ASPECTS OF THE BIOLOGY OF THE BUSHVELD SMALLSCALE YELLOWFISH (*LABEOBARBUS POLYLEPIS*): FEEDING BIOLOGY AND METAL BIOACCUMULATION IN FIVE POPULATIONS.

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SUMMARY

The general conservation status of freshwater ecosystems worldwide is poor and continues to decline at a rapid rate. This decline is a result of severe alteration of freshwater ecosystems caused by human activities. With an ever increasing human population as well as economic development, an increase in the demand for water is inevitable, as well as an increase in pollution to freshwater ecosystems. The sectors which are responsible for this are the domestic, agricultural, recreational and industrial sectors as they all depend on fresh flowing water. Aquatic ecosystems are heavily degraded on a global level by these human activities and impacts. South Africa is no different to the rest of the world. The quality of water in South African river systems has deteriorated due to increasing industrial, mining and agricultural activities in the catchments. Fish are often used as an indicator for ecological integrity as they are long-lived and therefore a good indicator of long-term exposure. Relatively little is known about *Labeobarbus polylepis* (Small-scale yellowfish) with reference as an indicator species as well as its feeding habits. This study consists of two components, namely the bioaccumulation of heavy metals by *L. polylepis* and the feeding biology of *L. polylepis.* Each of these components will be completed separate from one another.

The first component of this study was the assessment of the water quality from five river systems in South Africa, namely the Phongola, Assegaai, Elands and Komati rivers, as well as the Ngodwana Dam. The aims for this component of the study were to evaluate the overall health of the five different populations of *L. polylepis* and to obtain site specific metal bioaccumulation data for each locality. Suggestions and proposals for future monitoring and management of these waterbodies were also made.

The *L. polylepis* populations within these five systems were assessed, as it is believed that fish are a good indicator species for environmental conditions. The fish were sampled through the use of gill nets. The different tissues (liver and muscle) of *L. polylepis* bioaccumulated varying concentrations of the selected metals (Al, Cd, Cr, Cu, Mn, Ni, Fe, Pb, Zn) and it was apparent which tissue accumulated higher concentrations of a specific metal. The liver and kidneys of fish assist with the detoxification or elimination of toxic substances from the body, as a result, accumulated metals are concentrated here. The fish muscle is abundant on the fish

and can therefore be used to determine the human health risk if the fish are eaten. The liver samples generally showed higher concentrations than the concentrations found in the muscle samples. Muscle should always be included in biomonitoring programmes because it is the edible part of the fish and may result in metal toxicosis in humans. In addition to this, liver should be incorporated in all general biomonitoring programmes when assessing the extent of bioaccumulation of metals in fish. None of these metals appeared to have significantly high concentrations in any of the five populations. In comparisons made to other project conducted on other fish species, the metal concentrations found in this study were lower than those concentrations found in other projects.

In conclusion, this baseline study has determined the metal concentrations in the aquatic environment (owing to anthropogenic activities) that may be accumulated by fish populations and may affect their general health. Monitoring of these study areas should be continued, using results obtained from this study as a reference for the assessment of possible changes in the quality of the water of these five systems.

The second component of this study focuses on the feeding biology of *L. polylepis.* Generally very little is known about the feeding habits of *L. polylepis*. The primary diet of *L. polylepis* has been described as a combination of algae (winter months) and aquatic insects (summer months), but that *L. polylepis* also was able to take to snails, mussels, crabs and small fish. As a result of the prescribed feeding biology, speculation into its feeding habits described *L. polylepis* as an all round omnivore. The stomachs of the sampled fish were dissected and the contents removed and then identified. Food items were categorised into invertebrate taxa, detritus, fish remains and unidentifiable components. It was evident from the sampled stomach contents that *L. polylepis* feeds off the bottom, this is confirmed with the presence of corbiculidae and gomphidae in the diet. *L. polylepis* is most likely to feed on the algae found on the bottom or on other surfaces. It may also be concluded that algae and detritus are consumed as *L. polylepis* attempts to dig for food items. The consistent food variation within the diets per population suggests that *L. polylepis* is an opportunistic feeder with the ability to change it's diet in accordance with the availability of food. It can be concluded that *L. polylepis* feeds on algae in the quiet pools during the winter months, because most of the plant material available consists mainly of algae. During the summer months when more water is available in the systems, algae is less abundant and as a result, *L. polylepis* feeds on invertebrates as well as other small aquatic animals.

This study has provided a baseline understanding of the feeding habits and food preferences for *L. polylepis* found in five different systems, similar as well as different habitats at a similar sampling period (April 2006).

OPSOMMING

Die algemene bewaringstatus van varswater ekosisteme wêreldwyd is swak en voortdurende agteruitgang vind plaas teen 'n vinnige tempo. Hierdie agteruitgang is as gevolg van drastiese veranderinge aan varswater ekosisteme wat deur menslike aktiwiteite veroorsaak word. 'n Toename in die aanvraag na water asook die besoedeling van varswater ekosisteme is onafwendbaar as gevolg van die toename in menslike bevolkingsgetalle en ekonomiese groei en ontwikkelling. Die sektore wat verantwoordelik is vir die agteruitgang van die ekosisteme is meestel huishoudelik-, landbou-, industrieel- en die ontspanningssektore as gevolg van hul behoefte aan vars lopende water. Die genoemde menslike aktiwiteite en impakte veroorsaak dat akwatiese ekosisteme agteruit gaan op 'n wêreldwye vlak. Suid-Afrika is geen uitsondering nie. Die kwaliteit van die water in die Suid-Afrikaanse rivierstelsels het versleg as gevolg van die toename in industriële-, mynbou- en landbou- aktiwiteite in die onderskeie opvangsgebiede. Visse is meestal 'n goeie maatstaf vir die ekologiese integriteit aangesien hul leeftyd langer is en dus lang termyn blootstelling ontvang. *Labeobarbus polylepis* (Kleinskub geelvis) is relatief onbekend met verwysing na aanduider spesies asook hul voedings gewoontes. Hierdie studie bestaan uit twee komponente, nl. die bio-akkumulasie van swaar metale in *L. polylepis* en die voedingsbiologie van *L. polylepis*. Die onderskeie komponente sal appart voltooi word.

Die eerste komponent het bestaan uit die assessering van die kwaliteit van die water in vyf rivier stelsels in Suid-Afrika. Hierdie stelsels sluit in die Pongola-, Assegaai-, Elands-, en Komatiriviere asook die Ngodwana Dam. Die doel van hierdie komponent van die studie was om die algemene gesondheid van die vyf verskillende bevolkings *L. polylepis* te evalueer en om die metaal bio-akkumulaise data vir elke punt te bepaal. Voorstelle aangaande monitering en bestuur van die stelsels is ook gemaak.

Die *L. polylepis* bevolkings in elke sisteem is getoets aangesien visse as goeie indikator spesies van natuurlike kondisies gesien word. Kieu-nette is gebruik om die visse mee te vang. Verskillende konsentrasies van die gekose metale (Al, Cd, Cr, Cu, Mn, Ni, Fe, Pb en Zn) is gevind in verskillende weefsel (lewer en spier) van *L. polylepis*. Daar was 'n duidelike verskil tussen die akkumulasie van die verskeie metale in die verskeie weefsel. Die lewer en niere is toegerus om 'n vis te help om

van gifstowwe in die liggaam ontslae te raak. Dit veroorsaak dat die metale in die organe versamel. Die spiermassa van die vis beslaan die grootste gedeelte van die vis en is dus ‘n aanduiding van die gesondheidsrisiko betrokke indien mense dit sou eet. Hoër konsentrasies is oor die algemeen in die lewer monsters gevind as in die van die spiere. Die spierweefsel moet altyd in moniteringsprogrmme ingesluit word aangesien dit die eetbare deel van die vis verteenwoordig en metaal toxicosis tot gevolg kan hê. Wanneer daar na die graad van metaal bio-akkumulasie in visse wil gekyk word moet die lewer altyd ingesluit word in die moniteringsprogram. Nie een van die metale het beduidende hoë konsentrasies in die vyf bevolkings gehad nie. Tydens ‘n vergelyking met ander projekte wat op ander visspesies uitgevoer is, is die metaal konsentrasies in hierdie studie laer as die van die ander projekte.

Tydens die grondlyn studie is die metaal konsentrasies teenwoordig in die akwatiese omgewing (a.g.v. antropogeniese aktiwiteite) bepaal wat moontlik kan versamel in visbevolkings en hul gesondheid kan beinvloed.

Die tweede komponent van die studie fokus op die voedingsbiologies van *L. polylepis*. Oor die algemeen is baie min bekend aangaande die voedingsgewoontes van *L polylepis*. Die primêre diëet van *L. polylepis* word beskryf as ‘n kombinasie van alge (winter maande) en akwatiese insekte (somer maande), maar dat *L. polylepis* ook soms slakke, mossels, krappe en klein vissies eet. As gevolg van die genoemde eetgewoontes word *L. polylepis* as ‘n omnivoor beskryf. Die mae van die visse is gedissekteer en die inhoud is verwyder en geïdentifiseer. Die inhoud is gekategoriseer volgens invertebrate taxa, gruis, vis oorblyfsels en onidentifiseerbare komponente. Die teenwoordigheid van onder andere Corbiculidae en Gomphidae is bewys van die feit dat *L. polylepis* op die bodem voed. *L. polylepis* voed meestal op alge wat op die bodem of ander oppervlaktes voorkom. Nog ‘n afleiding wat gemaak kan word is dat alge en gruis ingeneem word wanneer daar na kos gegrou word vir ander voedsel. Die variasie in die dieet van die verskeie bevolkings is ‘n aanduiding dat *L. polylepis* opportunistiese voeders is met die vermoë om hul dieet te verander volgens die beskikbaarheid van kos. Daar kan ook afgelei word dat *L. polylepis* tydens wintermaande in stil poele op die alge voed aangesien die plant materiaal wat beskikbaar is meestal bestaan uit alge. Gedurende die somermaande wanneer meer water beskikbaar is, is alge nie so geredelik beskikbaar nie. *L. polylepis* voed dan op invertebrate en ander klein akwatiese diere.

Hierdie studie het basiese inligting aangaande die voedingsgewoontes en voedselvoorkeure van *L. polylepis* wat in vyf verskillende sisteme voorkom met ooreenstemmende sowel as verskillende habitatte tydens dieselfde versamelingsperiode (2006).

CHAPTER 1

1 General Introduction

1.1 Introduction

This study has is a component of a larger study being undertaken by a team of researchers. Taking this into consideration, this study as well as the complementary studies, which together will address the biology and ecology of the five populations of the Bushveld Smallscale yellowfish (*Labeobarbus polylepis*). This study specifically addresses two tasks, firstly an assessment of the bioaccumulation of heavy metals of five populations of the Bushveld Smallscale yellowfish (*L. polylepis*) and secondly an assessment of the feeding biology of five populations of the Bushveld Smallscale yellowfish (*L. polylepis*) from Mpumalanga, South Africa. These components have been undertaken separately in this study and are thus presented separately.

Assessment of the bioaccumulation of heavy metals of five populations of the Bushveld Smallscale yellowfish (L. polylepis).

The quality of water in South African river systems has deteriorated due to increasing industrial, mining and agricultural activities in the catchments (State of Rivers Report, 2001) This study was undertaken to assess the water quality from five river systems in South Africa, namely the Phongola and Assegaai rivers in the Phongolo River Catchment and the Elands and Komati rivers, as well as the Ngodwana Dam from the Inkomati River Catchment. The aim for this, the first component of the study was to evaluate the general health of the five different populations of *L. polylepis* and to obtain site specific metal bioaccumulation data for each population from each locality considered in the study. Suggestions and proposals for future monitoring and management of these waterbodies were also made.

In studies conducted by Phillips (1980) the ideal response of an indicator to environmental pollutants was illustrated: “The tissue concentration is a definite function of the environmental level and duration of exposure, yet when contaminant levels are lowered the body burden is also reduced.” Many factors govern the efficacy of indicators in presenting an estimate of prevailing environmental concentrations of substances (Groenewald, 2000). According to Hellawell (1986) some of the factors are not considered to be independent of one another, such as physiological condition and age; size and sexual maturity.

As the liver and kidneys of fish assist with the detoxification or elimination of toxic substances from the body, accumulated metals are concentrated here (El-Domiaty, 1987, Manahan, 1989, Ray et al., 1990). The organs/tissues within the body of an organism may act as temporary or permanent dumping sites (Groenewald, 2000). According to Groenewald (2000) bioaccumulation would most likely not occur if metals were eliminated rapidly, thus reducing the possibility of damage to the tissue. Biotransformation may occur in order to eliminate pollutants and this will result in the rates and routes of elimination of the metabolites being potentially different from the original pollutant (Spacie and Hamelink, 1985).

According to Heath and Claassen (1999) the fish muscle used is abundant on the fish and can therefore be used to determine the human health risk if the fish are eaten. The liver is a detoxicanting organ and has high loads of zinc, copper iron and cadmium, most fish livers are easy to dissect as a single organ and are large enough to supply sufficient tissue for metal determination (Heath and Claassen, 1999)

In conclusion, this baseline study has determined the metal concentrations in the quatic environment (owing to anthropogenic activities) that may be accumulated by fish populations and may affect their general health. Monitoring of these study areas should be continued, using results obtained from this study as a reference for the assessment of possible changes in the quality of the water of these five systems.

Assessment of the feeding biology of five populations of the Bushveld Smallscale yellowfish (*L. polylepis*).

The second component of this study focuses on the feeding biology of *L. polylepis.* Generally very little is known about the feeding habits of the Bushveld Smallscale Yellowfish (*Labeobarbus polylepis*). The primary diet of *L. polylepis* was described by Skelton (2001) as a combination of algae (winter months) and aquatic insects (summer months), but that *L. polylepis* was able to take to snails, mussels, crabs and small fish. As a result of this prescribed feeding biology, speculation into its feeding habits described *L. polylepis* as an all round omnivore**.** According to Le Roux and Steyn (1968), *L. polylepis* feeds off of the bottom and is most likely too feed on the algae found on the bottom or on other surfaces. Gaigher (1969) suggests that *L.*

polylepis has the ability to change its diet in accordance with the availability of food and thus considers this species as an optional feeder. *L. polylepis* feeds on algae in the quiet pools during the winter months, because most of the plant material available consists mainly of algae. During the summer months when more water is available in the systems, algae are less abundant and as a result, *L. polylepis* feeds on invertebrates as well as other small aquatic animals. Greater portions of detritus are often consumed by *L. polylepis* in the form of rotten pieces of roots, stems and leaves during these seasons (Gaigher, 1969). Gaigher suggest the reason for the intake of detritus is that it is taken in as *L. polylepis* attempts to feed on other organisms. Other components which can make up the diet of *L. polylepis* include water living invertebrates like craps, mussels, snails and vertebrates like fish, amphibian larvae and even small birds are part of the diet.

This study has provided a baseline understanding of the feeding habits and food preferences for *L. polylepis* found in five different systems, similar as well as different habitats at a similar sampling period (April 2006).

1.2 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 and the non-characteristic habitat in which this population occurs. 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, 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 Assagaai 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*. The locations of the sampling sites and an overview of the sites are presented in Figure 1-1.

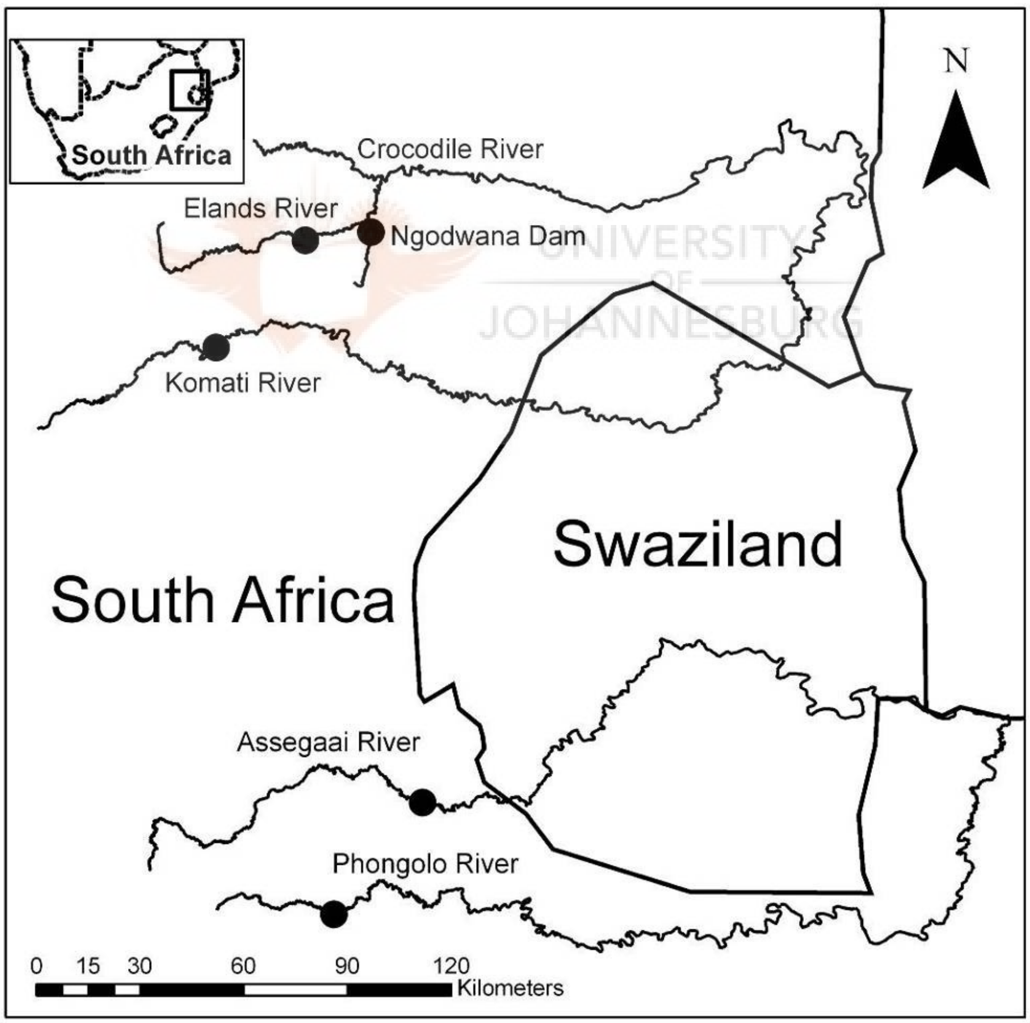

Figure 1-1. Graphical representation of the location of the sampling sites of the five *Labeobarbus polylepis* used in this study.

1.2.1 Collection

A minimum of twenty *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). Individuals where captured using an array of sampling techniques including seine nets, cast nets, electro-shocking, gill nets (mesh size 45mm – 95mm) 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.

System	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.*

1.3 Study Hypothesis

The study hypotheses established for this study concerns both the metal bioaccumulation component as well as the feeding biology component for all of the *L. polylepis* populations considered and are addressed separately. The study hypotheses for each component are as follows:

The research hypothesis for the metal accumulation component of the study has been established as: heavy metals will be accumulated by all of the populations of *L. polylepis* considered in this study and the levels of metals accumulated will be as a result of metals available in the environments.

Similarly the research hypothesis for the feeding biology component of this study has been established as: the diet composition assessment undertaken for *L. polylepis* in this study will indicate that this species is an insectivorous and a piscivourous predator.

1.4 Aim and Objectives

The aims and objectives of this study have been divided into two separate components. The first component of the study addresses the metal bioaccumulation of all the *L. polylepis* populations considered for the study. The second component of the study addresses the feeding biology of all the *L. polylepis* populations considered for the study. These two components will be addressed separately throughout the study. The metal bioaccumulation component will precede the feeding biology consistently throughout the study report. The aims of the two study components are as follows:

The aim of this component of the study was to determine the extent of metal bioaccumulation in *L. polylepis.* In order to achieve the aim the following objectives were set:

- Determine the extent of metal bioaccumulation the organs and tissues of *L. polylepis.*
- Determine the preferred order of bioaccumulation of the 9 selected metals in the liver and muscle of *L. polylepis.*

- Determine if there were any temporal differences in metal bioaccumulation between the selected sampling localities.

The aim of this chapter is 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. In order to achieve the aim the following objectives were set:

- Characterise the feeding biology of *L. polylepis* individuals
- Comparisons of inter-population and intra-population feeding characteristics.

1.5 Literature Review

According to Jackson and Coetzee (1982) large Cyprinids of the genus *Barbus* and *Labeo,* now including the recently established *Labeobarbus* genus are among the most valuable freshwater species in Africa. In addition to this, Jackson and Coetzee (1982) states that these fishes are greatly esteemed as a food source in most parts of tropical Africa, whereas in South Africa, only the two yellowfish species, namely *L. aeneus* and *L. kimberlyensis* that occur within the Orange-Vaal River catchment are among the most highly regarded of our indigenous freshwater angling species of South Africa (van Vuuren et al., 1989).

Yellowfish have been identified as a potentially important commercial species. According to FOSAF (2006), yellowfish are a highly valued angling species. This species is the basis upon which the Yellowfish flyfishing industry has been created. In an article published by Farmers Weekly (October, 2005), the angling tourism industry in the Vaal River has been valued at R1.2 billion per annum. Yellowfish have been provided as an alternative to alien angling species by the Conservation Departments (FOSAF, 2006).

According to Vlok (2000) a literature survey was compiled indicating how little information on yellowfish is published. The lack of research with regard to *Labeobarbus polylepis,* as well as the status of this species is however a concern that needs to receive urgent attention (Fouche, Angliss and Vlok, 2006). *Labeobarbus* is a distinctively big genus of carp-like (Cyprinus) fish with sucker like lips. The *Labeobarbus* genus is generally considered to be a cosmopolitan species as they are distributed all over South Africa. The mouth of the genus is terminal and with the point of the lower jaw directly beneath the beginning of the snout (Crass, 1964). In accordance with Jubb (1965; 1967), two different groups have been created from the nine *Labeobarbus* species, resulting from properties associated with the striations on the scales as well as with the fourth dorsal ray. The five *Labeobarbus* species comprising a single group include *Labeoarbus aeneus, Labeobarbus kimberleyensis, Labeobarbus marequensis, Labeobarbus polylepis,* en *Labeobarbus natalensis,* and they have characteristic smooth rays and length running striation on the scales (Skelton, 2001). *Labeobarbus marequensis* and *L. polylepis,* occur in the Limpopo province (Fouche, Angliss and Vlok, 2006). In a report about the status of the yellowfish populatons in the Gauteng province (2006), it had been stated that within the rivers of the three catchments within the boundaries of Gauteng Province, four yellow fish species occur. Namely *Labeobarbus marequensis* and *L. polylepis* occur naturally in the north-east flowing Crocodile–West / Marico System and the Olifants River (north east) systems whereas *L. kimberleyensis* and *L. aeneus* occur naturally in the westerly flowing Orange/Vaal River System. *L. aeneus* has not been found out of it's natural distribution in the Wilge River, Bronkhorstspruit or Elands Rivers in Gauteng to date, in spite of being erroneously introduced into dams in the upper tributaries of the Olifants River system in Mpumalanga during the 1970's. Although all five species still occur in the rivers of Gauteng, the increasing and rapid changes in land-use within these catchments may be a threat to populations of what already little is known about their integrity. Some of the increasing threats to these fish populations are water quality, fragmentation of habitat and migration routes as well as the destruction of spawning grounds and over utilization of the resource through subsistence and sport fishing.

Skelton (2001) indicates that *Labeobarbus polylepis* is a species which prefers the colder waters, as well as a relatively high altitude as they are not found at an altitude lower than 600m. *L. polylepis* has been known to prefer deep pools and flowing waters of permanent rivers and readily occurs in dams. This species occurs in the southern tributaries of the Limpopo system as well as in the, Incomati and Phongolo systems (Skelton, 2001). A large portion of the Limpopo Highveld as well as the south-western parts of Zimbabwe are drained by this river system. *L. polylepis* is also distributed in some of the tributaries of the Limpopo River, such as Groot Marico and the Crocodile tributaries, which all rise in the north-western Limpopo, as well as the Elands and the Olifant which drains central Limpopo. According to Jubb (1964), *L. polylepis* is not found in the tributaries of the Nuanetsi, Umwingwane and Tuli rivers, which all rise in Zimbabwe.

Crass (1964) suggest that *L. polylepis*, similarly with *L. natalensis,* is more limited to the upper course of the Natal rivers (Phongola system), but competition between *L. marequensis* in the lower regions of the rivers may be the causing factor for the limited distribution rather than ecological preference. The reason why *L. polylepis* does not develop the various mouth forms found in *L. marequensis* may possibly be attributed to interspecies competition. In accordance with Fouche, Angliss and Vlok (2006), the bushveld smallscale yellowfish, *Labeobarbus polylepis* does occur in the Limpopo province. The status of *L. polylepis* is considered to be extinct in the Letaba river system where the lowveld largescale yellowfish (*Labeobarbus marequensis*) is common.

Labeobarbus polylepis is identifiable because of the variable lips on the sub terminal mouth of the species as well as of the two pairs of barbells (Skelton, 2001).In a comparison between *L. polylepis, L. natalensis* and *L. marequensis,* the lower jaw of *L. natalensis* is usually shorter than *L. polylepis* and considerably shorter in *L. marequensis.*

The importance of *L. polylepis* is considerably significant in Mpumalanga as it is considered by the Mpumalanga Parks Board to be one of the most important species. This reason being that *L. polylepis* provides valuable aquatic ecosystem information which is then used to establish management scenarios of aquatic resources. In addition to all of this, angling enthusiasts target this species and as a result, many parts of the rivers within Mpumalanga are classed as fly-fishing waters. Natural geological formations which result in waterfalls act as boundaries for fish movement and as a result many *L. polylepis* populations within these rivers have historically been isolated. More recently however, due to primarily dams (e.g. the Nooitgedagt and Vygeboom dams), artificial boundaries exist which have isolated populations (Mulder *et al*., 2004). Crass (1964), Jubb (1967) and Mulder (1989) state that there is no 'rubber-lipped' variation within the *L. polylepis* species, but this 'rubber-lipped' variation was discovered by both Matthes (1963) and Du Plessis (1956) and again by Gaiger (1969), but only in the Elands River. This literature has resulted in a huge dispute over whether the *Varicorhinus* or 'rubber-lipped' variations actually exist within the species. These differences in the various populations of *L. polylepis* have recently been studied, and following new information from recent research projects (Austin *et. al.,* 2005) some long awaited biological components of the *L. polylepis* populations (Gaiger, 1969; Kleynhans, 1992) have been brought to light. In an honors project by Van der Bank and von Bratt (2004) the findings may suggest that the Elands River population of *L. polylepis* is genetically unique as it differs from other *L. polylepis* populations. The unpublished data from the masters project by Austin et al. (2005) has indicated that significant morphological differences between two *L. polylepis* populations occur.

This study comprises two independent components, namely an investigation into the metal bioaccumulation and an assessment of the feeding preferences of five populations of Bushveld-smallscale Yellowfish in South Africa. These two study components will be addressed separately throughout the study report with the metal bioaccumulation component preceding the feeding biology component consistently.

1.6 Metal Bioaccumulation

The rivers, streams and lakes of South Africa have been seriously affected by a number of factors (GWC Schulz and HJ Schoonbee, 1999). Mining and industry have been the cause of major pollutants which sometimes have irreversible changes and deterioration in the water quality and biology of such waters (Förstner and Prosi, 1979; Van Eeden and Schoonbee, 1996). As a result of an increase in industry, the aquatic environment is subjected to heavy and diverse pollutant loading (Ahokas et al., 1976). A need has arisen for an indication of what these impacts are placing on our rivers. Fish play an important role in that they are directly impacted by the conditions of the water in which they live. Fish as biological indicators can be of assistance in both the development of restoration and management concepts for rivers (Schiemer, 1994; Schiemer et al., 1999) Fish thus not only serve as a monitoring tool but also contribute practical value to the assessment of ecological integrity (Schiemer, 2000). The ecological conditions of a river can be indicated by fish because the various guilds integrate over their life cycle a wide range of riverine conditions (Copp, 1989; Gaudin, in press; Persat et al., 1995; Schiemer et al., 1991). All metals are found at varying levels in all ground and surface waters and are natural constituents of the environment (Martin and Coughtrey, 1982).

It has been documented that aquatic biota have the ability to accumulate pollutants such as metals and organic compounds (USEPA, 1991). The studies or methods monitoring the ability of fish organs and/or tissues to take up and retain pollutants such as metals or biocides is known as bioaccumulation (Roux, 1994). This can only be studied or monitored if the rate of elimination of the pollutants does not exceed their uptake (Spacie and Hamelink, 1985). The metals taken up may either be essential for bodily functions such as metabolism of aquatic organisms or non-essential and thus have no significant biological role (Prosi, 1979, Rainbow and White, 1989) The five potential routes for metals to enter a fish are via the food, non-food particles, gills, oral consumption of water and the skin (Nussey et al.,, 2000). The absorbed metals are transported to storage points (i.e. bone) via the blood or to the liver for transformation and/or storage ((Nussey et al., 2000). Pollutants transformed by the liver may be stored there, excreted by the bile or transferred back into the blood for possible excretion by either the gills or kidneys, or even stored in fat which is an extra hepatic-tissue (Heath, 1991). Thus, for a specific time after environmental exposure, the concentrations of metals found in different tissues depends on several dynamic processes all taking place concurrently ((Nussey et al., 2000). Metal uptake and toxicity in freshwater fish are largely influenced by the pH, Ca concentration, and alkalinity of the water (Alabaster and Lloyd 1980; Spry and Wiener 1991). For freshwater fish, the toxicity of heavy metals does not depend solely on the water chemistry, but also on temperature, the size and the condition of the organisms and the season (Shaw and Brown, 1974; Howarth and Sprague, 1978; Peres and Pihan, 1991; Vittozzi and De Angelis, 1991). The ability of metals to ultimately become toxic at some elevated levels can be attributed to the chemical characteristics of the metals (Rainbow, 1985). Abnormally high concentrations of non-essential metals have the ability to affect the organism's ability to excrete, sequester or otherwise detoxify themselves (Thorp et al, 1979).

Metals are readily dissolved in water and are taken up by aquatic organisms. Bioaccumulation is the process which causes an increased chemical concentration in an aquatic organism compared to that in water, due to uptake by all exposure. Bioaccumulation can thus be viewed as a combination of bioconcentration and food uptake. A high concentration of a metal in water does not involve direct toxicological risk to fish. Bioaccumulation is mediated to a large extent by both abiotic and biotic factors that influence metal uptake (Rajotte and Couture, 2003).

Metals are generally present in an oxidized form or are chemically bonded to proteins by strong bonds. This increases bioaccumulation of metals and reduces their excretion (Groenewald, 2000). Humans as well as other wildlife species consume fish as a dietary source, thus the monitoring of contamination of pollutants in fish provides a direct measure of the potential human and ecological health concerns associated with contaminant sources. The major problem however is that the fish are highly mobile and as such contamination cannot be related to a specific source.

In the aquatic environment metals can accumulate directly from the water and from dietary sources (Van Eeden, 1990; Maartens, 1994 and Mason, 1996). Maartens (1994) states that some authors believe uptake is from the diet while others believe it is from the water and others believe it is taken up from both sources. Both uptake routes can also occur simultaneously in the field (Maartens, 1994). According to Groenewald (2000), Van Eeden (1990) and Luus-Powell (1997) there are two routes by which metals can be accumulated, this in reference to the drinking of water and via the integument. As a result of this bio-accumulation, aquatic organisms are useful indicators of pollution (Luus-Powell, 1997). Heavy metals can be accumulated by aquatic organisms and then concentrated to amounts considerably higher than those found in the environment (Ferard et al., 1983). As a result of this, it is important to identify the pathways of accumulation of the heavy metals and their affinity to different tissues (Panchanathan and Vattapparumbil, 2006) Aquatic organisms can be used specifically to indicate chronic sub-lethal levels of metals in the environment, for which they are well suited (Barnhoorn, 1996). The more contaminated the water is the higher the metal concentrations will be in tissues of organisms (Luus-Powell, 1997 and Wepener 1997). Metals released into surface water accumulate in sediments through absorption and precipitation processes but can also be re-introduced in a bio-available form to fish and other aquatic biota through changing water quality (Coetzee, 1996).

Being exposed to elevated levels of metal concentrations found in an aquatic environment can then absorb the bioavailable metals directly from the environment, this absorption can take place via the gills and the skin or with the ingestion of contaminated food and water (Nussey, 1998). The blood stream then transports the metals and brings them into contact with various organs and tissues (Van der Putte and Part, 1982). According to Heath (1991), metal concentrations can be regulated by the fish to a certain extent, thereafter bioaccumulation will occur. Thus the ability of each organ to regulate or accumulate metals will be directly responsible for the total amount of metal accumulated in a specific organ and/or tissue (Nussey, 1998). In addition, the particular metal content can be influenced by physiological differences of each organ and tissue in the fish (Kotze, 1997)

1.7 Feeding Biology

This chapter focuses on the feeding biology of five separate populations of *Labeoberbus polylepis* from Mpumalanga. According to Mann (1992) the diet of some fish species can be analysed to determine the spatial distribution and ecological requirement of these species. Within freshwater fishes the greatest diversity of feeding types is among the benthivorous fishes (Gerking, 1994). A characteristic of these fishes are their sensorial appendices and inferior protractile mouths (Chao and Musick, 1977; Gerking, 1994). They prey on benthic invertebrates which are either near to or on the substrate, this feeding is done by burying the mouth into the substrate and swallowing part of the sediment (Hobson and Chess, 1986; Sazima, 1986; Soares *et al.*, 1993; Edgar and Shaw, 1995). According to Mann (1992) and Lechanteur and Griffiths (2002), indicated that the trophic structure of a fish community can be determined by the analysis of the diets of fish species within these communities. A dietary overlap of benthivorous fishes may be reduced owing to differences in behaviour, habitat and time of feeding, as well as the type and size of prey (Hobson and Chess, 1986; McCormick, 1995; Platell *et al.*, 1998).

Members of the Cyprinidae family form a rich diversified family, with feeding habits adapted to a broad variety of environments resulting in many specialisations. Within the family, the genus *Labeobarbus* has been described as comprising of facultative feeders, which have very distinct preferential diets but will readily adapt to another strategy if circumstances change (Matthes, 1963). This flexibility has been partly responsible for some misinterpretation in the literature concerning the diet of the genus *Labeobarbus.* It is through this study that we aim to get a better understanding of what the preferred diet of *L. polylepis* is.

CHAPTER 2

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

2.1 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. 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 and 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 liver and muscle tissues of *L. polylepis* and to determine if there were any temporal differences in metal bioaccumulation between the selected sampling localities.

2.2 Materials and methods

2.2.1 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, from the Assegaai River (Usutu Catchment), the Phongola River (Phongola Catcment) and the Elands and Komati Rivers and the Ngodwana Dam (all three from the Komati Catchment).

2.2.2 Field sampling

Twenty individual *L. polylepis* were sampled from the five different rivers between May 2006 and July 2006 using an array of sampling techniques which included seine nets, cast nets, electro-shocking, gill nets (mesh size 45mm – 95mm) 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 (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.

2.2.3 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 a 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 200µl 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 for 15 minutes at 10 – 40% power until the solutions appeared clear (fully digested) (Blust et al., 1988). After digestion, each of the samples was 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. The metal concentrations of each sample were calculated as follows:

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

2.2.4 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 $P<0.05$.

2.3 Results

The findings of the metal bioaccumulation experiment are presented here. The metal concentrations (Al, Cd, Cr, Cu, Fe, 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 2-1, Figure 2-2, Figure 2-3 and Figure 3-4. The summary statistics for all the bioaccumulation data are presented in Appendix A and Appendix B.

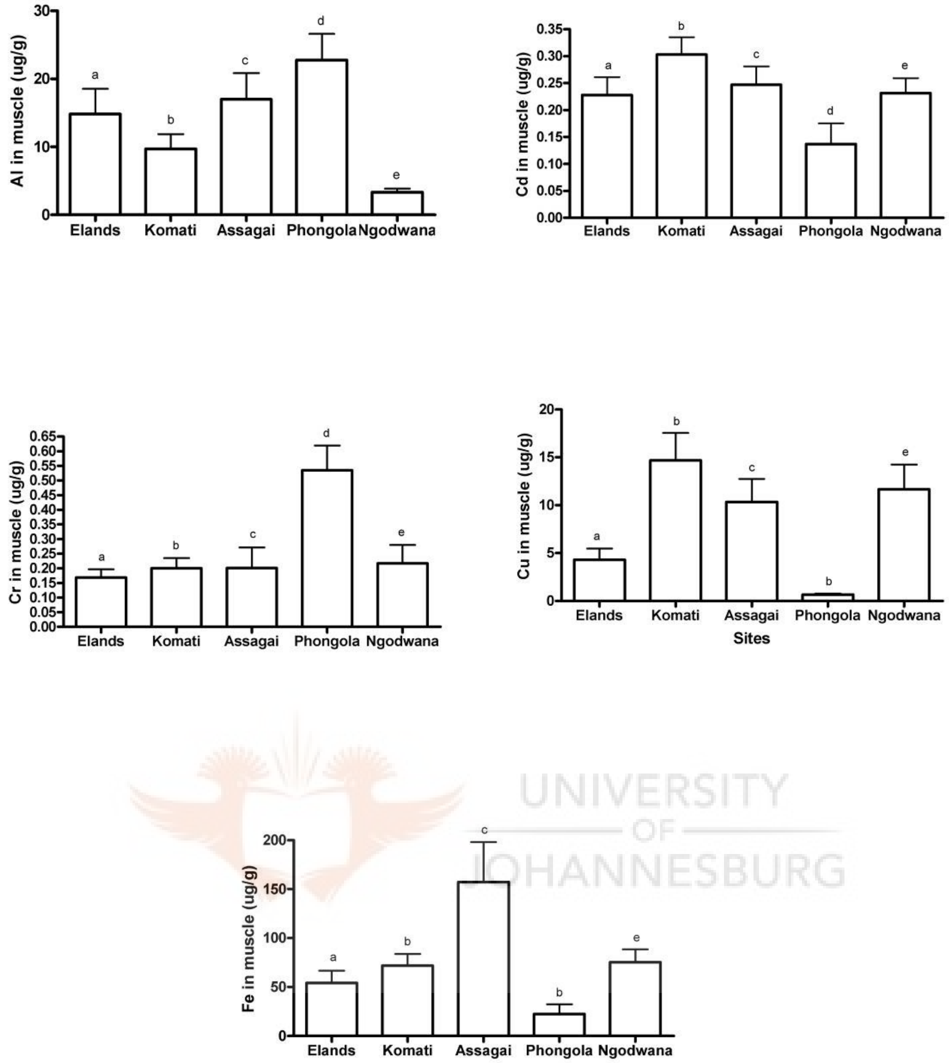

Figure 2-1: The mean metal concentration in the muscle from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant differences (P< 0.05).

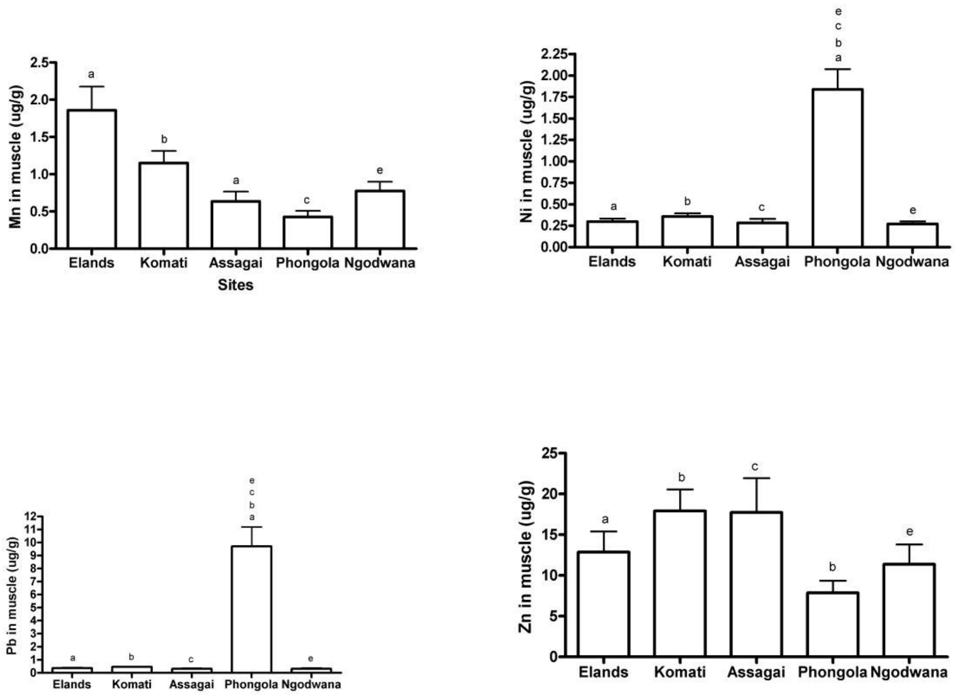

Figure 2-2: The mean metal concentration in the muscle from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant differences (P< 0.05).

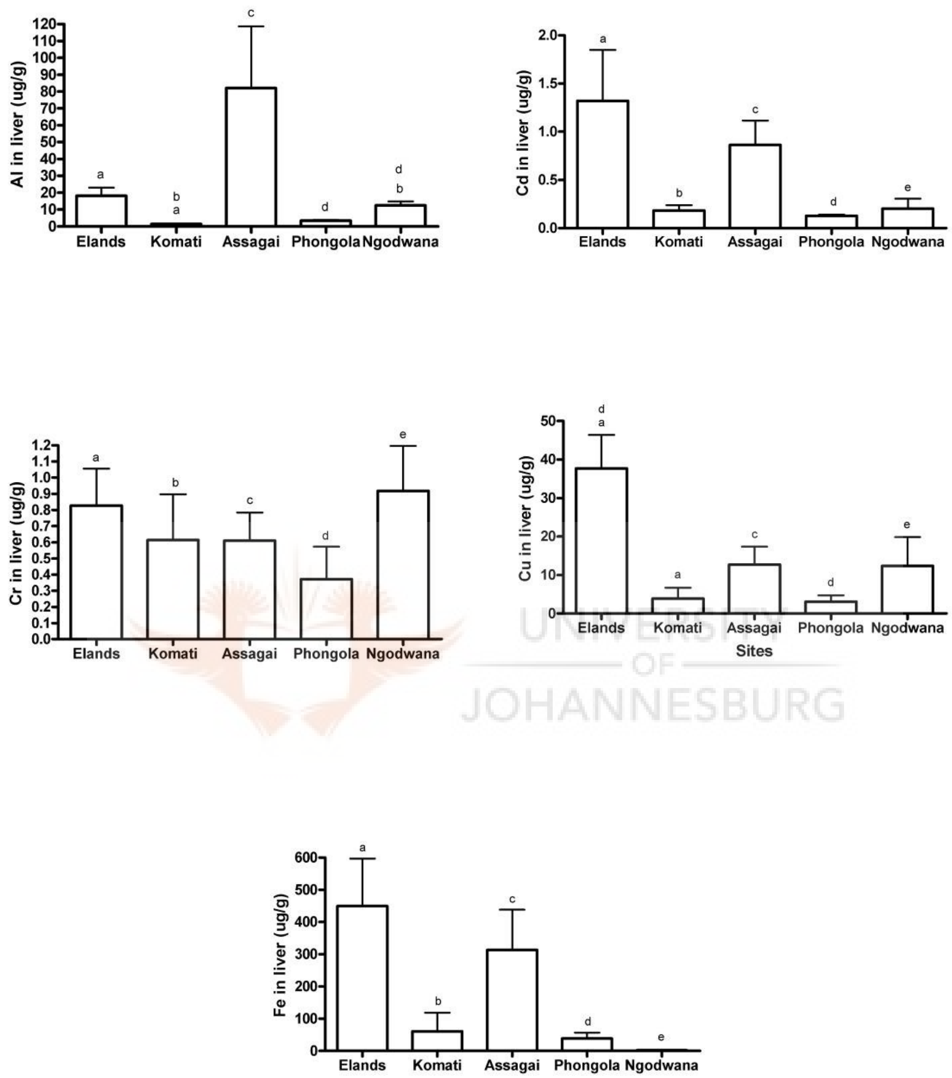

Figure 2-3: The mean metal concentration in the liver from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant difference (P< 0.05)

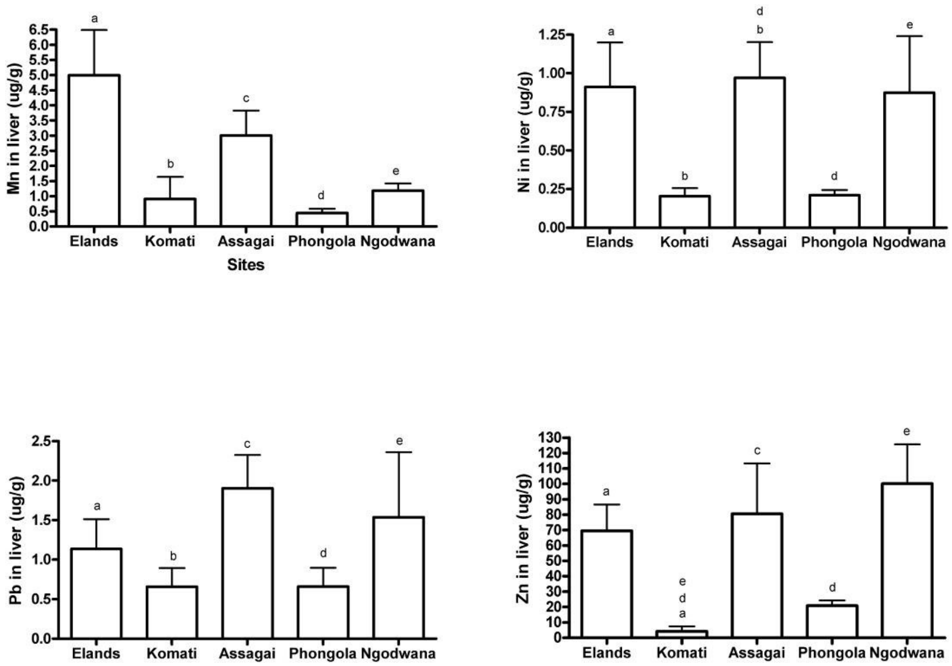

Figure 2-4: The mean metal concentration in the liver from *L. polylepis* at the different sampling areas in µg/g (dry mass). Common superscript is used to denote significant difference (P< 0.05)

2.3.1 Aluminium (Al)

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 2-3). 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 (82µg/g) 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 remaining Al concentrations from both tissue samples taken from the remaining four systems were significantly lower than the Assegaai River liver sample.

2.3.2 Cadmium (Cd)

Cadmium showed the highest bioaccumulation in the liver of *L. polylepis* in the Elands River and Assegaai River populations (Figure 2-3). 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 2-1). 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.

2.3.3 Chromium (Cr)

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 3-1). 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 2-1).

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 2). The highest Cr concentration found in the liver samples from this project was found in the Ngodwana Dam population (0.9 µg/g) (Figure 2-3) 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 2). This is an indication that all sampled systems for this project have low chromium concentration levels.

2.3.4 Copper (Cu)

The Cu bioaccumulation order in *L. polylepis* was highest in the liver samples of all populations except for the Komati River population (Figure 2-3), with significant differences ($P < 0.05$) of 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 2-1).

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 of 14.7 µg/g. In comparison with the copper concentrations found in the fish species in Table 2 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.

2.3.5 Iron (Fe)

Iron showed the highest bioaccumulation in the liver of *L. polylepis* in the Elands, Assegaai and Phongola rivers populations (Figure 2-3). 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 liver of the Elands River 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 higher in Fe concentration. A large variation in Fe concentrations was evident in both the muscle and liver samples.

2.3.6 Manganese (Mn)

The Mn bioaccumulation order in *L. polylepis* was highest in the liver sample taken from the Elands River population (Figure 3-4). 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 2-2). 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.

2.3.7 Nickel (Ni)

Nickel showed the highest bioaccumulation in the muscle of *L. polylepis* in the Phongola River population (Figure 2-2). 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 3-4). 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 (1.8µg/g). The Ni concentrations found in the muscle sample of 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 0.97µg/g being the highest.

2.3.8 Lead (Pb)

The order of bioaccumulation for Pb in *L. polylepis* was the highest in the liver for all the sampled populations except the Phongola River population which showed the highest Pb concentration in the muscle of all samples (Figure 2-2). A significant concentration 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 2-2). 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 (9.7µg/g). The remaining Pb concentrations for the four remaining *L. polylepis* populations were significantly lower and similar in concentration to one another.

2.3.9 Zinc (Zn)

The order of bioaccumulation for Zn in *L. polylepis* was the highest in the liver samples taken from the Ngodwana Dam (Figure 3-4). 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 2-2). 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 when comparing the Elands and Komati River populations, the Komati and Phongola River populations and 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 liver tissue (100.1µg/g) with the lowest Zn concentrations being recorded for the Komati River population (4.1µg/g).

2.4 Discussion

Bioaccumulation results of other bioaccumulation studies on indigenous South African fish species are presented in Table 2. 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, it 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 its solubility is very dependant on the pH. With a pH less than 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 establish a possible source for this. The comparisons made with two other fish species (Table 2) 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 its potential hazards to aquatic biota (Mayer et al., 1991; Barber and Sharma, 1998) as well added potential hazards to human beings (Groten and Van Bladeren, 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 and 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 and Part, 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 a difference in 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 2). The concentrations found in the liver were also lower than the Cr concentrations found in the liver of fish species from other systems (Table 2).

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, sythesis 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 in 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 establish a reason for this. The highest Cu concentration found in the liver samples was 37.7µg/g and when this is compared to the Cu concentrations found in other fish species from other systems (Table 2) 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 and 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 and 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 2) 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, 1989; 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 2) 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 2).

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 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 in the Komati and Phongola River populations that showed the highest Ni concentrations in the muscle samples. In addition to this, the highest overall Ni concentration was found in the muscle sample from the Phongola River. With reference to Table 2 it is noted that Seymore (1994) observed 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 2).

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 establish a reason for this. The comparisons made with the Pb concentrations found in the muscle samples of two different fish species (Table 2) 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). The toxicity of Zn to fish is dependent on dissolved oxygen concentrations, hardness, pH and temperature of the water (Skidmore, 1964; Buthelezi et al., 2000)

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 2), 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 2), 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 2: Historical data assessment of the levels of metals in fish found in South Africa (Nussey 1998, Groenewald 2000, Nussey et al., 2000, Robinson and 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

2.5 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.

CHAPTER 3

3 The feeding biology of five selected populations of *Labeobarbus polylepis* in South Africa.

3.1 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 600m (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 600m, 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 (Mulder et. al., 2004). This study forms a part of a research programme that has been established to study selected biological aspects 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, 1986). The *Labeobarbus* spp. have an alimentary canal which is made up of a pseudogaster, varying lengths of a mid gut and a simple hind gut (Eccles, 1986). 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, 1986). 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, 1986). 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 forms (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.

3.2 Materials and methods

3.2.1 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 1-1).

3.2.2 Collection of specimens

Twenty *L. polylepis* individuals were collected from each sampling locality between May and July of 2006. The individuals were captured using an array of sampling techniques including seine nets, cast nets, electro-shocking, gill nets (mesh size 45mm – 95mm) 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.

3.2.3 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 for 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.

3.2.4 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).

3.3 Results

Of the 100 stomachs examined in this study, none were empty. Table 3 presents the findings of the Frequency of Occurrence, 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 contained this food type in its 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 3-1. 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 2 and Figure 3-2, 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 3: Findings of the Frequency of Occurrence, 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

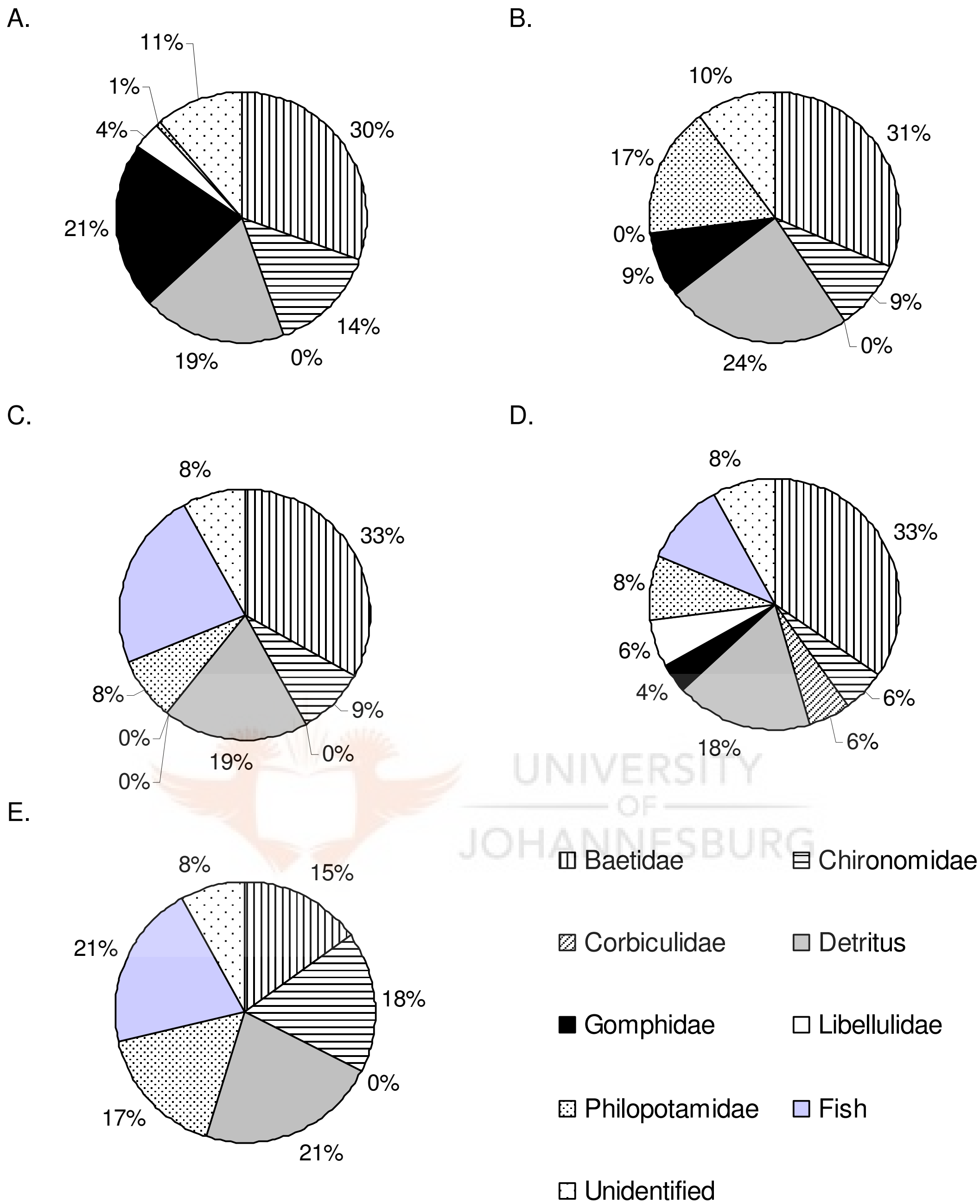

Figure 3-1: Graphical representations of the overall percentage of stomach contents of the *L. polylepis* populations sampled in the study (legend in the figure). Graphs represent the Elands (A), Komati (B), Phongolo (C) and Assagaai (D) rivers as well as the Ngodwana Dam (E).

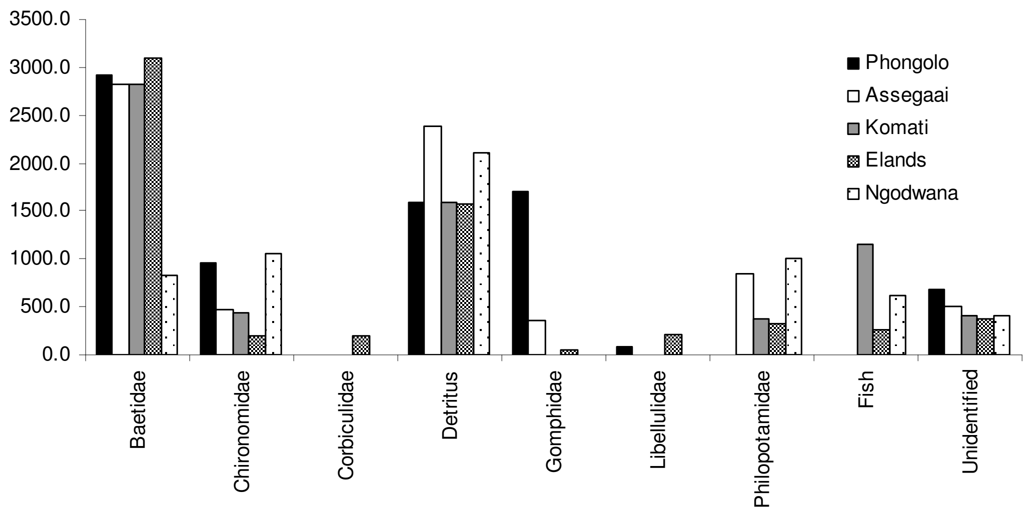

Figure 3-2: Graphical overview of the Importance Index results for each community of *L. polylepis* surveyed in this study.

Results of the multivariate statistical assessments (Figure 3-3 and Figure 3-4) 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 individuals. 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.

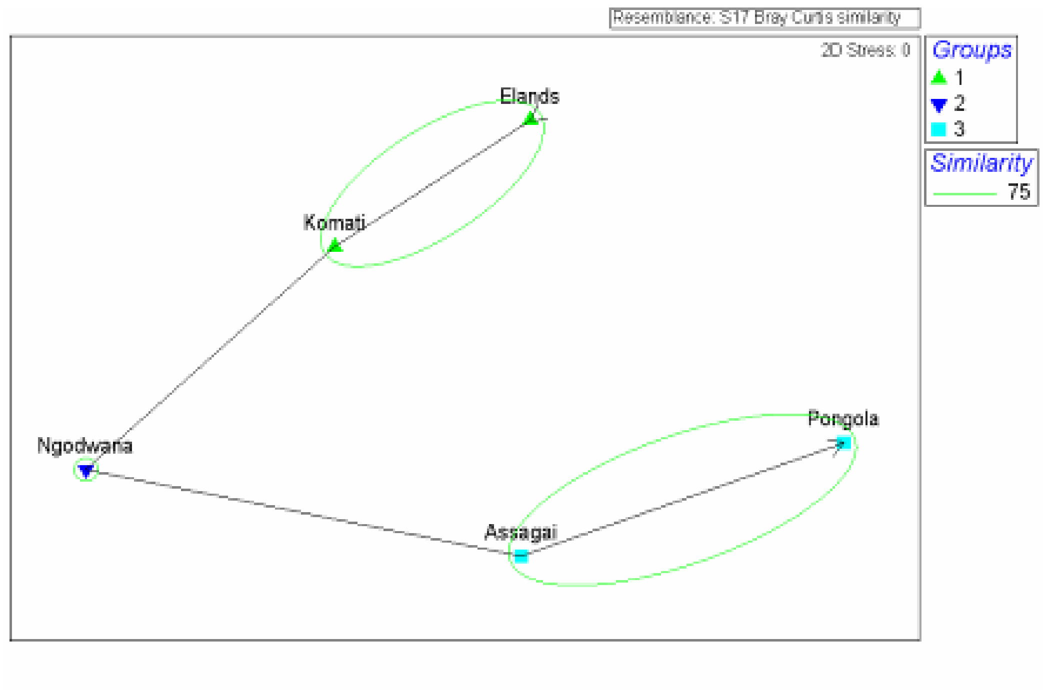

Figure 3-3: Bi-plots representing the NMDS ordination of the stomach content based on A. percentage contribution and B. Importance Index values (%) of the populations of *L. polylepis* assessed in this study. MDS of the raw data represented at a similarity cut off of 75%. Differences between group 1,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

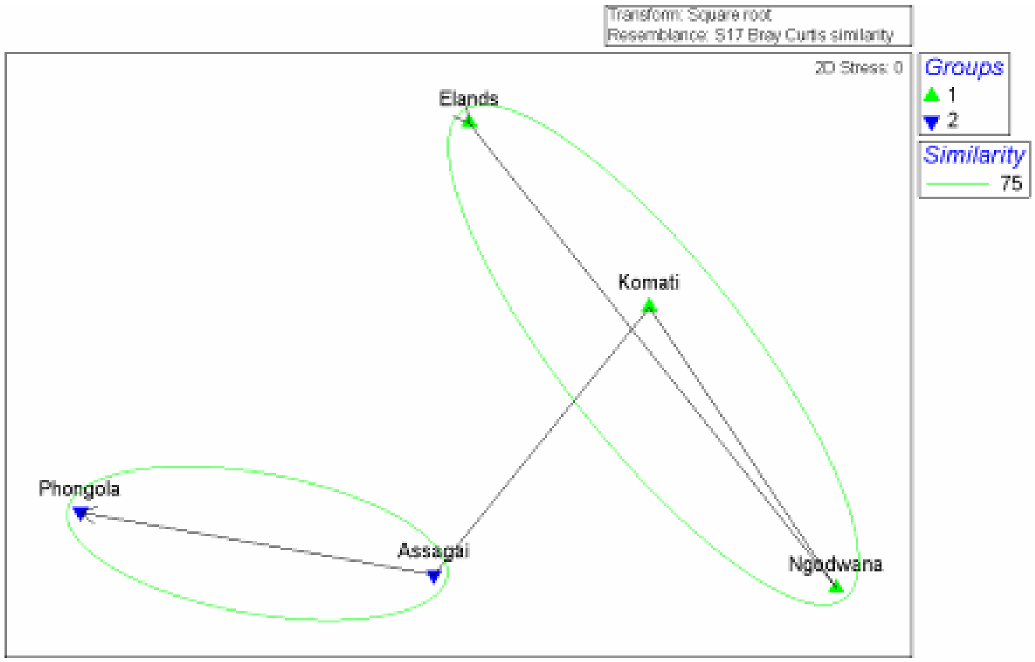

Figure 3-4: Multi-disciplinary Scaling (MDS) of the stomach content of the populations of L. polylepis assessed in this study. MDS of the square root transformed data represented at a similarity cut off of 75%. Differences between group 1 and 2 represented spatially in the graph.

3.4 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 Assagaai 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 600m 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, 2007b). 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 to 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) fed on 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.

3.5 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.

CHAPTER 4

4 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.

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. Additionally, 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 feeding biology of the 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. It is therefore suggested that isolated populations that are determined to be unique should be awarded with an individual conservation status and conserved and or managed accordingly.

CHAPTER 5

5 Recommendations

It is recommended that further research and studies be conducted on *L. polylepis* with reference to a greater variety in tissue samples analysed, the inclusion of more populations of *L. polylepis* with the inclusion of other fish species as well as sediment and water for determining bioaccumulation of metals. In the cases where metals appeared to be toxic or occur in sub-lethal concentrations, the source of these metals needs to be identified in future monitoring programmes and, if necessary, measures should be taken in order to reduce the levels thereof.

The diet composition for *Labeobarbus polylepis* is that of an insectivore and a piscivore. They are well adapted to forage in substrate to capture their prey as well as feed in different trophic levels on suspensions. The ability of the Smallscale yellowfish to capture other fish indicates the predatory nature of this fish. This fish is an opportunistic feeder, with the abilities to find, dig or hunt for prey. These feeding characteristics imply that *L. polylepis* feeds by sight. In order to get a better understanding of the feeding biology and food preferences of *L. polylepis,* more variables need to be considered. It would be beneficial to understand the feeding biology of this fish species if variables such as season, sampling time, length and sex were included. This would provide for a greater overall indication of the feeding biology. Considerations should also be made for the systems sampled, habitat, SASS and water physio-chemistry. Future programmes are recommended in order to build on to the information generated from this study.

Initially, following the outcomes of this study it is recommended that the approach adopted in this study should be expanded to consider the 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 for 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:

- 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.

CHAPTER 6

6 References

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Appendix A: 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 Lower Bound	95% Confidence Intervals Upper Bound	Minimum	Maximum
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 B: 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 Lower Bound	95% Confidence Intervals Upper Bound	Minimum	Maximum
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