	4.54	5.01	3.34	3.94	4.09
Post anal - caudal peduncle	4.17	2.00	1.73	1.90	1.45	1.82	2.03	1.44	2.08	1.85	4.40	4.01	1.88	2.11	2.12	1.00	2.07	1.85	1.54	1.73	1.61	1.75	3.73	1.64	1.67	1.21	1.07	1.74
Head length	9.62	5.13	3.93	4.40	3.58	4.17	5.41	3.77	5.65	4.02	11.93	10.16	4.26	4.20	4.59	2.59	4.83	4.03	4.13	3.78	4.17	4.10	9.35	3.98	4.03	3.00	3.11	3.51
Eye diameter	1.32	0.93	0.85	0.86	0.79	0.87	1.02	0.95	1.09	0.87	1.45	1.43	0.96	0.88	0.92	0.78	0.97	0.93	0.93	0.89	0.96	1.01	1.35	0.92	0.84	0.78	0.78	0.87
Snout length	2.20	1.53	0.92	1.21	0.90	0.98	1.33	0.97	1.50	0.96	3.69	3.12	1.10	0.91	1.07	0.60	1.34	0.97	1.07	0.85	0.96	0.96	2.70	0.85	0.87	0.69	0.71	0.75
Orbital preoperculum groove	2.26	1.05	0.71	0.83	0.71	0.82	1.16	0.64	1.16	0.75	2.78	2.50	0.78	0.75	0.91	0.51	0.99	0.79	0.88	0.72	0.75	0.78	2.37	0.87	0.71	0.59	0.51	0.66
Gape width	2.98	1.50	1.15	1.34	1.04	1.16	1.48	0.97	1.45	1.00	3.63	3.21	1.13	1.12	1.12	0.79	1.13	1.08	1.11	0.96	1.16	1.09	2.88	1.02	1.07	0.79	0.81	0.88
Gape height	3.38	1.84	1.43	1.53	1.27	1.46	1.99	1.26	1.87	1.39	4.43	3.57	1.48	1.46	1.54	1.04	1.71	1.47	1.35	1.43	1.48	1.35	3.21	1.32	1.42	1.05	0.96	1.20
Upper jaw length	3.97	2.13	1.56	1.81	1.44	1.66	2.13	1.50	2.34	1.49	5.03	4.11	1.68	1.69	1.84	0.94	2.07	1.61	1.65	1.45	1.64	1.60	4.00	1.52	1.62	1.24	1.17	1.40
Lower jaw length	2.90	1.54	1.06	1.17	0.95	1.15	1.52	1.06	1.54	1.11	3.93	3.14	1.19	1.09	1.23	0.70	1.33	1.20	1.25	1.04	1.17	1.13	2.91	1.07	1.07	0.84	0.82	0.98
Anterior barbel length	1.46	0.91	0.83	1.08	0.81	0.72	0.97	0.69	1.09	0.81	1.29	2.33	0.93	0.89	1.02	0.60	0.87	1.02	0.82	0.82	0.88	0.83	1.75	0.73	0.76	0.73	0.59	0.69
Posterior barbel length	1.81	0.95	0.81	0.85	0.92	0.70	1.07	0.96	1.15	0.99	1.74	2.23	0.94	0.97	1.02	0.68	0.95	0.93	0.97	0.92	0.85	1.06	1.74	0.65	0.84	0.60	0.79	0.64
Premaxilla - supraoccipital	5.00	2.41	2.09	2.16	1.81	2.13	2.84	2.01	2.85	1.98	6.25	5.02	2.11	2.15	2.44	1.28	2.45	1.99	2.06	1.96	1.98	2.23	4.79	1.98	1.97	1.66	1.60	1.76

Appendix E (continued): Table of the morphological measurements from the *L. polylepis* individuals from the Assegaai River (ND=No data).

Measurements	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
Fork length	29.83	39.78	36.02	36.67	32.92	31.29	34.04	31.18	34.54	28.04	23.10	28.80	34.20	35.21	29.19	29.93	30.70	36.18	28.87	37.42	30.65	37.89	32.93	31.02	35.05	32.10	38.35	34.30	27.24	5.77
Lateral line - dorsal fin	4.25	5.88	5.54	5.97	5.14	4.56	5.40	5.28	5.20	4.54	3.94	4.41	2.62	5.83	4.84	4.99	4.80	5.68	4.60	6.03	4.78	5.66	5.22	4.79	4.70	5.29	5.64	5.53	4.40	2.38
Lateral line - pelvic fin	1.88	2.64	2.95	2.87	2.22	2.05	2.53	2.73	2.48	2.19	1.65	2.07	2.61	2.89	2.03	2.29	2.10	2.54	2.07	2.90	2.19	2.52	2.35	2.11	2.30	2.40	2.63	2.65	1.79	2.38
Body width (before pectoral fins)	3.77	5.05	4.63	4.71	4.28	4.11	4.25	4.14	4.49	3.49	2.58	3.51	4.02	4.83	3.63	3.98	3.25	4.65	3.60	4.92	3.78	5.01	3.91	3.72	4.30	4.12	4.51	4.45	3.31	4.27
Eye diameter	1.15	1.38	1.39	1.47	1.41	1.12	1.16	1.17	1.38	1.17	1.16	1.08	1.41	1.20	1.12	1.26	1.23	1.35	1.20	1.25	1.21	1.25	1.19	1.16	1.13	1.22	1.24	1.28	1.08	1.17
Snout length	1.89	2.80	2.62	2.50	2.43	1.94	1.99	2.25	2.26	1.73	1.49	1.79	2.11	2.38	2.03	2.10	2.18	2.72	2.03	2.97	1.32	2.29	2.45	1.91	2.73	1.85	2.16	2.28	1.67	2.22
Orbital preoperculum groove	1.24	2.32	1.81	2.05	1.57	1.45	1.68	1.70	1.72	1.29	0.68	1.37	1.47	1.75	1.43	1.60	1.42	2.15	1.47	2.08	1.29	1.97	1.54	1.57	1.44	1.48	1.70	1.85	1.21	1.66
Gape width	2.25	3.29	3.14	2.94	2.68	2.32	2.73	2.86	2.79	2.20	1.57	2.41	2.63	2.94	2.31	2.24	2.32	3.57	2.44	2.91	2.44	3.28	2.56	2.78	2.80	2.58	ND	2.78	2.08	2.57
Gape height	2.31	3.97	3.82	3.60	2.80	2.63	2.94	3.12	3.31	2.29	1.53	2.60	2.83	3.32	2.67	2.86	2.68	3.86	2.51	3.94	2.73	3.20	2.91	2.83	3.11	3.22	ND	2.86	2.23	2.84
Upper jaw length	2.51	4.09	3.66	3.62	3.23	2.73	2.88	3.18	3.16	2.62	1.83	2.67	2.91	3.63	2.75	2.77	2.93	3.80	2.76	3.84	2.61	3.46	3.05	2.73	3.05	2.92	2.98	2.70	2.07	3.00
Lower jaw length	1.88	3.09	2.68	2.76	2.61	1.97	1.93	2.40	2.45	1.86	1.18	1.91	2.29	2.75	1.99	2.00	2.15	2.97	1.85	2.87	1.96	2.51	2.34	2.06	2.53	2.01	2.51	2.38	1.81	2.46
Anterior barbel length	1.10	1.25	1.85	1.76	1.32	1.36	1.44	1.60	1.40	1.33	0.48	1.16	1.41	1.76	1.57	1.27	1.34	1.31	1.35	1.92	1.26	1.91	1.76	1.27	1.74	1.46	1.47	1.63	1.27	1.68
Posterior barbel length	1.17	2.26	2.26	1.54	2.06	1.65	1.70	1.72	1.73	1.39	0.91	1.35	1.62	1.98	1.56	1.51	1.76	1.71	1.55	1.97	1.56	2.44	1.87	1.46	1.88	1.49	2.17	2.09	1.67	1.85
Premaxilla - supraoccipital	3.20	4.42	3.94	4.18	4.11	3.31	3.73	3.63	4.08	3.12	2.14	3.18	3.89	3.74	3.26	3.50	3.43	4.30	3.03	4.23	3.33	3.23	3.81	3.18	3.69	3.37	4.15	4.15	3.29	4.14
Origin of pectoral - premaxilla	6.77	9.78	9.08	6.57	7.92	6.89	7.53	4.43	8.11	6.43	4.48	6.35	7.56	8.50	6.83	6.85	6.70	8.72	6.54	8.69	7.14	8.39	7.49	7.16	8.73	7.41	9.00	7.82	5.95	7.87
Max dorsal spine length	5.15	6.20	5.19	5.91	5.51	7.79	5.49	5.42	5.35	4.55	4.46	4.59	5.65	5.17	4.62	4.83	5.17	5.72	5.01	5.73	4.78	5.81	5.32	5.10	5.48	5.36	6.46	5.89	4.28	6.04
Dorsal fin base length	3.36	4.74	4.31	4.09	3.74	3.90	4.02	4.02	4.12	3.27	3.03	3.42	3.96	3.82	3.49	3.78	3.82	4.11	3.55	4.58	3.53	4.16	3.76	3.76	4.01	4.03	1.39	3.87	3.31	4.23
Anal fin base length	2.00	2.86	2.81	2.64	2.27	2.17	2.66	2.47	2.55	2.00	1.67	2.11	2.54	2.42	2.05	2.31	2.21	2.68	2.03	2.74	2.09	2.66	2.21	2.27	2.46	2.30	2.54	2.26	1.76	2.90
Max anal spine length	4.39	6.66	6.78	6.48	5.56	4.71	5.97	5.84	5.93	3.97	4.14	4.28	5.05	5.82	4.07	5.43	4.29	6.34	4.63	5.93	4.55	6.22	4.76	4.43	5.83	5.78	7.07	5.78	3.65	6.13
Pectoral fin length	5.47	7.46	6.75	6.52	6.06	5.80	6.47	6.32	6.17	5.30	4.50	5.42	6.23	6.53	5.21	5.60	5.88	6.53	5.43	6.77	5.70	6.24	6.08	5.95	6.27	5.97	6.66	6.70	5.13	6.65
Prepectoral length	6.88	9.97	9.37	9.02	8.21	7.04	7.72	7.53	8.31	6.41	4.54	6.45	7.46	8.33	6.87	6.92	6.82	9.05	6.68	8.79	7.14	8.54	7.39	6.96	8.54	7.39	9.04	7.88	5.97	8.01
Prepelvic length	14.37	19.80	18.29	17.93	16.49	15.34	16.48	15.58	17.18	13.80	10.78	14.20	16.11	17.69	14.37	15.49	14.97	18.59	14.01	19.80	14.86	18.92	15.95	14.75	16.68	15.95	18.64	16.90	13.14	17.57
Preanal length	19.88	28.30	26.30	26.60	23.70	22.10	23.50	22.70	24.50	19.81	15.43	22.20	23.20	24.50	19.91	22.60	21.70	26.00	19.40	28.40	20.56	26.90	22.90	21.30	25.70	23.10	27.10	34.70	18.73	25.70
Anterior pelvic - origin of pectoral	7.70	10.64	9.77	9.52	8.24	8.05	9.13	7.96	8.80	7.27	6.38	7.27	8.13	9.30	7.26	8.09	7.53	9.00	7.30	9.81	7.76	9.79	8.53	7.85	8.65	8.29	9.94	8.73	7.22	9.48
Origin of pectoral - supraoccipital	5.20	7.44	6.65	6.62	6.21	5.31	5.98	5.73	6.03	5.02	3.79	4.89	5.61	6.57	5.11	5.51	5.48	6.71	5.17	6.90	5.47	6.91	5.86	5.67	6.76	5.97	6.88	5.83	4.74	6.04
Supraoccipital - origin of dorsal fin	11.05	14.58	13.35	13.92	12.01	11.42	12.55	11.30	12.40	10.45	7.65	10.54	12.08	13.40	11.08	12.01	11.02	13.41	10.84	14.93	11.45	14.05	12.71	11.02	12.15	12.26	13.75	12.99	9.90	13.11
Dorsal fin origin - pelvic origin	5.81	8.27	7.91	8.29	7.09	6.44	7.27	7.49	7.08	6.32	5.35	6.17	6.96	8.29	6.33	9.39	6.32	7.90	6.34	8.28	6.70	8.10	7.32	6.60	7.02	7.22	7.86	7.74	6.03	7.65
Dorsal fin origin - pectoral origin	5.71	11.82	10.73	11.50	9.20	9.05	9.70	9.43	9.76	8.15	6.47	8.56	9.86	11.23	8.48	6.35	9.29	11.00	8.94	11.65	9.41	11.09	10.08	9.02	9.29	9.92	10.93	10.16	8.31	10.90
Supraoccipital - pelvic origin	11.84	16.20	14.73	15.00	13.40	12.48	13.82	12.27	13.64	11.22	9.17	11.20	13.06	14.68	6.57	11.63	12.18	14.56	11.54	15.28	12.36	15.26	13.33	12.49	13.91	13.02	15.55	13.29	10.79	13.78
Anterior pelvic - post anal	8.79	10.74	9.88	10.47	9.03	9.16	10.21	10.02	9.77	8.07	6.59	8.15	9.99	10.16	7.62	8.97	6.59	10.15	7.84	9.84	5.37	10.71	9.41	8.31	10.20	9.34	11.08	9.45	7.70	10.44
Post anal - post dorsal	6.67	8.59	8.27	8.27	7.68	7.14	7.97	7.92	8.05	6.89	5.76	6.59	7.96	7.70	6.86	7.75	6.98	8.93	6.55	8.82	7.11	8.67	8.10	6.87	8.05	7.86	8.54	8.31	6.43	8.59
Pelvic fin base length	1.10	1.60	1.36	1.39	1.23	6.07	1.32	1.29	1.19	1.07	0.97	1.17	1.33	1.51	1.25	1.18	1.26	1.39	1.16	1.40	1.10	1.50	1.31	1.13	1.35	1.20	1.40	1.35	1.13	1.36
Post pelvic - post dorsal	5.43	6.95	7.20	7.35	6.41	5.99	6.86	6.81	6.69	5.48	4.86	5.63	6.60	7.42	5.30	7.86	6.02	6.98	5.90	7.39	5.88	7.43	6.50	6.08	6.70	6.48	7.22	7.30	5.60	7.51
Body width (before dorsal fin)	3.89	4.91	4.90	4.96	4.24	4.25	4.23	4.67	4.93	3.82	3.00	3.70	4.64	4.97	4.03	4.25	4.12	4.93	3.77	5.01	3.93	4.82	4.35	4.27	4.39	4.28	4.97	4.63	3.45	4.63
Caudal peduncle length	5.63	7.72	6.65	6.64	6.78	2.97	6.48	5.98	6.41	5.28	4.51	5.97	6.28	6.88	5.99	5.95	5.72	6.47	5.54	7.26	5.98	7.30	6.13	6.14	6.67	6.57	7.03	6.67	5.25	6.73
Caudal peduncle dept	2.58	3.43	3.39	3.48	3.29	1.36	3.11	3.27	3.09	2.71	2.28	2.67	3.16	3.34	2.78	2.82	2.83	3.33	2.71	3.62	2.68	3.50	3.32	2.93	3.07	2.95	3.25	3.37	2.52	3.31
Caudal peducle width	1.23	1.57	1.51	1.62	1.46	3.41	1.46	1.41	1.53	1.29	1.02	1.39	1.87	1.53	1.33	1.36	1.41	1.50	1.24	1.67	1.31	1.78	1.66	1.59	1.65	1.67	1.72	1.76	1.19	1.75
Caudal base length	2.94	4.35	3.88	4.07	3.64	3.40	3.61	3.81	3.66	3.22	2.48	3.17	3.54	3.72	2.94	3.35	3.56	3.93	3.27	4.22	3.06	4.22	3.60	3.39	3.49	3.26	3.98	3.80	3.04	3.74
Upper ray length	6.36	4.19	7.83	7.50	7.33	6.64	6.85	7.46	ND	6.19	5.95	6.39	7.50	7.14	6.09	6.70	7.37	7.43	6.79	7.51	6.95	7.73	7.24	7.05	7.49	7.02	7.56	7.48	6.08	7.55
Lower ray length	6.72	7.97	7.81	7.42	7.33	6.64	7.17	7.36	7.77	5.92	5.75	6.39	6.58	7.27	6.38	6.66	7.02	7.84	6.77	7.70	6.62	7.46	7.21	7.05	7.45	7.26	8.28	ND	5.99	7.61
Caudal fin max height	8.81	11.20	13.05	13.51	12.77	10.05	11.91	12.23	ND	9.73	10.03	10.41	11.44	10.29	8.56	12.07	11.97	13.43	11.33	13.59	ND	12.84	10.24	12.14	11.27	10.96	ND	ND	8.76	ND
Post dorsal - caudal peduncle	8.09	10.44	9.42	9.52	9.20	8.23	9.43	8.92	8.97	8.00	6.81	7.91	9.85	8.55	7.80	9.27	7.95	9.94	7.79	11.34	8.54	10.18	9.32	7.27	9.82	9.27	10.07	9.38	7.48	9.48
Post anal - caudal peduncle	2.95	4.03	3.28	3.26	3.51	3.10	3.34	2.72	3.19	2.43	2.39	2.88	3.30	3.42	3.19	3.09	2.63	3.27	2.77	3.69	3.29	3.80	3.29	3.02	3.08	3.48	2.67	3.52	2.43	3.23
Head length	6.87	9.58	8.73	8.98	8.18	6.92	7.68	7.60	7.93	6.49	4.55	6.43	7.52	8.34	6.72	7.06	7.07	8.94	6.67	8.96	6.82	8.35	7.74	6.95	8.34	7.13	8.64	8.17	6.12	7.94
Eye diameter	1.15	1.38	1.39	1.47	1.41	1.12	1.16	1.17	1.38	1.17	1.16	1.08	1.41	1.20	1.12	1.26	1.23	1.35	1.20	1.25	1.21	1.25	1.19	1.16	1.13	1.22	1.24	1.28	1.08	1.17
Snout length	1.89	2.80	2.62	2.50	2.43	1.94	1.99	2.25	2.26	1.73	1.49	1.79	2.11	2.38	2.03	2.10	2.18	2.72	2.03	2.97	1.32	2.29	2.45	1.91	2.73	1.85	2.16	2.28	1.67	2.22
Orbital preoperculum groove	1.24	2.32	1.81	2.05	1.57	1.45	1.68	1.70	1.72	1.29	0.68	1.37	1.47	1.75	1.43	1.60	1.42	2.15	1.47	2.08	1.29	1.97	1.54	1.57	1.44	1.48	1.70	1.85	1.21	1.66
Gape width	2.25	3.29	3.14	2.94	2.68	2.32	2.73	2.86	2.79	2.20	1.57	2.41	2.63	2.94	2.31	2.24	2.32	3.57	2.44	2.91	2.44	3.28	2.56	2.78	2.80	2.58	ND	2.78	2.08	2.57
Gape height	2.31	3.97	3.82	3.60	2.80	2.63	2.94	3.12	3.31	2.29	1.53	2.60	2.83	3.32	2.67	2.86	2.68	3.86	2.51	3.94	2.73	3.20	2.91	2.83	3.11	3.22	ND	2.86	2.23	2.84
Upper jaw length	2.51	4.09	3.66	3.62	3.23	2.73	2.88	3.18	3.16	2.62	1.83	2.67	2.91	3.63	2.75	2.77	2.93	3.80	2.76	3.84	2.61	3.46	3.05	2.73	3.05	2.92	2.98	2.70	2.07	3.00
Lower jaw length	1.88	3.09	2.68	2.76	2.61	1.97	1.93	2.40	2.45	1.86	1.18	1.91	2.29	2.75	1.99	2.00	2.15	2.97	1.85	2.87	1.96	2.51	2.34	2.06	2.53	2.01	2.51	2.38	1.81	2.46
Anterior barbel length	1.10	1.25	1.85	1.76	1.32	1.36	1.44	1.60	1.40	1.33	0.48	1.16	1.41	1.76	1.57	1.27	1.34	1.31	1.35	1.92	1.26	1.91	1.76	1.27	1.74	1.46	1.47	1.63	1.27	1.68
Posterior barbel length	1.17	2.26	2.26	1.54	2.06	1.65	1.70	1.72	1.73	1.39	0.91	1.35	1.62	1.98	1.56	1.51	1.76	1.71	1.55	1.97	1.56	2.44	1.87	1.46	1.88	1.49	2.17	2.09	1.67	1.85
Premaxilla - supraoccipital	3.20	4.42	3.94	4.18	4.11	3.31	3.73	3.63	4.08	3.12	2.14	3.18	3.89	3.74	3.26	3.50	3.43	4.30	3.03	4.23	3.33	3.23	3.81	3.18	3.69	3.37	4.15	4.15	3.29	4.14

Appendix F (continued): Table of the morphological measurements from the *L. polylepis* individuals from the Phongolo River (ND=No data).

Measurements	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P31	P32
Fork length	30.00	34.21	42.67	17.46	16.49	15.42	18.38	16.50	15.47	15.10	16.93	16.78	15.90	15.92	24.49	27.78	32.63	25.47	32.75	30.28	17.83	27.52	29.90	28.49	29.98	26.32	23.30	18.76	18.97	19.01	18.17
Lateral line - dorsal fin	4.69	5.34	6.63	2.54	2.45	2.28	2.54	2.56	2.17	2.15	2.42	2.38	2.09	2.17	4.17	4.25	4.87	3.76	4.74	4.29	2.93	4.23	4.28	4.23	4.27	3.98	3.47	2.71	2.67	2.70	2.96
Lateral line - pelvic fin	1.93	2.58	3.08	1.04	1.01	1.08	1.22	1.07	1.04	0.99	1.10	1.12	1.15	1.02	1.69	1.87	2.80	1.60	2.51	2.18	1.72	1.92	2.18	2.11	2.06	1.63	1.70	1.04	1.29	1.24	1.23
Body width (before pectoral fins)	3.69	4.54	5.47	2.15	1.81	1.83	2.09	1.71	1.54	1.53	1.99	1.76	1.74	1.63	3.00	3.26	3.95	2.98	4.01	3.66	2.26	3.26	3.56	3.57	3.95	3.00	2.67	2.07	2.06	2.00	2.14
Eye diameter	1.16	1.13	1.47	0.82	0.80	0.85	0.87	0.83	0.73	0.78	0.79	0.82	0.76	0.93	0.89	1.13	1.18	0.92	1.21	1.13	0.81	0.87	1.21	1.07	1.13	1.04	0.91	0.80	0.85	0.81	0.92
Snout length	1.69	2.46	2.95	1.04	0.98	0.93	1.18	0.79	0.97	0.84	1.03	0.97	0.85	0.97	1.56	1.89	2.14	1.62	2.27	1.96	1.08	1.80	1.77	1.97	2.28	2.02	1.14	1.18	1.04	1.12	0.95
Orbital preoperculum groove	1.51	1.74	2.26	0.89	0.71	0.67	0.83	0.94	0.78	0.72	0.78	0.69	0.67	0.71	0.87	1.45	1.75	1.14	1.68	1.58	0.85	1.04	1.29	1.81	1.51	1.21	1.11	0.91	0.79	0.89	0.66
Gape width	2.24	2.81	2.94	1.53	1.14	1.20	1.26	1.13	1.16	0.95	1.22	1.15	1.18	1.32	1.77	2.07	2.58	1.88	2.55	2.45	1.31	2.12	1.91	2.20	2.48	1.88	1.73	1.35	1.35	1.35	1.31
Gape height	2.58	3.08	3.73	1.70	1.43	1.44	1.67	1.37	1.38	1.31	1.44	1.52	1.30	1.48	1.70	2.57	2.74	2.16	3.00	2.76	1.72	2.10	2.52	2.52	2.51	2.24	1.97	1.44	1.52	1.84	1.48
Upper jaw length	2.67	3.42	4.19	1.53	1.51	1.40	1.70	1.30	1.41	1.23	1.49	1.40	1.27	1.37	1.99	2.60	3.12	2.26	3.13	2.72	1.52	2.36	2.65	2.74	2.77	2.41	2.10	1.68	1.71	1.83	1.60
Lower jaw length	1.98	2.39	2.88	1.18	1.93	0.92	1.19	0.86	0.92	0.84	1.07	1.10	0.96	1.13	1.24	1.96	2.34	1.49	2.31	2.11	1.11	1.52	1.96	1.95	2.23	1.78	1.57	1.21	1.11	1.31	0.97
Anterior barbel length	1.21	1.51	1.28	0.91	0.46	0.57	0.85	0.71	0.72	0.65	0.51	0.84	0.43	0.70	0.35	1.12	1.38	0.89	1.58	1.13	0.89	ND	1.56	1.63	1.40	1.14	1.36	0.68	0.85	0.78	0.39
Posterior barbel length	1.19	1.76	1.63	0.93	0.70	0.70	1.03	0.79	0.86	0.51	0.55	1.01	0.57	0.86	0.59	1.64	1.72	ND	1.73	1.40	1.02	ND	1.53	1.72	1.72	1.34	1.36	0.87	1.09	0.96	0.34
Premaxilla - supraoccipital	3.18	4.34	4.26	2.08	2.00	1.95	2.17	1.83	1.91	1.69	1.95	1.61	1.97	1.90	2.49	3.33	3.25	3.03	4.01	3.43	2.19	3.00	3.17	3.37	3.59	2.91	2.30	1.92	1.97	2.30	1.95
Origin of pectoral - premaxilla	6.44	8.48	7.72	4.26	3.60	3.53	4.09	3.63	3.48	3.46	3.88	3.73	3.57	3.64	4.98	6.24	7.72	5.72	7.97	7.01	4.12	5.73	6.68	6.57	7.30	6.17	5.54	4.13	4.15	4.25	3.97
Max dorsal spine length	4.40	5.35	6.13	2.99	2.58	2.54	2.69	2.66	2.56	2.29	2.75	2.73	2.45	2.61	4.09	4.67	4.80	4.26	5.04	4.46	3.08	4.53	4.54	4.36	4.60	3.96	3.60	2.98	2.95	2.86	2.92
Dorsal fin base length	3.47	3.97	5.69	2.03	1.92	1.67	2.07	1.86	1.73	1.73	1.92	1.83	1.93	1.76	2.99	3.40	3.59	2.95	3.85	3.54	1.92	3.33	3.26	3.46	3.85	2.91	2.67	1.94	2.18	2.29	2.01
Anal fin base length	2.23	2.69	3.91	1.16	1.13	1.04	1.27	1.08	0.98	0.95	1.16	1.11	1.17	1.08	1.69	1.99	2.45	1.80	2.62	2.29	1.14	2.04	2.38	1.91	2.17	1.88	1.61	1.28	1.27	1.29	1.36
Max anal spine length	3.97	6.48	5.95	2.34	2.16	2.22	2.31	2.12	2.17	1.61	2.37	2.30	1.92	2.03	3.61	3.72	4.07	3.72	5.21	4.90	2.28	4.01	3.74	3.70	3.87	3.55	3.02	2.40	2.48	2.59	2.60
Pectoral fin length	5.03	5.82	6.74	3.12	2.54	2.44	3.10	2.71	2.67	2.51	2.54	2.96	2.59	2.63	4.43	5.35	5.51	4.20	5.65	4.90	3.28	4.53	5.04	5.01	5.28	4.51	4.02	3.17	2.95	3.08	3.11
Prepectoral length	6.64	8.46	9.91	4.22	3.71	3.61	4.16	3.72	3.63	3.48	3.97	3.66	3.53	3.68	5.15	6.23	7.67	5.77	7.85	6.87	4.17	5.59	6.69	6.69	7.23	6.14	5.56	4.00	4.22	4.46	4.08
Prepelvic length	13.60	17.07	20.90	8.53	8.21	7.73	8.84	7.75	5.30	7.12	8.02	7.72	7.66	7.54	11.43	13.66	16.51	12.78	16.23	14.70	8.95	12.93	14.11	13.79	15.29	13.01	11.35	9.22	9.13	9.19	8.74
Preanal length	20.55	26.06	30.30	12.08	11.07	10.90	13.47	11.26	10.83	10.09	11.58	11.34	11.11	10.90	17.09	19.66	23.10	18.27	23.00	21.80	12.65	18.65	20.90	20.52	21.70	19.12	16.58	12.41	13.22	13.33	12.80
Anterior pelvic - origin of pectoral	7.40	9.02	11.73	4.54	4.27	4.41	4.81	4.42	3.88	3.81	3.93	4.21	3.72	3.68	6.44	6.91	8.36	6.21	8.37	7.78	4.28	7.59	7.63	7.06	6.94	6.30	6.06	4.57	3.51	4.54	4.67
Origin of pectoral - supraoccipital	5.06	6.45	7.99	3.25	2.71	2.77	3.15	2.78	2.58	2.64	2.90	3.01	2.69	2.76	4.22	4.98	5.88	4.57	5.97	5.24	3.24	4.60	5.17	5.20	5.63	4.75	4.27	3.21	3.26	3.46	3.20
Supraoccipital - origin of dorsal fin	10.26	12.28	15.28	6.40	5.61	5.65	6.66	5.85	5.07	5.27	5.87	5.97	5.41	5.64	8.60	9.83	12.82	9.19	12.26	11.27	6.90	8.80	10.53	10.36	10.94	9.98	8.98	6.74	6.88	7.06	6.73
Dorsal fin origin - pelvic origin	6.13	7.36	9.46	3.24	3.08	2.88	3.40	3.03	3.00	2.90	4.04	3.05	4.36	2.94	5.45	5.80	6.81	4.98	6.64	5.98	4.83	5.28	5.77	5.96	6.17	5.07	4.86	3.41	3.59	3.66	3.92
Dorsal fin origin - pectoral origin	8.35	9.72	11.74	4.60	4.59	4.39	5.62	4.81	4.09	4.26	3.08	4.66	2.86	4.21	7.81	8.34	10.62	7.50	10.24	8.81	5.42	8.29	8.33	8.52	8.96	8.45	7.29	5.54	5.52	5.65	5.71
Supraoccipital - pelvic origin	11.62	14.09	17.53	6.85	6.48	6.21	7.33	6.64	5.85	5.86	6.43	6.39	5.92	6.09	10.01	10.91	12.95	9.81	13.12	12.22	6.97	11.21	12.26	5.19	12.05	10.42	9.48	7.06	7.29	7.36	7.19
Anterior pelvic - post anal	7.36	10.32	12.06	4.84	4.86	4.61	5.54	4.56	4.56	4.32	4.84	4.78	4.41	4.47	7.75	8.60	9.61	7.42	8.75	9.34	5.42	8.17	9.06	8.01	8.49	8.09	7.12	5.50	3.58	5.58	5.46
Post anal - post dorsal	6.73	8.44	10.06	3.86	3.71	3.35	4.53	3.78	3.67	3.42	4.05	3.74	3.69	3.87	5.66	6.35	7.81	5.62	7.32	7.01	4.12	6.33	6.82	6.47	6.81	6.05	5.54	4.35	4.43	4.35	4.56
Pelvic fin base length	1.05	1.36	1.76	0.70	0.61	0.61	0.71	0.65	0.65	0.53	0.64	0.68	0.68	0.59	1.11	1.15	1.12	1.08	1.21	1.17	0.74	1.15	1.28	1.07	1.12	1.06	0.73	0.72	0.76	0.78	0.79
Post pelvic - post dorsal	5.81	6.90	8.75	3.21	3.07	2.41	3.49	3.01	2.94	3.25	3.63	3.55	3.33	3.53	5.68	6.74	6.74	4.93	6.85	5.96	3.76	5.75	5.57	5.68	5.92	5.27	4.66	3.31	3.37	3.85	3.97
Body width (before dorsal fin)	4.07	4.76	6.08	2.15	1.88	1.92	2.26	2.09	1.79	1.63	2.07	1.99	1.85	1.70	3.28	3.49	4.42	3.29	4.36	3.90	2.52	3.44	4.05	3.78	4.22	3.37	2.99	1.97	2.24	2.19	2.23
Caudal peduncle length	5.79	6.41	8.41	3.80	3.43	3.02	3.83	3.13	3.18	3.11	3.42	3.58	3.23	2.97	5.14	5.45	5.98	5.06	6.26	5.66	3.81	5.48	5.72	5.34	5.72	4.96	4.87	3.85	3.69	3.89	3.62
Caudal peduncle dept	2.68	3.26	3.77	1.56	1.31	1.27	1.73	1.40	1.34	1.23	1.41	1.54	1.35	1.39	2.46	2.50	2.78	2.18	3.09	2.61	1.71	2.27	2.56	2.38	2.64	2.35	2.18	1.56	1.62	1.82	1.77
Caudal peducle width	1.37	1.53	2.10	0.73	0.57	0.70	0.75	0.67	0.51	0.61	0.71	0.61	0.61	0.63	1.01	1.27	1.45	0.94	1.51	1.15	0.77	1.15	1.34	1.19	1.63	1.08	1.27	0.71	0.75	0.73	0.73
Caudal base length	3.07	3.51	4.48	1.71	1.54	1.46	1.86	1.56	1.53	1.47	1.67	1.61	1.55	1.53	2.70	2.86	3.44	2.55	3.54	3.08	1.81	2.78	3.14	2.89	3.15	2.68	2.23	1.90	1.90	1.96	2.08
Upper ray length	6.61	7.26	8.63	4.02	3.39	3.36	3.86	3.86	3.55	ND	3.35	3.57	3.41	3.36	6.14	6.55	6.74	ND	7.12	6.05	4.13	5.79	6.12	6.13	6.71	5.68	5.31	4.17	4.11	4.27	4.24
Lower ray length	6.54	7.21	7.47	4.02	3.35	3.39	3.89	3.76	3.65	3.26	3.26	3.60	3.29	3.74	6.14	6.53	6.74	5.62	7.16	6.28	3.97	5.66	6.27	6.11	6.50	5.78	5.25	3.77	3.92	4.11	4.14
Caudal fin max height	10.11	ND	13.94	5.76	5.20	4.73	5.83	4.50	5.39	ND	5.36	5.61	5.26	4.97	9.15	10.24	10.05	ND	9.73	7.21	5.91	9.21	8.50	8.78	9.79	6.89	7.63	6.36	ND	5.42	6.66
Post dorsal - caudal peduncle	8.18	10.25	12.78	5.03	5.08	4.07	5.13	4.41	4.76	4.38	4.88	4.90	4.65	4.72	7.03	7.63	9.38	7.32	8.35	8.70	4.98	8.10	8.28	7.79	8.00	7.41	6.32	5.32	5.67	5.56	5.71
Post anal - caudal peduncle	3.07	2.85	4.45	2.02	1.99	1.34	2.04	1.74	1.53	1.53	1.72	1.73	1.90	1.64	2.67	2.43	3.03	2.92	3.12	2.59	2.04	2.56	2.95	2.58	3.08	2.81	2.30	2.29	2.05	2.00	1.90
Head length	6.69	8.13	9.59	4.08	3.69	3.59	4.09	3.52	3.53	3.37	3.89	3.76	3.49	3.60	5.03	6.29	7.35	5.72	7.66	6.80	4.13	5.77	6.47	6.67	7.31	6.18	5.32	4.24	4.21	4.60	3.99
Eye diameter	1.16	1.13	1.47	0.82	0.80	0.85	0.87	0.83	0.73	0.78	0.79	0.82	0.76	0.93	0.89	1.13	1.18	0.92	1.21	1.13	0.81	0.87	1.21	1.07	1.13	1.04	0.91	0.80	0.85	0.81	0.92
Snout length	1.69	2.46	2.95	1.04	0.98	0.93	1.18	0.79	0.97	0.84	1.03	0.97	0.85	0.97	1.56	1.89	2.14	1.62	2.27	1.96	1.08	1.80	1.77	1.97	2.28	2.02	1.14	1.18	1.04	1.12	0.95
Orbital preoperculum groove	1.51	1.74	2.26	0.89	0.71	0.67	0.83	0.94	0.78	0.72	0.78	0.69	0.67	0.71	0.87	1.45	1.75	1.14	1.68	1.58	0.85	1.04	1.29	1.81	1.51	1.21	1.11	0.91	0.79	0.89	0.66
Gape width	2.24	2.81	2.94	1.53	1.14	1.20	1.26	1.13	1.16	0.95	1.22	1.15	1.18	1.32	1.77	2.07	2.58	1.88	2.55	2.45	1.31	2.12	1.91	2.20	2.48	1.88	1.73	1.35	1.35	1.35	1.31
Gape height	2.58	3.08	3.73	1.70	1.43	1.44	1.67	1.37	1.38	1.31	1.44	1.52	1.30	1.48	1.70	2.57	2.74	2.16	3.00	2.76	1.72	2.10	2.52	2.52	2.51	2.24	1.97	1.44	1.52	1.84	1.48
Upper jaw length	2.67	3.42	4.19	1.53	1.51	1.40	1.70	1.30	1.41	1.23	1.49	1.40	1.27	1.37	1.99	2.60	3.12	2.26	3.13	2.72	1.52	2.36	2.65	2.74	2.77	2.41	2.10	1.68	1.71	1.83	1.60
Lower jaw length	1.98	2.39	2.88	1.18	1.93	0.92	1.19	0.86	0.92	0.84	1.07	1.10	0.96	1.13	1.24	1.96	2.34	1.49	2.31	2.11	1.11	1.52	1.96	1.95	2.23	1.78	1.57	1.21	1.11	1.31	0.97
Anterior barbel length	1.21	1.51	1.28	0.91	0.46	0.57	0.85	0.71	0.72	0.65	0.51	0.84	0.43	0.70	0.35	1.12	1.38	0.89	1.58	1.13	0.89	ND	1.56	1.63	1.40	1.14	1.36	0.68	0.85	0.78	0.39
Posterior barbel length	1.19	1.76	1.63	0.93	0.70	0.70	1.03	0.79	0.86	0.51	0.55	1.01	0.57	0.86	0.59	1.64	1.72	ND	1.73	1.40	1.02	ND	1.53	1.72	1.72	1.34	1.36	0.87	1.09	0.96	0.34
Premaxilla - supraoccipital	3.18	4.34	4.26	2.08	2.00	1.95	2.17	1.83	1.91	1.69	1.95	1.61	1.97	1.90	2.49	3.33	3.25	3.03	4.01	3.43	2.19	3.00	3.17	3.37	3.59	2.91	2.30	1.92	1.97	2.30	1.95

Appendix G (continued): Table of the morphological measurements from the *L. natalensis* individuals from the Umvoti River (ND=No data).

Measurements	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24
Fork length	17.08	17.10	14.48	15.16	17.59	20.30	15.61	17.27	18.77	15.79	28.43	19.59	15.41	15.62	14.07	18.77	16.10	15.73	14.64	16.06	15.73	11.48	9.98	7.10
Lateral line - dorsal fin	2.70	2.87	2.52	2.46	2.90	3.43	2.62	2.98	3.26	2.62	3.40	3.38	2.71	2.40	2.52	2.86	2.78	2.64	2.15	2.82	2.62	1.83	1.72	0.96
Lateral line - pelvic fin	1.05	1.27	0.99	1.01	1.21	1.46	1.05	1.20	1.29	1.08	1.60	1.33	1.06	0.90	1.05	1.16	1.16	1.15	1.97	0.90	1.11	0.79	0.69	0.41
Body width (before pectoral fins)	1.89	2.04	1.63	1.92	1.98	2.23	1.79	2.17	2.13	1.77	2.69	2.19	1.80	1.73	1.61	1.85	1.85	1.76	1.59	1.91	1.77	1.31	1.16	0.82
Eye diameter	0.79	0.82	0.80	0.73	0.81	0.97	0.81	0.82	0.94	0.77	1.04	0.88	0.76	0.83	0.75	0.82	0.87	0.81	0.74	0.80	0.74	0.65	0.57	0.51
Snout length	1.19	1.27	1.04	1.11	1.38	1.45	1.16	1.39	1.31	1.16	1.83	1.51	0.96	0.83	0.91	0.87	0.79	1.10	0.98	0.84	0.97	0.65	0.52	0.42
Orbital preoperculum groove	0.63	0.65	0.61	0.62	0.77	0.91	0.70	0.65	0.77	0.59	1.04	0.92	0.57	0.61	0.69	0.66	0.60	0.58	0.60	0.62	0.66	0.43	0.38	0.27
Gape width	1.33	1.38	1.06	1.13	1.28	1.52	1.12	1.29	1.40	1.07	1.90	1.67	1.27	1.16	1.14	1.51	1.25	1.40	1.09	1.28	1.29	0.93	0.93	0.62
Gape height	1.37	1.22	1.20	1.20	1.12	1.46	1.15	1.03	1.38	1.12	1.93	1.68	1.06	1.07	1.23	1.26	1.15	1.35	1.02	1.24	1.20	0.95	0.88	0.62
Upper jaw length	1.35	1.69	1.09	1.42	1.63	1.85	1.59	1.65	1.64	1.41	2.39	1.85	1.52	1.37	1.42	1.44	1.35	1.49	1.35	1.54	1.49	0.95	0.83	0.71
Lower jaw length	0.98	1.11	0.63	1.03	1.00	1.41	1.00	0.97	1.10	0.81	1.49	1.21	0.86	1.01	0.96	0.90	0.98	1.06	0.91	0.88	0.90	0.66	0.61	0.39
Anterior barbel length	0.73	0.61	0.46	0.50	0.69	0.75	0.59	0.63	0.84	0.61	0.90	0.83	0.72	0.54	0.59	0.67	0.77	0.66	0.56	0.69	0.67	0.40	0.42	ND
Posterior barbel length	0.73	0.81	0.52	0.74	0.69	1.00	0.72	0.73	0.90	0.51	1.97	0.70	0.83	0.73	0.80	0.91	0.84	0.64	0.73	0.88	0.82	0.71	0.46	0.32
Premaxilla - supraoccipital	1.87	2.45	1.99	2.23	2.20	2.12	2.17	2.29	2.33	2.05	2.86	2.55	1.59	1.73	1.70	1.70	0.78	1.73	1.41	1.74	1.64	1.06	1.14	0.79
Origin of pectoral - premaxilla	3.76	3.69	3.35	3.44	3.85	4.33	3.37	3.86	4.11	3.77	5.09	4.46	3.44	3.59	2.74	3.72	3.65	3.44	3.26	3.71	3.47	2.62	2.30	0.80
Max dorsal spine length	2.99	3.41	2.80	2.90	3.13	3.81	3.41	3.14	3.40	3.13	4.19	3.39	3.09	2.71	2.98	3.44	3.69	3.22	3.13	3.13	3.26	2.03	1.89	1.58
Dorsal fin base length	2.04	2.11	1.83	1.84	2.07	2.47	1.87	2.21	2.23	1.83	2.79	2.23	1.83	1.84	1.70	2.08	2.10	1.91	1.83	1.95	1.89	1.44	1.17	0.85
Anal fin base length	1.35	1.14	1.22	1.14	1.39	1.74	1.00	1.57	1.45	1.12	2.10	1.73	1.28	1.33	0.98	1.11	1.23	1.16	1.17	1.27	1.10	0.71	0.68	0.54
Max anal spine length	2.53	2.79	2.37	2.58	2.71	4.03	2.71	2.78	3.21	2.88	4.54	3.65	2.81	2.25	2.19	2.95	2.81	3.03	2.28	2.62	2.77	1.60	1.62	1.15
Pectoral fin length	3.19	3.17	3.02	2.87	3.49	3.95	2.96	3.31	3.67	3.37	4.48	3.65	3.15	2.97	2.70	3.12	3.18	3.10	2.80	3.13	3.11	2.07	1.86	1.36
Prepectoral length	3.69	4.04	3.38	3.56	4.13	4.51	3.81	4.06	4.39	4.04	5.56	4.74	3.71	3.63	3.51	3.68	3.73	3.55	3.38	3.83	3.64	2.69	2.40	1.82
Prepelvic length	7.98	8.22	7.18	7.41	8.54	10.01	7.77	8.39	9.13	7.50	11.51	9.79	7.71	7.83	6.98	7.84	8.11	7.59	7.32	7.82	7.78	5.67	4.81	3.54
Preanal length	11.24	11.63	10.30	10.94	12.17	14.61	11.07	12.07	12.73	11.16	16.56	14.13	11.02	11.27	10.12	11.62	11.73	11.13	10.54	11.13	11.22	8.01	6.96	5.03
Anterior pelvic - origin of pectoral	4.33	4.27	3.76	3.88	4.56	5.50	4.14	4.52	5.09	3.98	6.28	5.49	4.14	4.17	3.63	4.16	4.27	4.03	4.01	4.02	4.37	2.98	2.17	1.86
Origin of pectoral - supraoccipital	3.16	3.10	2.63	2.70	3.33	3.67	2.84	3.18	3.35	2.96	4.33	3.49	3.00	2.88	2.72	2.97	2.81	2.79	2.67	2.99	2.84	2.23	1.81	1.41
Supraoccipital - origin of dorsal fin	6.52	5.92	5.23	5.85	6.50	8.11	6.05	6.43	7.29	5.71	9.30	7.24	6.24	6.37	5.71	6.06	5.92	5.66	5.79	6.21	6.04	4.38	4.03	2.71
Dorsal fin origin - pelvic origin	3.76	3.99	3.25	3.25	3.92	4.65	3.52	3.95	4.23	3.49	5.33	4.52	3.41	3.29	3.40	3.82	3.78	3.59	3.15	3.61	3.45	2.61	2.20	1.39
Dorsal fin origin - pectoral origin	5.26	5.28	4.25	4.58	5.49	6.61	4.77	5.33	5.84	4.80	7.29	6.11	4.83	4.79	4.54	5.19	5.11	4.84	4.73	5.11	4.97	3.36	2.97	2.08
Supraoccipital - pelvic origin	6.94	6.73	5.93	6.21	7.36	8.13	6.16	7.03	7.64	6.35	9.59	8.25	6.33	6.59	5.91	6.62	6.52	6.37	5.98	6.41	6.77	4.87	4.02	2.98
Anterior pelvic - post anal	4.59	5.11	4.63	4.57	5.58	5.94	4.52	5.45	5.50	4.48	6.78	5.96	4.69	5.03	4.30	5.05	4.73	4.78	4.56	4.90	4.86	3.30	3.00	2.18
Post anal - post dorsal	3.99	4.13	3.64	3.75	4.13	4.89	3.73	4.05	4.31	3.85	5.80	4.78	3.69	3.55	3.29	4.06	3.70	3.69	3.51	3.72	3.39	2.70	2.30	1.58
Pelvic fin base length	0.76	0.79	0.69	0.69	0.76	0.90	0.85	0.76	0.78	0.70	1.01	0.77	0.70	0.67	0.70	0.79	0.71	0.71	0.61	0.69	0.79	0.54	0.44	0.31
Post pelvic - post dorsal	3.67	3.73	3.01	3.37	3.73	2.70	1.94	3.76	4.16	3.38	2.99	4.24	3.41	3.06	3.28	3.68	3.59	3.38	3.08	3.58	3.45	2.42	2.07	1.37
Body width (before dorsal fin)	1.97	2.12	1.78	1.94	2.39	4.47	3.26	1.97	2.24	1.86	5.04	2.50	2.07	1.76	1.64	1.94	1.95	1.83	1.64	2.00	1.79	1.36	1.09	0.67
Caudal peduncle length	3.47	3.33	2.76	2.91	3.76	3.78	3.15	3.32	3.73	3.13	4.52	3.68	3.01	2.96	2.39	3.26	3.11	2.95	2.67	3.48	2.62	2.21	1.91	1.33
Caudal peduncle dept	1.67	1.79	1.46	1.55	1.66	1.81	1.57	1.69	1.85	1.65	2.12	1.93	1.56	1.54	1.46	1.72	1.58	1.61	1.46	1.75	1.58	1.16	1.02	0.67
Caudal peducle width	0.66	0.69	0.65	0.62	0.66	0.86	0.64	0.72	0.69	0.54	0.77	0.69	0.57	0.52	0.46	0.68	0.64	0.56	0.55	0.61	0.59	0.31	0.35	0.10
Caudal base length	2.08	2.08	1.83	1.86	2.22	2.35	1.81	2.09	2.32	1.96	2.84	2.29	1.84	1.75	1.67	2.03	1.96	1.85	1.77	1.83	1.88	1.35	1.29	0.78
Upper ray length	4.39	4.08	4.11	4.00	4.14	4.90	4.07	4.33	5.01	4.39	5.77	4.39	ND	3.85	4.10	4.15	4.04	ND	4.41	4.01	4.58	2.90	ND	2.07
Lower ray length	4.22	4.29	4.07	3.92	4.11	5.14	3.78	3.78	4.88	4.21	ND	4.83	4.17	3.78	3.83	3.97	4.21	4.27	ND	4.13	4.48	2.73	2.81	ND
Caudal fin max height	5.87	5.12	5.52	5.17	4.68	6.50	4.95	4.95	5.91	4.08	ND	5.35	ND	5.63	6.41	4.78	5.21	ND	4.07	4.81	5.37	3.55	3.30	ND
Post dorsal - caudal peduncle	4.43	4.51	2.91	3.85	5.07	5.10	4.38	4.56	4.98	4.24	6.58	5.56	4.08	4.01	3.77	4.63	3.96	4.08	3.79	4.34	4.42	3.27	2.72	1.73
Post anal - caudal peduncle	1.86	1.70	0.90	1.17	1.54	1.82	1.53	1.45	2.04	1.32	2.36	1.59	0.91	1.26	1.04	1.67	1.40	1.42	1.05	1.70	1.26	1.08	0.86	0.49
Head length	3.70	3.83	3.21	3.46	3.92	4.32	3.65	3.97	4.30	3.70	5.40	4.50	3.47	3.67	3.46	3.61	3.63	3.52	3.28	3.80	3.57	2.60	2.31	1.78
Eye diameter	0.79	0.82	0.80	0.73	0.81	0.97	0.81	0.82	0.94	0.77	1.04	0.88	0.76	0.83	0.75	0.82	0.87	0.81	0.74	0.80	0.74	0.65	0.57	0.51
Snout length	1.19	1.27	1.04	1.11	1.38	1.45	1.16	1.39	1.31	1.16	1.83	1.51	0.96	0.83	0.91	0.87	0.79	1.10	0.98	0.84	0.97	0.65	0.52	0.42
Orbital preoperculum groove	0.63	0.65	0.61	0.62	0.77	0.91	0.70	0.65	0.77	0.59	1.04	0.92	0.57	0.61	0.69	0.66	0.60	0.58	0.60	0.62	0.66	0.43	0.38	0.27
Gape width	1.33	1.38	1.06	1.13	1.28	1.52	1.12	1.29	1.40	1.07	1.90	1.67	1.27	1.16	1.14	1.51	1.25	1.40	1.09	1.28	1.29	0.93	0.93	0.62
Gape height	1.37	1.22	1.20	1.20	1.12	1.46	1.15	1.03	1.38	1.12	1.93	1.68	1.06	1.07	1.23	1.26	1.15	1.35	1.02	1.24	1.20	0.95	0.88	0.62
Upper jaw length	1.35	1.69	1.09	1.42	1.63	1.85	1.59	1.65	1.64	1.41	2.39	1.85	1.52	1.37	1.42	1.44	1.35	1.49	1.35	1.54	1.49	0.95	0.83	0.71
Lower jaw length	0.98	1.11	0.63	1.03	1.00	1.41	1.00	0.97	1.10	0.81	1.49	1.21	0.86	1.01	0.96	0.90	0.98	1.06	0.91	0.88	0.90	0.66	0.61	0.39
Anterior barbel length	0.73	0.61	0.46	0.50	0.69	0.75	0.59	0.63	0.84	0.61	0.90	0.83	0.72	0.54	0.59	0.67	0.77	0.66	0.56	0.69	0.67	0.40	0.42	ND
Posterior barbel length	0.73	0.81	0.52	0.74	0.69	1.00	0.72	0.73	0.90	0.51	1.97	0.70	0.83	0.73	0.80	0.91	0.84	0.64	0.73	0.88	0.82	0.71	0.46	0.32
Premaxilla - supraoccipital	1.87	2.45	1.99	2.23	2.20	2.12	2.17	2.29	2.33	2.05	2.86	2.55	1.59	1.73	1.70	1.70	0.78	1.73	1.41	1.74	1.64	1.06	1.14	0.79

Appendix H: Overview of the descriptive statistical assessment of the metals found in the muscle of the five *Labeobarbus polylepis* populations included in this study.

Metals	Population	N	Mean	SD	Std. Error	95% Confidence Intervals		Minimum	Maximum
						Lower Bound	Upper Bound		
Al	Elands	19	14.817	16.306	3.741	6.958	22.677	0.02	59.17
	Komati	20	9.713	9.700	2.169	5.173	14.252	1.09	45.16
	Assegai	20	16.979	17.362	3.882	8.853	25.105	1.31	65.51
	Phongola	20	22.794	17.021	3.806	14.827	30.760	3.95	74.96
	Ngodwana	17	3.327	2.192	0.532	2.200	4.454	0.96	10.19
Cd	Elands	19	0.228	0.145	0.033	0.158	0.299	0.03	0.54
	Komati	20	0.303	0.143	0.032	0.236	0.369	0.09	0.58
	Assegai	20	0.247	0.150	0.034	0.177	0.317	0.02	0.61
	Phongola	20	0.137	0.168	0.038	0.058	0.216	0.02	0.76
	Ngodwana	17	0.231	0.116	0.028	0.172	0.291	0.1	0.56
Cr	Elands	19	0.169	0.122	0.028	0.110	0.228	0	0.56
	Komati	20	0.200	0.157	0.035	0.126	0.273	0.03	0.68
	Assegai	20	0.201	0.314	0.070	0.054	0.348	0	1.31
	Phongola	20	0.535	0.374	0.084	0.360	0.709	0.05	1.59
	Ngodwana	17	0.217	0.258	0.063	0.084	0.350	0	0.76
Cu	Elands	19	4.303	5.127	1.176	1.832	6.774	0.25	17.14
	Komati	20	14.682	12.792	2.860	8.695	20.668	0.82	38.32
	Assegai	20	10.341	10.739	2.401	5.315	15.366	0.14	41.17
	Phongola	20	0.659	0.512	0.115	0.419	0.898	0.04	1.74
	Ngodwana	17	11.655	10.644	2.582	6.183	17.128	0.63	39.03
Fe	Elands	19	54.164	54.529	12.510	27.882	80.446	0.27	198.58
	Komati	20	71.910	53.037	11.859	47.087	96.732	2.08	161.66
	Assegai	20	157.263	182.487	40.805	71.856	242.670	2.74	640.65
	Phongola	20	22.460	44.184	9.880	1.781	43.138	0.55	196.94
	Ngodwana	17	75.242	54.358	13.184	47.293	103.190	8.89	169.72
Mn	Elands	19	1.859	1.382	0.317	1.193	2.526	0	4.98
	Komati	19	1.149	0.714	0.164	0.805	1.494	0	2.53
	Assegai	20	0.636	0.575	0.129	0.367	0.905	0.11	2.08
	Phongola	20	0.425	0.368	0.082	0.252	0.597	0.06	1.23
	Ngodwana	17	0.776	0.492	0.119	0.524	1.029	0.12	1.77
Ni	Elands	19	0.298	0.155	0.035	0.224	0.373	0.06	0.63
	Komati	20	0.357	0.164	0.037	0.280	0.433	0.16	0.73
	Assegai	20	0.282	0.216	0.048	0.181	0.383	0.04	0.82
	Phongola	20	1.838	1.049	0.235	1.347	2.328	0.13	3.53
	Ngodwana	17	0.271	0.117	0.028	0.211	0.331	0.12	0.52
Pb	Elands	19	0.351	0.226	0.052	0.242	0.460	0.03	0.71
	Komati	20	0.461	0.168	0.038	0.382	0.540	0.19	0.73
	Assegai	20	0.313	0.227	0.051	0.207	0.419	0.04	0.93
	Phongola	20	9.717	6.671	1.492	6.595	12.839	0.04	24.5
	Ngodwana	17	0.315	0.239	0.058	0.192	0.438	0.02	0.82
Zn	Elands	19	12.874	11.045	2.534	7.551	18.198	0.03	37.05
	Komati	20	17.932	11.824	2.644	12.398	23.465	0.65	41.15
	Assegai	20	17.712	18.976	4.243	8.831	26.593	1.31	76.85
	Phongola	20	7.843	6.751	1.510	4.683	11.002	1.64	24.23
	Ngodwana	17	11.372	10.037	2.434	6.211	16.532	0.02	36.43

Appendix I: Overview of the descriptive statistical assessment of the metals found in the livers of the five *Labeobarbus polylepis* populations included in this study.

Metals	Population	N	Mean	SD	Std. Error	95% Confidence Intervals		Minimum	Maximum
						Lower Bound	Upper Bound		
Al	Elands	19	18.141	21.270	4.880	7.889	28.393	3.34	79.59
	Komati	20	1.387	1.842	0.412	0.525	2.249	0.11	5.88
	Assegai	19	82.033	159.713	36.641	5.054	159.012	1.69	567.86
	Phongola	19	3.345	2.629	0.603	2.078	4.612	0.59	9.19
	Ngodwana	19	12.401	10.604	2.433	7.289	17.512	3.04	49.96
Cd	Elands	19	1.321	2.300	0.528	0.212	2.430	0.21	10.33
	Komati	20	0.183	0.243	0.054	0.069	0.297	0.00	0.98
	Assegai	19	0.864	1.097	0.252	0.335	1.392	0.08	3.85
	Phongola	19	0.127	0.071	0.016	0.092	0.161	0.01	0.30
	Ngodwana	19	0.205	0.446	0.102	-0.009	0.420	0.00	1.69
Cr	Elands	19	0.828	0.997	0.229	0.348	1.309	0.15	4.59
	Komati	20	0.614	1.268	0.284	0.020	1.208	0.06	5.88
	Assegai	19	0.609	0.767	0.176	0.239	0.979	0.00	2.95
	Phongola	19	0.371	0.879	0.202	-0.053	0.794	0.01	3.93
	Ngodwana	19	0.919	1.211	0.278	0.335	1.503	0.17	5.62
Cu	Elands	19	37.697	38.044	8.728	19.360	56.034	0.60	126.86
	Komati	20	3.915	12.477	2.790	-1.925	9.754	0.07	56.75
	Assegai	19	12.670	20.658	4.739	2.713	22.627	0.76	66.96
	Phongola	19	3.076	7.275	1.669	-0.430	6.583	0.21	31.82
	Ngodwana	19	12.373	32.843	7.535	-3.457	28.203	0.33	142.08
Fe	Elands	19	449.847	642.478	147.395	140.183	759.512	0.60	2536.35
	Komati	20	60.781	258.855	57.882	-60.367	181.929	0.00	1160.34
	Assegai	19	313.107	545.314	125.104	50.274	575.940	0.22	1701.54
	Phongola	19	38.173	81.503	18.698	-1.111	77.456	0.27	366.30
	Ngodwana	19	1.593	2.540	0.583	0.368	2.817	0.00	11.09
Mn	Elands	19	4.997	6.498	1.491	1.866	8.129	0.43	28.32
	Komati	20	0.906	3.234	0.723	-0.608	2.419	0.00	14.50
	Assegai	19	3.004	3.586	0.823	1.276	4.733	0.09	11.03
	Phongola	19	0.441	0.622	0.143	0.141	0.741	0.10	2.84
	Ngodwana	19	1.181	1.051	0.241	0.675	1.687	0.15	4.49
Ni	Elands	19	0.912	1.252	0.287	0.309	1.516	0.16	5.74
	Komati	20	0.204	0.233	0.052	0.095	0.313	0.01	0.98
	Assegai	19	0.970	1.011	0.232	0.483	1.457	0.08	3.29
	Phongola	19	0.211	0.143	0.033	0.142	0.280	0.06	0.55
	Ngodwana	19	0.874	1.595	0.366	0.105	1.643	0.17	7.30
Pb	Elands	19	1.134	1.651	0.379	0.339	1.930	0.14	7.65
	Komati	20	0.658	1.047	0.234	0.168	1.148	0.07	4.90
	Assegai	19	1.903	1.843	0.423	1.015	2.792	0.06	6.59
	Phongola	19	0.659	1.028	0.236	0.164	1.155	0.00	4.80
	Ngodwana	19	1.537	3.589	0.823	-0.193	3.267	0.22	16.29
Zn	Elands	19	69.454	74.805	17.162	33.399	105.509	0.06	317.26
	Komati	20	4.138	15.316	3.425	-3.030	11.306	0.00	68.86
	Assegai	19	80.516	142.816	32.764	11.681	149.351	2.46	493.27
	Phongola	19	20.871	14.873	3.412	13.703	28.039	3.61	70.62
	Ngodwana	19	100.131	111.429	25.563	46.424	153.837	6.44	480.90

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