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Conservation of tigerfish, *Hydrocynus vittatus*, in the Kruger National Park with the emphasis on establishing the suitability of the water quantity and quality requirements for the Olifants and Luvuvhu rivers

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Hydrocynus vittatus Castelnau, 1861, commonly known as tigerfish, is a flagship species widely distributed in the North Eastern region of South Africa, and are easily identified by the public. This species is actively targeted and utilised by angling and subsistence fishing communities and also used as indicator species by resource and water quality managers to transfer ecosystem related information to the public. Tigerfish therefore has a high ecological, economical and social value to South Africans. Unfortunately, they are lost through habitat changes caused by water extraction, pollution and obstructions like dams and weirs. Tigerfish depend greatly on the available natural habitats to breed, feed and function appropriately. A slight change in the environment may cause depletion of the overall population. Tigerfish are considered rare in South Africa and are classified as a protected species. Scientific studies of all aspects of tigerfish biology are therefore vitally important to understand what quality habitat is required for its successful survival. This information is necessary to development a conservation plan for tigerfish in South Africa. The ecological and economic importance and current conservation status of the tigerfish lead to the current project undertaken by researchers from the Centre for Aquatic Research (CAR) in the Department of Zoology, University of Johannesburg and Water Research Group (WRG), Unit for Environmental Sciences and Management, North West University.

Historically tigerfish were prevalent in all 6 major rivers in the Kruger National Park (KNP) and areas on the western border of the Park. Recent surveys have shown that the distribution of this protected species is drastically reduced. The development of a management strategy to protect tigerfish within the Kruger National Park rivers is therefore of utmost importance. As a top predator tigerfish bio-magnifies pollutants and the risk that these pollutants pose are greater to them than to the lower trophic levels. A single study on metal levels in the Olifants River is the only information on levels of contamination in tigerfish. The levels of organic and inorganic substances together with the information on population structures and reproductive status will provide valuable insight into whether exposure to these contaminants has an influence on the general health of tigerfish populations in the KNP. This study addressed all the factors that might influence the health and conservation status of tigerfish. The upper catchments of all the rivers that run through the KNP are subjected to mining as well as intensive agricultural activities with high contamination potential. This tigerfish project was conducted on request from the KNP Scientific Services who identified the management of tigerfish within the borders of the KNP

as a conservation priority. The study dealt with questions on the sufficiency of the current ecological water allocation for the Olifants River in terms of aquatic species requirements in the system as well as individual and population health.

OBJECTIVES AND AIMS

AIM 1

Determine the current distribution of tigerfish in the Olifants and Luvuvhu Rivers within the KNP.

AIM 2

Determine the biological requirements of Kruger National Park tigerfish.

AIM 3

Determine whether the environmental water allocation for the Olifants and Luvuvhu Rivers is sufficient to support a healthy tigerfish population.

AIM 4

Determine the factors that might limit the current distribution of tigerfish in the Olifants River in the KNP, including water quality and habitat modification.

AIM 5

A) Propose a management strategy for the conservation of tigerfish in the KNP with emphasis on mitigating measures to stimulate tigerfish populations to return to their original natural habitats. B) Validation and consolidation of the use of tigerfish as indicator species of quality and quantity related Threshold of Potential Concern (TPC) in the Olifants and Luvuvhu Rivers.

METHODOLOGY

Four sites were selected along the Olifants River as it flows through the KNP with the fifth site at the confluence of the Letaba and the Olifants River in the Olifants River Gorge. An additional site was selected in the Letaba River just before its confluence with the Olifants River. Four sites were selected along the Luvuvhu River as it flows through the KNP towards Mozambique. The first site was where the river enters the KNP opposite an informal rural settlement and the last site before the confluence of the Luvuvhu and the Limpopo Rivers.

Water and sediment quality

Physico-chemical water parameters were taken *in situ* at each sampling site in both rivers. Samples were frozen and transported to the laboratory for further analysis. After thawing samples suspended metal, chemical and turbidity analyses were done using standard techniques. High and low flow (HF, LF) surveys were done in 2009 (LF only), 2010 and 2011 (HF only). Sediment samples were analysed for the levels of inorganic and organic pollutants, percentage organic carbon and grain size. The Community Bureau of Reference

(CBR) extraction procedures were used for the separation of metals. Certified reference materials (CRM) were used to test the analytical efficiency and for quality control. Pooled dried sediment samples from each site were analysed for organochlorine pesticides (OCPs) with a gas-chromatograph (GC) employing standard techniques. Quality assurance and quality control was achieved by using a corresponding standard.

Habitat

Different biotope diversities were evaluated in the current including instream and marginal vegetation, and GSM (gravel, sand, mud). A fish habitat assessment was conducted to describe the fish refuge potential at each of the sampling sites.

Macro-invertebrates and Fishes

The sampling of the Olifants and Luvuvhu Rivers was done over two consecutive LF seasons. The macro-invertebrate composition at all the sites on both the Olifants and Luvuvhu Rivers were determined and assessed. The Fish Response Assemblage Index (FRAI) was compiled. Standard techniques were employed in both cases. Sites were used that had been previously sampled and those that had a Reference Frequency of Occurrence (FROC). Representative habitat biotopes were sampled employing approved fish sampling techniques. Histopathology assessments were done to establish the health of selected fish species from both river systems. Flow-dependent habitat type preferences by fishes of the Olifants River were done using a spatial habitat modelling exercise, fish community structure assessment and a desktop evaluation of habitat preferences. The effects of altered flow dependent habitat types on fish communities were done with a flow-stress assessment. Fish communities sampled in the habitats were used to determine different community structures. Multivariate statistical procedures and Geographical Information Systems (GIS) modelling procedures were used to evaluate the habitat and flow preferences of the fish communities.

Fish Health Assessment

The condition factor was determined after sampling and the hepato-, gonado- and spleno-somatic indexes calculated for each species. Histopathology analyses were done on gill, liver, kidney and gonad samples. Otoliths were prepared for age determination.

Bioaccumulation

Levels of Cu, Mn, Pb, Cd and Hg in muscle tissue were determined with ICP-OES and ICP-MS using standard techniques for sample preparation and analysis. The DDT congeners – p,p'-DDE, o,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, o,p' and p,p'-DDT (Sum ΣDDTs), hexachlorobenzene (HCB), α-, β-, γ and δ-hexachlorocyclohexane (HCH) isomers (Sum ΣHCHs), the chlordanes (ΣCHLs) – cis- and trans chlordane (cChl, tChl), its oxidised form, i.e. oxychlordane (OxC), and heptachlor (HC) and its break down products cis- and trans were also determined.

Biomarker responses

A gram tigerfish liver and muscle were mixed with Hendrickson stabilising buffer, and stored in liquid nitrogen for biomarker analysis. The remaining portions of the axial muscle were frozen in for further analysis. Values were obtained for biomarkers of exposure and effect.

Statistical analyses

Data were analysed by one-way ANOVA, with sites as variables. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. Post-hoc multiple comparisons between sites were made using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine significant differences ($p<0.05$). Univariate diversity indices were used to assess community structures, species richness and diversity. Primer Multivariate Software was used to analyse invertebrate and fish community similarities and groupings, and clusters to represent community response. Multidimensional scaling was carried out to show similarity groupings of the sample sites. The analysis of similarities (ANOSIM) test was used to show significant groupings in the cluster and MDS diagrams.

Principle Component Analysis (PCA) was done to assess the spatial patterns associated with water and sediment quality, bioaccumulation in fish tissue, biomarker responses and fish community structures. A Redundancy Analysis (RDA) assessment was carried out to determine the factors that were responsible for the groupings calculated in the PCA.

RESULTS AND DISCUSSION

THE OLIFANTS RIVER

Water and sediment quality

None of the in situ water quality variables recorded displayed any definite spatial trends at the five sites in the Olifants River. The Letaba River had lower conductivity levels than the Olifants River and temperatures ranged between 16 and 29°C within surveys. The pH levels remained relatively constant throughout at all sites and surveys. Conductivity reflected a variation during HFs and LFs with higher values during low flows. Almost all the in situ water quality parameters fell within the target water quality range (TWQR) for aquatic ecosystems. Nutrient levels remained fairly low throughout the study and were indicative of mesotrophic conditions. A slight increase in nitrate levels would cause the Olifants River to become eutrophic. The very high sulphate levels measured in the Olifants River was probably caused by coal mining and industrial activities in the upper catchment.

Lower concentrations of Cr, Fe, Zn, Pb, Mn and Ni were present compared to previous studies. The levels of Zn and Cu were higher than in previous studies with Al, Mn, Ni, Ag, Se, Ca, K and Na higher in the Letaba River. Metal concentrations from the

suspended solids in the water column of the Olifants and Letaba Rivers were higher for most metals compared to dissolved metal concentrations. No clear spatial patterns were observed in the Letaba and Olifants Rivers but clear temporal differences were evident. The Aquatic Toxicity Index (ATI) developed for the Olifants River to interpret the water quality was applied. An ATI score above 60 is acceptable and the ATI scores for the Olifants and Letaba Rivers did not go below 70 with scores for sites on the Olifants River ranging between 73 and 87, and scores for the Letaba River ranging between 72 and 87.

No spatial trends in total metal concentrations were observable for any of the metals in sediments of the Olifants and Letaba Rivers. The total metal concentrations measured in sediment were very similar to historical metal concentrations in the Olifants River. Spatial differences existed between the Olifants River and the Letaba Rivers. Temporal differences in metal concentration were only found in the sediments of the Olifants River.

The number of organochlorine contaminants tested for varied from 6 to 21 out of the 22 selected. The sediments of the Letaba River contained low organochlorine concentrations during both flow periods. During the high flow the sediments were dominated by a high organic content. The organochlorine pesticides were associated with fine sediment particles. The sediment in the Olifants River during the LF period was dominated by medium sand with cis-Chlordane, Endrin and heptachlor associated with it.

Habitat preference and flow requirements for fishes

The macro-invertebrate communities changed from a fair state in 2001 to a seriously modified state in 2009 and a poor state in 2010. The average numbers per taxa decreased downstream, differed between the two surveys and showed temporal and spatial variation. Water abstraction and elevated salt levels in the Olifants River negatively affected the macro-invertebrate community, diversity and abundance decreased. The Fish Response Assemblage Index (FRAI) showed that there is a large number of species absent and some species in low abundance. The recent high rainfall in high-flow periods flushed the system providing better water quality and general habitat for fish species. The results show some temporal and spatial variation in fish community structures. The habitats accommodated five groups of fish species with preferences for specific flow-depths. Tigerfish has a high preference for only two habitat types, i.e. deep (>1200 mm) fast flowing (>0.8 m/s) conditions. It also prefers relatively deep (>700 mm) no flow to fast flowing (0-1.35 m/s) habitat types. Important cover features for the species include water column and possibly over hanging vegetation. At flows of 17.5 m^3/s for the dry season the availability of fast flowing habitats is 45% (observed data) and 24% (modelled data). Sufficient maintenance habitats for all rheophilic species are then available. Below a discharge of 4.9 m^3/s the availability of fast flowing categories reduce to critical levels for both observed and modelled flows. The indicator rheophilic fishes would then be forced to take up refuge in un-preferred

habitat types. At <2 m^3/s the fast flowing habitat types for the indicator fishes reduce to unacceptably low availabilities.

Fish health assessment

Selected target organs of *H. vittatus* and *Labeobarbus marequensis* from the Olifants River have normal histological liver, kidney and gill structures and the alterations identified, had no observable effect on physiological function. No histological alterations were identified in any gonad samples.

Bioaccumulation in *H. vittatus*

A considerable variation in the metal bioaccumulation in tigerfish, as reflected in historic data was confirmed. The bioavailability of water and sediment-bound contaminants were influenced by a multitude of variables within the water column and sediment, i.e. physical, chemical and biological factors. Metal bioavailability to benthic dwelling fish showed a positive relationship in Cu, Ni and Zn bioaccumulation. There were distinct higher concentrations of bio-accumulated OCPs in the low flow periods. The ΣDDTs (*o,p'*- and *p,p'*-DDE, DDD, DDT) were the most abundant organochlorine pesticide and was measured in all samples. DDT isomers were present in the order of DDE>DDT>DDD. There were clear flow-related influences on the DDT bioaccumulation with ΣDDTs concentrations higher than the 1000 ng/g maximum allowable residue level in edible fat as prescribed by the European Union (EC 2005). The levels of total DDTs in the Olifants River were higher when compared to results of previous studies. The HCHs were next highest with the isomers decreasing in concentration δ>β>α>γ for all surveys except for the Letaba River.

Biomarker response in *H. vittatus*

The lower AChE activity and increased MT and CYP1A activities recorded in *H. vittatus* liver tissue indicated fish responses to metals and organic chemicals during this survey. The biomarkers of anti-oxidant effect showed lipid and protein breakdown during specific conditions. The lipid and protein catabolism coincided with higher energy consumption and availability during this period.

THE LUVUVHU RIVER

Water and sediment quality

All in situ water quality variables measured in the Luvuvhu River fell within the TWQR for aquatic ecosystems. Spatial trends were observed for temperature pH and conductivity, with an increase in all these variables as the river flows through the park. No spatial and temporal patterns in physico-chemical variables and metals were present. Concentrations of dissolved Al exceeded the TWQR during all surveys at all sites. Lead (Pb) and Zn exceeded the TWQR at different sites during specific surveys while all the other metals where at lower levels at the different sites. Spatial and temporal patterns were not general. Metal concentrations in suspended matter were higher than in dissolved form for most metals.

There were notable temporal patterns in metal concentrations with variations during the different surveys. The ATI scores associated with water quality variables ranged between 55 and 87. There were distinct spatial and temporal variations with the highest ATI scores recorded during the low flow and a trend of improved water quality was found as the river flows through the park. The high ammonium and orthophosphate levels predominantly influenced the ATI scores at sites in the Luvuvhu River.

The percentage organic matter in sediment at all sites ranged between 0.45% and 5.68% with no spatial or temporal trends observed. Metal concentrations also showed no spatial or temporal trends. The bioavailability of metals differed between sites. The total metal concentrations and physical sediment characteristics at the different sites revealed temporal differences. Twenty-one of the 22 organochlorine compounds tested for were present in the sediment of the Luvuvhu River. Only o,p'-DDT was not measured in sediments from any of the sites during both surveys. Trace amounts of the organic contaminants were present. The least number of organic contaminants present was 13 and the most 18 of the 22 compounds studied.

Habitat

The dominant velocity-depth classes and biotope diversities were for invertebrates included riffles, backwaters, bedrock, sedges, reeds, grasses, slack water and channels. Fish habitats identified were slow-deep, fats-deep, slow-shallow and fast-shallow.

Macro-invertebrates and fishes

The macro-invertebrate communities were in a seriously modified state in 2009 (Class E/F) and in a fair/good state (Class C/B) for the 2010 period compared to a natural state/class in 2001. The overall decrease in organism abundance is of concern and is probably caused by increased upstream anthropogenic activities. Marked spatial and temporal trends are visible and the same as in the Olifants River. Fish communities within the Luvuvhu River showed the same trends. A large number of fish species were absent, and species sampled are in low abundance. The fish communities have temporal trends similar to those found in the Olifants River. On a special scale the FRAI scores decreased from the upstream to downstream sites. The increased abstraction and utilization water for agricultural and domestic use tend to decrease flow volumes, especially in low-flow periods. The fish communities and assemblages in the Luvuvhu River are therefore no longer in a natural state.

Fish Health Assessment

The light microscopy analysis showed normal histological structures and function in the liver and kidneys of two fish species studied. The observed histological alterations had no serious effects.

Bioaccumulation in *H. vittatus*

The concentrations of Cd, Cu, Mn and Zn have decreased during the study period whereas the rest of the metals remained constant over the sampling period. There were no significant temporal changes in bioaccumulation of individual metals. Except for Al all metals were lower in tigerfish when compared to the Olifants River bioaccumulation results. There were no significant differences in lipid OCP content of the muscle tissue between the two flow periods. The temporal OCP bioaccumulation patterns reflected OCP usage and run-off patterns. All the measured OCPs are significantly higher during the low flow period. This suggests that input from diffuse sources has a longer residence time in the environment resulting in bioaccumulation. The highest recorded levels of ΣDDTs in fish from South African freshwater systems were measured during LF. DDT application for malaria vector control in the upper catchment of the Luvuvhu River is the probable reason for this phenomenon. The low DDE:DDT ratio indicates that the DDT exposure is a mixture of recent DDT application and historical levels. The high chlordane, lindane, Endrin and Aldrin concentrations is probably the result of wide-spread use of OCPs in the upper reaches. The Dieldrin found in sediment samples did not bioaccumulate in tigerfish muscle.

Biomarker response in *H. vittatus*

The biomarker responses in liver tissue of *H. vittatus* indicated that there are responses to metal (increased MT) and organochlorine (increased CYTP450) levels. The ROS protective mechanisms were activated and this is reflected in the lower lipid break down products that are formed. These are energy consuming processes as displayed in the significant increase in energy consumption.

CONCLUSIONS

Water and sediment quality

The physico-chemical quality and metal concentrations in the Olifants, Letaba and Luvuvhu Rivers are influenced by flow conditions with more than 50% of the variation in the water quality data demonstrating these influences. Only 16% of the variation in the data can be explained by river specific factors influencing the water quality of the three rivers studied. Low flow conditions are characterised by increased DO, pH and electrical conductivity. The majority of metals (both dissolved and suspended) are associated with high flow conditions together with increased turbidity and nutrient levels. Dissolved Cu, Se and Zn were notably higher in the Olifants River than in the Luvuvhu River. Anthropogenic activities in the Luvuvhu River system modifies water quality and elevated metals in both the Olifants and Luvuvhu Rivers are likely caused by mining activities in the Bushveld complex and land erosion respectively. Water hardness in the Olifants River was much greater as reported in previous studies and resulted in lower concentrations of many metals in the water. The

Olifants River sediments were fine and rich in inorganic components with high metal concentrations, while the Luvuvhu system sediments consisted of course sand and gravel. The influence of flow attributed to 20% in the variation of the data on sediments during high flow periods in the Olifants River. Although the majority of metals were in the inert residual fraction of the sediment, some metals occurred in high proportions in the bioavailable acid-soluble and reducible fractions. These metals have an increased potential for biological uptake and therefore could pose a risk to aquatic biota. Organochlorine pesticide concentrations in sediments of the Olifants and Luvuvhu Rivers were dependent on the flow conditions and associated physical characteristics of the sediments. The highest cis-chlordane and heptachlor concentrations were present in medium sand sediments. Dieldrin was recorded in sediments at all sites in the Luvuvhu River. Concentrations are very similar to OCP concentrations measured in sediments from industrial sites in the Vaal triangle and much lower than in the Phongola floodplain.

Biological assessment of the Olifants and Luvuvhu Rivers

Invertebrates and Fish

A comprehensive grouping of invertebrates the rivers on both temporal and spatial levels occurred. The Luvuvhu River communities grouped separately from the Olifants River communities during surveys, but both Luvuvhu flow periods grouped together. The Olifants River macro-invertebrate communities differ in terms of the two flow periods and in terms of the Luvuvhu River communities. There was a very clear temporal, and a small spatial variation in invertebrate community structures in both the rivers sampled. These groupings can be attributed to the effects of increased run-off during the 2010 rainy season in the Olifants and Luvuvhu Rivers. The system was flushed and thereby creating more favourable conditions for the macro-invertebrate community. There is little spatial and temporal variation in the fish communities for both rivers. The fish population in the Olifants River Gorge was the same in all the surveys.

Fish health assessment of *H. vittatus* populations from the Olifants and Luvuvhu Rivers

Although both the Olifants and Luvuvhu Rivers are polluted by anthropogenic activities, the semi-quantitative histological assessment results indicate that the fish sampled were in good health based on macroscopic and microscopic observations respectively. All hisotology index values for the species studied were within a normal range.

Metal and organic bioaccumulation in *H. vittatus* in the Olifants and Luvuvhu Rivers

The tigerfish bioaccumulation patterns of elevated Cu and oxy-Chlordane levels in the Letaba and Olifants Rivers and high concentrations of DDTs, HCHs, Lindane, Co as well as Al in the Luvuvhu River clearly showed that site and survey specific conditions were responsible for the metal and organic bioaccumulation. Acid volatile sulphides (AVS) played

an important role in influencing the availability of sediment-bound metals within aquatic systems.

Biomarker response of *H. vittatus* in the Olifants and Luvuvhu Rivers

The higher metal and OCP exposures in tigerfish from the Olifants and Luvuvhu Rivers resulted in increased oxidative stress with chronic effects. Biomarker responses in tigerfish did not differ much between the two river systems and provided valuable information on the stress levels demanding higher energy reserves in the individuals sampled.

Factors that might possibly limit the distribution of *H. vittatus* in the Olifants River

Tigerfish were present in all sites in the Luvuvhu River confirming that it is currently a good reference site for tigerfish. Healthy tigerfish were present at all the sites in the Olifants River, even above Mamba Weir. Very young tigerfish were sampled at sites 1-4 with very low abundance. It shows that the tigerfish recently returned to upstream areas probably because of recent consistently high rainfall with higher flow and better water and sediment quality. Very high densities of a large size range were present at the confluence of the Olifants and Letaba Rivers. Pansteatitis was also not observed in tigerfish. The main factors influencing the limited distribution of tigerfish in the Olifants River are probably water quantity, availability, and lack of suitable habitat.

RECOMMENDATIONS

The use of tigerfish as an indicator species for water quality and quantity in the KNP

Tigerfish do respond to the presence of low levels of pollutants. Their highly mobile nature enables them to avoid exposure to debilitating stressors and since one of the key criteria for the choice of a bioindicator is that it should represent the ambient conditions, the tigerfish may not be an ideal indicator species for water quality. However, results from the flow assessment done as part of this study clearly showed that tigerfish have very specific flow and habitat requirements, thus making them an excellent indicator species of water quantity. Furthermore, all fish species from the Olifants River have identifiable habitat preferences that were successfully used to evaluate the effects of reduced flows. Low flow discharges of approximately 17 m^3/s in the Olifants River may begin to show higher levels of stress in fish due to reductions in habitat diversity and abundances. Below a flow of 4.9 m^3/s the resulting reduction in flow dependent habitat types would become severe. Future monitoring protocols should observe and evaluate the impact of reduced flows in the Olifants River after events of extreme low flow. The synergistic effects of increased stress levels of populations in the Olifants River, due to other impacts, e.g. water quality stressors for during extreme low flow periods is unknown and should be evaluated.

Proposed management strategy for the conservation of *H. vittatus* in the KNP

Although these minimum flows fall into the minimum flow ranges of the currently available instream flow requirements for the Olifants River the current threshold for the drought flows may be too low and should be increased to a minimum of 5.0 m^3/s. During these low flow periods the local tigerfish populations would be maintained for a few months in slow-deep refuge areas. Population health has to be monitored during and after such events to ensure survivability of the population.

Thresholds for Potential Concern (TPCs) for river health in the KNP

The current KNP TPCs for EC are 1200 µS/cm and TDS of 800 mg/ℓ for the Olifants River. These are extreme ranges, and thought to be too high. To be in line with the requirement of the TWQR for freshwater systems and apply results from this study, it is recommended that the current TPC for the Olifants River for EC be lowered to 1000 µS/cm and TDS values to 700 mg/ℓ. The EC TPC value for the Luvuvhu River is currently 800 µS/cm, with a TDS of 520 mg/ℓ. These values are high when compared to historic data and the values from the present study. An EC TPC of 600 µS/cm for the Luvuvhu River, with a TDS of 420 mg/ℓ.

The current TPC for fish communities is described as follows: “the fish present ecological state (PES) per river reach should not drop one biological condition class (A-F) or show a continuous negative trend in the biological integrity categories (metrics) established for each river”. These TPCs (fish EC) are outdated and are based on the Fish Assessment Integrity Index (FAII) (Kleynhans, 1999. FRAI is now the accepted index regarding the RHP, and replaced the FAII (Kleynhans *et al*., 2007). It is thus proposed that the current Fish community TPC be amended to include the use of FRAI rather than FAII. The threshold lowering of a biological condition class is regarded as a suitable TPC and should thus be retained. Based on the findings from the present study the Luvuvhu River has dropped one biological condition class. This is a concern that should receive urgent attention from KNP managers.

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LIST OF ABBREVIATIONS

ABM	Active biomonitoring
ACh	Acetylcholine
AChE	Acetylcholinesterase
ASPT	Average Species per Taxon
BCh	Butyrylcholine
BChE	Butyrylcholinesterase
BCR	Community Bureau of Reference
BSA	Bovin Serum Albumin
BSS	Buffered Substrate Solution
CAT	Catalase activity
CCT	Collision Cell Technology Gas
CYP1A	Cytochrome P4501A
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
Ea	Available Energy
Ec	Consumed Energy
EC	Electrical conductivity
EDTA	Ethylene Diamine Tetraacetic Acid
EROD	7-Ethoxyresorufin-*O*-Deethylase
FRAI	Fish Response Assessment Index
GHB	General Homogenising Buffer
HF	High Flow
ICP-MS	Inductively Coupled Plasma-Mass Spectrometer
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometer (Also known as Atomic Emission Spectrometer)
INT	p-IodoNitro-Tetrazolium Chloride
KNP	Kruger National Park
LF	Low Flow
LP	Lipid Peroxidation
Ma	Mass After
Mb	Mass Before
MDA	Malondehyde Content
MFO	Mixed Function Oxygenase System
MT	Metallothionein
NWA	National Water Act
PAH	Polycyclic Aromatic Hydrocarbon
PC	Protein Carbonyl Formation
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated Dibenzodioxins

PHH	Polycyclic Halogenated Hydrocarbon
PNS	Peripheral Nervous System
POP	Persistant Organic Pollutant
PPB	Potassium Phosphate Buffer
RDA	Redundancy Analysis
r.p.m	rotations per minute
ROS	Reactive Oxygen Species
SASS	South African Scoring System
SOD	Superoxide Dismutase
TDS	Total Dissolved Solids
TOP	Threatened or Protected Species
TPC	Threshold of Potential Concern
TWQG	Target Water Quality Guidelines
TWQR	Target Water Quality Requirements
W	Watts

1 INTRODUCTION AND AIMS

1.1 Introduction to *Hydrocynus vittatus*

African freshwater fish are an important natural source of protein and provide 21% of the total protein intake on the continent (Revenga et al., 1998). Locals are dependent on inland fish as either a source of food or a means of income; for this reason fish have great significance in the life of mankind, especially for those living in poverty within the immediate vicinity of fish populations (FAO, 2005). Fish not only plays a major role as a protein source for local rural communities, but also promotes the tourism industry in terms of recreational and sport game fishing. *Hydrocynus vittatus* Castelnau, 1861, commonly known as tigerfish, is one of the most important freshwater fish species in Southern Africa because of its economic and livelihood value (Smit et al., 2009). Species such as the tigerfish depend greatly on their natural habitats to breed, feed and function appropriately. A slight change in a fish's environment may cause depletion of the overall population. It is thus vitally important that studies are done to gain an understanding of all aspects of the various species in order to protect habitats and the species therein. This is particularly true for the tigerfish, a species recently included on South Africa's protected species list (DEAT, 2007).

As a result of its ecological and economic importance, as well as its current conservation status, the tigerfish has been the focal point of four different research projects undertaken by researchers from the Centre for Aquatic Research (CAR) in the Department of Zoology at the University of Johannesburg and Water Research Group (WRG) in the School of Environmental Sciences at North West University. One of the first aspects highlighted by these projects was the paucity of information available on particular aspects of tigerfish biology as well as information on specific populations of. The aim of this review is to provide an in-depth review on all available literature on tigerfish research in Southern Africa and also to highlight the existing gaps in our knowledge of this species. This includes a look into the history, classification, biogeography, genetics and conservation of tigerfish as well as their biology and how they are impacted on by humans.

Tigerfish: Past and Present

Hydrocynus vittatus (Figure 1) is a dominant species in many African rivers and lakes (Griffith, 1975) and an important freshwater piscivorous predator in Africa (Jackson, 1961; Lewis, 1974; Winemiller & Kelso-Winemiller, 1994). Although not found in the coastal rivers of Angola, the Kunene and Kafue Rivers, Lake Malawi and the rivers of

Kenya (Bell-Cross, 1965-66; Skelton, 2001), this species is well distributed throughout Southern Africa including the Zambezi River, Okavango River and Delta, Limpopo River system and the lowveld reaches of coastal systems south to the Phongolo (Skelton, 2001). Tigerfish are important in both commercial and recreational fisheries in the Zambezi River and Okavango/Chobe Rivers and in Lake Kariba (Griffith, 1975; Winemiller & Kelso-Winemiller, 1994).

Figure 1. One of the authors with a trophy size tigerfish, *Hydrocynus vittatus*, caught in the Luvuvhu River as part of this study.

The history of the genus *Hydrocynus*

Fossil assemblages atypically yield tigerfish skeletons as they are too delicate to be preserved (Stewart, 1997). However, *Hydrocynus* spp. are represented in the Neogene fossil record (Stewart, 2001) as their distinctive teeth preserve well (Trapani, 2008). The location of all fossil records, for *Hydrocynus* spp., is illustrated in Figure 2. According to Schwartz (1983), *Hydrocynus* spp. are principally represented by teeth although elements of jaws have also been discovered. In the Senegal River finds of *Hydrocynus* spp. include four vertebrae, one tooth, a jaw fragment, a keratohyal and a hyomandibular bone (Van Neer, 2008).

Although no findings have been recorded in Miocene or pre-Miocene North African sites, they are known from central and East African sites suggesting that they possibly evolved in the pre-Pliocene east-to-west flowing rivers (Stewart, 2001). The

oldest evidence for tigerfish is a tooth found in the Lake Albert Rift Basin (Van Neer, 1992). Although length construction by a single tooth was deemed difficult as tooth size varies depending on age and jaw placement, this author was under the impression that the fish was medium sized (< 50 cm standard length). Other *Hydrocynus* spp. fossils (Figure 2) were found in Lakes Albert (Greenwood et al., 1966) and the Lusso Beds of the Lake Edward Rift Basin (Stewart, 1990).

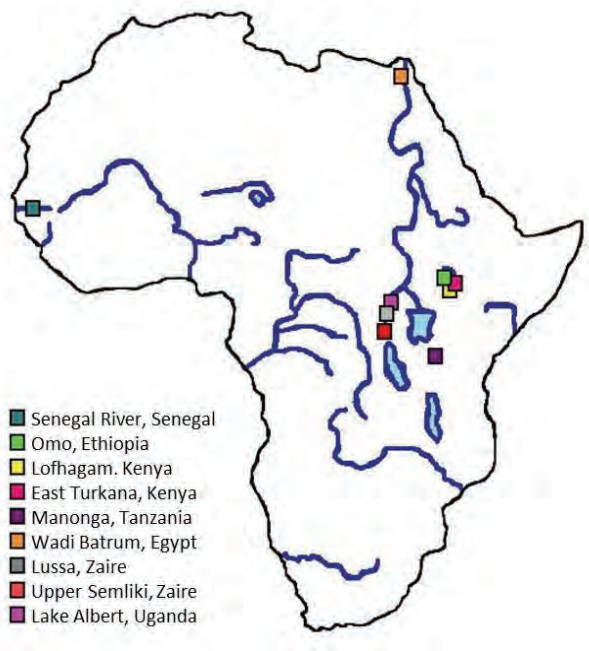

Figure 2. Distribution of *Hydrocynus* spp. in Africa between the Late Miocene and Early Pleistocene.

Systematics and taxonomy

Although tigerfish have been around for many years, there have been many problems regarding the classification of this genus and the species therein. The Characidae are a large family of freshwater fish, indigenous to two continents, namely Africa and South America. While family names are not meant to be assigned to clades unless relationships have been undeniably determined (Weitzman & Malabarba, 1998), Alestidae was included in the Characidae family with no cladistic analysis to substantiate its placing (Murray & Stewart, 2002). Brewster (1986) reviewed *Hydrocynus* using polarity (not cladistics), concluding that *Alestes sensu strict* (*s.str.)* should be assigned as the sister group of *Hydrocynus* and found no characters to sustain an association between Bryconaethiops and *Alestes s.str.* as determined by Géry (1968). Not taking any of Brewster's (1986) conclusions or

suggestions into account, and commencing a separate study all together, Géry (1995) suggested Alestidae be split into two subfamilies, Alestinae (comprising Alestini and Petersiini) and Hydrocyninae. These results show that *Hydrocynus* is not as closely related to *Alestes*, completely contradicting Brewster (1986). A cladistic analysis of Neotropical characids by Ortí (1997) revealed that *Hydrocynus* is closer in relation to the tribe Petersiini than to *Alestes* and thus concluded that *Alestes* should be placed in the sister position to Petersiini and *Hydrocynus*. Murray and Stewart (2002) studied the relationships between *Alestes*, *Brycinus* and *Hydrocynus* by examining various morphological characteristics (soft anatomy, jaws, ventral skull and suspensorium, orbitosphenoid tube, dorsal cranium preopercular bone, postcranial elements and caudal fin). These authors concluded that Alestidae is monophyletic, that Hydrocyninae should not be considered a valid subfamily, and that *Hydrocynus* (and possibly *Bryconaethiops*) should be included in the Alestidae.

Based on the above, Alestidae currently include the genera *Alestes*, *Brycinus*, *Bryconaethiops* and *Hydrocynus*. Tigerfish belong to the genus *Hydrocynus*. There are five species of these specialised, ferocious predators (Skelton, 2001). *Hydrocynus brevis* Günther, 1864 is found in the Nilo-Sudan to Upper Guinea regions; *H. tanzaniae* Brewster, 1986 occurs in the Ruaha and Rufiji River systems of Tanzania (Gagiano, 1997); and *H. goliath* Boulenger, 1898 is limited to the Oubangui River and the upper and central Congo basin (Brewster, 1986). *Hydrocynus vittatus* Cuvier, 1819 and *H. forskahlii* Cuvier, 1819 are included in this genus but their taxonomic placement has been a subject of controversy among scientists for many years (Brewster, 1986; Paugy & Guegan, 1989; Skelton, 1990; 2001).

When reviewing the *Hydrocynus* spp., Brewster (1986) concluded that *H. vittatus* was the same species as *H. forskahlii*. According to Skelton (1990), Brewster (1986) based her study entirely on preserved, museum specimens, thus failing to take into consideration the colour/pigment diversity of the two species. This author also failed to show key points of similarity or differences and did not present the evidence on which her final decision was based (Skelton, 1990).

Based on morphological validation, Paugy and Guegan (1989) stated *H. vittatus* and *H. forskahlii* were not the same species and in fact both were present in the Niger system. According to these authors, *H. forskahlii* has a shorter head, slimmer body, more advanced placement of the dorsal fin, greater distance between the adipose and dorsal fins, additional lateral line scales and extra gill rakers on the first gill arch. *Hydrocynus vittatus* also differs by possessing a black adipose fin and a black tip on the dorsal fin. Paugy and Guegan (1989) took their analysis a step further, also assessing the parasites of the two different tigerfish. Both *H. forskalii*

with *H. vittatus* were host to different monogenean species of the genus *Annulotrema*. Taking all these data into account these authors suggested that the two tigerfish were in fact separate species and that *H. forskalii* and *H. vittatus* were the central African and Southern African tigerfish, respectively. A recent study by Goodier et al. (2011) further endorsed the rejection of the synonymising of *H. vittatus* and *H. forskahlii* based on molecular grounds. These authors found genetic evidence of phylogenetic divergence between the two aforementioned species representing a deep Miocene cladogenesis event in the evolution of *Hydrocynus*.

Biogeography

Historical African waterways were once interconnected, permitting the wide dispersal of ancestral fauna (Greenwood, 1983). Bell-Cross (1965-66) hypothesised that the Kasai River (a tributary of the Congo River), was the dispersal route for tigerfish from the Congo basin into the Upper Zambezi headwaters, and onto the southernmost population of Phongolo. Moore et al. (2007) also hypothesised that *H. vittatus* originated in the Congo basin and subsequently dispersed in a southerly direction to southern Mozambique, and to Mpumalanga Province and KwaZulu-Natal Province in South Africa. Cotterill (2006) estimates this invasion of tigerfish into the Upper Zambezi and adjacent rivers to have occurred relatively recently (during the Pleistocene period). According to Cotterill and Goodier (2009), *Hydrocynus* spp. have dispersed east across the African Rift Valley only three times. The first in the Lower Zambezi, along the Gwembe and/or Luangwa graben; the second from the White Nile (south-west Sudan) into the Omo drainage (including Lake Turkana) reaching Lake Chamo in south-west Ethiopia; and the third into Tanzania's Rufiji-Ruaha drainage basin (possibly along a Congo tributary across Lake Tanganyika). Skelton (1994) believes that there is a lack of evidence to prove the theory of north to south migrations and instead hypothesised that the modern distribution of fishes is a result of the drainage evolution within that region.

A study on the drainage evolution of Central Africa (Figure 3) by Stankiewicz and De Wit (2006) stated that North Africa was mostly below sea level pending the end of the Cretaceous period (65 million years ago). Hereafter, an intricate sequence of uplifts and stream captures created the African river basins we recognize today. These authors stated that the drainage evolution in the Palaeocene period started when the Okavango, Kalahari and Zimbabwe (OKZ) axis beheaded the Limpopo River, in turn transforming the Okavango, Cuando and Upper Zambezi into a landlocked system. Simultaneously, the watershed separating the Congo Basin from

the rivers draining into the newly formed Atlantic Ocean moved eastwards. During the Pliocene period the Rufiji River was beheaded, Chambeshi and Luangwa became landlocked, Lualaba was reversed, and the Congo Basin was landlocked awaiting the breach of the watershed to the Atlantic Ocean. In the Pleistocene period, the Chambeshi was captured by the Kafue and Luapula Rivers while the Luangwa and Upper Zambezi was captured by the Lower Zambezi. Thereafter, further captures of the Cuando and Kafue Rivers generated the model observed today.

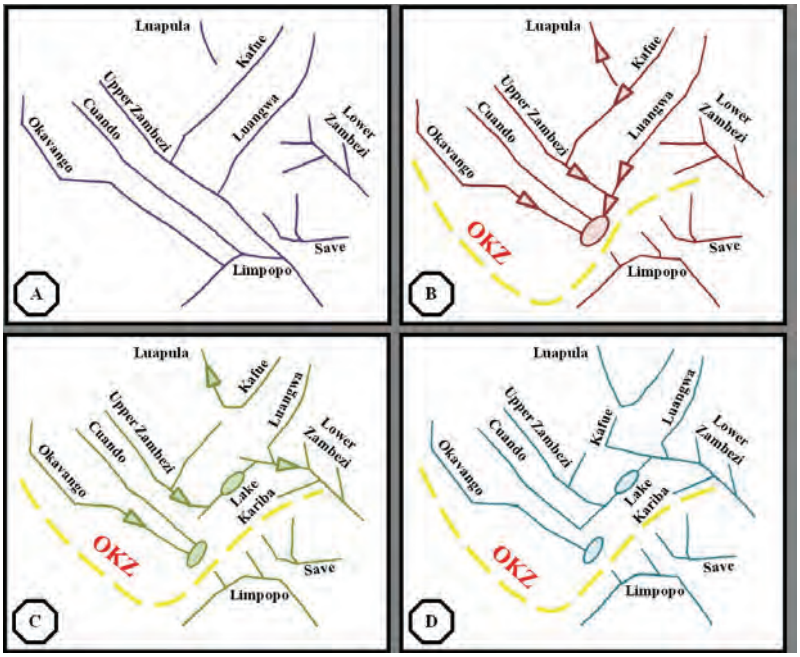

Figure 3. Drainage evolution model of the Zambezi, Okavango and Limpopo, south of the Congo Basin during the (A) Cretaceous (> 65 Ma), (B) Palaeocene (34-65 Ma), (C) Pleistocene (1 Ma), and (D) the present (modified from Stankiewicz and De Wit 2006); *OKZ = Okavango, Kalahari and Zimbabwe axis.*

Whichever way the tigerfish were distributed, their distribution pattern and geographical changes of land surfaces has led to the isolation of populations over time. According to Ayala (1982), this isolation is a principal cause of both phenotypic and genotypic differences amongst various populations of a species.

Genetics

To our knowledge there are only three known published genetic studies on tigerfish. The first, an electrophoretic analysis, was done by Kotzé et al. (1998) comparing the genetic variation of tigerfish from the Upper Zambezi (ZAM) and Olifants (OLI) River. This study revealed the OLI tigerfish population had higher genetic variation than ZAM. These authors thus concluded that OLI represents the most suitable stock for

use in artificial propagation. However, they did not include any of the other tigerfish populations in Southern Africa in their study (e.g. Okavango, Incomati, Phongolo, Limpopo and Mozambican systems). Thus it is not known how these populations' heterozygosity would compare. For this reason it is not yet safe to say that OLI tigerfish would be the best population to be used as brood stock.

The second electrophoretic analysis by Soekoe et al. (2009) yields information about the quantity and pattern of genetic variation in tigerfish of the Okavango Delta (OKA), comparing this information to the previous study by Kotzé et al. (1998), mean heterozygosity was lowest in OKA and highest in OLI. These authors stated the cause of this low variation to be a founder effect instigated when the Okavango and Zambezi rivers became separated. Another possible explanation would be that the Okavango is a more stable system and therefore large variation might not be required by individuals for survival.

The latest genetic analysis (Goodier et al., 2011), using mtDNA sequence data, provides the first complete molecular phylogeny of *Hydrocynus*, incorporating all extant described species with representative coverage. This analysis included five species of *Hydrocynus* (*H. forskahlii, H. brevis, H. goliath, H. tanzaniae, H. vitattus*) from 23 principal rivers within 15 geographically isolated drainage basins throughout sub-Saharan Africa. This study reveals two modes of speciation in *Hydrocynus*, allopatry by dichopatric speciation (ancestral species isolated across a new geographical barrier) and/or peripatric speciation (founders disperse across an existing barrier with subsequent divergence, as supported by the chrono-biogeographic strategy (Hunn & Upchurch, 2001, Crisp et al., 2011).

Goodier et al. (2011) found the presence of five previously unknown lineages (A-E), all with independent evolutionary histories initiated in the Plio-Pleistocene. Lineage A, an unknown species complex, was found in the Congo Basin (Kwango, main Congo, upstream Kisangani and Lulu River). Lineage B, C and D, all *H. vittatus sensu stricto* (s.s.) were found in the Lake Tanganyika tributary (Lufubu River), Congo and Zambian Congo (Lake Mweru, Lake Bangweulu, Dja River), and Zambian Congo (Luapula River, Lake Mweru, Lake Bengweulu, Chambeshi River) respectively. Lineage *E. forskahlii* complex was found in Sanaga River and West Cameroon (Sanaga River). Goodier et al. (2011) further states Complex D to be a sister species to *H. vittatus* and Complex E a sister species to *H.forskahlii*. Except for Group E in the Sanaga river, all new lineages discovered occur in sympatry with at least one described species of *Hydrocynus*. It is, however, not yet clear how/why these sympatric lineages exist, therefore Goodier et al. (2011), highlights the need for further studies (morphological, ecological and behavioural).

Phylogeography

Recent research by Goodier et al. (2011) shows that evolutionary events in tigerfishes are attributed to a spatio-temporal drainage evolution, isolating ancestral populations in new habitats or opened up dispersion prospects expanding their range. This study rendered various shared haplotypes between populations and thus brought about new information on the dispersal patterns of tigerfish and the past connections of the systems in which they reside. Upon analysing tigerfish in the Congo Basin, *Hydrocynus. s.s.* appear to have disbursed from the south. Furthermore, results show numerous populations of *Hydrocynus* share haplotypes across immense distances in this Basin. This indicates that *H. goliath* and *H. vittatus* were either previously connected, and/or experienced major dispersions in more recent past. Results also showed that the Okavango and Upper Zambezi Rivers tigerfish share a haplotype, confirming recurrent connection amongst the Okavango and Upper Zambezi systems (Bell-Cross, 1965).

Conservation

According to Skelton (1987), *H. vittatus* was not listed in the Red Data Book of Fishes, thus Gagiano (1997) concluded that there was no need for concern about, or protection of this species. The latter author did, however, state that the status of this species may differ from one system to another due to factors such as loss of habitat, water quality and overexploitation. More recent literature shows this status to no longer be true. Numbers have declined in many rivers due to overfishing, water extraction, pollution and obstructions such as dams and weirs (Steyn et al., 1996; Skelton, 2001). This has resulted in tigerfish being placed on the South African protected species list (DEAT, 2007). Conversely, according to the IUCN Red List of Threatened Species (Azeroual et al., 2009), *H. vittatus* are common and plentiful with a wide distribution and therefore listed as a species of least concern in central, eastern, north eastern, southern and western Africa. Surprisingly, this decision was made after stating that the tigerfish are depleted by heavy fishing pressure and protected in some reserves in Southern Africa (Azeroual et al., 2009). Azeroual et al. (2009) also suggest that local gillnet and riverine fisheries need to be managed in conjunction with the construction of fish-ways around weirs and dams.

Hence, although the conservation status of tigerfish in Africa and Southern Africa in particular is in dispute, it is clear that this species is overfished and under pressure in various parts of our continent. It might thus be better rather to err on the side of caution when it comes to the conservation of tigerfish in Africa.

Tigerfish Biology

Natural reproduction

Although the exact locality of tigerfish spawning is not known (Kenmuir, 1972), it has been reported that spawning takes place amongst aquatic vegetation on flooded river banks (Gaigher, 1970; Steyn et al., 1996) in shallows upstream of rivers (Badenhuizen 1967) and floodplains (Gaigher, 1967; 1970). Spawning behaviours seems to vary between populations. Jackson (1961) reports an excessively short spawning period for all members of the order Ostariophysi. Bell-Cross (1965-66) found ripe running males in the Upper Zambezi during October while mature females were only caught in November. In an attempt to determine the spawning time of this species, netting and underwater observations were used in an attempt to find eggs or fry. Unfortunately by mid-December there was no sign of either. The duration of the breeding season is speculated to be as long as five months and is said to correlate with the river flow (Kenmuir, 1972), usually taking place during times of flood (Gaigher, 1970; Bowmaker, 1973; Kenmuir, 1972).

Spawning behaviour of tigerfish in the Okavango seems to be different to that of other systems as Merron and Bruton (1988) believed that spawning took place before, and not during flooding. As tigerfish mostly rely on flooding to spawn, environmental factors (e.g. drought) and human manipulation of systems (e.g. induced flooding) may interfere with this natural behaviour. If a female waits too long to spawn, it will lead to the atrophy of her eggs (Steyn, 1987). Egg atrophy was reported by Bowmaker (1973) in Mwede, and by Langerman (1984) in Lake Kariba.

Maturity of tigerfish not only differs between populations but also between sexes. In Lake Kariba males ripen before females (Kenmuir, 1972). The same pattern was true for tigerfish from the Olifants River in the Kruger National Park as Du Preez and Steyn (1992) found that males were already mature in April and October and some in ripe-running condition while females were less developed.

The start of female maturity between different systems ranges over lengths of between 260-522 mm. Female maturity seems to vary amongst populations and have been reported to commences at a length of 360 mm in the Incomati River (Gaigher, 1975), 260 mm in Lake Kariba (Langerman, 1984), 420 mm in the Okavango River (Van Zyl 1992) and 522 mm in the Okavango Delta (Gerber et al., 2009). Males mature at smaller sizes (170-451 mm) with male maturity taking place at 200 mm in the Incomati River (Gaigher, 1975) and Lake Kariba (Langerman, 1984), 170 mm in the Okavango River (Van Zyl, 1992) and 451 mm (TL) in the Okavango Delta (Gerber et al., 2009). From the above it is clear that tigerfish from

Lake Kariba and the Okavango Delta mature at the smallest and largest sizes, respectively, from all populations studied thus far.

Female fecundity is extremely high with one large female (650 Forked Length (FL)-700 FL) capable of producing approximately 800 000-1 000 000 eggs (Van Loggerenberg, 1983; Skelton, 2001). Males have high sperm counts which is a distinctive feature of stream spawners (Steyn, 1993), but low sperm motility (Steyn & Van Vuren, 1991). Unfortunately tigerfish are not able to capitalise on their high fertility due to factors such as unsynchronised maturity and uneven sex ratios (Steyn, 1987). These problems drastically reduce the chance of successful spawning and thus fertilisation of the females. Uneven sex ratios have been reported in Lake Kariba by Kenmuir (1972) where the female to male ratio was 1.35:1 in non-breeding seasons and 1:4 in peak seasons. Langerman (1984) reported a female to male ratio of 1:1.8 in the same system. Unsynchronised maturity has been stated in many publications and almost seems to be the norm for this species.

Ageing

Despite the importance of tigerfish, few aging studies are available for this species, and the information that is available focuses on scale age and does not take otoliths into account. According to Griffith (1975) the management of this species has been hindered by this lack of knowledge. It is important to determine the best ageing techniques per species in order to establish the age structures of various populations. This eliminates any errors in the age-based assessment of the growth and mortality rates of a species, and allows proper species management (Kanyerere et al., 2005; Kimura et al., 2006). Although sectioned otoliths are considered to be the most appropriate hard tissue for growth and age determination in sub-tropical and tropical fishes (Beamish & McFarlane, 1987), currently only a single study has been done using otoliths while all previous age and growth estimates of *H. vittatus* in Africa have been done entirely on scales (Griffith, 1975). The adoption of an age-determination method should be preceded by an age-validation technique, to determine accuracy (Beamish & McFarlane, 1983). Age validations may, however, be too time-consuming or expensive, and therefore many studies attempt to determine process errors in the form of errors in precision and accuracy (Campana, 2001). As of today, no age validation has been done for any species of tigerfish. Thus all ages are relative age estimates.

Tropical fish (e.g. tigerfish) are more difficult to age as the ring formation on their bones depends on food availability, type of food and breeding unlike temperate

fishes that depend only on temperature (Guma'a et al., 1984). Therefore, if the annularity of the formation of growth rings on bony structures is not verified per species, age estimation might be inaccurate (Bishai & Abu Gideiri, 1965; De Bont, 1967; Blake & Blake, 1977).

Guma'a et al. (1984) studied the reliability of ageing three bony structures of the tigerfish from Sudan and concluded that the opercula and vertebra had the highest ageing reliability, while the scales were least dependable due to their ability to constantly regenerate. These authors tested two ageing methods, namely scales (Bagenal, 1978) and opercular bones (Craig, 1974). The results of this study showed that tigerfish render a predictive equation of L = 58.747 + 43.786 R_S (r = 0.97) and L = 23.901 + 20.337 R_O (r = 0.83) for the scale and opercular bone methods, respectively. Guma'a et al. (1984) concluded that the opercular bones were reliable but the scales were not, due to their regenerative capacity.

Ageing studies carried out in the Upper Zambezi (Hastings, 1971) and Lake Kariba (Balon, 1971; Kenmuir, 1972) assumed that regular marks seen on the scales were annuli. Not one of these studies took into account the time taken for these marks to be deposited onto the scales, nor the cause of their deposition (Griffith, 1975). For this reason, Griffith (1975) assessed the regularity, timing and cause of the mark depositions on the scales of tigerfish in Lake Bangweulu to validate scale-ageing techniques for this species. Kenmuir (1972) and Griffith (1975) found that these regular marks were formed between November and January, coinciding with the spawning period. The latter author thus believed that these checks may function as annuli in age assessments of mature fish if their age and maturity are known.

Based on the length frequency method of growth, Kenmuir (1972) established that fish of two years and three years old had lengths of approximately 30 cm and 38 cm, respectively, and that the older the fish the larger the variability of lengths. Although Balon (1971) found that older males appeared to have a faster growth rate than females, Kenmuir (1972) did not notice this difference in males and females of up to the five years old and unfortunately did find many males beyond this class to prove or disprove Balon's (1971) statement. Kenmuir (1972) also states that large tigerfish have a more rapid growth rate than average and that slower growing fish have less chance of reaching large sizes

Gerber et al. (2009) compared the scales, and whole and sectioned lapillus otoliths to determine the best method for use in the ageing of this species in order to ensure ageing accuracy**.** The most appropriate method for ageing *H. vittatus* was found to be the sectioned lapillus otoliths. The ageing study of Gerber et al. (2009) showed that male tigerfish did not disappear from populations at a young age, as

previously thought and in fact lived for 20 years while females lived for up to 16 years.

Tooth replacement

Several studies are available on tooth replacement in characins (Monod, 1950; Petrick, 1967; Roberts, 1967; Kenmuir, 1972; Gaigher, 1975; Tweedle, 1982; Brewster, 1986). Evidence of tigerfish replacing their teeth has been around for many decades. Petrick (1967) reported that tigerfish do in fact possess replacement teeth in both their upper and lower jaws and tried to discover how these teeth rotate into position to replace the lost teeth. This author went on to state that although replacements in the upper jaw are highly likely to become erect and move upward and into the functional tooth's place, the replacements in the lower jaw lay in such a way that it seemed far too complex for them to be able to do the same. Monod (1950) and Weitzman (1962) also doubted that the so-called replacement teeth of the lower jaw were actually able to perform a replacement function. Begg (1972) found dozens of tigerfish teeth at the bottom of a tank where he kept eight large tigerfish for a month. This author thus dried a skull of *H. vittatus* and found cavities below the palate of the fish that contained canines which he referred to as the replacement teeth.

Toothless specimens have been caught by anglers and tigerfish have also been found to contain what is assumed to be their own teeth in their stomach contents (Begg, 1972; Kenmuir, 1972). Gagiano (1997) found three teeth in the stomach contents of one of his specimens. He assumed that the teeth were swallowed by the individual in the replacement process and that low catch frequencies of tigerfish with no teeth may indicate replacement to be a swift process which is imperative for a predator that depends on its teeth for survival. Tweedle (1982) observed a tigerfish with loose teeth and commented that they were easily removed with only light finger pressure. This author also collected a tigerfish specimen, 400 mm in length, and weighing 740 g with unusually small teeth protruding only 3 mm from its gums, and assumed that these had been recently replaced and that replacement takes place simultaneously.

Probably the most compelling evidence of tooth replacement in tigerfish comes from Gaigher (1975) who caught 31 tigerfish from which one noteworthy individual stood out. Although all fish were of similar size, this one had small teeth compared to the large well-defined teeth of all other individuals. An X-ray analysis of the head of all 31 individuals revealed that all but this one still possessed replacement teeth

embedded in their jaws, yet again indicating that the small teeth were due to them having been newly replaced. The upper, lower, right and left jaws teeth of this specific individual were also all identical. Thus the author concluded that tigerfish replace all of their teeth simultaneously. This was also noted by Kenmuir (1972) and Gagiano et al. (1996).

Gagiano et al. (1996) studied tooth replacement of tigerfish from the Olifants and Letaba Rivers in the Kruger National Park. He documented that the first ever replacement happens at approximately 6-7 months of age and is completed within 3-5 d. The teeth of the tigerfish also adapt according to the prey they are feeding on during their different prey-cycle stages. Fry at lengths of between 10 mm and 25 mm have conical teeth which are replaced with tricuspids at lengths of 25-35 mm and again substituted with conicals when the diet becomes increasingly piscivorous (Skelton, 2001).

Food and feeding

Kenmuir (1975), Mhlanga (1997) and Takano and Subramaniam (1998) studied the feeding habits of tigerfish and tigerfish fingerlings from Lake Kariba. Before the introduction of the kapenta (*Limnothrissa miodon* Boulenger, 1906) in 1967 and 1968, tigerfish fed largely on Cichlidae and Characidae. This species, however, showed a preference for the kapenta and thus a dietary shift took place (Mhlanga, 1997).

Although mainly piscivorous, tigerfish also feed on insect and zooplankton at different stages of their lives. Bell-Cross (1965-66) studied tigerfish from the Upper Zambezi River system. This author stated that fish less than one year old fed on zooplankton, crustaceans, insects and juvenile fish; fish older than two years (18-50 cm) fed on adult fish smaller than 10 cm; and fish > 50 cm (23.18 kg) fed on fish that grew > 10 cm as adults. Kenmuir (1975) states that five-day-old tigerfish larvae of ± 5 mm feed on zooplankton while 40-50 mm fish feed on insects and fish and 60-70 mm individuals become almost entirely ichthyophagous.

In Gagiano's (1997) M.Sc. dissertation on the Olifants River tigerfish, invertebrates were found in 84% of fish sampled and in fish of up to 320 mm (SL), thus he concluded that fish did not play a major part in this population's food consumption pattern and that there was no clear-cut change to an exclusively ichthyophagous diet. This same author also found no correlation between length classes and feeding preference of tigerfish from the Olifants and Letaba populations. Although the size of the tigerfish prey increases in direct proportion to its body size

(Adebisi, 1981), their maximum prey size is approximately 40% thereof (Takano & Subramaniam, 1998). Prey fish are usually taken from the side and swallowed whole and head first (Skelton, 2001).

Bell-Cross (1965-66) also noted a variation in tigerfish feeding behaviour with changes in water flow. During low flow (June-November), fish aged two years and older assembled in/near-fast moving water preying on congregations of small fish. In high water seasons (December-January), floodplains were the habitat of choice as small species breed in shallower water. During the high-water seasons in April and May tigerfish congregate where the receding floodwaters from plains flow into large rivers bringing back the smaller species.

Microscopic biology

Coetzee et al. (1991) studied the stomach wall of tigerfish from the Caprivi and noted distinct differences in this species compared to other vertebrates. These dissimilarities include the mucosa which is made up of four layers, the epithelial layer, gastric glands, lamina propria and muscularis mucosae. Narrow, columnar cells abundant with mucous granules make up the epithelial layer. Gastric glands consist of pepsinogenic cells of non-uniform height, and contain tubulovesicles and microvilli. The lamina propria and muscularic muso were both found to include five different basally located, granulated cell types. The submucosa consists of loose connective tissue, serosa of mesothelium and a tunica muscularis made up of inner circular and outer longitudinal layers. These authors went a step further and did an immunocytochemical analysis which confirmed CCK (gastrin/cholecystokinin) and VIP (vasoactive intestinal polypeptide) immunoreactivities in the gastric glands. Finding VIP and CCK is a first in Alestidae (then Characidae) as a previous study on 11 teleost species by Langer et al. (1979) showed no immunoreactivity in the Characidae studied.

Another histological and ultrastructural analysis was also done, this time by Geyer et al. (1996), on the hepatopancreas of the tigerfish from the Caprivi. This study shows the liver to have irregular lobules which are split by the exocrine pancreas and its connective tissue. Spherical/oval hepatocytes, two to three layers thick, possess centrally located nuclei with highly discernible nucleoli. Smooth and rough endoplasmic reticulum, free polysomes and mitochondria are found in abundance in the cytoplasm of these hepatocytes. Found throughout the liver is exocrine pancreatic tissue containing spherical, basally located nuclei with prominent

nucleoli and rough endoplasmic reticulum and secretory granules. This tissue is encapsulated by endothelium and isolated from the parenchyma via a sinusoid.

Ecology

Ecological studies have been done on tigerfish from the Incomati River system (Gaigher 1970), Lake Kariba (Kenmuir, 1972), Upper Zambezi (Bell-Cross, 1965-66; Thorstad et al., 2003; Økland et al., 2005), and the Okavango System (Merron & Bruton, 1995).

In the main river and tributaries of the Upper Zambezi, the distribution of *H. vittatus* is more dependent on behaviour inhibition (such as fear of being cut off from main habitat) than on physical factors such as food and oxygen availability or temperature fluctuations (Bell-Cross 1965-66). Interestingly, this author also states that the Ngonye falls are not a permanent physical barrier for tigerfish migrations as the river level rises in the rainy season reducing the height of the falls. Tigerfish are present in the tributaries of the west bank (Chobe River and Lungwebungu) and east bank (Kabompo and west Lunga).

Gaigher (1970) studied the ecology of tigerfish in the Incomati River system and found them only in the warmer waters of the lowveld sections and common in the Incomati River up to Komatipoort and in the Sabie River up to the eastern border. This author attributed the absence of tigerfish above the weirs to a major hailstorm in 1964 that wiped out whole tigerfish populations. Gaigher (1970) also found that tigerfish migrated downstream to Mozambique to spawn and upstream at the end of the rainy season following *Labeo cylindricus* Peters, 1852.

Kenmuir (1972) found that tigerfish fry occurred in higher densities at the river lake interface of Lake Kariba and stayed in shoals near the surface during the day and further descended into the depths by night. Juveniles (30-60 mm) occupy marginal areas with suitable vegetation cover (although tigerfish are sometimes near vegetation they have never actually been recorded under it; see Økland et al., 2005) while larger fish (60-80 mm) revert to open water habitats (Skelton, 2001).

In the Okavango, tigerfish are restricted to the perennial swamp and riverine floodplains. Possible inclination to these areas may include this species' preference for large, clear, fast-flowing habitats or its sensitivity to change and therefore preference for more stable habitats (Merron & Bruton, 1995).

Thornstad et al. (2003) studied the movements and habitat utilisation of three different fish species in the Upper Zambezi River. When these authors compared radio-tagged tigerfish (n = 15), to *Oreochromis andersonii* Castelnau, 1861 and

Serranochromis robustus Günther, 1864, tigerfish movements were recorded as being 4 to 16 times higher than those of the other two species. This was, however, not the case for all tracked tigerfish as half of the individuals remained permanently within their defined home ranges. The other half of the tigerfish tracked showed that they were resident for periods of time but not only in one locality; on average they moved 18 784 m between localities.

Parasites

Along with the *Annulotrema* spp. studied by Paugy and Guegan (1989), mentioned earlier, not many studies emphasise tigerfish parasites. Boomker (1994) studied the nematodes of tigerfish (*H. vittatus*) from the Crocodile and Olifants Rivers (Kruger National Park). This research proved tigerfish to be a new host for *Contracaecum* spp. larvae. This author found that larger fishes (e.g. catfish and tigerfish) are major paratenic hosts for this species of larvae. Tigerfish of the Crocodile and Olifants Rivers were host to between 90 and 266 and 31 and 42 larvae, respectively. Both systems showed 100% prevalence of the *Contracaecum* spp. larvae. Boomker (1994) attributed this high prevalence of larvae to the abundance of the intermediate host in dams compared to streams/rivers or the final host, piscivorous birds, being present in great numbers. New host records were also found in tigerfish of the Crocodile River where *Spinitectus* sp. and *Paracamallanus cyathopharynx* both showed 50% prevalence. The latter of these two species was recorded in tigerfish for the first time in South Africa. Boomker (1994) is of the opinion, however, that this should be considered an accidental parasite of the tigerfish.

According to Christison (1998) six *Annulotrema* spp. have been recorded for *H. vittatus*. These were from Tanzania (*Annulotrema magna* Paperna 1973; *A. ruahae* Paperna 1973; *A. nili ruahae* Paperna 1979; *A. pikei ruahae* Paperna 1979); Mali (*A. pikoides* Guegan, Lambert and Birgi 1998); Ghana/Uganda; and Southern Africa [*A. pikei* (Price, Peebles and Bramford 1969)]. The eggs of these monogeneans have no filaments and are thus thought to be released directly into the water (Christison 1998). Christison (1998) thus hypothesises that this release is synchronized to the tigerfish spawning period, ensuring their transmission as during this time tigerfish inhabit shallower, calmer water. This same dissertation states that although high infestations of *Annulotrema* spp. are common in tigerfish, histological sections reveal that the pathology they cause is limited and not life threatening. At lower infestation levels these parasites are seen to be site-specific as to the gill arch they choose and

the section which they occupy; at higher intensities, however, site preference becomes less uniform (Christison, 1998).

As part of her Ph.D. thesis, Reed (2003) studied myxosporean parasites in fish from the Okavango Delta over the period 1998 to 2001. This author was the first to record the presence of *Myxobolus hydrocyni* Kostoingue and Togoebaye 1994, in the Okavango. A total of 51 tigerfish were caught, all ranging between 100 mm and 740 mm. All *M. hydrocyni* were found in the gill arches and opercula of *H. vittatus* at a prevalence of 22%.

Humans and tigerfish

Angling stress

Despite tigerfish being protected, they are a high-profile species economically due to their popularity as a sport fish. Although the sport-fishing industry encourages anglers to practise catch-and-release angling, no studies have been done on the effect this practice may have on this species or any other freshwater game species in Africa. The effect of catch-and-release angling on tigerfish is of utmost importance as the ultimate success of this type of angling depends on the survival of the fish by minimising injury and mortality (Bartholomew & Bohnsack, 2005). Following high-intensity anaerobic exercise, various studies have shown that once captured, the blood lactate levels in fish are elevated. This may possibly be associated with delayed mortality (Ferguson & Tufts, 1992; Van Raaij et al., 1996). Due to this observation, Smit et al. (2009) studied the use of blood lactate as a biomarker for angling-induced metabolic stress in tigerfish and examined the relationship between angling time and blood lactate levels. These authors analysed the landing time, handling time, body mass, total length and blood from 66 anaesthetised fish. A strong, positive correlation (r^2 = 0.607) was seen between the landing time and body mass of landed fish as well as significant elevations in blood lactate levels subsequent to angling, regardless of angling time. These results led the authors to propose that longer angling time significantly increases physiological stress, in turn possibly impacting on the breeding success and mortality of tigerfish.

Ecotoxicology

Within fish communities, piscivorous fish have the highest mercury concentrations indicating the presence of possible bioaccumulation (Phillips et al., 1980; Wren et al., 1983). Thus top predators, such as tigerfish, are more susceptible to pollutants

compared to species in lower trophic levels. Organic and inorganic contaminants continuously infiltrate water systems as a result of numerous harmful practices such as mining, agriculture and pest control, to name a few. Even though this is a well-known fact, and tigerfish are especially susceptible, little information is available on contaminant levels in this species, as only four studies using this species have been published in Southern Africa.

The first study was on the environmental and health implications of DDT-contaminated fish from the Phongolo Flood Plain (Bouwman et al., 1990). These authors sampled *Hydrocynus vittatus, Oreochromis mossambicus* Peters, 1852 and *Eutropius depressirostris* Peters, 1852 finding low levels of DDT in the fillets of all three species. The results of this study showed that tigerfish had the highest levels of DDT; the authors attributed these higher DDT levels to the fact that tigerfish are piscivorous predators and potamodromous causing bioaccumulation and possible exposure to areas with higher localized contamination, respectively. Thus Bouwman et al. (1990) stated that the body burden in tigerfish is not a true reflection of local conditions although they are essential indicators of system contamination. Fish downstream showed lower DDT levels and its by-products which Bouwman et al. (1990) believed to be due to photodecomposition, adsorption into clay/organic sediment and biological decomposition.

The second study was a preliminary investigation of selected metal concentrations in tigerfish from the Olifants River in the Kruger National Park (Du Preez & Steyn, 1992). The concentrations of Fe, Zn, Pb, Ni, Cu, Cd and Mn were analysed by atomic absorption spectrophotometry. Axial muscle, gill, stomach, intestine, liver, gonads and body fat comprised the tissues studied. According to Du Preez and Steyn (1992), metals were detected in all tissues in varying concentrations, demonstrating disparity of accumulation in fish. The highest concentration of Cd, Mn, Ni and Pb was found in the stomach, Zn the gonads and Cu the liver. Bioaccumulation factors were also generally low (< 100) suggesting low bioavailability of metals.

The third study tested the mercury concentrations in three species of fish namely *H. vittatus*, *Sargochromis condringtonii* Boulenger, 1908 and *Limnothrissa miodon* from Lake Kariba (Mhlanga, 2000). This study showed that tigerfish (a piscivore), had the highest mercury content of all species tested. Leggett et al. (1991) reported the detection of no mercury in water samples from the same study area, thus, Mhlanga (2000) hypothesised food as the major source of mercury in fish. Further reiterating the possibility of bioaccumulation, the fish eagle, *Haliaeetus*

vocifer Daudin, 1800, one of the few predators of the tigerfish, had high liver mercury concentrations (66-395 mg/kg dry wt.) within the same system (Douthwaite, 1992).

The fourth study by Ikingura and Akagi (2003), used species from various trophic levels to determine total mercury (THg) and methylmercury (MeHg) levels in fish from Tanzanian hydroelectric reservoirs. THg levels in non-piscivorous fish were two to six times lower (5.9-61.8 µg/kg wet wt.) than those found in piscivores (21.8-143 µg/kg wet wt.). Of six species studied the tigerfish they identified as *Hydrocynus vittatus* (possibly *H. tanzaniae*) had the highest mercury levels (21-143 µg/kg wet wt.), with larger fish having higher mercury concentrations. Between 56% and 100% of the THg detected was MeHg. According to Weiner and Spry (1996), > 75% of accumulated mercury, from muscle tissue, in freshwater fish is the organic form resulting in further effectual transfer into the fish by direct uptake from water and through the food chain. Rogers et al. (1995) attributes higher Hg levels in fish to flooding which increases the decomposition of submerged organic matter, thus amplifying microbial activity. According to Ikingura and Akagi (2003), the duration of elevated Hg levels is hard to predict. Mercury levels in non-predatory species may only revert back to pre-impoundment levels after 10-15 years after floods while the levels in predatory species were still increasing (Verdon et al., 1991).

Recently Wepener et al. (2012) studied the current exposure levels of tigerfish to organohalegens in the Pongolapoort Dam, South Africa. These authors tested for the presence of DDT, PCB, HCB, HCH, PBDE and CHLs in tigerfish muscle. Their results showed that the historical use of DDT and the current use of HCBs were reflected in the bioaccumulation patterns of these pesticides by tigerfish. Wepener et al. (2012) further concluded that the seasonal variation of the organochlorine pesticides found in the tigerfish could be attributed to the lipid reserve status of the tigerfish, rather than changes in organic pollutant run off.

Health

In the only study to date on the health status of any tigerfish population, McHugh et al. (2011) did a histology-based health assessment of *H. vittatus* from the Pongolapoort Dam, South Africa. These authors found that although relative high levels of DDT was present in the tigerfish muscle (also see Wepener et al. 2012), and liver, kidney and gill alterations did occur, the fish studied were all in a healthy state. This study provided valuable baseline information on the histology of tigerfish and their cellular response to pollutants such as DDT.

Induced reproduction

The Transvaal Directorate: Nature and Environmental Conservation previously attempted to artificially breed tigerfish for restocking purposes. However, since tigerfish are sensitive to being transported over long distances, and their breeding biology was not known, these attempts were unsuccessful (Gaigher, 1967; Van Loggerenberg, 1983).

Van Loggerenberg (1983) found that female tigerfish do not become sexually mature in captivity and need to be stripped, fertilised and hatched in order for a breeding programme to be successful. In order to understand the way in which this species reproduces naturally, Steyn et al. (1996) embarked on a study of tigerfish reproductive biology and in doing so identified some major factors explaining why artificial reproduction was never a success. These factors include unsynchronized maturation, short breeding seasons and discrepancy of the number of males and females available.

To overcome these predicaments and facilitate the synchronization of spawning and gamete availability a technique for sperm cryopreservation was developed (Steyn & Van Vuren, 1991) ensuring that sperm would always be available as and when it was needed. Steyn (1993) established the physiochemical characteristics of tigerfish sperm allowing for the establishment of an artificial insemination and fertilization protocol. Steyn et al. (1996) went on to successfully induce the reproduction and development of tigerfish and thus made it possible for populations of this species to be restocked. Despite the availability of this information there has since been no record of any population that has been restocked with artificially bred tigerfish.

Conclusion

Most of the publications on tigerfish available in the literature are limited to specific populations. Out of 10 different river systems studied, 26% of the tigerfish research was done in Lake Kariba and 16% in the Upper Zambezi River (Figure 4), and information available on all other systems studied comprised only between 2% and 7%. To date the most popular subjects studied for this species are their ecology, predation, age and growth, genetics, parasites and reproduction (Figure 5). Because tigerfish are a protected species in South Africa, it is imperative that conservation managers have a broad knowledge and understanding of this species. For this reason further in-depth studies are needed encompassing, among many others, the health, genetics, spawning behaviour, age, growth, maturity and the effects of toxins,

pollution and other anthropogenic influences on this species. Only once we have a full understanding of the biology and behaviour of a species will it be possible to implement proper management programmes to ensure the long-term survival of this protected species.

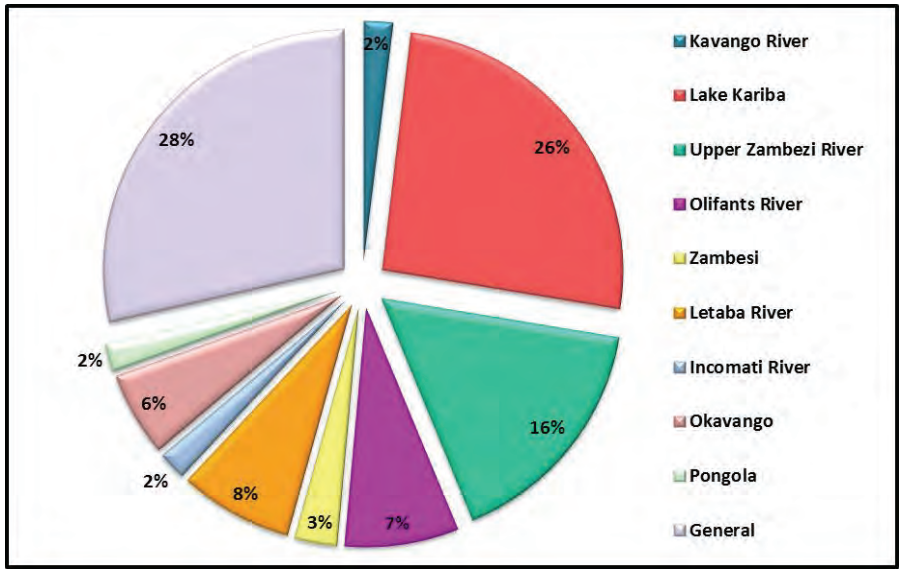

Figure 4. Breakdown of the 86 publications dealing with *Hydrocynus vittatus* here reviewed by locality of the populations studied.

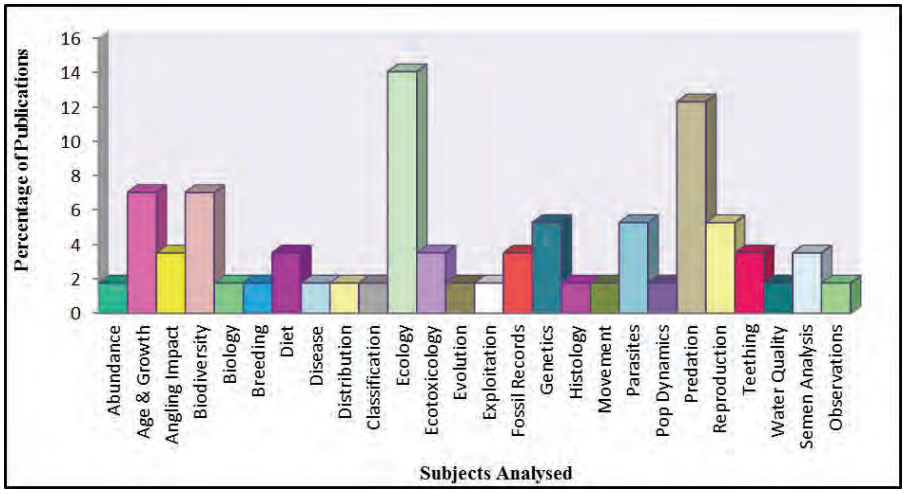

Figure 5. Breakdown of the 86 publications dealing with *Hydrocynus vittatus* here reviewed by research topic.

1.2 Introduction to the Olifants River

The Olifants River, originating in the Bethal-Trichardt area (Coetzee et al., 2002) is the largest catchment in the Kruger National Park and occupies a total 54 805 km2 (Du Preez & Steyn 1992). It initially flows northwards before flowing eastwards; passing through Kruger National Park (Figure 6) before finally entering into Mozambique (Coetzee et al. 2002). The Olifants River passes through the Bushveld

Complex, which is known to possess the largest deposits of chromium, vanadium and platinum group metals on earth (Von Gruenewaldt & Merkle, 1995; Clarke et al., 2009). In parts, the basal sequence is dominated by nephelinites and volcaniclastics, forming part of the Lebombo Group of the Mesozioc Karoo Supergroup (De Bruiyn et al., 2005). The Olifants River is known to lie on a number of dyke swarms (Jourdan et al., 2006). From the entrance of the Olifants River into KNP it passes through the following geological formations and dyke swarms: Orpen Gneiss, Timbivati Gabbro, the Clarens Formation, and the Mashikiri, Letaba and Sabie River Formations before finally leaving the park through the Jozini formation which lies on the Kaapvaal Craton (De Bruiyn et al., 2005).

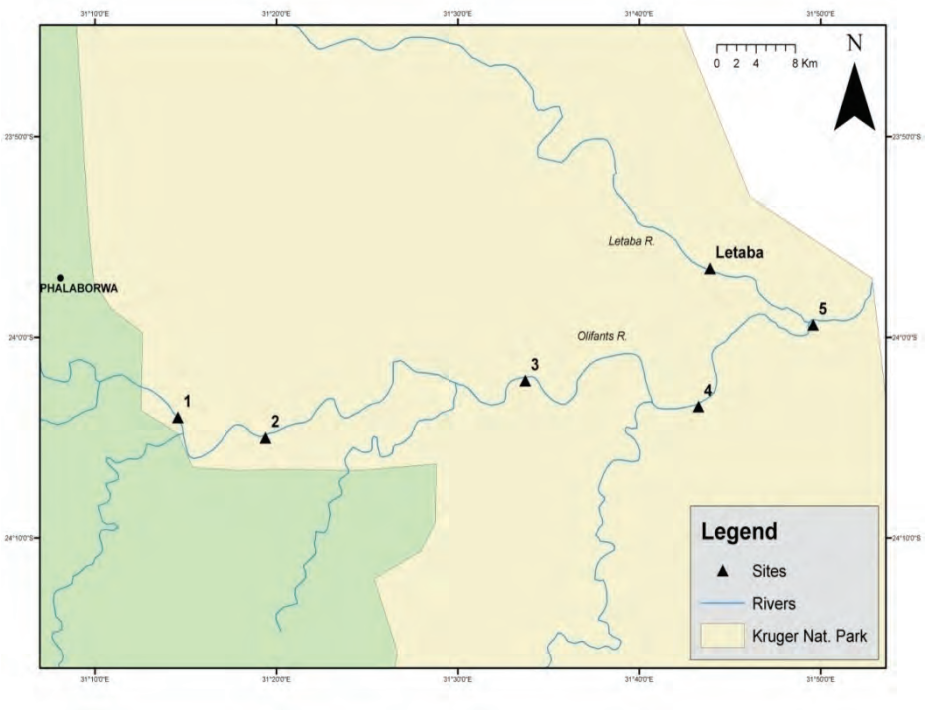

Figure 6. Map of the Olifants River in Kruger National Park, with sampling sites used during the study.

The Olifants River is regarded as one of the most polluted rivers in South Africa (Seymore et al., 1995; Kotze et al., 1999; Avenant-Oldewage & Marx, 2000a), with numerous mining, industrial, agricultural and urban activities in its catchment. The Witbank-Highveld coal field in the upper reaches of the Olifants River is known to discharge mine water directly into streams without pre-treatment causing the local acidification and regional salinisation of the river (Van Zyl et al., 2001). Anglo-coal operates in Witbank from Goedehoop, Greenside, Kleinkopje and Landau mines. Although Anglo coal uses recycled water from water reclamation ponds, they have been given permission to release 177 tons of sulphate per year into the Olifants River

which may result in local acidification and regional salinisation (Cloete, 2008). Also present in the Witbank area are numerous steelworks including Highveld Steel, Ferro Metals and Trans Alloys (Coetzee et al., 2002). There is also a petrol depot, two paint factories and a brewery in this area (Coetzee et al., 2002). Other industries that affect the water quality of the upper Olifants River are six of the eight thermal power stations in the country, 37 coal mines, six brick mines, 17 sand mines, four felsite mines, seven clay mines, domestic effluent and sewage treatment works which overload the river with nutrients (Coetzee et al., 2002). In the Phalaborwa area there are extensive mining and industrial activities, which releases large quantities of sulphates (Wepener et al., 1999) and heavy metals into the river through mining effluent (Seymore et al., 1994, Seymore et al., 1995) and dust that results from mining activities (Wepener et al., 1999). This has been found to affect the water quality of the lower Selati River which flows into the Olifants River (Seymore et al. 1994; 1995). Other factors influencing the distribution of heavy metals in the lower Olifants River are silts which are deposited in the Phalaborwa Barrage, and released during periods of high flow, thus affecting turbidity, dissolved oxygen (DO) and the influx of metals into the system (Wepener et al.; 1999).

More recent studies conducted on the Olifants River have focused on lipid oxidation within fish and crocodiles as a result of the fish and crocodile deaths in the Loskop Dam and in the Olifants gorge in the Kruger National Park (Huchzermeyer et al., 2011). Pathology, histopathology and blood-smear examinations of fish in the Kruger National Park during the 2008 mass crocodile mortalities showed changes consistent with fish suffering from lipid autoxidation which has been described in the literature for rainbow trout (*Onchorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and bluefin tuna (*Thunnus thynnus*). This lipid autoxidation is consistent with a Vitamin E deficiency and is unlikely to be normal in wild-caught fish. Fish severely affected by lipid autoxidation would become easy prey for predators, possibly even before a mass mortality of fish is noticed (Huchzermeyer et al., 2011). The author suggested that lipid autoxidation might be caused by anthropogenic pollutants entering the Olifants River system affecting the primary production and availability of Vitamin E in the aquatic ecosystem. Such excessive pro-oxidant challenges are likely to affect the entire food chain. Increased nutrients and the presence of large impoundments along the Olifants River, like Loskop Dam and Massingir Dam, have caused the proliferation of some species like sharptooth catfish (*C. gariepinus*) and Mozambique tilapia (*O. mossambicus*). The large impoundments mentioned above contributed to the abundant availability of excessively fat fish for predators to feed on. Depleted antioxidants (Vitamin E) and excessive fat in the fish

may have led to crocodiles having insufficient protection against the fish lipids consumed and precipitated the development of pansteatitis in the crocodiles (Huchzermeyer *et al.*, 2011).

Despite all the studies referred to above that indications that the Olifants River and the organisms living in it is not in a healthy state, the Olifants' River Health Report (Balance et al., 2001) describes the catchment as a whole as being in a 'fair to good state'. The section that lies within the KNP was described as being in a fair state. Rashleigh et al. (2009) found that within the KNP, there was no loss of species, but species assemblages were changing. The findings of this study concurred with the conclusions reached by the RHP (Balance et al., 2001). In a study done by Roux (2001) within the KNP, a high biodiversity in biological communities was found. However, it was reported that flow changes had led to assemblage differences and that sufficient water quality and quantity should be present to support species and communities. Conversely, the Olifants River has recently been classified as one of the most threatened river systems in South Africa (Kotze, 1997; Balance et al., 2001; Van Vuuren, 2009; Heath et al., 2010). Based on the above, it can be said that the Olifants River is a river under stress, and this study will attempt to ascertain how these impacts relate to the biological communities present.

1.3 Introduction to the Luvuvhu River

The Luvuvhu River catchment occupies a total of 5941 km^2, originating in the Soutpansberg Mountains. It flows from south-eastern Soutpansberg for 200 km (Angliss et al., 2001), running along the foothills of the Lebombo Mountain range in the lower reaches of the river (Botha & De Wit, 1996), and forms part of the larger Limpopo System, joining the Limpopo at Pafuri (Angliss et al., 2001) (Figure 7). The eastern limb of the Bushveld complex (as previously discussed) touches the southern parts of the Luvuvhu water management area (EWISA, 2007). It has a mean annual precipitation of 608 mm and a mean annual evaporation of 1 678 mm (Kleynhans, 1996; Angliss et al., 2001). There are a variety of different soil types in the Luvuvhu catchment, from alluvial soils, sands and gravel, acidic sandy loamy and gravelly to sandy, sandy loamy and clayey soils. The geology varies from sedimentary rocks in the north to metamorphic and igneous rocks in the south (EWISA, 2007). The geological types it passes through varies from sandstone, shale, grit, conglomerate, quartzite and basalt to gneiss (sandstone, quartzite and shale), granite, and gneiss-granite with dolerite intrusions (Angliss et al., 2001). The Luvuvhu River passes through many different geological regions including the pre-Karoo Basement, the Karoo Supergroup which is dominated by sedimentary rocks and the Karoo

Supergroup which is dominated by igneous rocks (Botha & De Wit, 1996). At the confluence of the Luvuvhu and Limpopo Rivers, near Pafuri, the river passes through the Malonga Formation which can be subdivided into calcerous conglomerate sandstone with intercalculated red, mottled siltstone and sandstone (Botha & De Wit, 1996). The Eastern-most outcrops south of Pafuri are red or grey calcareous marls and large hardpan calcrete horizons (Botha & De Wit, 1996). It passes through a Gona-re-Zhou region and is calcerous sedimentary rock from the calcerous post-gondwanan succession (Botha & De Wit, 1996). The weathering profile shows decalcified parent material with silcrete/ferruginised zone and hard ferricrete developed patchily overlain by a layer of unconsolidated rounded clasts and surficial red/yellow sand (Botha & De Wit, 1996). Also present are strongly rubified sand and rounded clasts and fragments of yellowish decalcified parent sandstone quartz (Botha & De Wit, 1996).

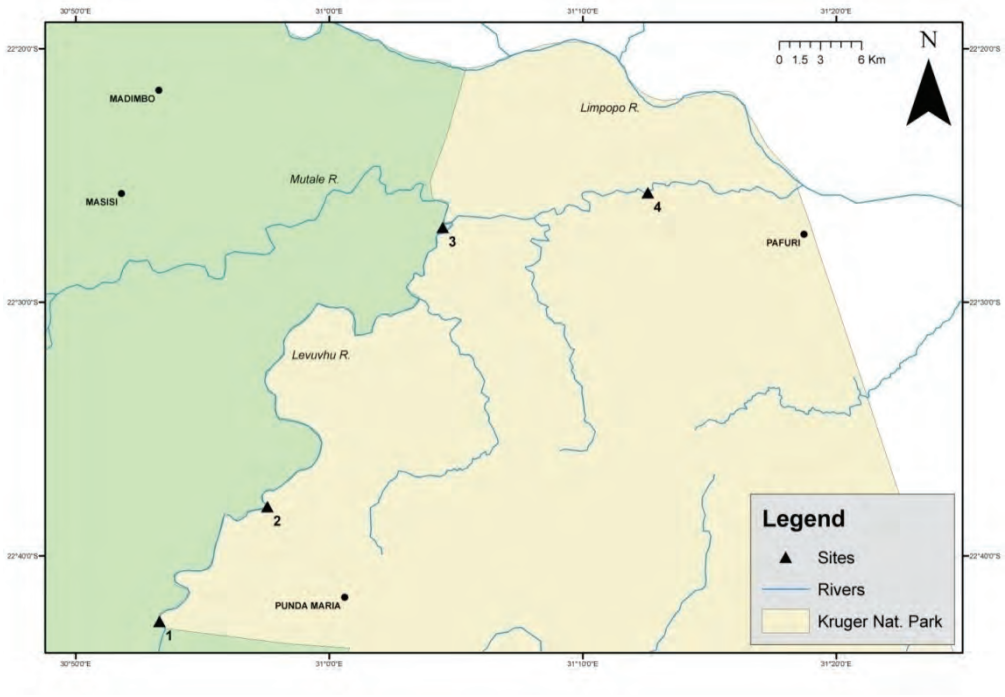

Figure 7. Map of the Luvuvhu River in Kruger National Park, with sampling sites used in this study.

The area is known to have few industry and mining impacts, however there are two mines in the Luvuvhu River catchment, those being the Tshikondeni Coal Mine and the Geocapro Magnesite Mine (Angliss et al., 2001; EWISA, 2007) and there are also gold mines along the Klein Letaba River (Angliss et al., 2001) which joins the Luvuvhu River. The area is highly used for agriculture and forestry (Kleynhans, 1996), where many of these actions threaten bank stability and lead to

erosion. Organic pollutants such as phthalates, which are widely used as industrial chemicals and are released into rivers through effluent discharges, leaching from waste dumps and diffuse sources of pollution, and such pollution has been found in the Luvuhvu River (Fatoki et al., 2010). Dichloro-diphenyl-trichloroethane (DDT) is also largely used in this area as a preventative measure for malaria (Van Dyk et al., 2010), and was shown by Van Dyk et al. (2010) to possess endocrine disrupting properties that might affect the local human population.

According to Fouche et al. (2005) the ever-increasing rural populations settling in these areas will in future place increasing demands on riverine ecosystem resources through various subsistence activities such as doing laundry, ploughing the fields and collecting wood in the riparian zone. Pesticide usage and water extraction by commercial farmers will further add to the degradation of this ecosystem's integrity (State of Rivers Report, 2001; Fouche et al., 2005). The construction of the Albasini Dam and the Nandoni Dam in the middle catchment has led to increased abstraction and flow regime disruption (State of Rivers Report, 2001; Fouche et al., 2005). These impoundments are deemed necessary in order to provide irrigation water to farmers and domestic water to residents but the consequent adverse effects on the ecological integrity of the Luvuvhu River are not known.

These developmental factors will result in more and more pressure on the Luvuvhu River system and ultimately on the biological communities within the system. In terms of the ecological status regarding the biological communities of Luvuvhu River, the RHP describes the river on a catchment scale as being in a ‘fair to natural condition’ (State of Rivers Report, 2001). The assessment was further broken down into reaches, and the river reach within the Kruger National Park (KNP) was seen to be in a natural pristine state. Kleynhans (1996) did a study on flow-related problems within the Luvuvhu. He concluded that although river conditions are said to be pristine and its biological communities in good shape, aquatic biota would increasingly be negatively affected by flow-related problems as more and more water would be abstracted for irrigation, commercial and domestic use within the catchment. In various technical reports (Fouche et al., 2005) the biological communities in terms of assemblages were described as being in a natural state, although some species assemblage problems and population decreases were identified. In the State of Rivers Report (2001) the biological communities of the Luvuvhu River are also described as being in a natural state, concurring with the RHP report. Overall, most literature therefore supports the conclusions made by the RHP report (State of Rivers Report, 2001). However, no recent studies have been

published in terms of the RHP and although it would seem that the Luvuvhu River reach within the KNP is in a natural state, the increased pressures mentioned earlier could cause this to change.

1.4 Rational for use of specific endpoints

Water quality is used to describe the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses, and for the protection of the health and integrity of aquatic ecosystems. Many of these properties are controlled or influenced by components that are either dissolved or suspended in water as a result of either natural or anthropogenic input, or both (DWAF, 1996). The accepted RHP approach will be followed. A series of water samples were collected from the aquatic ecosystems associated with the study area at selected sites. In addition during the collection process certain *in situ* water quality variables were assessed including: oxygen concentration and saturation, conductivity, pH and temperature. The collected water samples were analysed for a range of nutrients, salts and metals.

Sediment quality influences an important abiotic compartment as they represent the ultimate repository for many chemical contaminants in the freshwater environment. Sediments also provide habitats for many aquatic organisms. The objective of monitoring bulk sediment chemistry is to detect and describe spatial and temporal changes of these sediments pollutants. Monitoring of pollutant levels in sediments is a widely accepted means of measuring the condition of the benthic habitat and is a powerful tool for the evaluation of spatial and temporal effects of anthropogenic and natural disturbances (Wepener & Vermeulen, 2005). The singular use of sediment pollutant loading to assess the condition of the benthic habitat or to guide the decision-making process is not recommended since other factors, such as water quality and sediment grain size, can also affect habitat quality. The objective of monitoring sediment grain size composition is to detect and describe spatial and temporal changes of the benthic environment. The availability of sediment contaminants is often correlated with the grain size composition of the benthic medium; sediments contaminants are more easily adsorbed onto small grain sediment surfaces. Likewise, grain size information may explain the temporal and spatial variability in biological assemblages; changes in sediment grain size often affect an infuanal organism's ability to build tubes, capture food, and escape predation.

Habitat quality is an important part of an ecosystem structure and function as it forms the physical template of the ecosystem. If the habitat quality is affected, it will

have an effect on the whole system's integrity. When the habitat diversity is extensive and un-impacted, the biotic community tends to be in a healthy state. In this study the habitat quality and diversity were assessed by applying the methods described by Dallas (2007) for macroinvertebrates and fish.

Bioaccumulation: Measurements of chemical such as metals by direct chemical analysis in water and sediment are limited in reliability (Smolders et al., 2004). Consequently, after the initial suggestion by Goldberg (1975), many studies have utilised living organisms to assess metal levels (i.e. through the process of bioaccumulation) in the environment (Wepener et al., 2012). Chapman (1997) and Rainbow (2007) stress that at present bioaccumulation studies are used to provide information on contaminant-specific bioavailability, assist in identifying possible causative agent(s) of toxicity, and relate body burdens to food chain accumulation values relative to secondary poisoning or biomagnification. These authors caution against the application of bioaccumulation to identify potential toxicity caused by metals as toxic reactions are related to a threshold concentration of metabolically available metal and not to total accumulated metal concentration. Therefore the bioaccumulation results that are presented should be seen as a biological measure of metal bioavailability within the study area.

Biomarker analysis: To overcome the shortcoming of bioaccumulation studies only providing information on biological exposure, increasing research is conducted to evaluate the causal relationships between pollutant exposure and measurable biological effects in aquatic organisms. Consequently, biomarkers, and in particular applying a suite of biomarkers, are more frequently being implemented to assess the general health of organisms in stressed ecosystems and as a measure of environmental health (Van der Oost et al., 2003). Wepener (2008) suggests that in order for biomarker application to be effective the choice of biomarkers is important. Primary responses are rapid and reversible responses at a (intra)cellular biochemical level, secondary responses are generally physiological changes which take more time to occur in organisms and tertiary responses are the least reversible, occur at the highest level of biological organization and have the longest lasting effect. It is for this reason that biomarkers are selected to reflect both measures of exposure and effect. Generally those responses at cellular level must be complemented with assessments at higher levels of biological organization, e.g. fish health assessment and fish community assessment. In this study two types of biomarkers were selected, i.e. biomarkers of exposure and effect. Biomarker responses of exposure; Acetylcholinesterase (AChE – pesticide exposure), cytochrome P450 activity

(CYP450 = PAH and organic chlorine exposure), metallothioneins (MT – metal exposure) and effects; malondialdehyde (MDA), catalase (CAT) activity, superoxide dismutase (SOD) activity and protein carbonyls (PC), all indicative of oxidative stress, cellular energy allocation (CEA) and condition index (CI) indicative of energetic disturbances were applied in this study.

Fish health assessment index were applied using the Fish Health Index (Avenant-Oldewage, 2001). This index is based on a macroscopic technique that applies a range of external appearance features, haematological parameters, parasitic infestation and internal organ features to derive a health score for fish. The score derived for fish from affected areas are related to the reference health status to quantify the measure of health deterioration.

Histopathology was applied to detect any cellular damage ensuing from stressor exposure using accepted international practices (Hinton, 1994). This analysis is based on a microscopic technique that is used to assess the response of organs and tissues to environmental stressors. Cells are the first biological structures that will show visible pathological changes due to exposure to stressors. This technique is used as a measure of effect and to a limited degree a measure of exposure, e.g. histopathology of testis in the presence of endocrine disrupting chemicals. A range of different tissues were utilised, i.e. gill (the first site of environmental – biological toxicant interaction), liver (internal detoxification site) and gonads (indicators of endocrine disruption and potential population effects).

Macroinvertebrate community structure: Aquatic macroinvertebrate assemblages and communities offer a good reflection of the prevailing flow regime and water quality in a river (Thirion, 2007). As such, aquatic macroinvertebrates have been used to assess the biological integrity of stream ecosystems with relative success throughout the world (Rosenberg and Resh, 1993; Barbour et al., 1996), more commonly than any other biological group (O'Keeffe and Dickens, 2000). For South African circumstances, the current index being used to determine and assess the status of riverine macroinvertebrates is the SASS5 protocol developed by Dickens and Graham (2002). The index is based on the presence/absence of particular macroinvertebrate families, and their perceived sensitivity to water quality changes (Dickens & Graham, 2002). This index has undergone several upgrades, but Version 5 is currently in use. It is an accredited protocol that is a biological index of water quality (Ferreira et al., 2008). From this, a classification system was developed by Dallas (2007) which takes into account historical SASS5 scores to form biological bands and as such ecological classes. The ecological category was created from the

biological bands by aligning the SASS5 score and the Average Species per Taxon (ASPT). There was a unique biological band graph for each ecoregion, as historical SASS5 data from each ecoregion were used to create the ecoregion-specific bands. When assessing the results for the SASS5 protocol, both the ASPT and the SASS5 score itself must be taken into account and interpreted in terms of the reference conditions for that river reach, section or site. The ASPT is generally more accurate as an indicator of macroinvertebrate community health, and as such is examined more closely (Dickens & Graham, 2002). It must also be mentioned that the habitat assessment of each site plays a large role in the interpretation of the SASS5 results. The habitats must be rated and then the results assessed based on what habitat was available.

Fish community structure: The use of the attributes of fishes in the assessment of the environmental condition of ecosystems is widely incorporated in the management of freshwater ecosystems (Belpaire et al., 2000; Karr, 1981; Kleynhans, 1999). The multi-metric approach of assessing the attributes of fish communities incorporates information from individual, population and community levels into a single, ecological-based index, reflecting the overall condition of the aquatic ecosystem. In this assessment fish was comprehensively sampled at all selected sites during the survey using active and passive netting techniques, as well as the use of electro-narcosis or commonly termed electro-shocking, where applicable, to collect fish. The fish data were evaluated by the Fish Response Assessment Index (FRAI) (Kleynhans et al., 2007). This index is applicable in all freshwater ecosystem components of the study and is the current index of choice utilised by the RHP (Kleynhans & Louw, 2006). Following the assessment of each driver and response component the lines of evidence (outcomes of each component assessment) were integrated into an current aquatic ecosystem integrity state (EcoStatus) score using the EcoClassification methodology (Kleynhans & Louw, 2006). Furthermore the approach adopted to assess the fish community structures of the different sites is based on the approach implemented by Cyrus et al. (2000). Their approach is to let the community “tell their own story” before attempting to determine how well environmental parameters matched the community patterns. Non-parametric multivariate analyses of community data, based on among-sample similarity matrices, draw inferences only from its ranks. These methods consequently lack model assumptions and therefore have a general validity of application. In contrast to univariate analyses (e.g. ANOVA, regression), multivariate procedures consider each taxon to be a variable and the presence/absence or abundance of each taxon to be an attribute of a site or time (Cyrus et al., 2000). Subtle changes in the community composition across sites,

which are generally masked when the characteristics of a site are combined into a single value, are more likely to be detected by multivariate procedures. Spatial and temporal trends in fish community composition can therefore be displayed by using multivariate methods of data analysis (Clarke & Warwick, 1994).

Fish flow dependent habitat requirement: The flow regimes of most of South Africa's river systems have been altered beyond recognition due to impoundments and excessive water abstraction (Davies et al., 1993; Davies & Wishart, 2000). Alterations in the flow regimes of rivers have been documented to have a negative impact on the conservation status of numerous aquatic organisms including fishes (Skelton, 2000). Understanding the potential impacts of flow regulation and habitat degradation on the biota continues to be a pressing challenge for river scientists. Fish are key components of river ecosystems and are important indicators of their ecological state (Kleynhans et al., 2005). They are particularly sensitive to changes in flow and temperature at critical phases of their life history such as spawning, migration and during early growth and development (Larinier, 2000; Friedl and Wüest, 2002). Understanding the role of flow-dependent habitat variables in regulating fish population dynamics is essential for effective conservation and management of fishes and the systems in which they occur.

1.5 Project Aims

As discussed earlier, tigerfish (*Hydrocynus vittatus*) are widely distributed in the north eastern region of South Africa and are considered to be useful flagship species, thus a species of fish which are easily identified with by the public and widely used by ecosystem managers to relate important ecosystem related information to the man on the street. This species is actively targeted and utilised by various angling and subsistence fishing communities throughout this part of the country, and also used as indicator species by resource managers. As a result tigerfish has a high ecological, economical and social value to South Africans. Although valuable, very little is known about this charismatic species, and unfortunately, before we have the chance to fully understand some of the biological attributes of this species we are losing it due to water extraction, pollution and obstructions like dams and weirs (Steyn et al., 1996; Skelton, 2001). Tigerfish are considered to be rare in South Africa and as of 2008 are classified as a protected species alongside great white sharks and the coelacanth. Despite the fact that this fish hold an important profile as economic and ecologic important species, published information is only available for certain aspects of their biology and also only from specific populations (see review earlier). Historically tigerfish were prevalent in all 6 major rivers in the Kruger National Park and even in

areas on the western border of the Park. Recent surveys have shown that the distribution of this protected species has drastically been reduced. It is thus important that a management strategy is developed for the protection of this iconic fish within the Kruger National Park. Tigerfish are one of the few indigenous top predator fish species of South Africa. It is well documented that top predators biomagnify pollutants and that the risk that these pollutants pose are greater to them than to the lower trophic levels. Notwithstanding this, there is a paucity of data on the levels of contaminants in this species with the only South African study being limited to metal levels in the Olifants River tigerfish population (Du Preez & Steyn, 1992). The levels of these organic and inorganic substances together with the information on population structures and reproductive status will provide valuable insight into whether exposure to these contaminants has an influence on the general health of tigerfish populations in the Kruger National Park. This study will thus specifically address all the factors that might influence the health and conservation of the tigerfish. The upper catchments of all the rivers that run through the KNP are subjected to mining as well as intensive agricultural activities and aquatic organisms are at risk due to environmental exposure to these contaminants. This project on the conservation of tigerfish in the Kruger National Park was conducted on request from the KNP Scientific Services who has identified the management of tigerfish within the borders of the KNP as a conservation priority. The project also addressed the very important question on whether the current ecological water allocation for the Olifants River is sufficient not just in terms of the absence or presence of species, but also the individual and population health of the fish present in the system. With this in mind, this project aimed to:

1: Determine the current distribution of tigerfish in the Olifants and Luvuvhu Rivers within the Kruger National Park.

2: Determine the biological requirements of Kruger National Park tigerfish.

3: Determine whether the environmental water allocation for the Olifants and Luvuvhu Rivers is sufficient to sustainably support a healthy tigerfish population.

4: Determine the factors that might limit the current distribution of tigerfish in the Olifants River in KNP, including water quality and habitat modification.

5: A) Based on the results of this study propose a management strategy for the conservation of tigerfish in the KNP with emphasis on mitigating measures to stimulate tigerfish populations to return to their original natural habitats. B) Validation and consolidation of the use of tigerfish as indicator species of quality and quantity related Threshold of Potential Concern (TPC) in the Olifants and Luvuvhu Rivers.

2 MATERIAL AND METHODS

2.1 Site selection

Olifants River

Four sites were selected along the Olifants River as it flows through the KNP to assess the change in metal concentrations from the theoretically more polluted western to the eastern boundary. An additional site was selected in Letaba River and one at the confluence of the Letaba and the Olifants River (Site 5) in the Olifants River Gorge to determine the contribution of the Letaba River to the state of the Olifants River. The physico-chemical parameters of the sites were determined separately, to determine whether or not the pollutant concentration decreases down the longitudinal gradient of the river. Results from tigerfish from the Olifants River were pooled due to permit restrictions on the number of samples permissible. Site 1 (S24° 03’ 58.7’’ E31° 14’ 35.2’’) is located at Mamba Weir on the western boundary as the Olifants River enters the KNP (Figure 8). Although this site is below a weir, there is sufficient habitat for fish and macroinvertebrate communities to thrive, and as such habitat availability should not be a factor influencing abundances. Site 2 (S24° 05’ 07.2’’ E31° 19’ 16.3’’) is below an old ranger station, and is a section of river where the riverbed is predominantly a mixture of sand and bedrock (Figure 9). As such, it provides many channels and habitat availability is ideal for fish and macroinvertebrate communities. Site 3 (S24° 02’ 06.7’’ E31° 33’ 55.9’’) is considered to be a habitat type that is representative of the majority of this Olifants River reach (Figure 10). It has a wide macro-channel, with slow-flowing micro-channels that are predominantly sand based. Here, it is expected that biological community abundances and diversity will be lower, as river flow and depth are uniform and as such habitat diversity is low. Site 4 (S24° 03’ 14.7’’ E31° 43’ 50.5’’) is just upstream of the new DWA gauging weir. It represents a relative diversity of habitats, and moderate species diversity and abundance is expected (Figure 11). Site 5 (S23° 59’ 25.2’’ E31° 49’ 33.3’’) is located at the confluence of the Olifants and Letaba River in the Olifants River Gorge (Figure 12). This is an important site, as it is in this area where the crocodile mortalities referred to earlier have been occurring. The Letaba River site (S23° 56’ 32.9’’ E31° 43’ 53.5’’) is located in the Letaba River before its confluence with the Olifants River (Figure 13). This is a comparative site to the Olifants River sites sampled. Flow rate and volume is low, but habitat diversity is high and as such biological community diversity should be high.

Figure 8. Site 1 at Mamba Weir as the Olifants River enters into the Kruger National Park (Google Earth).

Figure 9. Site 2 as the river flows eastwards through the Kruger National Park (Google Earth).

Figure 10. Site 3 situated further east than Site 2, as the river flows eastwards through the Kruger National Park (Google Earth).

Figure 11. Site 4 situated before the confluence of the Olifants and Letaba Rivers (Google Earth).

Figure 12. Site 5 situated at the confluence of the Olifants and the Letaba Rivers (Google Earth).

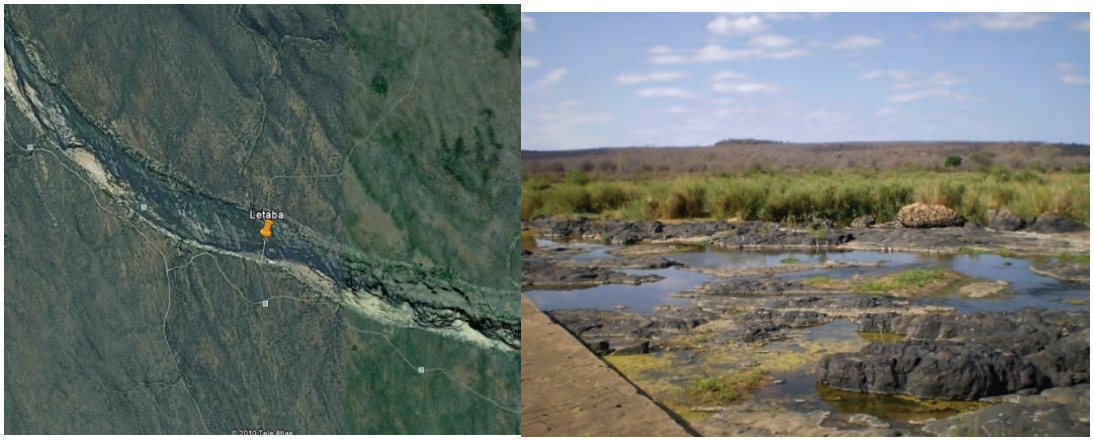

Figure 13. Letaba site, situated along the Letaba River, before the confluence of the Letaba and Olifants Rivers (Google Earth).

Luvuvhu River

Four sites were selected along Luvuvhu River as it flows through KNP towards Mozambique (Figure 7). The physico-chemical water quality parameters were measured at each of the sites to determine whether there is a change as the water flows from the western to the eastern boundary of the KNP. Site 1 (S22° 42' 34.6'' E30° 53' 19.6'') is located where the Luvuvhu River enters the KNP and is opposite an informal rural settlement. The Makuya Nature Reserve is to the north on the western bank, and from here onwards the Luvuvhu River runs through protected areas. The biotopes here are all present, and species diversity and abundances should be high (Figure 14). Site 2 (S22° 38' 05.3'' E30° 57' 33.5'') is located downstream from Site 1 before entering Lanner Gorge (Figure 15). Here the Luvuvhu River flow starts to slow down and the river broadens with large pools and channels present. Site 3 (S22° 27' 04.3'' E31° 04' 47.7'') is downstream of the confluence of the Mutale River and Luvuvhu Rivers (Figure 16). Site 4 (S22° 25' 40.5'' E31° 12' 34.0'') is located downstream of Site 3 before the confluence of the Luvuvhu and the Limpopo River (Figure 17).

Figure 14. Site 1 situated on the Luvuvhu River as the river enters the Kruger National Park (Google Earth).

Figure 15. Site 2 situated east of Site 1, as the river flows through the Kruger National Park (Google Earth).

Figure 16. Site 3 situated just before the confluence of the Luvuvhu and Mutale Rivers (Google Earth).

Figure 17. Site 4 situated just before the confluence of the Luvuvhu and Limpopo Rivers (Google Earth).

2.2 Water quality

Physico-chemical water parameters such as conductivity (µS/cm), total dissolved solids (TDS; mg/L), DO (both percentage saturation and concentration), temperature (°C), and pH were taken *in situ* at each sampling site in the different sites. The

measurements were taken using the following instruments: Cyberscan D0100-Conductivity/TDS meter, Cyberscan D0100- Dissolved Oxygen/temperature meter and Waterproof pHScan pH meter. Sub-surface samples for water metal analysis, and suspended particle metal analysis, were collected from the sites in triplicate in acid-washed polypropylene bottles. These samples were frozen in an Engel 42 L field laboratory fridge-freezer (Sawafuji Electric co. Ltd. 54605420100) and were transported back to the laboratory for further analysis.

Dissolved and suspended metal analysis

The water samples were allowed to defrost and reach room temperature. Cellulose nitrate filter paper (0.45 µm mesh size) was pre-weighed and placed on a glass fibre filter. Sample (99 mL) was filtered, the filtrate was acidified with 2 mL 65% suprapur nitric acid, mixed with 1 mL of indium (In; internal standard chosen because it is rare and possesses few interferences) and decanted into 15 mL Falcon tubes for metal analysis.

Pre-weighed filter paper with residue was rolled into pre-weighed 15 mL Falcon tubes, ensuring that the filter paper was not damaged and placed in a drying oven at 60°C to dry. Once dry, the filter paper was re-weighed and placed in Teflon bombs with 9 mL 30% suprapur HCl and 3 mL 65% suprapur HNO_3 and allowed to be digested in a Milestone Ethos microwave for 45 minutes at 1 000 W and 200°C. The samples were placed in 50 mL glass volumetric flasks and made up to volume with ultrapure water and 500 µL In. The following metals were determined on a radial inductively coupled plasma optical emission spectrometer (ICP-OES; Spectro Arcos FSH12) with the necessary procedural blanks and quality control standards: Fe, Mg, Na, Ca, K, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb and Al. Metals that were below detection on the ICP-OES, as well as As and Se were analysed on an axial inductively coupled plasma mass spectrometer (ICP-MS; X-series II) with H_2/He collision cell technology gas (CCT) injection to reduce argon oxide (ArO) and Se interferences, and the r^2 value taken note of.

Chemical and Turbidity Analysis

The water samples were allowed to defrost and reach room temperature. The samples were tested in triplicate for sulphate (SO_4^{2-}), chloride (Cl), orthophosphate (PO_4^{2+}), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and turbidity (measured in FAU) using a Merck Pharo 100 Spectroquant and the appropriate test kits (Merck photometric test kits).

2.3 Sediment

Sediment samples for inorganic and organic pollutant analyses, percentage organic carbon and grain size were collected from each site in triplicate using acid-washed polypropylene bottles. Excess water was removed from the samples and the samples frozen in an Engel 42 L field laboratory fridge-freezer (Sawafuji Electric co. Ltd. 54605420100) and transported back to the laboratory for further analysis.

Sediment Particle Size Distribution

The sediment particle grain size was determined using an Endecott mechanical shaker with a set of Endecott sieves with different mesh diameters. These grain meshes divided the particles into 4000 µm, 2000 µm, 500 µm, 212 µm, 53 µm and <53 µm. The sieves were stacked from the largest size on top to the smallest size, with a final collection pan at the bottom. The samples were then weighed and added to the sieve on top and sieved for approximately 15 minutes. Afterwards, the sediment retained by each sieve was measured and the percentage composition of each particle size was calculated. The particle sizes were classified according to Cyrus et al. (2000): gravel (>4000 µm), very coarse sand (4000-2000 µm), coarse sand (2000-500 µm), medium sand (500-212 µm), fine sand (212-53 µm) and mud (<53 µm).

Organic Carbon Content

Approximately 1 g of dried sediment was weighed out and placed in pre-weighed, acid-washed ceramic crucibles. The samples were then transferred into an incinerator at 600°C for 6 hours. They were allowed to cool and were re-weighed to determine the inorganic carbon mass. The organic carbon percentage was determined using the following calculation:

% Organic Carbon Content = [(Mb-Ma)/Mb] x100%

Where Mb is mass before incineration and Ma is mass after incineration.

Metal analysis

In aquatic ecosystems changes in pH, salinity, redox potential, microbial activity and particulate matter in sediments affect the bioavailability of metals (Chandra Sekhar et al., 2003). Selective extraction can be used to extract the metals from one mineral

phase (Sandoval et al., 2001) and thus Community Bureau of Reference (CBR) extraction procedures have become of great importance in ecological assessments, allowing for the separation of metals which are bioavailable (acid soluble), less bioavailable (reducible), the least bioavailable (oxidizable) and non-bioavailable. Sediment samples were defrosted and placed in pre-weighed acid-washed glass bottles. The wet mass of the sediment was determined. Samples were placed in the drying oven at 60°C for approximately three days, removed and allowed to cool. The dry mass of the sediment was determined. Samples underwent BCR extraction as follows:

Stage 1: Approximately 1 g of each sample was weighed out in triplicate and placed in acid-washed 50 mL polypropylene tubes. Acetic acid (40 mL of 0.11 M, CH_3COOH) was added to each tube and to a procedure blank. Samples were allowed to extract for 16 h, and then centrifuged (Sigma 2-15 centrifuge) for 10 min. Supernatant was decanted into 50 mL volumetric flasks and made up to volume with 500 µL In and ultrapure water. Sediment was washed with 20 mL ultrapure water, centrifuged (Sigma 2-15 centrifuge) and the supernatant discarded.

Stage 2: Forty mL 0.1 M hydroxylamine hydrochloride (NH_2OH HCL) was added to each tube and to the procedure blank. Samples were allowed to extract for 16 hours and the residue separated from the extract as described above, the supernatant placed in 50 mL volumetric flasks, and made up to volume using 500 µL In and ultrapure water.

Stage 3: Acid stabilised 30% peroxide (10 mL, H_2O_2) solution was added to each sample and to the procedure blank, and allowed to digest at room temperature for an hour with occasional swirling. Samples were then covered and digested for a further hour in an 85°C hot water bath before uncovering and continuing to heat the samples until the liquid volume was reduced to a few millilitres.

Stage 4: Ammonium acetate (40 mL of 1 M, CH_3COONH_4) was added to each tube and the procedure blank and allowed to extract for 16 hours. The extract was separated from the residue as described above and the

supernatant placed in 50 mL volumetric flasks and made up to volume using 500 µL In and ultrapure water.

Stage 5: The residue was dried, and approximately 0.5 g was weighed out, placed in Teflon bombs and 9 mL of 30% HCl and 3 mL of 65% HNO_3 added to each bomb. The samples were allowed to be digested in a Milestone Ethos microwave for 45 minutes at 1 000 W and 200°C and then decanted into 50 mL polypropylene volumetric flasks. The bombs were washed twice with ultrapure water, the washings decanted into the volumetric flasks and made up volume with 500 µL In and ultrapure water. Samples from each stage of extraction were filtered using 0.45 µm filter paper and analysed on the ICP-OES and ICP-MS with the necessary procedural blanks and quality control standards. The following metals were determined on the ICP-OES (Spectro Arcos FSH12): Fe, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb and Al. Those metals that were below detection on the ICP-OES, as well as As and Se were analysed on the ICP-MS (X-series II) with CCT injection to reduce ArO, Se and choride ion (Cl^-, from the HCl) interferences and the r^2 value taken note of. The concentration in µg/g of dry weight was determined using the following calculation:

(Conc. metal µg/g) = [(conc. reading (µg/L) – blank) x (dilution/dry weight)] / 1000

Quality Assurance and Quality Control

Certified reference materials (CRM) were used to test the analytical efficiency. Two sets of sediment CRM (SL-1; IAEA and SARM-51; MINTEK) were extracted and analysed according to the prescribed methods. The percentage recoveries of the certified values were acceptable and ranged between 80 and 110% (Table 1) and therefore no correction factors were applied.

Table 1. Total metal (mg/kg) extracted from two certified reference materials, the certified metal concentrations (mg/kg) and the percentage recovery of the experimental procedure. All values represented as mean ± standard deviation.

Element	CRM SL-1 (IAEA)			SARM 51 (MINTEK)		
	Experimental values	Certified values	Recovery (%)	Experimental values	Certified values	Recovery (%)
Fe	67010± 2679	67400± 1700	99.42	145000± 6333	183600	78.98
Cu	31.19± 0.81	30± 6	104.0	254.6± 16.8	268	95.00
Mn	3617± 120	3460± 160	104.5	1896± 58	2100	90.28
Pb	35.51± 2.42	37.7± 7.4	94.20	4915± 213	5200	94.52
Cr	115.0± 8.2	104± 9	110.6	471.6± 18.5	509	92.65
Cd	0.2327 ±0.0008	0.26± 0.05	89.51	~	~	~
Zn	229.2± 12.4	223± 10	102.8	2072± 52	2200	107.5

Acid volatile sulphides

The purge-and-trap method was used for AVS determination, as described by Leonard et al. (1993). The sediment sample size was approximately 10 g wet weight with 5 mL of 1 N hydrochloric acid added. The reaction time for the method was 60 min. The limit of detection was 0.05 mm S/g dry weight. The diffusion method (Brouwer and Murphy, 1994), employed a 45 mm vial containing 10 mL of full term (SAOB) inserted inside a 30 ml scintillation vial which contained the sediment sample (1 g wet weight) and 4.5 mL of 0.9 N hydrochloric acid. After adding the hydrochloric acid to the sediment sample, the 20 mL vial was capped and placed on a rotary shaker for 60 min at 150 rpm. The sulphide in the SAOB was measured with an Orion sulphide ion selective electrode. Simultaneously extracted metals was determined by removing the overlying supernatant liquid with a syringe, filtering it through a 0.45 µm membrane filter into acid washed 10 mL polypropylene tubes. Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the extraction solution were measured by means of a Thermo X-series 2 quadrupole-based ICP-MS instrument. The SEM/AVS ratios (RM) are calculated by the following formula:

$$RM = SEM_M / AVS$$

Where:

- SEM_M is the molar amount of the metal M that was released by the extraction.
- AVS is the molar amount of sulphide determined in the trapping solution.

Organics analysis

Sample preparation

Analyses were undertaken on pooled sediment samples with one replicates from each site.

Dried sediment (typically around 2 g) was precisely weighted into an extraction thimble, 6 g copper powder was added, mixed with the sediment and the mixture was spiked with internal standards (10 ng CB 143 and 2 ng ε-HCH). Samples were extracted for 2 hours by hot Soxhlet with 100 mL mixture of acetone/hexane (1/3, *v/v*). The extract was evaporated and cleaned by passing through a cartridge filled with 8 g of acid silica (H_2SO_4, 44% w/w) and topped with 3 g copper powder. From the cartridge, pollutants were eluted with 20 ml hexane and 15 mL DCM. The eluate was evaporated to dryness and redissolved in 100 µL iso-octane (Covaci et al., 2005).

Gas chromatography analysis

Analysis of organochlorine pesticides (OCPs) was carried out with a gas-chromatography (GC) equipped with ^{63}Ni electron capture detector (GC-ECD: Shimadzu GC-2014, Kyoto, Japan). An ENV-8MS capillary column (30 m length×0.25 mm i.d., 0.25 µm film thickness; Kanto Chemical Co., Japan) was used for separation. One µL of each sample was injected in splitless mode. The GC oven temperature was programmed from 100°C held for 1 min, ramped at 12°C/min to 180°C, then at 4°C/min to 240°C, and finally at 10°C/min to 270°C and held for 5 min. The temperatures of injector and detector were 250°C and 320°C, respectively. Helium was used as the carrier gas with a flow rate of 1.0 mL/min and nitrogen as the make-up gas at a flow rate of 45 mL/min.

Quality assurance and quality control

The OCPs were identified by comparing their retention time with reference to the corresponding standard. The concentrations of the target analytes were quantified from the peak area of the sample to that of the standard peak area. The correlation coefficients (r^2) for the calibration curves were all greater than 0.995. For each set of 10 samples, a procedural blank and spiked blank were run to check for interference and cross-contamination. The mean recovery of OCPs for the spiked blanks was 90±11%. Spiking experiments using fortified samples, *O. niloticus* at 5 ng g^{-1} of the

composite standards showed recovery ranged from 70 to 110% for all OCPs. To further test the precision and accuracy of the analytical method, the standard reference material SRM 1947 (Lake Michigan Fish Tissue) was analyzed using the same procedures. Accepted recoveries ranged from 75% to 115% with RSD less than 12% were obtained.

The following OCPs were included in the analysis: The DDT congeners – p,p'-DDE, o,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, o,p' and p,p'-DDT (the sum expressed as ΣDDTs), hexachlorobenzene (HCB), α-, β-, γ and δ-hexachlorocyclohexane (HCH) isomers (the sum expressed as ΣHCHs), the chlordanes (ΣCHLs) – cis- and trans chlordane (cChl, tChl) and its oxidised form, i.e. oxychlordane (OxC) and heptachlor (HC) and its break down products cis- and trans nonachlor (TN, CN).

2.4 Habitat

Habitat and habitat availability is an important component when evaluating biological community strength. As with most other aquatic fauna, macro-invertebrate communities are largely influenced by the habitat diversity present within an aquatic ecosystem. Therefore, in the present study, different biotope diversities were evaluated including stones in current (riffle, run, boulder rapid, bedrock, chute, cascade), stones out of current (backwater, slackwater, pool, bedrock), instream vegetation, marginal vegetation and GSM (gravel sand and mud). Each of these biotopes were scored on a scale from 0 to 5, with 0 being absent, 1 rare, 2 sparse, 3 common, 4 abundant and 5 entire (Dallas, 2005).

A fish habitat assessment was conducted to provide a measure of the fish refuge potential associated with each of the sampling sites. This assessment characterises the fish habitats into four velocity-depth classes (including slow-deep, slow-shallow, fast-deep and fast-shallow habitat class, where fast is greater than 0.3 m/s, slow is less than 0.3 m/s, deep is greater than 0.3 m and shallow is less than 0.3 m) and associated cover present at each of the habitats (Dallas, 2005). All of these were quantified on a scale from 0 to 5, with 0 being absent, 1 rare, 2 sparse, 3 common, 4 abundant and 5 entire (Dallas, 2005). Measuring these various habitat types are an essential component in the interpretation of the fish integrity, as they can influence (by either creating or restricting) the fish populations and communities that are present within each sampling site.

2.5 Macroinvertebrates

The sampling of the Olifants and Luvuvhu Rivers was done in over two consecutive low flow seasons. The Olifants River surveys were from 07/10/2009-13/10/2009 and from 16/10/2010-22/10/2010. The Luvuvhu River surveys were from 07/09/2009-12/09/2009 and from 19/09/2010-24/09/2010. The surveys were conducted in the same manner for both of the rivers, starting at the sites where the river enters the KNP, and working our way downstream finishing at the site closest to where the rivers leave the KNP.

The macroinvertebrates for all the sites on both the Olifants and Luvuvhu Rivers were collected and assessed following the SASS5 protocol (Dickens & Graham, 2002). Each biotope (stones, vegetation and gravel, sand and mud) was sampled following the protocol using a standard SASS5 net. The *Aquatic Invertebrates of South African Rivers* by Gerber and Gabriel (2002) were used to identify the various representatives of the invertebrate families.

2.6 Fishes

The sampling of fish was carried out following the standard techniques used as part of the Fish Response Assemblage Index (FRAI) (Kleynhans et al., 2007). The various biotopes were identified and sampled. These biotopes are as follows: fast shallow, slow shallow, fast deep and slow deep. It must be mentioned that at some sites it was not possible to sample all the habitat biotopes for safety reasons, due to the presence of Nile crocodiles (*Crocodylus niloticus*) and hippopotamus (*Hippopotamus amphibious*). At all sites on the Olifants and Luvuvhu Rivers, fish were sampled using various techniques. Where possible, a Samus electro-shocker was used for a set time period (Figure 18a). A seine net 30 m long and 1.5 m deep with 16 mm mesh size was used in areas that were deemed safe (Figure 18c). Standard-size cast nets were also used, measuring cast per unit effort (Figure 18b). In addition at each site, rod-and-reel techniques were used to sample tigerfish (*Hydrocynus vittatus*) (Figure 18d, e). In addition to tigerfish, largescale yellowfish (*Labeobarbus marequensis*) and leaden labeo (*Labeo molybdinus*) were sampled for histopathological assessment and sediment bioaccumulation studies respectively in the Olifants River, whilst the redeye labeo (*Labeo cylindricus*) was used for comparative histopathology in the Luvuvhu River.

Figure 18. Electro-shocking (a), cast netting (b), seine netting (c) and rod and reel techniques (d&e) to sample fish in the Olifants and Luvuvhu Rivers.

For the FRAI assessment, historical data were consulted to establish which sites had been previously sampled, and which had a Reference Frequency of Occurrence (FROC) (Kleynhans et al., 2007). This was done in order to ascertain whether the sections of the rivers were to be assessed as one unit or to be broken down into separate units. The Olifants River is classified as a lowland river for the section that runs through the park, and as such one FRAI was applied to it. For the Luvuvhu River, the section where the river enters the park up until Lanner Gorge is classified as a lower foothill river (Sites 1 and 2).The section below the gorge up until the confluence with the Limpopo River, is classified as a lowland river (Sites 3 and 4). A FRAI was thus done for the section above the gorge (lower foothill) and for the section below the gorge (lowland river).

2.7 Flow requirements for fishes

To evaluate the preferences of availability of flow-dependent habitat types by fishes of the Olifants River a spatial habitat modelling exercise, fish community structure assessment with emphasis on flow dependent habitat use and a desktop evaluation of habitat preferences by Olifants River fishes was carried out. To evaluate the effects of altered flow dependent habitat types on fish communities of the Olifants River a flow-stress assessment was carried out.

Habitat modelling

The site selected for this portion of the study included a representative reach of the Olifants River (Site 2, see Figure 6). This portion of the Olifants River contains diverse and abundant pool, riffle and rapid habitat types with a range of fish cover features that is dominated by gravel and sand substrates in the slow flowing pool and backwater areas and bedrock, boulder and cobble substrates in the fast flowing areas. The diversity and abundance of flow-dependent habitat types or units of a reach of the Olifants river were characterised by spatially modelling the reach to generate a series of digital terrain models using ArcPAD® (8.0) on a hand held Trimble. Each habitat unit was selected and mapped according to the unique velocity-depth class (Kleynhans et al., 2005), surface flow type, actual velocities measured in m/s and substrate types. Depth was measured using a measurement stick in centimetres (accurate to 0.5 cm). Velocities were measured using a calibrated OTT flow meter using triplicate readings. The mean velocities were used in the analyses. Substrate type considerations included; silt, sand, gravel, cobble, boulder and bedrock types. Surface flow types monitored included barely perceptible flow, smooth and turbulent flows and undular breaking standing waves. Fish cover habitats including undercut banks and root wads, cover where water depth allowed for sufficient cover for the species, overhanging vegetation and substrate types including the occurrence of substrates such as cobble and boulder beds that are preferred by some species, associated with each segment were documented. The data collected was used to generation three-dimensional digital terrain models of the study area that will be used in the assessment.

Fish community structure

After the habitat units were defined, the fish communities of the study area were comprehensively sampled in a manner that would allow for later comparison to the habitat units. Fish using a range of techniques including fishing nets, electro-fishing and targeted angling methods. The netting techniques included the use of a medium sized seine net with two 30 m wings and a 2 m deep bag manufactured with 35 mm meshed sardine net. This net was used to scoop fish out of areas less than 2.5 m deep with sluggish slow or no flows. Gill net segments consisting of various mesh sizes including segments of 22 mm, 35 mm, 57 mm, 72 mm 90 mm and 120 mm mesh were used in deep slow flowing areas where relevant. Fyke nets made with 28 mm mesh, containing two traps separated by a 700 mm by 12 m wing were deployed in deep areas of the study area over night. Electrofishing techniques incorporating

the use of a battery operated SAMUS electro-fisher were used to sample fish in relatively shallow (<1.2 m) pool, backwater, rapid and riffle habitats. The catch per unit effort (CPUE) for each sampling method was documented and included in the fish community assessment. Table 2 presents a list of the fishes expected to occur in the study area based on the expected frequency of occurrence (FROC) (Kleynhans et al., 2007), the abbreviations used in the study to represent species and a summary of the available habitat preference information for species (Kleynhans et al., 2005)

Table 2. Fishes expected to occur in the Olifants River within the Kruger National Park and habitat preference information for species (Kleynhans et al., 2005, 2007).

			Velocity-depth preference				Flow	Cover preference				
Species	Common name	Abr.	FD	FS	SD	SS		OV	AV	UB	SUB	WC
Anguilla marmorata	Giant mottled eel	AMAR	-	-	4.4	-	2.8	-	-	3.9	4.2	-
Anguilla mossambica	Longfin eel	AMOS	3.4	3.3	3.4	-	2.8	-	-	4.1	4.9	-
Barbus annectens	Broadstriped barb	BANN	-	-	5	-	2.8	-	-	-	-	4.7
Barbus afrohamiltoni	Hamilton's barb	BFRI	-	-	4.7	4.3	2.8	-	-	-	-	4
Brycinus imberi	Imberi	BIMB	-	-	4.7	-	3	-	-	-	-	4.7
Labeobarbus marequensis	Largescale yellowfish	BMAR	4.1	4.4	4.4	3.4	3.2	-	-	-	4.5	4.1
Barbus rapax cf. Matozi	Papermouth	BMAT	-	-	4.7	4	3	-	-	-	4.1	4.2
Barbus paludinosus	Straightfin barb	BPAU	-	-	3.9	3.9	2.3	4.2	3.6	-	-	3.5
Barbus radiatus	Beira barb	BRAD	-	-	4.7	5	2.8	4.7	-	-	-	-
Barbus toppini	Beira barb	BTOP	-	-	3.3	4.3	1.1	4.7	-	-	-	-
Barbus trimaculatus	Threespot barb	BTRI	-	-	3.9	3.2	2.7	3.9	-	-	-	-
Barbus unitaeniatus	Longbeard barb	BUNI	-	-	5	4.3	2.3	4.6	-	-	-	-
Barbus viviparus	Bowstripe barb	BVIV	-	-	-	4.8	2.3	4.9	3.2	-	-	-
Clarias gariepinus	Sharptooth catfish	CGAR	-	-	4.3	3.4	1.7	-	-	-	-	-
Chiloglanis paratus	Sawfin rock catlet	CPAR	4.2	4.9	-	-	3.2	-	-	-	4.9	-
Chiloglanis pretoria	Shortspine rock catlet	CPRE	4.3	4.9	-	-	4.8	-	-	-	4.9	-
Chiloglanis swierstrai	Lowveld rock catlet	CSWI	-	4.7	-	-	4.8	-	-	-	4.9	-
Glossogobius giuris	Tank goby	GGIU	-	-	-	4.6	1.7	-	-	-	4.9	-
Hydrocynus vittatus	Tigerfish	HVIT	3.6	-	4.7	-	2.7	3.4	-	-	-	4.9
Labeo congoro	Purple labeo	LCON	5	-	5	-	3.3	-	-	-	5	3.4
Labeo cylindricus	Redeye labeo	LCYL	3.4	4.8	-	-	3.1	-	-	-	4.9	-
Labeo molybdinus	Leaden labeo	LMOL	3.3	4.3	3.7	-	3.3	-	-	-	4.7	-
Labeo rosae	Rednose labeo	LROS	-	-	4.7	-	2.5	-	-	-	5	-
Labeo ruddi	Silver labeo	LRUD	-	-	4.7	-	2.9	-	-	-	4.7	-
Micralestes acutidens	Silver robber	MACU	-	-	4.3	4.3	3.1	3.1	-	-	-	4
Mesobola brevianalis	River sardine	MBRE	-	-	4.3	4.2	1.1	-	-	-	-	5
Marcusenius pongolensis	Bulldog	MMAC	-	-	4.2	3.7	3	3.8	-	5	-	-
Oreochromis mossambicus	Mozambique tilapia	OMOS	-	-	4.6	3.8	0.9	-	-	-	-	3.9
Opsaridium peringueyi	Southern barred minnow	OPER	3.2	-	3.3	-	4.9	-	-	-	-	4.4
Petrocephalus wesselsi	Southern churchill	PCAT	-	-	4.7	4.3	2.8	3.3	-	5	-	-
Pseudocrenilabrus philander	Southern mouthbrooder	PPHI	-	-	-	4.3	1	4.5	-	3.2	-	-
Schilbe intermedius	Silver catfish	SINT	-	-	5	-	1.3	-	-	-	-	4.7
Synodontis zambezensis	Brown squeaker	SZAM	-	-	5	-	1.8	-	-	5	-	-
Tilapia rendalli	Redbreast tilapia	TREN	-	-	4.9	3.9	1.8	4.3	4.1	-	-	-

Note:
Flow intolerance rating guide: intolerant (score >4), moderately intolerant (score >3-4), moderately tolerant (score >2-3) and tolerant (score 1-2).
Cover preferences: overhanging vegetation (OV), aquatic macrophytes (AV), bank undercut (UB), substrate (SUB) and water column (WC).

Fish habitat preference

Two approaches were used to evaluate the habitat preferences of the fish communities, including the use of multivariate statistical procedures using observed data and Geographical Information Systems (GIS, ARCView 9.3) modelling procedures using historical habitat preference data (Table 2) (Kleynhans et al., 2005). The multivariate statistical procedures used in the study included ordination techniques that operate on the original fish community data sets (Van den Brink et al., 2003). This allows for the direct interpretation of the community structures of fish in terms of the taxa obtained in the study in relation to habitat variables. These techniques allow for the assessment of complex responses or changes in community structures obtained in the study and then when combined with Monte Carlo permutation testing, the statistical significance of hypothesised differences in the community structures can be tested (Van den Brink et al., 2003). Initially, the ordination approach allows for the expression of fish community structures between sampling locations without the need for correlating environmental or explanatory data. In this approach the variation of the composition of fish species is optimised to reflect the underlying structure of the data set. Thereafter, the largest part of the total variance of the data sets were used to establish a first latent variable and then a second were established that relies on the largest part of the remaining variance in the data set (Van den Brink et al., 2003). These two latent variables were used to construct ordination diagrams forming two axes. Samples (sites) and taxa are initially presented in the diagram as points at the location of the values on the latent variables. Samples with nearly identical or similar taxa compositions are located close together while samples located far apart represent those samples that have differing compositions of taxa (Van den Brink et al., 2003). When explanatory environmental data which included habitat data in this case is included, bi-plots that present arrows which point in the direction of higher values where correlations between the environmental variables and the sites occur (Van den Brink et al., 2003). In this study direct or constrained analyses were undertaken which involves overlaying captured variance of the explanatory environmental variables onto fish samples and taxa ordination diagrams. The linear response mode used to achieve this is a redundancy analyses (RDA), a derivative of principle component analyses (PCA) using the Canoco version 4.5 software package. In this study this procedure was used to establish a preference rating list of species to specific habitat types.

Historical habitat preference information of fishes occurring in study area were included by modelling the suitability of the habitat units observed in the study to historical species habitat preference information (Kleynhans et al., 2005). In this assessment the preferences of fishes to velocity depth classes (fast-deep, fast shallow, slow deep and slow

shallow), substrates and cover features were integrated through multiplication with substrate and cover feature preferences of species used as a weighting factor. The resulting habitat preference scores were superimposed onto the spatial habitat model generated for the reach of the Olifants River.

Flow-stress assessment

Once habitat preference ratings of fishes were established, these data were used to interpret environmental flow assessment stress ratings generated by observed and modelled data for the study area. The flow-stress assessment approach implemented in the study incorporated the use of the Revised Desktop Reserve Model (RDRM), an updated version of the Desktop Reserve model which takes into account hydrology, hydraulic and ecological characteristics of a region (Hughes and Hannart, 2003; Hughes, 2006; Hughes & Louw, 2010). This approach follows the Habitat Flow-Stressor Response methodology (HFSR) (Hughes, 2006). Two approaches were used to evaluate the environmental flows (EF) of the study area including the use of observed data and modelled data by the recently developed RDRM. Observed data were generated during a hydraulic survey to the Olifants River from 16 to 22 September 2011. For the observed hydraulics assessment, the observed cross-section and a synthesised rating curve were used. The rating curve was determined using the measured discharge and average depth and an estimated high flow data point. The rating curve coefficients computed are:

a = 0.200, b = 0.500 and c = 0.250 for Q = a * y b + C – where Q is the flow rate (m3/s) and y is the average flow depth (m).

Although sites for environmental flow (EF) studies that conform to the uniform flow assumption (i.e. equal longitudinal energy, water surface and channel bed gradient) are selected. The site selected for this EF study included multiple channels with different average water depths and velocities to allow for the evaluation of flow dependent habitat types for fishes. This negatively affects the confidence of the outcomes of the study. To address the confidence a modelled EF study without using the observed data was also undertaken and the outcomes were compared. The rating curve was calibrated within the hydraulic sub-model for use by the ecological sub-model. The parameters calibrated were: Manning n (min, max & shape factor) and Gradient (min, max & shape factor) (Hughes, 2006).

Furthermore, only 1 transect or hydraulic data point for the rating curve (i.e. flow rate and average depth) was undertaken and the confidence in the hydraulic analysis was poor

due to the complexity of the site. However, in order to provide some indication of the flow-stress information and EF requirements computed by the RDRM, two assessments of the site were undertaken. The first assessment determined the EF requirements of the site using the RDRM and no observed hydraulic data. The second assessment determined the EF requirements of the site using the RDRM and the surveyed hydraulic data. In each case, the flow class frequency distribution of the hydraulic results was produced.

Only the natural hydrology was used for the flow-stress assessment and it was obtained from the previous EF study on the Olifants system. The hydrology used was a summation of the Olifant 15 Ecological Water Requirement (EWR) site located upstream of the study area, within the same Department of Water Affairs quaternary catchment for the period 1920 to 1989. The maximum low flow discharge was computed using the separated baseflow option and the 20^{th} percentile point on the baseflow duration curve. For the modelled hydraulics assessment, the hydraulic inputs into the hydraulic sub-model were:

1. Geomorphological Zone – E
2. Flood Region – 7
3. Valley Slope – measured from Google Earth – 0.0008
4. Catchment Area – approximated to 50758 km^2

It is noted that geomorphological zones are related to valley slopes. In this case, the measured valley slope falls within the range of a geomorphological zone F (slope range 0.0001 to 0.001) but the value of 0.0008 is also close to the upper limit of geomorphological zone E. Little differences in the hydraulic parameters in the E and F zones occur so the geomorphological zone was subsequently changed to an F in order to be associated with the measured valley slope.

2.8 Fish Health Assessment

All fish specimens were transported to a nearby field laboratory for processing. The body mass and the total length of each fish were recorded. The fish were killed by severing the spinal cord anterior to the dorsal fin. A ventral incision was made to expose the visceral organs where after a standard necropsy was performed. Any macroscopic abnormalities were noted.

The liver, gonad and spleen masses were recorded to calculate the hepato-somatic index, the gonado-somatic index and the spleno-somatic index respectively for each fish. The body mass and length measurements were used to calculate a condition factor per fish (Carlander, 1969).

A gill, liver, kidney and gonad sample was collected for histopathological analysis. These tissue samples were fixed in 10% neutrally buffered formalin (gills, livers and kidneys) for 48 hours and in Bouins solution (gonads) for 24 hours. Following fixation, the tissue samples were washed in tap water and dehydrated in rising concentrations of ethanol before the samples were cleared in Xylene and imbedded in paraffin wax. The samples were sectioned at 5 µm and prepared for light microscopy analysis using standard techniques for Haematoxylin and Eosin staining.

Prepared slides were assessed by two assessors for increased objectivity using a multi-headed light microscope. The histological alterations identified were semi-quantified using the protocol applied by Van Dyk et al. (2009a), adapted from Bernet et al. (1999). In brief, for each alteration identified, a score value, indicating the severity of the occurrence of the alteration, and an importance factor, indicating the pathological importance of the alteration, were assigned. The score value and importance factor for each alteration was multiplied to obtain an index value. The various index values per organ were summed to provide an organ index value per fish. The respective organ indices were added per fish to provide a Fish Index representing the overall histological response identified per fish.

Otolith sections were used for ageing, according to the methods of Gerber et al. (2009). Left and right lapillus otoliths were removed from all *H. vittatus*, cleaned; air dried and stored in 25 mL McCartney bottles. Otoliths were prepared for sectioning following standard techniques (Wischniowski & Bobko, 1998) and then sliced using a double-bladed diamond-edged otolith saw. Cut sections were mounted on microscope slides using DPX mountant to enhance the section of the clarity of the sections. The sections were then viewed under transmitted and growth rings were counted. The second lateral line scale was taken from L. marequensis and then dried between two clean microscope slides. The scales were viewed using Nikon Profile Projector model 6CT2 at 20x magnification and a 30 cm diameter viewing screen and the growth rings were counted (Gerber et al., 2009).

2.9 Bioaccumulation

Metal analysis

Muscle samples were allowed to defrost at room temperature. Approximately 2 cm^3 was sectioned and placed into 25 mL Falcon tubes. Tubes and samples were placed in the drying oven at 60°C for 3-7 days until the samples were completely dry. Approximately 0.5 g of the dried sample was accurately weighed to 3 decimal places and placed in Teflon bombs where 7 mL 65% suprapur nitric acid and 1 mL 30% suprapur H_2O_2 were added to each sample. Samples were digested in a Milestone Ethos microwave and made up to 50 mL using 500 µL In and ultrapure water. The samples were filtered using 0.45 µm filter paper

and placed in 15 mL falcon tubes and analysed on the ICP-OES and ICP-MS. The following metals were determined on the ICP-OES (Spectro Arcos FSH12): Fe, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb and Al. Those metals that were below detection on the ICP-OES, as well as As and Se were analysed on the ICP-MS (X-series II) with CCT injection to reduce ArO and Se interferences and the r^2 value taken note of. The concentration in µg/g of dry weight was determined using the following calculation:

(Conc. Metal µg/g) = [(conc. reading (µg/L) – blank)x(dilution/dry weight)]/1000

Quality assurance was carried out using European mussel tissue reference material (ERMI-CE278), supplied by Industrial Analytical. Recoveries were acceptable ranging between 84 and 110% (Table 3).

Table 3. Metal (µg/g) extracted by the H_2O_2 extraction method from a certified reference material for muscle tissues (n = 3), the certified metal concentrations (µg/g) and the percentage recovery of the experimental procedure. All values represented as mean ± standard deviation.

Element	Experimental Value (µg/g)	Certified Value (µg/g)	Recovery (%)
Cu	10.4 ± 1.2	9.45	110
Mn	8.05 ± 0.90	7.69	105
Pb	1.67 ± 0.13	2.00	84
Cd	0.329 ± 0.030	0.348	95
Hg	0.206 ± 0.025	0.196	105

Organic pollutants

Tigerfish muscle tissue (10 g) were homogenized with anhydrous sodium sulphate and placed into acetone/hexane pre-washed extraction thimble. The samples were extracted in a Soxtherm S306AK Automatic Extractor System (Gerhardt, Germany) for 6 h with 150 ml mixture of hexane:acetone (3:1 v/v). The extracts were concentrated to approximately 2 ml using rotary vacuum evaporator, which then diluted to 10 ml with hexane. An Aliquot of 20% of the extract was taken for gravimetric lipid determination and the rest was subjected for clean-up process after solvent evaporation (Covaci et al., 2008). It was performed on a glass column packed with 6 g of activated florisil topped with anhydrous sodium sulphate. Elution was carried out using 80 ml of hexane containing 25% (v/v) diethyl ether. The effluent was concentrated to about 2 ml and then to near dryness under gentle nitrogen flow. The extract was redissolved in 100 µl n-decane and transferred to GC-vials for analysis.

The GC and quality assurance methods that were used are described under the sediment organic analysis (Section 2.3). The same OCPs as in sediments were included in the muscle tissue analyses: The DDT congeners – p,p'-DDE, o,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, o,p' and p,p'-DDT (the sum expressed as ΣDDTs), hexachlorobenzene (HCB), α-, β-, γ and δ-hexachlorocyclohexane (HCH) isomers (the sum expressed as ΣHCHs), the chlordanes (ΣCHLs) – cis- and trans chlordane (cChl, tChl) and its oxidised form, i.e. oxychlordane (OxC) and heptachlor (HC) and its break down products cis- and trans nonachlor (TN, CN). All concentrations are expressed in ng/g lipid mass.

2.10 Biomarker responses

Approximately 1 g each of tigerfish liver and muscle were placed in cryotubes, mixed with Hendrickson stabilising buffer (Wepener et al., 2005) and placed in liquid nitrogen for biomarker analysis. The remaining portions of the axial muscle were removed and frozen for further analysis. Dissection boards and tools were rinsed with 99.8% ethanol between dissections.

Approximately 0.2 g of collected liver tissue were placed in Eppendorf tubes labelled A and B respectively, and 0.2 g of muscle tissue was placed in an Eppendorf tube labeled as C. The sample in eppendorf A was homogenized on ice in 200 µL of General Homogenizing Buffer (GHB), centrifuged at 10 000 r.p.m. (Sigma 2-15 centrifuge) for 10 minutes at 4°C and aliquots of the supernatant taken for SOD, CAT, AChE, PC, LP and CYP450 activity analysis. The sample in Eppendorf B was homogenized on ice in 600 µL Tris-sucrose Buffer (Tris) and used solely for MT analysis. The sample in Eppendorf C was homogenized on ice in 200 µL ETS Buffer and used solely for CEA analysis.

Acetylcholinesterase

The methodology for AChE analysis was adapted from Ellman et al. (1961). The following chemical solutions were added to 24 of the 96 wells in a microtitre plate:

- 210 µL of Potassium Phosphate Buffer (PPB)
- 10 µL of s-Acetylthiocholine iodide
- 10 µL Ellmans' (2,2'-Dinitro-5,5'dithio-dibenzoic acid) reagent

The sides of the well were lightly tapped to ensure homogeneity, and the plate was covered with the plate lid and allowed to incubate at 37°C for 5 minutes. After incubation, 5 µL GHB was added to the first three wells as a procedure blank. 5 µL of sample was added to the other wells in triplicate so that there were 7 samples being read. The sides of the plate were lightly tapped to ensure mixing and the plate was read immediately at 405 nm, using an

automated microplate reader (Elx800-Universal microplate reader; BioTek instruments, USA), in 1 minute intervals over a 6 minute time period. The protein content was determined separately using the method of Bradford (1976), where the absorbance was measured at 630 nm and bovine serum albumin (BSA) used as a standard. Protein content is determined because each biomarker concentration is measured in activity per milligram protein.

Cytochrome P450 Activity

Cytochrome P450 activity was determined using a DetectX P450 demethylating fluorescent activity kit (Arbor Assays, K011-F1) where the samples were first diluted with assay buffer in a 1:6 ratio and the samples read using a Multi-Detection microplate reader (Synergy HT; BioTek instruments, USA). Protein content was determined using the method of Bradford (1976).

Metallothioneins

The method for MT analysis was adapted from Viarengo et al. (1997; 1999) for analysis on invertebrates using the modification as indicated by Atli and Canli (2008) and Fernandes et al. (2008). The samples were homogenised in 3:1 ratio of MT Tris homogenising buffer, and were centrifuged at 72 500 r.p.m (Biofuge stratus, Heraeus instruments) at 4°C for 20 minutes. Five hundred µL of cold (4°C) absolute ethanol and 40 µL of chloroform were added to 500 µL of the supernatant, and vortexed to ensure homogeneity. These samples were then centrifuged at 7 000 r.p.m (Sigma 2-15 centrifuge) (4°C) for 10 minutes. Three further volumes of cold ethanol were added to the mixture, vortexed and incubated at -20°C for 4 hours until a pellet formed. The supernatant was decanted and the pellet washed twice with 1 mL of washing buffer (87% ethanol, 1% chloroform, 12% homogenising buffer), after which it was vortexed and centrifuged at 3000 r.p.m (Sigma 2-15 centrifuge) (4°C) for 20 minutes. The pellet was dried using compressed air, and the pellet resuspended in 300 µL of Tris-Ethylene diamine tetraacetate (EDTA) and vortexed. Ellman's reagent (5,5' dithio-bis (2-nitrobenzoic acid); DTNB; 210 µL) and 15 µL of homogenising buffer were added to the first three wells as a procedure blank in triplicate. Ellman's reagent (210 µL) and 15 µL supernatant were added in triplicate per sample and the samples incubated at room temperature for 15 minutes. The absorbance of samples was read at 412 nm using an automated microplate reader and the protein content determined using the method of Bradford (1976).

Cellular Energy Allocation (CEA)

The method for CEA analysis was adapted from De Coen and Janssen (1997) and De Coen and Janssen (2003), for which protein content, glucose content, lipid content and electron transport system (ETS) activity were determined. 100 µL supernatant (as described previously) was further diluted, using 400 µL ETS buffer and 400 µL ultrapure water, and all analyses carried out on ice.

Available Energy Reserves (E_a)

Protein was determined using the method of Bradford (1976). Carbohydrate was determined using a glucose content test kit (GOD-PAP 1 448 668, Roche) and glucose standard (C FAS 759 350, Roche) at 560 nm with an automated microplate reader. Total lipids were extracted following the method of Bligh and Dyer (1959) using tripalmitin as a standard, where 250 µL supernatant was added to 500 µL chloroform and vortexed. Methanol (500 µL) and 250 µL ultrapure water was added to this solution, vortexed and then centrifuged at 4°C for 10 mintues at 7 250 r.p.m (Sigma 2-15 centrifuge). One hundred µL of the organic phase was placed in glass tubes and a blank prepared from 100 µL chloroform. Sulphuric acid (H_2SO_4; 500 µL) was added to each tube and the tubes covered with foil and incubated at 200°C for 15 minutes. One mL of ultrapure water was added to each tube and the samples allowed to cool down. Two hundred and forty five µL of each sample and the blank was added in triplicate to polyethylene microtitre plates and the sample absorbancies were read at 360 nm using an automated microplate reader.

Energy Consumption (E_c)

The cellular respiration rate (energy consumption) was determined by measuring the ETS activity. The samples were centrifuged at 7 250 r.p.m (Sigma 2-15 centrifuge) for 10 minutes at 4°C. Twenty five µL of supernatant of ETS buffer was placed in the first 3 wells in a microplate as a procedure blank. Twenty five µL of supernatant from each sample was placed in triplicate on a microplate with a maximum of 5 samples per plate. Buffered substrate solution (BSS; 0.3% (v/v; 75 µL) Triton X-100, and Tris-HCl), 25 µL NAD(P)H solution and 50 µL p-IodoNitro Tetrazolium violet/chloride (INT) was added to each well and the samples read kinetically at 490 nm at 20°C at 1 minute intervals over a 5 minute period using an automated microplate reader.

Cellular Energy Allocation (CEA)

The energy reserves were converted into energetic equivalents using the enthalpy of combusion values as indicated by De Coen and Janssen (1997), where these values were 17 500 mJ/mg glycogen, 39 500 mJ/mg lipid and 24 000 mJ/mg protein. The E_c was determined using the theoretical stochiometric relationship that indicates that for each 2 μmol of formazan formed, 1 μmol of oxygen is consumed in the ETS system. The amount of oxygen was transformed into energetic equivalents using an average oxyenthalpic equivalent of 484 kJ/mol O_2. The total energy budget was calculated using the following equation:

$CEA= E_a-E_c$
Where: $E_a = E_{glucose}+E_{lipid}+E_{protein}$
$Ec=E_{ETS}$

Superoxide Dismutase (SOD)

The methodology for SOD was adapted from Greenwald (1989) where 3 mL Tris Buffer was added to each sample and the reaction initiated by adding 25 μL pyrogallol solution and the samples read on a Multi-Detection microplate reader (Synergy HT; BioTek instruments, USA).

Catalase Activity (CAT)

The methodology for CAT was adapted from Cohen et al. (1970). While working on ice, 15 µL of the homogenate from Eppendorf A supernatant was placed in an Eppendorf with 60 µL 0.01 M Catalase Phosphate Buffer (CAT PP buffer; pH 7.0) and centrifuged at 10 000 r.p.m. (Sigma 2-15 centrifuge) for 10 minutes at 4°C. GHB (10 µL) was added in triplicate to the microtitre plate as a procedure blank and 10 µL of each supernatant was added to a microtitre plate in triplicate (maximum of 15 samples per plate). H_2O_2 (93 µL) was added to each well, once all of the wells had been filled the plate was tapped gently on the side and allowed to incubate at room temperature for 3 minutes. Sulphuric acid (H_2SO_4; 19 µL) was added to each well to stop the reaction, followed immediately by the addition of 130 µL 2 mM potassium permanganate ($KMnO_4$) to measure the amount of unreacted $KMnO_4$ spectrophotometrically at 409 nm using an automated microplate reader. The protein content was measured using Bradford reagent (Bradford 1976). Catalase Activity was expressed as µmol H_2O_2/mg protein/minute.

Lipid Peroxidation (LP)

The methodology for LP determination was adapted from Ohkawa et al. (1979) as modified by Üner et al. (2006). Twenty five μL of supernatant from each sample was placed in an acid washed glass tube where 50 μL 8.1% sodium dodecyl sulphate (SDS), 375 μL acetic acid, 375 μL thiobarbituric acid, and 175 μL ultrapure water was added to each tube. The tubes were placed in a hot water bath at 95°C for 30 minutes, thereafter it was allowed to cool down to room temperature. Ultrapure water (250 μL), and 1 250 μL of butanol-pyridine solution (15:1) was added to each sample, vortexed and centrifuged at 4 000 r.p.m (Sigma 2-15 centrifuge) for 10 minutes at room temperature. Two hundred and forty five μL of samples and the blank were added in triplicate to the microtitre plate and read at 540 nm using an automated microplate reader. Protein content was determined following the method of Bradford (1976).

Protein Carbonyls (PC)

The methodology for PC was adapted from Parvez and Raisuddin (2005) as assayed by Levine et al. (1990) and modified by Floor and Wetzel (1998). Supernatant (500 μL) was added to 500 μL 2,4-Dinitrophenylhydrazine (DNPH) and incubated for an hour at room temperature, during which time it was vortexed every 10-15 minutes. Trichloroacetic acid (6%; 500 μL) was added to each sample in order to precipitate the proteins, and was centrifuged at 24 166 r.p.m (Biofuge stratos, Haraeus instruments) for 3 minutes. The supernatant was discarded and the pellet washed three times and resuspended in 1 mL ethanol in order to remove the free reagent. The samples were allowed to stand for 10 minutes before centrifugation and the subsequent removal of supernatant. Guanidine hydrochloride (400 μL) was added to each sample in order to make the proteins soluble and allowed to stand at room temperature for 15 minutes. The samples were centrifuged at 38 666 r.p.m (Biofuge stratos, Haraeus instruments)for 5 minutes in order to remove any trace of insoluble material and the sample read in triplicate at 366 nm using an automated microplate reader and the proteins determined following the method of Bradford (1976).

2.11 Statistical analyses

Univariate analyses

The variations in each assessment endpoint 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. When the ANOVA revealed significant differences, post-hoc multiple comparisons between sites were made

using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine which values differed significantly. The significance of results was ascertained at $p<0.05$ (Zar, 1996).

Various univariate diversity indices have been used to assess community structure, as they may emphasize the species richness or equitability components of diversity to varying degrees. Indices that were used were the Shannon-Weiner diversity index (*H*'), which incorporates both species richness and equitability components (Clarke & Warwick, 1994), species richness, which compares the numbers of species present for any given number of individuals, Pielou's evenness index (*J*') and Margalef's index (*d*).

Multivariate analyses

The statistical community analysis of data was carried out using Primer Multivariate Software (Clarke & Warwick, 1994). For the analysis of the invertebrate and fish communities, presence/absence data was used. To display the community similarities and groupings, cluster analysis was done to represent community response in the form of a dendrogram. Multidimensional scaling was also carried out to show the correlation and similarity groupings of the sample sites, and from this the sites were grouped together to show their similarities. The analysis of similarities (ANOSIM) test was carried out to show that the results obtained and the groupings displayed via the community response in the cluster and MDS diagrams were statistically significant.

In this study Principle Component Analysis (PCA) (Canoco for Windows Version 4.53) statistical package was used to assess the spatial patterns associated with water and sediment quality, bioaccumulation in fish tissue, biomarker responses and fish community structures (Ter Braak & Smilauer, 2004). The PCA is based on a linear response model relating species and environmental variables (Van den Brink et al., 2003). Results of the ordination are a map of the samples being analysed on a 2 dimensional basis, where the placements of the samples reflect the dissimilarities or similarities between the samples; in this case the sampling sites. To determine which factors were responsible for the structure or groupings obtained in the PCA a Redundancy Analysis (RDA) assessment was carried out. A RDA is a derivative of a PCA with one additional feature which allows for the selection of the driving variables which are intended to be overlaid onto the PCA. The values entered into the RDA analysis are not the original data but the best-fit values estimated from a multiple linear regression between each variable in turn and a second matrix of complementary biological or environmental data. The RDA plots are interpreted through 2-dimentional bi-plots that present the similarities or dissimilarities between the samples analysed (Shaw, 2003).

3 THE OLIFANTS RIVER

3.1 Water quality

Physico-chemical characteristics

None of the in situ water quality variables recorded (Table 4) displayed any definite spatial trends at the five sites in the Olifants River. The Letaba River consistently had lower conductivity levels than the sites in the Olifants River. It was also evident that the lower conductivity from the Letaba River was responsible for decreasing the conductivity at site 5. The temperatures ranged between 16 and 29°C and within surveys stayed constant throughout the sites. Temperatures reflected the time of the year in which sampling was undertaken with water temperatures much higher during LF periods (i.e. late spring) than the HF periods (in late autumn). The pH levels remained relatively constant throughout the sites and the surveys with the Letaba River with slightly lower values. Conductivity also reflected the types of flow with LF surveys having higher conductivities than during the HF surveys. All the in situ water quality parameters fell within the target water quality range (TWQR) for aquatic ecosystems (DWAF, 1996) with the exception of DO in the Letaba River (i.e. 73%).

Wepener et al. (1999) ascribed the high conductivity values in the Olifants River to land erosion, overgrazing, removal of riparian vegetation and ploughing which causes an increase in turbidity and thus conductivity. The Phalaborwa Barrage captures most of the suspended sediments from the Olifants River and releases its water and suspended material during high flow periods into the Olifants River (Buermann et al., 1995; Wepener et al., 1999). However, it may be assumed that sediments are released from the Barrage in small quantities throughout the year with subsequent increases the turbidity of the river below. There were noticeable increases in conductivity up until Site 3, possibly due to the inflow of the Klasere River, which may also be a source of increased turbidity and thus conductivity. The conductivity decreased at Site 4, which may be attributed to suspended material settling into the sediments as a result of the river broadening and reduced velocity (Vannote et al., 1980).

The nutrients (ammonium, nitrate, nitrite and orthophosphate) levels remained fairly low throughout the study (Table 4). Orthophosphate and chloride concentrations reflected flow conditions with increased concentrations during LF periods. Conversely turbidity and COD increased during HF periods and decreased during the LF periods. Elevated nitrate levels found during all surveys are indicative of mesotrophic conditions and a slight increase in nitrate levels would cause the Olifants River to become eutrophic. Evidence of the increased nutrients was evident in the extensive filamentous algae growth observed. The increased nutrients in the Olifants River have been attributed to input from fertilizer plants and sewage treatment works in the upper catchment (Seymore et al., 1994).

Table 4. Physico-chemical variables measured at 5 sites in the Olifants River and one site in Letaba River during two consecutive high and low flow periods between 2009 and 2011. NS represents no sample available.

Sample	Temperature °C	pH	Oxygen %	Oxygen mg/L	Conductivity µS/cm	TDS mg/L	Ammonium mg/L	Chloride mg/L	COD mg/L	Nitrate mg/L	Nitrite mg/L	Phosphate mg/L	Sulphate mg/L	Turbidity NTU
OLI-S1-09LF	21	8.39	92.6	7.85	502	251	0.05	124.00	NS	0.64	0.01	0.23	128.00	13.00
OLI-S2-09LF	22.3	8.42	93.6	8.11	1019	523	0.09	150.00	NS	0.70	0.01	0.31	109.00	4.00
OLI-S3-09LF	23.7	8.36	111.5	9.8	2000	525	0.14	125.00	NS	1.00	0.01	0.03	89.00	11.00
OLI-S4-09LF	25.1	8.4	120.6	9.79	1216	565	0.05	100.00	NS	1.00	0.00	0.17	98.00	6.00
OLI-S5-09LF	24.5	8.48	117.2	9.89	498	251	0.08	101.00	NS	0.60	0.01	0.02	120.00	4.00
Letaba-09LF	23.8	7.48	73.8	6.11	515	263	0.14	178.00	NS	0.90	0.01	0.03	45.00	13.00
OLI-S1-10HF	NS	NS	NS	NS	NS	NS	0.1 ± 0.01	19.33 ± 3.38	17.23 ± 0.82	1.07 ± 0.31	0.01	0.14 ± 0.05	98.3 ± 14.17	21 ± 2.52
OLI-S2-10HF	NS	NS	NS	NS	NS	NS	0.09 ± 0.01	24 ± 2.52	20.67 ± 1.14	0.88 ± 0.14	0.01	0.16 ± 0.05	107.3 ± 32.87	21.33 ± 2.85
OLI-S3-10HF	NS	NS	NS	NS	NS	NS	0.08 ± 0	19.67 ± 7.31	16.87 ± 1.02	0.96 ± 0.14	0.01	0.1 ± 0.04	95.33 ± 11.46	20.33 ± 0.67
OLI-S4-10HF	NS	NS	NS	NS	NS	NS	0.24 ± 0.15	22 ± 0.58	21.3 ± 1.75	1.06 ± 0.06	0.02 ± 0.01	0.13 ± 0.06	69.3 ± 8.21	29 ± 2.52
OLI-S5-10HF	NS	NS	NS	NS	NS	NS	0.08 ± 0.01	17 ± 4.91	19.75 ± 1.85	1.49 ± 0.39	0.11 ± 0.06	0.11 ± 0.03	222 ± 99.95	30.5 ± 2.52
Letaba-09LF	NS	NS	NS	NS	NS	NS	0.1 ± 0.02	23.55 ± 6.45	22.05 ± 0.55	1.08 ± 0.14	0.02 ± 0.02	0.1 ± 0.01	21.5 ± 2.5	27.5 ± 4.5
OLI-S1-10LF	18.5	8.46	104.8	9.74	601	571	0.07 ± 0.02	40.33 ± 3.84	17.6 ± 0.71	0.67 ± 0.22	0.01	0.18 ± 0.03	96.33 ± 27.91	13.67 ± 0.88
OLI-S2-10LF	23.1	8.48	102.8	8.68	611	580	0.11 ± 0.03	34 ± 5.03	27.87 ± 3.34	2.08 ± 0.79	0.01	0.18 ± 0.05	153.3 ± 84.92	15 ± 2.89
OLI-S3-10LF	24.1	8.2	104.1	8.65	639	128	0.29 ± 0.13	45.33 ± 9.82	36.47 ± 8.62	2.53 ± 1.24	0.03 ± 0.02	0.16 ± 0.08	173.3 ± 96.91	17 ± 0.58
OLI-S4-10LF	21.7	8.43	101.7	9.16	655	619	0.21 ± 0.05	46.33 ± 4.18	25.43 ± 1.04	0.81 ± 0.22	0.01	0.1 ± 0.02	110.3 ± 40.4	14.33 ± 1.2
OLI-S5-10LF	24.2	8.16	118.8	9.29	136.9	131	0.09 ± 0.03	44.33 ± 6.12	27.53 ± 1.67	0.84 ± 0.11	0.01	0.06 ± 0.01	177.3 ± 84.78	14 ± 2.52
Letaba-09LF	28.3	7.97	113.9	8.43	132	123	0.1 ± 0.02	67.67 ± 11.72	40.9 ± 3.27	2.09 ± 0.36	0.01	0.11 ± 0.03	34 ± 9.61	14.67 ± 1.76
OLI-S1-11HF	17	8.53	96.7	8.83	380	NS	0.02 ± 0	14 ± 3.46	21.23 ± 0.24	NS	0.01	0.11 ± 0.06	97.67 ± 19.84	23.67 ± 3.33
OLI-S2-11HF	17	8.53	100.3	9.31	373	NS	0.01 ± 0.01	10 ± 3	21.23 ± 0.47	NS	0.01	0.2 ± 0.08	141.7 ± 11.55	21.33 ± 2.85
OLI-S3-11HF	16.9	8.66	101.5	9.66	379	NS	0.14 ± 0.03	9.67 ± 1.45	19.27 ± 1.16	NS	0.01	0.02 ± 0.01	156 ± 13.53	24.67 ± 4.48
OLI-S4-11HF	18.7	8.67	95.1	8.6	377	NS	0.23 ± 0.05	9.67 ± 1.86	21.5 ± 0.29	NS	0.01	0.05 ± 0.01	144 ± 15.95	34.33 ± 10.35
OLI-S5-11HF	17.6	8.53	104	9.9	373	NS	0.17 ± 0.03	13.67 ± 4.06	21.3 ± 0.92	NS	0.01	0.06 ± 0.01	137.3 ± 20.85	22.33 ± 0.33
Letaba-09LF	17.2	8.26	98	9.26	220	NS	0.19 ± 0.01	9.33 ± 1.86	18.5 ± 1.56	NS	0.01	0.05 ± 0.01	122.3 ± 33.14	14 ± 1

Sulphate levels in the Olifants River remained very high throughout the study period when compared to the Letaba River. The high sulphate levels have been attributed to coal mines in the upper catchment, open cast mining outside the Park and other industries in the catchment upstream of the KNP which increase exposed sulphur deposits (Wepener et al., 1999; Cloete, 2008; De Villiers & Mkwelo, 2009). Noticeably the chloride concentrations were higher in the Letaba River than in the Olifants River. It was also evident that the Olifants River water quality had a major influence on the lower Letaba River water quality (e.g. sulphates, pH and conductivity) during the 2011 HF survey as the high flows pushed water into the Letaba.

Metal concentrations

Dissolved Al concentrations increased as the Olifants River flows through the park during the LF2009 and HF2010 surveys and were highest during the 2010 surveys (Table 5). Sites from the LF2010 survey had the highest Al concentrations and the lowest Al concentrations were measured during the HF2011 survey. All Al concentrations exceeded the TWQR (10 µg/L) for aquatic ecosystems (DWAF, 1996). There were no spatial and temporal trends in the dissolved As and Cd concentrations. With the exception of the 2010 surveys the concentrations of these metals were below the TWQR. No spatial trends were observed for dissolved Cr concentrations during any of the surveys. The Cr concentrations were lowest during the LF 2009 and HF 2011 surveys, while the highest Cr concentrations (Site 3 – LF2010 and Site 4 – HF2010) exceeded the TWQR. Concentrations of dissolved Co showed similar trends to Cr in that they were lowest during the LF2009 and HF2011 surveys and were substantially higher during the 2010 surveys. Dissolved Cu concentrations from all surveys except LF 2010 showed a spatial trend as Cu concentrations increased downstream from west to east through the park. There was a slight decrease from 2009 to 2011. Sites 4 and 5 (LF2010) and Sites 2 and 3 (HF2011) had Cu concentrations that were below detection limits. The Cu concentrations were above the TWQR at Sites 2 and 5 (LF2009) and Site 6 (HF2010), Cu concentrations exceeded the chronic effect value (CEV) at Site 5 during LF2009. Dissolved concentrations of Fe showed no spatial or temporal trends. Concentrations of Fe were however lowest during the LF 2009 and HF2011 surveys. The Pb concentrations showed no spatial trends but were highest during HF2010 followed by LF2009, HF2010 and HF2011 had the lowest concentrations. All sites had Pb concentrations that exceeded the TWQR and CEV and concentrations at Sites 2, 4 and 6 exceeded the acute effect value (AEV). All sites except Site 5 during LF2009 had Pb concentrations exceeding the TWQR and Sites 2, 3 and 6 exceeded the CEV. Sites 3, 4 and 5 during LF 2010 had Pb concentrations exceeding the TWQR. Pb concentrations at all sites during HF 2011 were below the TWQR.

Table 5. Mean ± standard error of the dissolved metal concentrations in water from 5 sites in the Olifants River and one site in Letaba River during two consecutive high and low flow periods between 2009 and 2011. All concentrations are expressed as µg/L and mg/L and BD represents samples below detection limits.

Site and survey	Al µg/L	As µg/L	Cd µg/L	Ca mg/L	Cr µg/L	Co µg/L	Cu µg/L	Fe µg/L	Pb µg/L	Mg mg/L	Mn µg/L	Ni µg/L	K mg/L	Se µg/L	Ag µg/L	Na mg/L	U µg/L	Zn µg/L
OLI-S1-09LF	40.79	0.93	0.04	28.95	0.47	0.25	1.07	13.48	1.57	179.99	0.83	1.00	32.75	2.6	0.18	692.97	-	4.01
OLI-S2-09LF	43.74	1.43	0.26	59.79	0.77	0.48	1.44	30.97	2.75	187.46	1.42	1.46	32.18	2.81	0.49	684.8	-	3.36
OLI-S3-09LF	50.53	1.16	0.32	47.99	1.40	0.47	1.34	39.20	8.13	177.31	1.53	1.33	41.81	2.55	0.4	754.44	-	6.95
OLI-S4-09LF	56.20	0.49	0.07	34.31	0.39	0.13	0.60	21.53	1.22	88.55	1.11	0.79	15.27	1.44	0.41	283.88	-	3.02
OLI-S5-09LF	64.70	0.73	0.03	50.48	0.53	0.21	3.17	27.20	1.15	155.65	1.54	1.31	34.57	2.7	0.25	610.38	-	6.19
Letaba-09LF	80.24	1.07	0.22	62.56	0.85	0.39	0.96	30.08	2.47	159.61	8.15	1.78	67.43	3.99	0.58	1066.85	-	2.38
OLI-S1-10HF	63 ± 9.85	0.72 ± 0.12	15.14 ± 7.57	15.97 ± 1.59	4.44 ± 3.05	10.89 ± 5.37	0.57 ± 0.13	44 ± 6.43	12.71 ± 6.14	12.64 ± 1.3	3.91 ± 1.53	1.31 ± 0.06	3.49 ± 0.13	1.35 ± 0.06	11.73 ± 5.81	18.14 ± 1.5	5.27 ± 1.82	BD
OLI-S2-10HF	80 ± 10.12	0.91 ± 0.02	22.63 ± 0.2	15.44 ± 0.29	9.58 ± 1.96	15.9 ± 0.67	0.97 ± 0.12	72.33 ± 11.32	18.62 ± 0.53	15.22 ± 0.1	5.88 ± 0.32	1.91 ± 0.43	4.49 ± 0.35	1.63 ± 0.12	17.27 ± 0.62	24.58 ± 0.34	4.08 ± 0.85	3.9 ± 3.9
OLI-S3-10HF	77 ± 7.81	0.73 ± 0.27	7.71 ± 7.71	20.85 ± 1.81	3.44 ± 3.12	6.06 ± 5.84	0.41 ± 0.18	41.33 ± 7.69	7.03 ± 6.49	15.67 ± 0.07	2.40 ± 1.68	1.19 ± 0.21	4.04 ± 0.4	1.52 ± 0.14	6.43 ± 6.24	23.06 ± 0.29	5.88 ± 2.27	BD
OLI-S4-10HF	80 ± 19.09	1 ± 0.06	22.82 ± 0.19	18.24 ± 1.24	15.08 ± 3.39	16.63 ± 0.65	0.98 ± 0.03	115.33 ± 38.75	19.16 ± 0.52	14.89 ± 0.12	8.37 ± 2.06	1.94 ± 0.09	4.8 ± 0.2	1.85 ± 0.15	17.87 ± 0.6	22.71 ± 0.73	3.17 ± 0.95	22.51 ± 12.7
OLI-S5-10HF	80.67 ± 12.73	0.75 ± 0.14	15.34 ± 7.67	15.37 ± 3.21	6.87 ± 3.64	11.57 ± 5.68	1.13 ± 0.52	62 ± 17.95	13.26 ± 6.38	12.88 ± 2.04	4.2 ± 1.8	1.93 ± 0.58	4.14 ± 0.19	1.47 ± 0.15	12.36 ± 6.09	22.58 ± 0.44	4.22 ± 1.93	BD
Letaba-10HF	100.5 ± 4.5	0.84 ± 0.07	22.81 ± 0.27	12.78 ± 1.08	11.79 ± 1.27	16.56 ± 0.92	1.82 ± 0.3	73 ± 16	19.1 ± 0.73	8.6 ± 0.22	6.27 ± 0.02	2.66 ± 0.38	4.21 ± 0.15	1.33 ± 0.08	17.81 ± 0.85	21.91 ± 0.2	3.24 ± 1.36	19.3 ± 9.3
OLI-S1-10LF	163.67 ±78.17	0.42 ± 0.05	BD	12.08 ± 0.88	0.59 ± 0.08	0.28 ± 0.01	0.07 ± 0.07	36.67 ± 3.84	0.62 ± 0.02	24.22 ± 3.26	1.19 ± 0.03	0.89 ± 0.11	3.16 ± 0.52	2.06 ± 0.25	0.22 ± 0.01	36.88 ± 5.67	7.84 ± 0.03	BD
OLI-S2-10LF	127.67 ± 48.49	0.74 ± 0.3	4.75 ± 4.74	14.93 ± 1.42	6.30 ± 4.12	3.97 ± 3.7	0.49 ± 0.18	36 ± 2.52	0.91 ± 0.32	23.29 ± 2.72	4.32 ± 3.61	2.60 ± 1.13	3.9 ± 0.26	2.22 ± 0.65	4.176 ± 3.91	36.77 ± 4.23	7.46 ± 0.23	2.02 ± 0.2
OLI-S3-10LF	73 ± 40.04	1.41 ± 0.15	14.24 ± 0.02	20.21 ± 4.56	25.96 ± 24.77	11.36 ± 0.05	0.75 ± 0.75	34.67 ± 18.1	1.56 ± 0.03	20.14 ± 1.08	11.58 ± 0.06	3.11 ± 1.6	7.54 ± 4.47	3.7 ± 0.5	11.98 ± 0.05	33.19 ± 5.72	6.97 ± 0.01	BD
OLI-S4-10LF	152.33 ± 70.85	1.24 ± 0.03	14.17 ± 0.02	15.22 ± 0.77	4.03 ± 2.81	11.10 ± 0.08	BD	41.33 ± 3.93	1.45 ± 0.05	29.48 ± 1.59	11.43 ± 0.12	1.55 ± 0.08	4.86 ± 0.25	3.86 ± 0.18	11.76 ± 0.07	45.04 ± 2.74	7.01 ± 0.01	BD
OLI-S5-10LF	68 ± 5.86	1.24 ± 0.1	14.15 ± 0.07	14.42 ± 2.71	3.20 ± 1.1	11.05 ± 0.28	BD	112 ± 53.11	1.52 ± 0.04	28.18 ± 2.97	11.58 ± 0.36	1.67 ± 0.07	4.99 ± 0.4	3.76 ± 0.27	11.703 ± 0.26	40.25 ± 5.98	7 ± 0.01	BD
Letaba-10LF	179.67 ± 132.84	0.41 ± 0.01	0.04 ± 0.04	14.08 ± 0.57	6.69 ± 6.14	0.28 ± 0	0.81 ± 0.41	43.67 ± 21.85	0.57 ± 0.07	17.25 ± 0.61	4.47 ± 2.78	1.63 ± 0.5	5.1 ± 0.69	2.5 ± 0.06	0.403 ± 0.21	45.5 ± 0.24	7.95 ± 0.03	BD
OLI-S1-11HF	36.67 ± 7.54	0.17 ± 0.01	BD	8.41 ± 0.59	0.32 ± 0.03	0.24 ± 0.01	0.04 ± 0.04	19.33 ± 2.73	0.58 ± 0.01	17.59 ± 0.13	0.99 ± 0.1	1.69 ± 0.58	3.13 ± 0.37	1.71 ± 0.15	0.2 ± 0	26.11 ± 0.85	7.31 ± 0.03	1.22 ± 0.99
OLI-S2-11HF	39.67 ± 10.67	0.47 ± 0.32	4.72 ± 4.72	10.7 ± 0.72	0.52 ± 0.27	3.84 ± 3.6	BD	15.67 ± 4.67	0.83 ± 0.27	17.55 ± 0.28	4.32 ± 3.52	0.73 ± 0.32	2.57 ± 0.15	2.11 ± 0.65	4.03 ± 3.83	24.26 ± 0.37	7.11 ± 0.07	BD
OLI-S3-11HF	30.33 ± 3.67	0.15 ± 0.03	BD	10.99 ± 1.99	0.26 ± 0.01	0.24 ± 0	BD	16.67 ± 5.17	0.59 ± 0.01	17.90 ± 0.08	1.04 ± 0.17	0.6 ± 0.04	2.72 ± 0.13	1.73 ± 0.04	0.2 ± 0	24.63 ± 0.39	7.05 ± 0.03	BD
OLI-S4-11HF	37 ± 6.66	0.19 ± 0.09	BD	8.98 ± 0.86	0.30 ± 0.03	0.26 ± 0.01	0.07 ± 0.07	16.67 ± 1.86	0.61 ± 0.01	17.28 ± 0.35	1.11 ± 0.17	0.8 ± 0.34	2.67 ± 0.13	1.58 ± 0.2	0.21 ± 0	24.39 ± 0.33	6.92 ± 0.02	BD
OLI-S5-11HF	29 ± 4.73	0.27 ± 0.05	BD	10.09 ± 1.07	0.29 ± 0.02	0.26 ± 0.01	0.12 ± 0.12	16.33 ± 2.91	0.6 ± 0.01	15.92 ± 2.1	1.28 ± 0.08	0.77 ± 0.11	2.47 ± 0.17	1.84 ± 0.17	0.19 ± 0	23.27 ± 1.35	6.91 ± 0.01	BD
Letaba-11HF	36.67 ± 8.41	0.06 ± 0.01	BD	6.28 ± 0.25	0.26 ± 0.03	0.27 ± 0.01	1.15 ± 0.04	20.33 ± 2.73	0.58 ± 0.01	7.95 ± 0.17	2.71 ± 0.47	0.96 ± 0.14	2.1 ± 0.12	0.52 ± 0.17	0.18 ± 0	18.82 ± 0.82	6.89 ± 0	BD

Dissolved Mn concentrations showed trends similar to Cr and Co with the first and last surveys, with lower concentrations compared to both 2010 surveys. The Mn concentrations were all well below the TWQR at all sites during all surveys. Dissolved Ni concentrations stayed consistent throughout all surveys and showed no spatial or temporal trends. The same was found for Se concentrations. Sites 1, 2, 3, 5 and 6 for the LF2009 survey and all sites during the LF 2010 survey were above the TWQR. During the HF surveys all sites except Site 2 (HF 2011) were below the TWQR. Dissolved Ag concentrations showed a similar trend as Cr, Co and Mn in that the first and last surveys had lower concentrations than the two 2010 surveys. Dissolved concentrations of U were not measured during the first survey (LF2009) but concentrations remained constant during the study. The U concentrations did show a slight spatial trend with U concentrations decreasing as the Olifants River flows through the park. All sites during LF 2009 had Zn concentrations above the TWQR and Sites 1, 3 and 5 had concentrations above the CEV. Sites 2, 4 and 6 (HF2010) had Zn concentrations above the TWQR and CEV. The Zn concentrations at Site 2 (LF2010) were above the TWQR. Dissolved concentrations of Zn from all other sites and surveys were below detection limits.

The macro elements displayed very similar temporal results. Dissolved Ca concentrations were highest at all sites during LF2009 and decreased toward the HF2011 survey. The Mg concentrations were substantially higher during LF2009 compared to the other surveys. Apart from the first survey, the Letaba River site had the lowest dissolved Mg concentrations of all sites from all surveys. Concentrations of K were substantially higher during the LF2009 survey but remained consistent throughout the next three surveys. Concentrations of Na showed a similar trend as the other salts with LF2009 having substantially higher concentrations than the remaining surveys, Na concentrations remained similar through these surveys.

Concentrations of Co and As are highest at Site 2 and concentrations of Cr, Fe, Cd, Zn and Pb were higher at Site 3 and could possibly be due to inflow from other tributaries such as the Klasere, which may cause the remobilization of heavy metals from the sediment, or the geological contributions from the area. The remobilization of sediments and influx of solids from rainfall events during the highflow events are evident in the high TDS and conductivity of the Olifants River. The concomitant high DO could further cause the oxidizable fraction of metals (see Section 2.2) in these sediments to become bioavailable. Possible reasons for high Fe concentrations at Sites 3 and 4 are due to weathering of the basalt formations in the underlying geology (Seymore et al., 1994). The results from this study differ to those found in a study by Seymore et al. (1994) and Wepener et al. (1999), in that concentrations of Cr, Fe, Zn and Pb in the current study are lower than those found in the mentioned studies (Table 6). These metals were also highest at Site 3 as was found in

the study by Seymore et al. (1994). Concentrations of Zn and Cu between sites differ greatly to observations by Wepener et al. (1999; 2000) who found that concentrations at Site 4 were higher than concentrations at Site 1, where for the current study the opposite was found. The concentrations of Al, Mn, Ni, Ag, Se, Ca, K and Na were higher in the Letaba River than in any site in the Olifants River, possibly affecting fish caught at the confluence of the two rivers. The observed levels of Mn and Ni are lower than those in a study by Seymore et al. (1994) (Table 6), where the Mn and Ni concentrations were highest at Site 1 in the Olifants River while this study the concentrations were highest at Sites 3 and 2 respectively.

Table 6. Historical dissolved metal concentrations (µg/L) at selected sites in the Olifants River. NS represents metals not sampled.

Reference	Site and Month	Dissolved metal concentrations µg/L											
		Cd	**Cr**	**Ni**	**Pb**	**Fe**	**Cu**	**Mn**	**Zn**	**K**	**Ca**	**Mg**	**Na**
Du Preez and Steyn (1992)	Balule October 1990	BD	NS	185 ±58	355 ±89	2285 ±643	70±35	45 ±17	1075 ±573	NS	NS	NS	NS
Seymore et al. (1994)	Whole River October 1991	NS	9.6	16	178	440	32	38	128	24.2	43.8	73	104
Grobler et al (1994)	Phalaborwa Barrage Dec 1990	NS	38	NS	NS	82	NS	15	104	NS	NS	NS	NS
Marx and Avenant-Oldewage (1998)	Mamba & Balule November 1994	NS	NS	NS	20	NS	NS	NS	43.5 ±21.8	NS	NS	NS	NS
Kotze et al. (1999)	Mamba 1994-1995	NS	NS	NS	NS	NS	22	NS	NS	NS	NS	NS	NS
Avenant-Oldewage and Marx (2000a)	Mamba & Balule November 1994	NS	3	NS	NS	147.5 ±18.5	4	5 ±4	NS	NS	NS	NS	NS
Wepener et al. (2000)	Mamba & Balule 1990-1992	NS	NS	NS	NS	NS	17.65 ±1.25	NS	87.8 ±13.6	NS	NS	NS	NS

Metal concentrations in suspended matter

Metal concentrations from the suspended solids (Table 7) found in the water column of the Olifants and Letaba Rivers were higher for most metals when compared to dissolved metal concentrations. LF2009 had the highest concentration of Pb and the lowest concentrations of As, Co, Mn and Se when compared to the other surveys. HF2010 had the highest concentrations of Al, Cr, Co, Cu, Fe, Mn, Ni and Zn and the lowest concentrations of Ag and U. LF 2010 had the highest concentration of Cd and the lowest concentrations of Al, Co, Fe, Pb and Ni. HF 2011 had the highest concentrations of Se and U and the lowest concentrations of Cd, Cr, Cu and Zn. No spatial trends were observable, except that Site 5 at the confluence had lower metal concentrations during most of the surveys when compared to the other sites from that specific survey.

The PCA biplot for both physico-chemical parameters and metal concentrations in the Olifants and Letaba Rivers (Figure 19) show no clear spatial patterns as the Letaba River surveys always group with Olifants River sites of the corresponding survey. However, there are clear temporal differences as the surveys group separately. The 2010 high flow survey is separated furthest from all the other surveys due in part to higher concentrations of the following suspended metals; Co, Cd, Al, Mn, Fe, Cr, Cu, Zn and Ni (refer to Table 7). Sites from the LF2009 period grouped together and separate from other surveys due to higher dissolved salt concentrations (refer to Table 5), namely; Mg, Na, K and Ca, as well as higher TDS values (refer to Table 4). Sites from the LF2010 survey are grouped and different to other surveys based on the in situ water quality data (refer to Table 4). Sites from the 2011 high flow survey grouped and are separate from other surveys due to increased dissolved and suspended U concentrations, and higher suspended metal concentrations of As and Se (refer to Table 5), as well as due to increased concentrations of ammonium, sulphates and higher turbidity and COD. There are also notable differences between high flow and low flow periods. Low flow periods are associated with in situ water quality variables (refer to Table 4) and higher concentrations of dissolved salts, whereas high flow periods are associated with higher dissolved (refer to Table 5) and suspended (refer to Table 7) metal concentrations. Seymore et al. (1994) and Wepener et al. (1999) found that the water quality in the Olifants River is strongly related to rainfall and therefore flow. The release of water with high suspended matter loads from the Phalaborwa Barrage results in input of and are yet to be released. It was assumed in the current study that the sources of Na, K, SO_4, Mg and Cl resulted from mining in the Phalaborwa area and higher up in the catchment (De Villiers & Mkwelo, 2009).

To interpret the water quality in terms of its suitability to sustain healthy fish populations, the Aquatic Toxicity Index (ATI) that was developed for the Olifants River (Wepener et al. 1992), was applied to the data. The ATI scores for the Olifants and Letaba Rivers (Table 8) did not go below 70 at any of the sites during any of the surveys. According to the index classification system developed by Wepener and Vermeulen (1999) an ATI score above 60 is regarded as acceptable. Scores for sites on the Olifants River ranged between 73 and 87, and scores for the Letaba River ranged between 72 and 87 (Figure 20). There was very little change in ATI scores between surveys. ATI scores were highest at the first three sites during the HF survey of 2011 and ATI scores were similar between sites during the other surveys.

Table 7. Suspended metal concentrations (mean ± standard error, n=3) from water samples collected from 5 sites in the Olifants River and one site in Letaba River during two consecutive high and low flow periods between 2009 and 2011. All concentrations are expressed as µg/g dry mass and BD represents samples below detection limits.

Sample	Al µg/g	As µg/g	Cd µg/g	Cr µg/g	Co µg/g	Cu µg/g	Fe µg/g	Pb µg/g	Mn µg/g	Ni µg/g	Se µg/g	Ag µg/g	U µg/g	Zn µg/g
OLI-S1-09LF	10278 ± 150.7	4.02 ± 0.05	0.07	52.34 ±0.65	1.04 ± 0.01	18.2 ± 0.12	2288 ± 55.68	5 ± 0.03	22.97 ± 0.07	6.97 ± 0.04	0.17 ± 0.12	2.43 ± 0.01	-	52.88 ± 0.15
OLI-S2-09LF	155 ± 2.15	4.56 ± 0.23	0.12 ± 0.01	60.98 ± 3.71	0.79 ± 0.02	21.14 ± 0.2	361 ± 36.38	3.47 ± 0.04	13.54 ± 0.24	12.31 ± 0.16	0.18 ± 0.12	4.46 ± 0.05	-	50.79 ± 0.61
OLI-S3-09LF	23592 ± 20.52	1.63 ± 0.04	0.19	33.1 ± 0.27	0.49 ± 0	21.34 ± 0.11	7427 ± 25.32	3.21 ± 0.19	9.32 ± 0.03	11.38 ± 0.06	0.17 ± 0.12	2.17 ± 0	-	45.05 ± 0.22
OLI-S4-09LF	20879 ± 202.4	2.1 ± 0.07	0.13	51.37 ± 0.61	0.75 ± 0.01	20.28 ± 0.35	21155 ± 452.3	49.79 ± 6.19	9.52 ± 0.05	6.23 ± 0.08	0.17 ± 0.12	2.34 ± 0.02	-	84.06 ± 0.84
OLI-S5-09LF	67.75 ± 3.31	2.3 ± 0.03	0.09	52.33 ± 2.21	0.68 ± 0.01	18.27 ± 0.03	283 ± 4.79	2.94 ± 0.03	9.35 ± 0.12	13.07 ± 0.14	0.18 ± 0.12	3.44 ± 0.02	-	36.24 ± 0.23
Letaba-09LF	17579 ± 496.8	3.86 ± 0.05	0.2	68.28 ± 1.31	1.83 ± 0.01	21.49 ± 0.16	11951 ± 2379	5.44 ± 0.03	77.54 ± 0.88	8.52 ± 0.01	0.17 ± 0.12	2.77 ± 0.03	-	54.42 ± 0.31
OLI-S1-10HF	87023 ± 26140	17.08 ± 5.18	6.9 ± 3.07	326.2 ± 102	1195 ± 1171	86.26 ± 22.54	70863 ± 20751	1.13 ± 1.13	9826 ± 5100	32.1 ± 11.16	14.3 ± 10.46	BD	BD	182.8 ± 30.17
OLI-S2-10HF	135884 ± 34162	23.03 ± 9.71	7.59 ± 1.78	404.4 ± 148.4	5304 ± 5287	168.3 ± 63.34	109148 ± 32152	2.29 ± 0.2	8913 ± 2027	72.54 ± 16.78	11.77 ± 7.58	0.06 ± 0.06	BD	433.2 ± 108.16
OLI-S3-10HF	99336 ± 16687	22.95 ± 4.58	13.19 ± 0.81	435.1 ± 40.19	27.64 ± 5.94	127.3 ± 15.61	85006 ± 17002	0.23 ± 0.23	9597 ± 4691	43.59 ± 19.25	12.11 ± 1.88	BD	BD	458.8 ± 194.91
OLI-S4-10HF	90299 ± 18133	20.86 ± 4.13	7.98 ± 2.61	376 ± 71.66	5530 ± 3424	114.5 ± 25.42	76142 ± 16898	0.32 ± 0.26	9648 ± 3421	40.09 ± 10.91	10.84 ± 10.23	BD	BD	274.3 ± 84.17
OLI-S5-10HF	120453 ± 31032	17.15 ± 12.38	6.85 ± 2.75	348.8 ± 155.1	2278 ± 5263	116.4 ± 39.03	81297 ± 25770	0.78 ± 0.92	10379 ± 4088	39.06 ± 22.59	9.15 ± 4.93	1.81 ± 1.21	BD	237.2 ± 85.12
Letaba-10HF	107792. ± 54362	30.43 ± 23.17	8.37 ± 3.87	455.9 ± 328.8	8792 ± 8786	135.2 ± 65.61	86131 ± 48237	1.6 ± 1.6	8018 ± 6086	58.48 ± 44.81	9.49 ± 1.61	BD	BD	346.2 ± 110.78
OLI-S1-10LF	4116 ± 2119	16.85 ± 1.04	12.45 ± 0.11	194 ± 38.4	BD	41.79 ± 6.98	1059 ± 1059	BD	106.6 ± 26.91	BD	BD	12.45 ± 12.45	BD	110.8 ± 32.12
OLI-S2-10LF	10607 ± 5670	15.59 ± 1.29	12.32 ± 0.23	235.3 ± 116.6	0.76 ± 0.76	68.59 ± 7.78	5861 ± 4598	BD	181.7 ± 70.11	BD	BD	BD	BD	142.2 ± 47.34
OLI-S3-10LF	4876 ± 501	27.33 ± 2.99	12.62 ± 0.29	231.3 ± 34.72	BD	58.05 ± 16.93	1086 ± 168	BD	203.6 ± 1.12	BD	BD	BD	BD	135.1 ± 22.82
OLI-S4-10LF	5299 ± 1644	33.68 ± 1.81	0.44 ± 0.11	58.78 ± 18.79	1.05 ± 0.13	32.26 ± 8.21	3210 ± 1731	2.98 ± 1.1	133.5 ± 12.52	BD	0.68 ± 0.68	3.88 ± 1.87	1.02 ± 0.12	55.7 ± 25.72
OLI-S5-10LF	2164 ± 385	34.83 ± 8.04	0.6 ± 0.34	35.11 ± 3.19	1.32 ± 0.97	6.56 ± 6.56	1217 ± 661	2.99 ± 1.41	158.1 ± 25.39	BD	30.38 ± 16.15	12.23 ± 9.79	0.82 ± 0.16	53.6 ± 30.56
Letaba-10LF	16723 ± 985	7.55 ± 0.79	12.03 ± 0.47	129.61 ± 7.91	BD	55.7 ± 7.18	12286 ± 1114	BD	460.2 ± 86.01	BD	BD	BD	BD	179 ± 30.39
OLI-S1-11HF	14602 ± 2040	15.94 ± 2.09	0.2 ± 0.04	44.32 ± 11	3.49 ± 0.47	12.35 ± 2.39	9965 ± 1453	1.85 ± 0.24	125.1 ± 19	BD	22.04 ± 3.42	0.78 ± 0.3	1.02 ± 0.17	38.35 ± 12.68
OLI-S2-11HF	12506 ± 1352	17.85 ± 2.74	0.07 ± 0.01	54.94 ± 20.04	2.82 ± 0.36	10.56 ± 3.66	8745 ± 1329	2.8 ± 1.27	1025 ± 11.73	BD	24.15 ± 8	BD	0.99 ± 0.14	23.56 ± 3.74
OLI-S3-11HF	13856 ± 4405	21.22 ± 4.72	0.12 ± 0.05	78.37 ± 9.85	2.66 ± 0.46	12.94 ± 5.22	9763 ± 3508	1.83 ± 0.92	101.6 ± 27.29	24.67 ± 24.67	21.04 ± 4.33	BD	1.69 ± 0.73	37.76 ± 10.6
OLI-S4-11HF	13912 ± 1459	13.62 ± 0.64	0.03 ± 0.01	31.58 ± 4.04	2.18 ± 0.35	16.26 ± 4.64	9712 ± 1422	2.06 ± 0.64	108.9 ± 11.43	BD	10.77 ± 3.19	0.01 ± 0.01	1 ± 0.05	11.2 ± 3.41
OLI-S5-11HF	15744 ± 2196	10.72 ± 0.59	0.05 ± 0.03	21.7 ± 4.08	2.37 ± 0.28	20.55 ± 5.43	10868 ± 1830	5.47 ± 2.32	104.9 ± 12.72	BD	28.41 ± 7.32	1.75 ± 1.72	0.9 ± 0.19	33.58 ± 14.83
Letaba-11HF	15359 ± 770	9.4 ± 0.52	0.04 ± 0.02	25.16 ± 0.97	2.01 ± 0.14	20.52 ± 3.71	11427 ± 824	4.8 ± 0.56	146.7 ± 22.37	BD	36.44 ± 0.49	BD	0.29 ± 0.02	27.54 ± 3.38

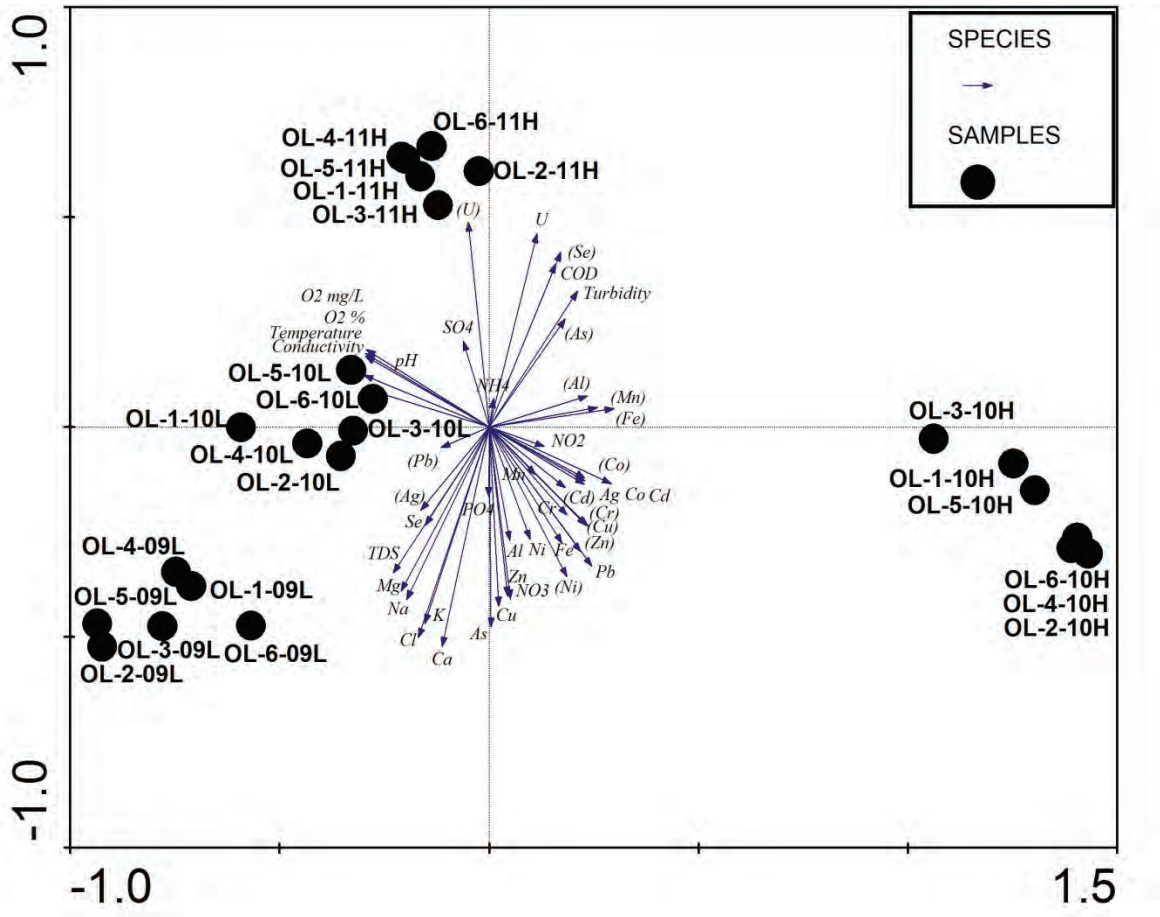

Figure 19. PCA biplot for the Olifants and Letaba Rivers indicating spatial and temporal patterns of physico-chemical parameters, dissolved and suspended (in parentheses) metal concentrations. The biplot describes 79% of the variation in the data, where 63% is displayed on the first axis, while 16% is displayed on the second axis.

A slight spatial trend can be observed along the Olifants River with average ATI scores increasing as the river flows through the park from Site 1 on the western border to Site 5 (Site 1: 80.86, Site 2: 78.98, Site 3: 80.14, Site 4: 77.92 and Site 5: 83.38) at the eastern border of the park. Even though the Letaba River had a lower average score (79.48) it seemed to have little or no influence on the water quality at the confluence (Site 5). The lowest scores (Table 8) for individual variables in the Olifants River were almost entirely due to increased turbidity (NTU), with the lowest scores for turbidity ranging from 46 to 58. Increased ammonium concentrations also contributed to the lowering of scores at Sites 3 and 4 during the various surveys. Increased levels of orthophosphates and K with scores of 47.5 and 46 brought the scores down for Site 2 and Site 6 during the LF2009 survey respectively. All of the above factors namely increased Ammonium, orthophosphate and K concentrations combined with turbidity to bring down overall scores. Metal concentrations

had no effect on lowering the ATI scores and this was in contrast to the ATI scores for similar sites in the Olifants and Letaba Rivers from 1990 to 1992 (Wepener et al., 1999).

Table 8. Individual ATI scores and corresponding lowest rating scores for sites on the Olifants and Letaba Rivers during all surveys of the study.

Sampling Site	Index score	Lowest Rating
OLI-S1-09LF	80	Turbidity **(56)**
OLI-S2-09LF	76.77	Orthophosphates **(47.5)**
OLI-S3-09LF	79.85	Turbidity **(58)**
OLI-S4-09LF	83	Turbidity **(64)**
OLI-S5-09LF	86.55	Turbidity **(68)**
Letaba-09LF	72.48	Potassium **(46),** Turbidity **(56)**
OLI-S1-10HF	77.9	Turbidity **(52)**
OLI-S2-10HF	77.6	Turbidity **(52)**
OLI-S3-10HF	80.12	Turbidity **(52)**
OLI-S4-10HF	73.03	Turbidity **(48),** Ammonium **(50)**
OLI-S5-10HF	78.98	Turbidity **(48)**
Letaba-10HF	78.78	Turbidity **(50)**
OLI-S1-10LF	79.22	Turbidity **(50)**
OLI-S2-10LF	77.31	Turbidity **(56)**
OLI-S3-10LF	77.97	Ammonium **(45),** Turbidity **(54)**
OLI-S4-10LF	77.46	Ammonium **(54.5),** Turbidity **(56)**
OLI-S5-10LF	86.4	Turbidity **(56)**
Letaba-10LF	83.74	Turbidity **(56)**
OLI-S1-11HF	86.3	Turbidity **(52)**
OLI-S2-11HF	84.22	Turbidity **(52)**
OLI-S3-11HF	82.61	Turbidity **(50)**
OLI-S4-11HF	78.19	Turbidity **(46)**
OLI-S5-11HF	81.58	Turbidity **(52),** Ammonium **(51.3)**
Letaba-11HF	82.92	Turbidity **(56)**

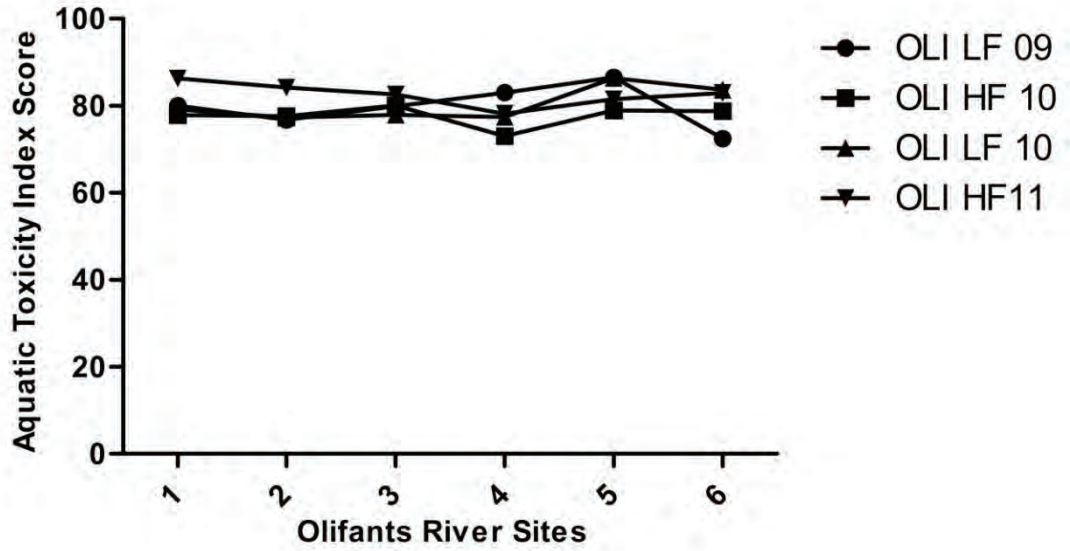

Figure 20. Aquatic toxicity index (ATI) rating scores of water quality at all sites along the Olifants and Letaba Rivers during all surveys.

3.2 Sediment

Physical characteristics

The moisture content of sediments from the Olifants and Letaba River sites during all surveys (Table 9) remained similar; between 20 and 30%, except Site 4 during the HF2010 survey (40.5%). The percentage organic matter (Table 9) in sediments from all sites ranged from 0.41 to 8.59%. Sediments collected during the HF2011 had higher organic content than the other surveys. During the low flow 2009 survey the organic content at most sites was low. However, Sites 1 and 3 had slightly higher organic content and were classed as moderately low. Organic content for most sites (2, 5 and Letaba River) were classed as low and during the during the HF2010 survey, while organic content at Sites 1 and 3 were moderately low. Site 4 however, had a high amount of organic content. During the LF2010 survey Sites 1 to 3 had low organic content, Sites 5 and Letaba were slightly higher with moderately low organic content, with Site 4 again having the highest organic content (medium). The Letaba River site had low to moderately low organic content throughout the surveys. The particle size distribution of the sampled sediments from the selected sites during the various surveys (Table 9) had a predominantly small grain size, i.e. < 500 µm, except for Site 4 during the low flow 2009 survey and the Letaba River site during all surveys.

Metals tend to have a greater bonding capacity to silty soils with high organic content (Kwon & Lee, 2001) which was found mainly at Site 4. The second most abundant particle

size was very fine sand, and this, coupled with the medium sand may result in sediments from the Olifants River having a great adsorption capacity for metals (Kwon & Lee, 2001). Letaba River sediments are dominated by gravel and this, coupled with a low organic content could result in the higher concentrations of metals observed in water samples from this site.

Table 9. Percentage moisture-, organic content and particle size distribution from selected sites on the Olifants and Letaba Rivers during the four surveys.

	Moisture content (%)	Organic content (%)	Particle size (μm)					
Sample			>4000	>2000	>500	>212	>50	>0
OLI-S1-09LF	-	1.02	4.19	5.56	9.44	43.64	19.99	17.17
OLI-S2-09LF	-	0.41	0.21	0.56	3.98	52.63	31.09	11.52
OLI-S3-09LF	-	1.18	0.19	0.19	6.26	59.86	25.05	8.46
OLI-S4-09LF	-	0.73	0.37	0.29	67.99	29.40	1.89	0.07
OLI-S5-09LF	-	0.93	0.14	0.08	0.78	47.34	41.71	9.95
OLI-S6-09LF	-	0.49	47.80	14.33	31.31	5.04	1.35	0.13
OLI-S1-10HF	24.30	2.00	3.27	5.71	7.34	32.50	33.80	17.38
OLI-S2-10HF	18.45	0.72	0.00	1.80	6.43	49.76	35.39	6.62
OLI-S3-10HF	17.33	1.55	0.00	6.69	15.74	37.08	31.38	9.11
OLI-S4-10HF	40.48	8.59	7.99	13.50	24.59	11.38	18.34	24.20
OLI-S5-10HF	18.65	0.47	0.00	5.91	6.41	43.86	38.30	5.52
OLI-S6-10HF	14.83	0.56	8.16	14.01	52.65	13.17	7.31	4.71
OLI-S1-10LF	19.31	0.53	12.09	6.61	27.59	42.92	5.71	5.08
OLI-S2-10LF	23.03	0.69	1.58	5.84	14.92	50.30	21.23	6.12
OLI-S3-10LF	21.98	0.48	3.88	5.55	25.45	45.18	13.85	6.09
OLI-S4-10LF	21.41	2.23	3.66	6.66	28.74	25.56	29.38	6.00
OLI-S5-10LF	22.73	1.14	5.47	8.81	37.58	28.49	11.81	7.84
OLI-S6-10LF	22.81	1.79	24.69	17.44	38.41	8.11	6.20	5.15
OLI-S1-11HF	28.05	3.80	3.44	5.94	12.54	36.95	31.53	9.60
OLI-S2-11HF	29.55	3.25	6.05	7.89	9.41	21.17	41.96	13.52
OLI-S3-11HF	25.58	2.28	3.40	6.55	14.13	29.59	34.68	11.65
OLI-S4-11HF	27.40	1.51	0.00	6.14	6.96	22.33	55.63	8.94
OLI-S5-11HF	23.74	0.86	1.43	5.73	11.32	45.75	26.04	9.72
OLI-S6-11HF	22.11	1.21	12.80	14.54	42.03	14.77	10.43	5.43

Metal concentrations

No spatial trends in total metal concentrations were observable for any of the metals in the Olifants and Letaba Rivers (Appendix A1). Total Al, As and Pb concentrations showed a temporal trend with highest during high flow periods, while the Letaba River had higher total Al, Fe, Se and Ag concentrations during low flow periods. The LF2009 survey had the highest total Cu, Pb and Mn concentrations and the lowest concentrations of Cd, Cr and Co. The HF2010 survey had the highest total Cd, Co, and U concentrations and the lowest concentrations of Pb. Total Ni concentrations remained similar at all sites and surveys

throughout. Total Zn concentrations showed no spatial or temporal trends, but were higher at Sites 1 and 3 during the LF2010 survey.

The total metal concentrations measured in this study were very similar to historical metal concentrations at similar sites in the Olifants River (Table 10). The results indicate that flow has a major influence on the total metal concentrations with lower concentrations during the high flow periods due to the remobilisation of metals from the sediments.

The spatial results for sequential extraction (surveys combined) are depicted in Figure 21 and Figure 22. Metals in the acid-soluble (A) and reducible fractions (B) are considered to be biological available and as they become oxidised (C) and ultimately inert (D) the bioavailability decreases (Baeyens et al., 2003). Site 1 had the highest bioavailability of Cu, Ni, and Zn, and the lowest bioavailability of Mn. Site 2 had the highest bioavailability of Co, Cu and Mn, and the lowest bioavailability of Ag, Al and Se. Site 3 had the highest bioavailability of Ag and Al, and the lowest bioavailability of Mn. Site 4 had the highest bioavailability of Ag, Al, Cr, Fe and Mn while Site 5 had the highest bioavailability of Ag, Al, Cd and Se, and the lowest bioavailability of Cu and Mn. The Letaba River had the highest bioavailability of Mn, and the lowest bioavailability for all the other metals Ag, Al, As, Cd, Cr, Co, Fe, Ni, U and Zn. All the Olifants River sites had similar bioavailibilities of U. Notably the Cu bioavailability decreased as the Olifants River flowed through the Park.

Table 10. Historical total sediment metal concentrations at selected sites in the Olifants River.

Reference	**Site and Month**	**Concentration metals in sediment (µg/g dry weight)**							
		Cd	**Cr**	**Ni**	**Pb**	**Fe**	**Cu**	**Mn**	**Zn**
Seymore et al. (1994)	Whole River October 1991	X	30	21	5	16040	14	194	20
Marx & Avenant-Oldewage (1998)	November 1994	X	X	X	20	X	X	X	67.5 ±1.5
Kotze et al. (1999)	Mamba 1994-1995	X	X	X	X	X	21	X	X
Avenant-Oldewage & Marx 2000b	November 1994	X	182 ±77	X	X	33855 ±625	29.5 ±19.5	493 ±118	X
Wepener et al. 2000	Mamba and Balule 1990-1992	X	X	X	X	X	25 ±0.3	X	41.2 ±7.5

The temporal sequential extraction data (Figure 23; Figure 24) are based on the combined site data for each flow period in the Olifants and Letaba Rivers. The LF2009 survey had the highest bioavailability of Ag, Al, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn. The HF2010 survey had the highest bioavailability of Cd and Fe. The LF2010 survey had the highest bioavailability of U. The HF2011 survey had the lowest bioavailability of Ag and Al. The bioavailability of Cd during all surveys was high in relation to total concentrations. The bioavailability of Al, Cr, Pb and Zn decreased with successive surveys.

The PCA biplot (Figure 25) indicates spatial differences between the Olifants River and the Letaba River with all sites from the various surveys on the Letaba River grouping together. This is due to the coarse sand (CS), very coarse sand (VCS) and gravel fractions comprising a higher percentage of the total grain size distribution (refer to Table 9). The total concentrations of Al, Cd, Ni and Zn are lower in the Letaba River compared to the Olifants River. There are no major groupings that indicate spatial patterns differences in the Olifants River based on the total metal concentrations and the grain size distributions. However there are temporal differences as the 2009 survey was separate from the 2010 and 2011 surveys. This grouping was due to the higher concentrations of Cr, Cu and Pb and to a lesser extent Ag and Mn. The sediments in the Letaba River did not show any temporal differences.

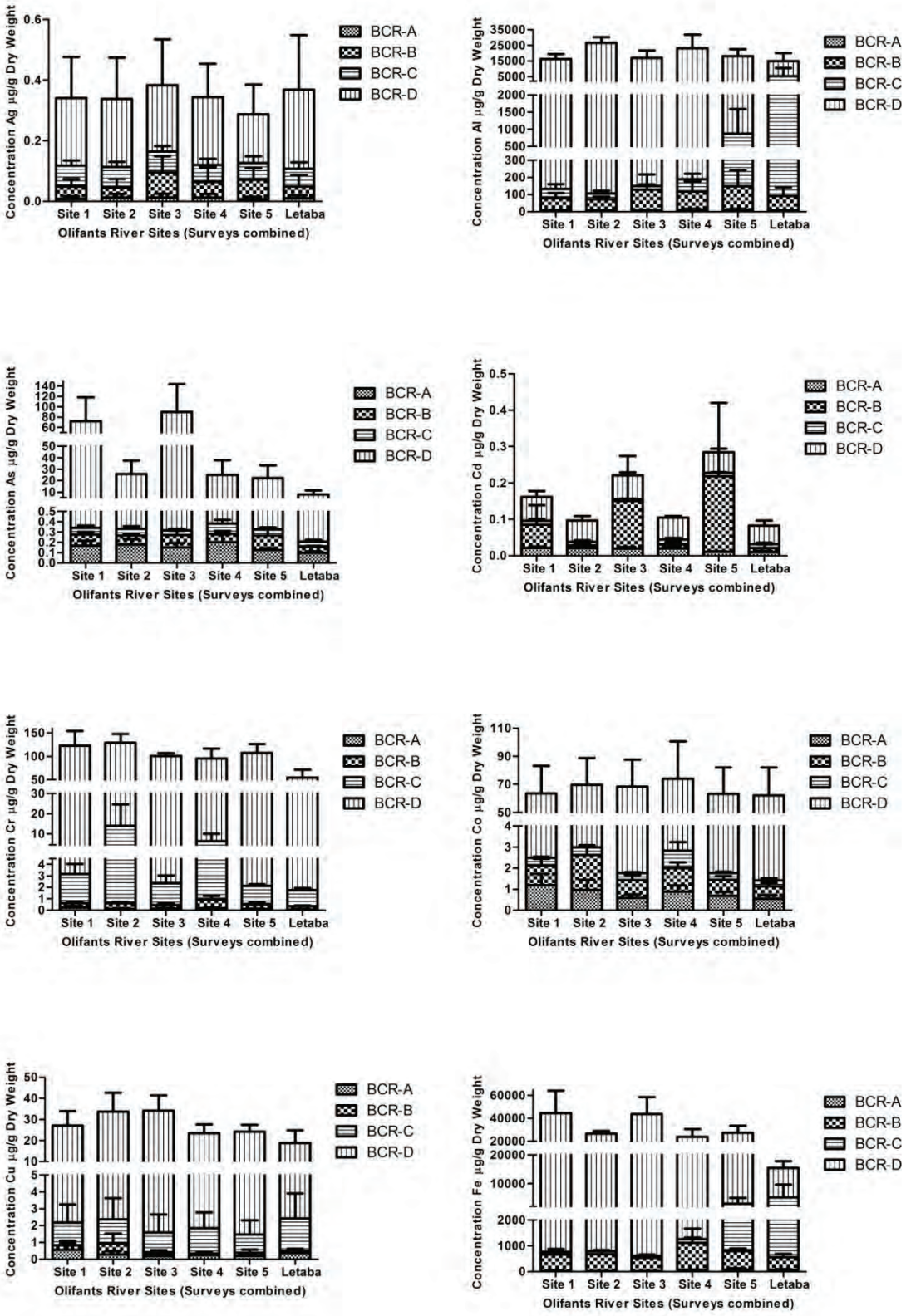

Figure 21. Metal concentrations (µg/g dry mass) in various fractions of sediment collected from sites on the Olifants and Letaba Rivers. Data from the four surveys were combined per site. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

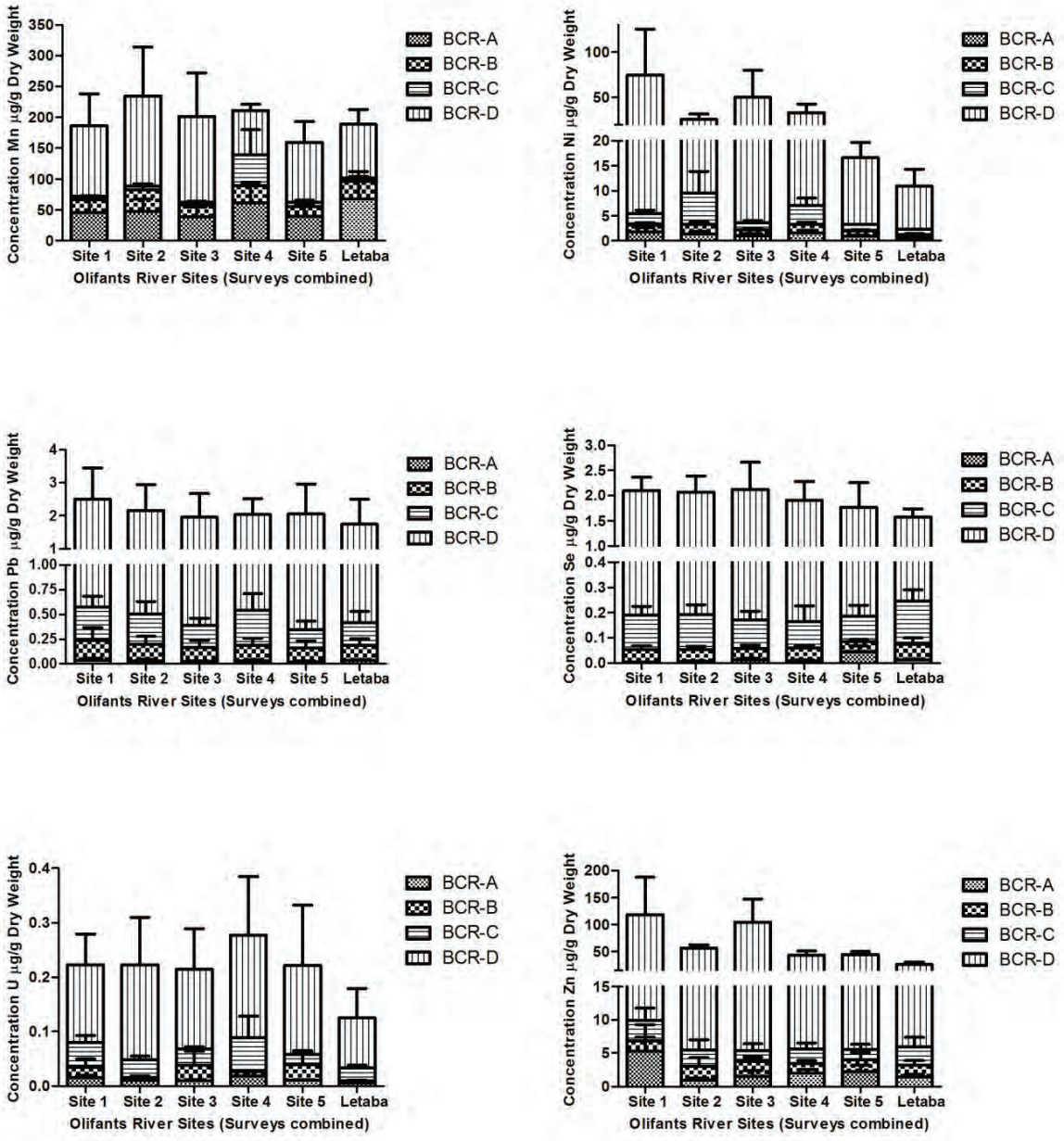

Figure 22. Metal concentrations (µg/g dry mass) in the various fractions of sediment collected from sites on the Olifants and Letaba Rivers. Data from the various surveys were combined per site. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

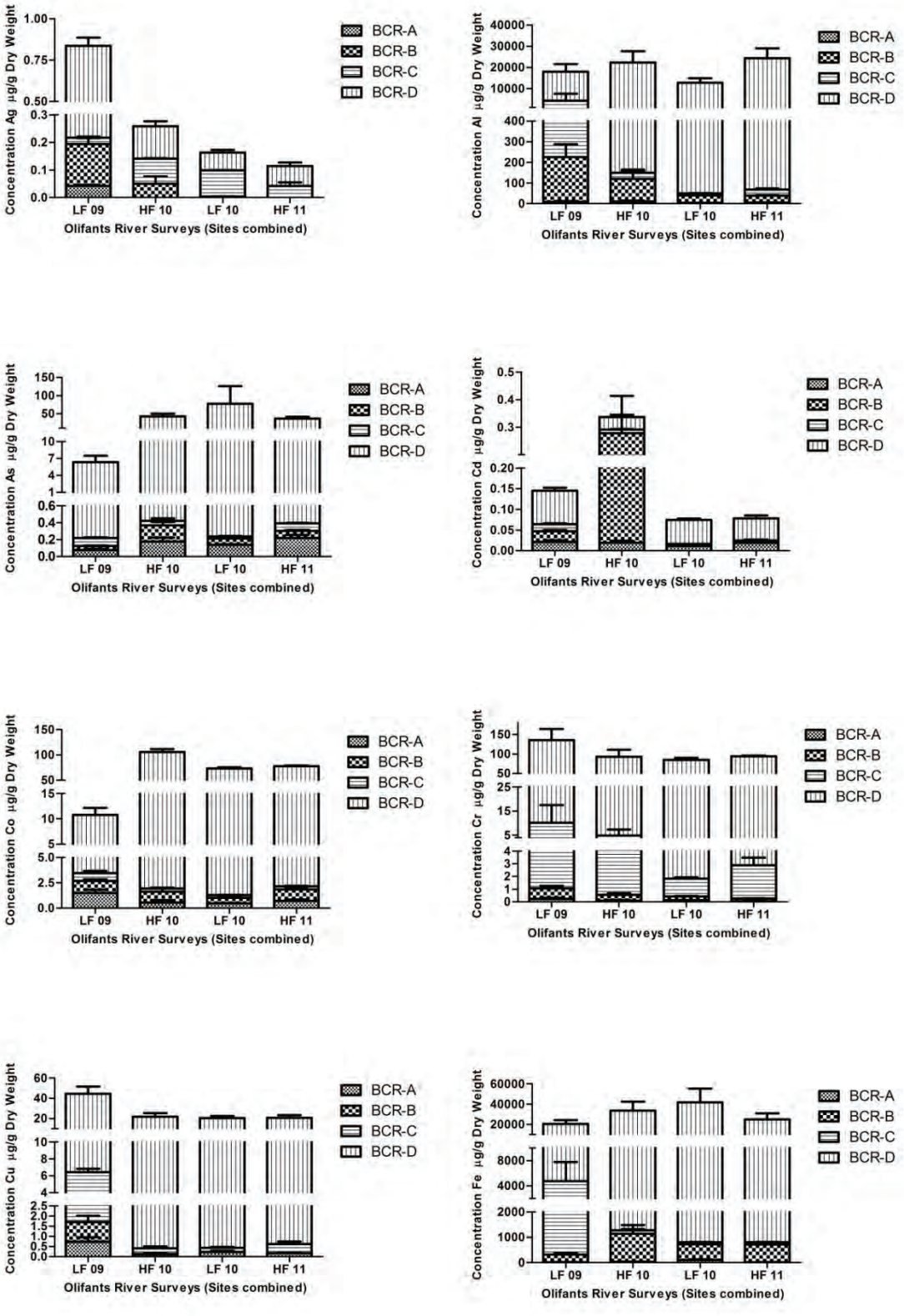

Figure 23. Metal concentrations (µg/g dry mass) in the various fractions of sediment collected during the four different surveys on the Olifants and Letaba Rivers. Data from the various sites were combined per survey. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

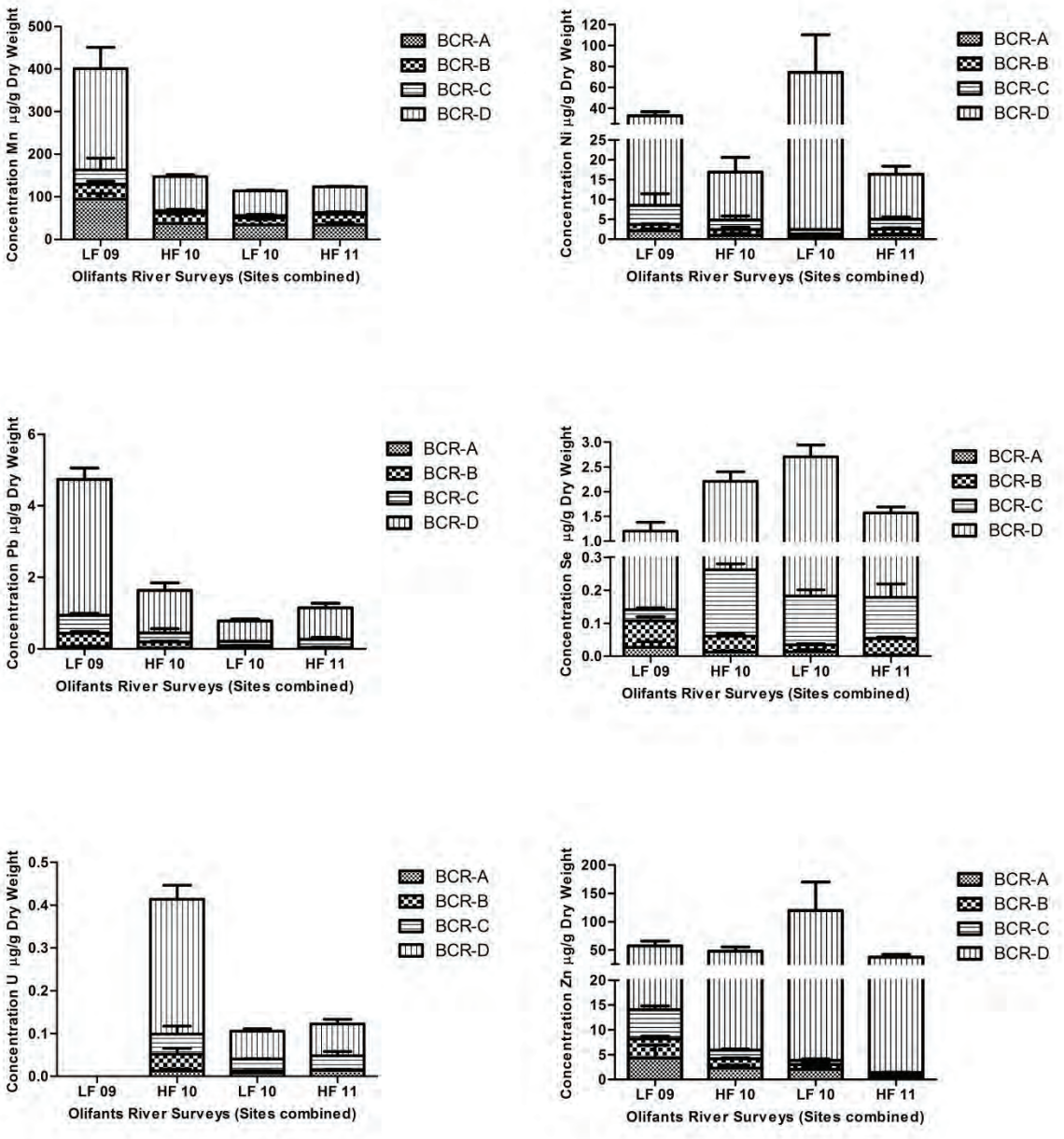

Figure 24. Metal concentrations (µg/g dry mass) in the various fractions of sediment collected during the four different surveys on the Olifants and Letaba Rivers. Data from the various sites were combined per survey. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

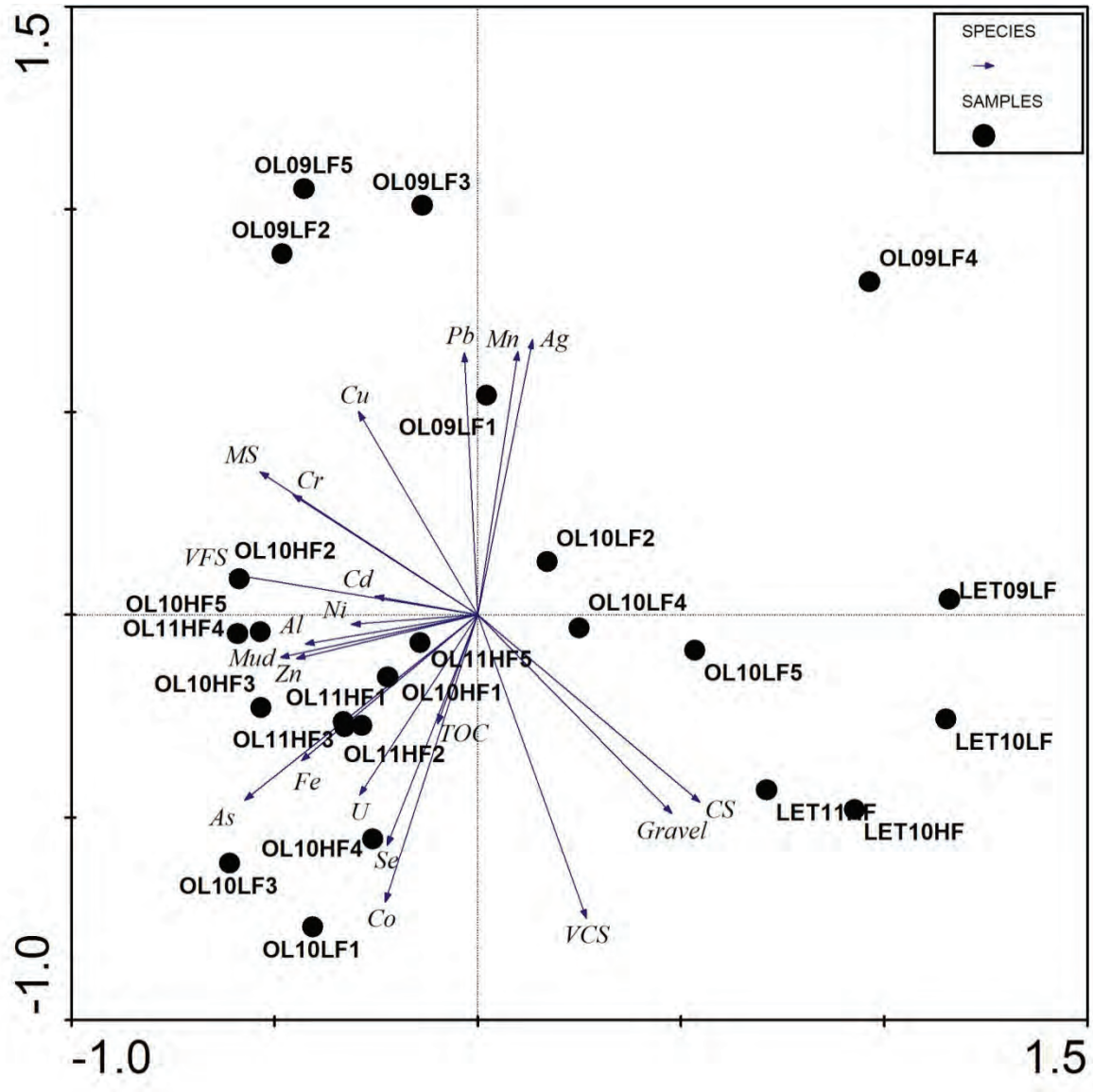

Figure 25. PCA biplot for Olifants and Letaba Rivers indicating temporal and spatial patterns based on physical charateristics and metal concentrations in sediments. The biplot describes 58.4% of the variation in the data, where 33.4% is displayed on the first axis, while 25% is displayed on the second axis.

Organic contaminant concentrations

The organic contaminant concentrations sampled in sediments from the Olifants and Letaba Rivers during the LF2010 and HF2011 surveys are presented in Table 11. Only six of the 22 organochlorine contaminants tested for were found at sites during the LF2010 survey whereas 21 of the 22 were present at sites during the HF2011 survey. During the LF2010 survey trace amounts of Heptachlor, cis-Chlordane and p,p'-DDD were found at Site 1. At Site 2 o,p'-DDE, p,p'-DDE and p,p'-DDD were found. Sites 3 and 5 had trace amounts of Heptachlorine and cis-Chlordane and Site 4 had the most organic contaminants present

during this period (5 of the 6 contaminants). The Letaba River had trace amounts of α-HCH, p,p'-DDE and p,p'-DDD. During the HF2011 survey only p,p'-DDE was measured at Site 2. The Letaba River site again only had trace amounts of 3 contaminants, i.e. trans-Chlordane, p,p'-DDE and p,p'-DDT. Site 1 had 4 of the tested organic contaminants, i.e. α-HCH, cis-Chlordane, p,p'-DDE and p,p'-DDT. Sites 3, 4 and 5 had the most organic contaminants present with 19.

The PCA biplot (Figure 26) is an excellent representation of the variation in spatial and temporal organochlorine concentrations and physical charateristics of the sediments in the Olifants and Letaba Rivers. On the first axis almost 84% of the variation is explained by spatial differences between the Olifants and Letaba River sediments and temporal variation between HF and LF sampling periods in the Olifants River. The second PC axis also explains some of the temporal variation in Olifants River sediment concentrations.

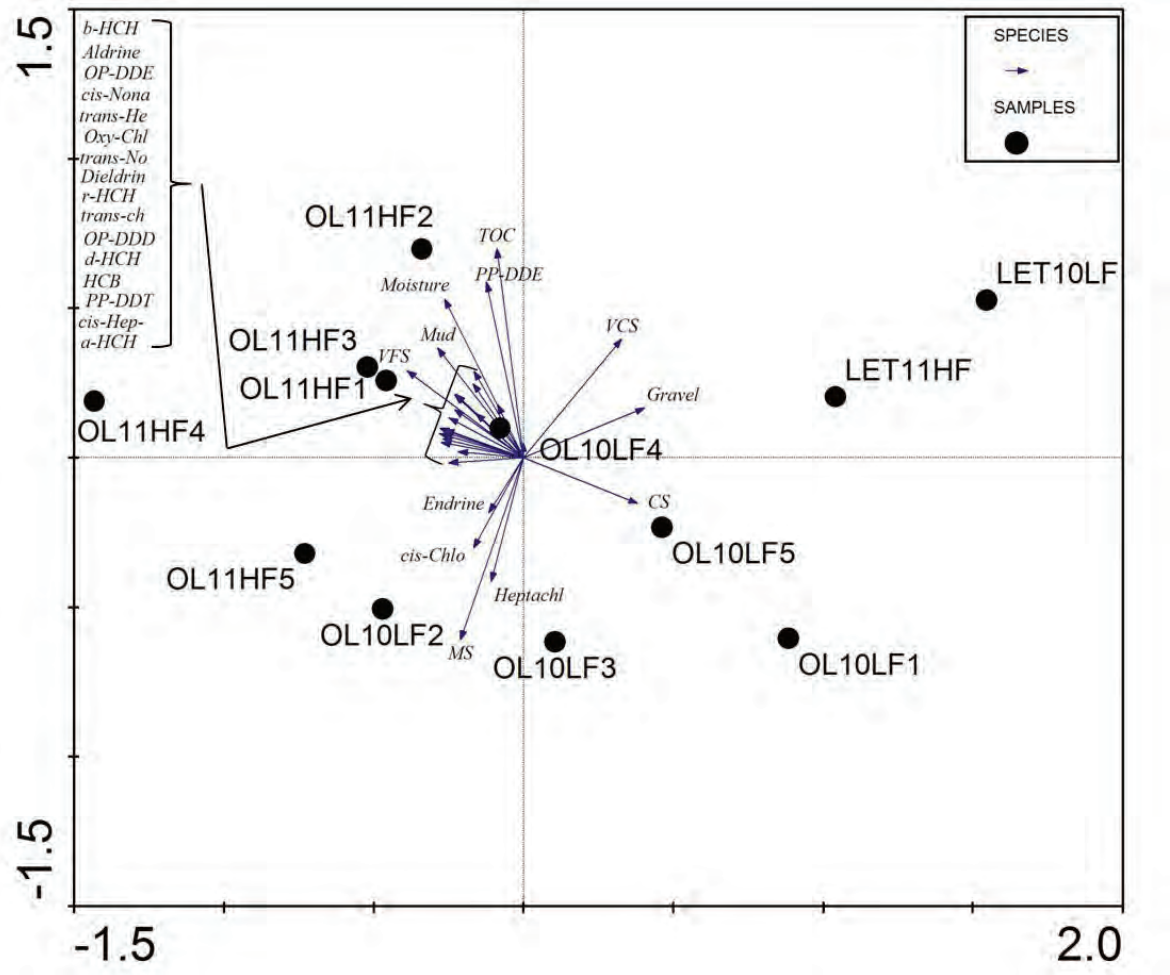

Figure 26. Spatial and temporal PCA biplot for physical sediment charateristics and organochlorine concentrations in sediments of the Olifants and Letaba Rivers. The biplot describes 83.9% of the variation in the data, where 65.8% is displayed on the first axis, while 18.1% is displayed on the second axis.

Table 11. Organic contaminant concentrations (ng/g dry weight) in sediments collected from the Olifants and Letaba Rivers for the Low flow 2010 and High flow 2011 surveys.

	Organic contaminants ng/g dry weight										
Sample	a-HCH	HCB	b-HCH	r-HCH	d-HCH	Heptachlor	Aldrine	Oxy-Chl	cis-Hep-epox	trans-Hep-epox	OP-DDE
OLI-S1-10LF	-	-	-	-	-	0.18	-	-	-	-	-
OLI-S2-10LF	-	-	-	-	-	-	-	-	-	-	0.16
OLI-S3-10LF	-	-	-	-	-	0.42	-	-	-	-	-
OLI-S4-10LF	-	-	-	-	-	0.24	-	-	-	-	0.36
OLI-S5-10LF	-	-	-	-	-	0.24	-	-	-	-	-
OLI-S6-10LF	0.17	-	-	-	-	-	-	-	-	-	-
OLI-S1-11HF	0.18	-	-	-	-	-	-	-	-	-	-
OLI-S2-11HF	-	-	-	-	-	-	-	-	-	-	-
OLI-S3-11HF	0.20	0.03	0.20	0.22	0.24	0.24	0.14	0.16	0.19	0.18	0.42
OLI-S4-11HF	0.20	-	0.25	0.13	0.24	0.23	0.16	0.13	0.18	0.18	0.15
OLI-S5-11HF	0.20	-	0.19	0.07	-	0.24	0.16	0.12	-	0.18	0.24
OLI-S6-11HF	-	-	-	-	-	-	-	-	-	-	-

	Organic contaminants ng/g dry weight										
Sample	trans-chlordane	trans-Nonane	cis-Chlordane	PP-DDE	Dieldrin	OP-DDD	Endrine	OP-DDT	cis-Nonach	PP-DDD	PP-DDT
OLI-S1-10LF	-	-	0.12	-	-	-	-	-	-	0.25	-
OLI-S2-10LF	-	-	-	0.22	-	-	-	-	-	0.25	-
OLI-S3-10LF	-	-	0.20	-	-	-	-	-	-	-	-
OLI-S4-10LF	-	-	0.18	0.47	-	-	-	-	-	0.31	-
OLI-S5-10LF	-	-	0.17	-	-	-	-	-	-	-	-
OLI-S6-10LF	-	-	-	0.31	-	-	-	-	-	0.26	-
OLI-S1-11HF	-	-	0.12	0.25	-	-	-	-	-	0.57	-
OLI-S2-11HF	-	-	-	0.33	-	-	-	-	-	-	-
OLI-S3-11HF	-	0.23	0.18	0.38	0.31	0.42	-	-	0.16	0.11	0.64
OLI-S4-11HF	0.16	0.28	0.17	0.27	0.28	0.29	-	-	0.16	0.09	0.60
OLI-S5-11HF	-	0.18	0.15	0.23	0.24	0.19	0.33	-	0.29	0.07	-
OLI-S6-11HF	0.06	-	-	0.23	-	-	-	-	-	-	0.25

The sediments of the Letaba River are dominated by course material and low organochlorine concentrations during both flow periods. During the high flow the sediments were dominated by fine particles with a high organic content. The majority of the organochlorine pesticides were associated with these sediments. The sediment in the Olifants River during the low flow period was dominated by medium sand with cis-Chlordane, Endrin and heptachlor associated with the sediments.

3.3 Habitat

Habitat assessment is extremely important when monitoring biological community strength. This is due to the fact that it must be known whether species are absent due to habitat loss or habitats not being present, or other drivers such as water quality deterioration, flow reductions, exotic species, etc. For the purpose of this study, a habitat assessment of the macro invertebrate and fish habitats was done according to Dallas (2005) and the RHP. This method is used specifically for the indices employed with the RHP, and as such was used for this study as the same indices and techniques were implemented. This method does not give an overall ecological class rating, but allows for the interpretation of data when SASS5 is implemented for macroinvertebrates and FRAI is implemented for fish. As such, they will not be discussed directly, but referred to when the macroinvertebrate and fish communities are discussed in the following sections. The habitat assessments are displayed below in Table 12 and
Table 13.

Table 12. The dominant velocity-depth classes and biotope diversities observed in this study for each site on the Olifants River during the low flow 2009 survey as determined using method of Dallas (2005).

	Site 1 (Mamba)	(Site 2) Tseri	Site 3 (Fig Tree)	Site 4 (Balule)	Site 5 (Gorge)	Letaba River
Invertebrate habitat						
Stones in current	4	5	4	3	4	1
Stones out of current	2	1	2	1	3	3
Vegetation	2	2	2	2	2	3
GSM	4	3	4	3	3	4
Fish habitat						
Slow-deep	4	5	3	1	5	3
Fast-deep	4	4	1	3	3	0
Slow-shallow	3	2	4	4	3	4
Fast-shallow	4	4	4	4	4	3
0=absent, 1=rare, 2=sparse, 3=moderate, 4=abundant and 5=very abundant						

Table 13. The dominant velocity-depth classes and biotope diversities observed in this study for each site on the Olifants River during the low flow 2010 survey as determined using method of Dallas (2005).

	Site 1 (Mamba)	(Site 2) Tseri	Site 3 (Fig Tree)	Site 4 (Balule)	Site 5 (Gorge)	Letaba River
Invertebrate habitat						
Stones in current	3	5	4	2	4	1
Stones out of current	2	2	2	2	2	3
Vegetation	2	2	2	2	2	4
GSM	4	3	4	3	3	4
Fish habitat						
Slow-deep	3	5	4	1	5	4
Fast-deep	2	3	2	2	4	0
Slow-shallow	3	2	4	5	3	4
Fast-shallow	4	4	4	2	4	2
0=absent, 1=rare, 2=sparse, 3=moderate, 4=abundant and 5=very abundant						

3.4 Macroinvertebrates

According to the RHP (Balance et al., 2001), the macroinvertebrate communities of the Olifants River within the KNP were in a fair state during the 2001 River Health programme surveys. When using the methodology and classification suggested by Dallas (2007), the SASS5 results obtained from this study (Table 14) show that the communities are in a seriously modified state for the 2009 period (Class E) and a poor state (Class D) for the 2010 period. The river reach within the KNP lies within one ecoregion, and therefore the data are depicted on one graph (Figure 27). The highest SASS5 score was 142 for Site 1 during the LF2010 survey period and the lowest was 51 for Site 5 during the LF2009 survey. The highest ASPT was 6.34 at Site 3 for the 2010 survey period, the lowest being 4.25 for Site 5 for the 2009 survey period. The Letaba Comparative Site had low ASPT scores of 4.55 for the 2009 survey period and 5 for the 2010 survey period. It must be mentioned that although the Letaba River was a comparative site, the habitat availability of the SASS5 biotopes was very low, and as such it was expected that low scores would be obtained. All biotopes were present, but in low concentrations.

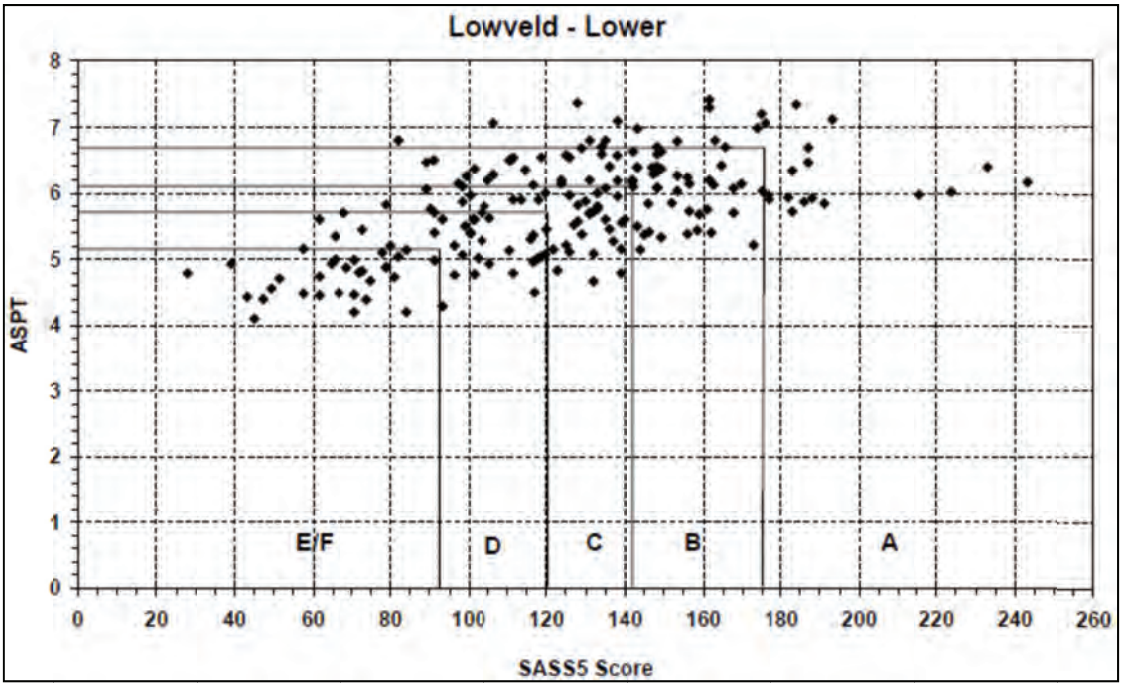

Figure 27. Biological bands for the Lowveld Lower Zone, calculated using percentiles from historical data (Dallas, 2007).

Table 14. SASS5 scores and ASPTs and consequent ECs for all sites on the Olifants River for both 2009 and 2010 low flow sampling surveys.

	SASS5 score	ASPT	EC
1OLI09	99	5.82	D
2OLI09	94	5.22	D
3OLI09	88	5.18	E/F
4OLI09	85	5.31	E/F
5OLI09	51	4.25	E/F
1LET09	50	4.55	E/F
1OLI10	142	5.91	C/B
2OLI10	126	5.73	C
3OLI10	121	6.34	C/D
4OLI10	119	5.17	C/D
5OLI10	108	5.4	D
1LET10	115	5	D

It is interesting to note that the highest or lowest SASS5 score does not always correlate with the highest and lowest ASPT. ASPT is based on sensitivities and this is why ASPT is a more appropriate measure of macroinvertebrate community strength. In general, the scores decreased downstream, and differed from the 2009 and 2010 period which shows temporal and spatial variation (Figure 28, Figure 29).

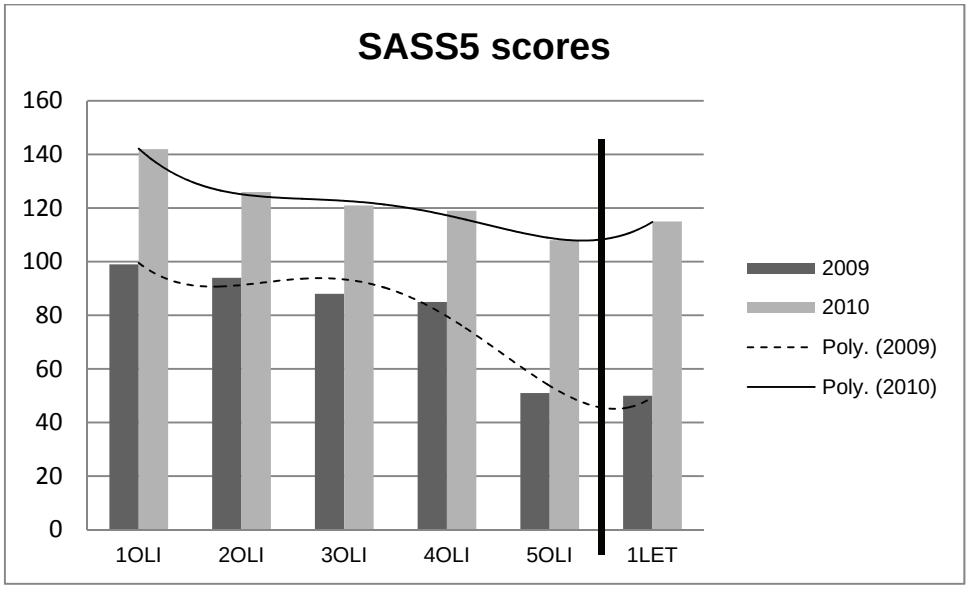

Figure 28. SASS5 scores for all sites on the Olifants River for both low flow survey periods.

A general spatial trend of decreasing SASS5 scores per site was seen moving downstream. This was evident for both the 2009 and 2010 periods. A temporal trend was also seen, as the 2009 period yielded lower scores than the 2010 period. It is interesting to note that each

site responded in a similar way for the 2009 period and the 2010 period. The trendlines in Figure 28 show an almost linear response for all sites with the increase in the scores from period to period. The ASPT scores follow the same trends (Figure 29). The only site that does not is the Letaba Comparative Site, but this is a different river, and as such would therefore not follow the same trend. What is expected is to see a decrease in the severity of these trends, as the ASPT is a more accurate way of interpreting the SASS5 results. This does occur, but the temporal and spatial trends are evident, and as such must be looked into. The trends mentioned above are important as they can be compared to the fish communities for the Olifants River.

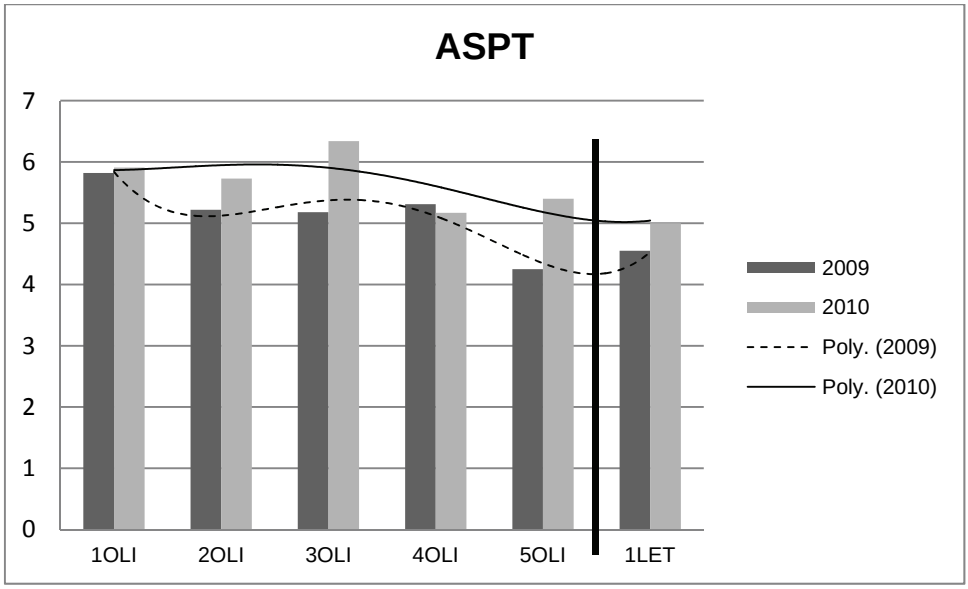

Figure 29. Average Species per Taxon (ASPT) scores for all sites on the Olifants River for both low flow survey periods.

The effects of high-flow volumes on the system during the high-flow period of 2010, can be seen with the macroinvertebrate communities. The system received a flushing of sorts, and as macroinvertebrates are short-lived and can respond quickly to favourable conditions, their numbers and species richness increased accordingly. It also shows that the river and sites in question have similar habitats and respond to similar habitat conditions. The *in situ* water quality variables also show an improvement in quality for the 2010 period, especially regarding the EC values. This would be a driver for the communities to respond in a positive way, and shows that the system has improved somewhat. What needs to be discussed is why the invertebrate scores have decreased in general since the last comprehensive RHP survey. It has been previously explained in terms of the effects of upstream abstraction and pollution on the fish communities. The same effects will be seen with the macroinvertebrate communities, but just on a different scale. Mantel et al. (2010) showed that

macroinvertebrate communities in the Western Cape and Mpumalanga were adversely affected by small dams, which diminished flow and increased the effect of adverse water quality on communities. These studies were based on the upper reaches of rivers, but a similar result would emerge for rivers in lower reaches, especially as they become larger and slower moving. The abstraction of water in the Olifants River has been well documented as previously mentioned, and if we combine the effect of water abstraction with adverse water quality, the macroinvertebrate community diversity and abundance will drop. From the water quality data explained previously and from Balance et al. (2001) we know that the Olifants River has elevated salt levels and salinisation is taking place. Lerotholi (2005) showed that an increase in salinity had an adverse effect on macroinvertebrate communities in Eastern Cape Rivers. These affects are also described by Bunn and Davies (1992) and Marshall and Bailey (2004). Water abstraction from the Olifants River means lower flow volumes, which leads to habitat loss and loss of available biotopes for macroinvertebrate communities. Lower flow volumes will also concentrate salts and increase salinisation within the Olifants River. If these impacts on the Olifants River are combined, their ultimate effects on the macroinvertebrate communities will be amplified, and this could explain why the SASS5 scores were low in 2009, and why SASS5 scores rose when river flow increased during the high-flow period of 2010 (Figure 28).

3.5 Fish Response Assemblage Index

All species sampled and expected within each habitat biotope for each site as per the FRAI (Kleynhans et al., 2007) are listed in Table 15 and Table 16. There are a large number of species absent, and some species sampled are in low abundance. It can be seen that the LF2009 period of sampling yielded fewer fish species and lower abundances than the LF2010 period. As mentioned above, some habitats were not sampled fully, especially SD, and this could account for some of the absent species. All the habitats that were available were sampled, so the absent species can either be attributed to adverse conditions within the river due to anthropogenic stressors, or a sampling error. This can be further explained by comparing the *Barbus* spp. sampled for both periods. For the LF2009 period, only *Barbus trimaculatus* and *B. viviparus* were sampled. For the LF2010 period, *B. eutaenia, B. paludinosus, B. trimaculatus, B. unitaeniatus* and *B. viviparus* were sampled. According to FRAI, these species have a high preference for SS and SD habitat. SS was sampled wherever it was available, and SD whenever it was safe. This means that these species should be present, as they were present in SS habitats during the 2010 period. These species were therefore included in the FRAI assessment, as they should be present in habitats sampled. The Anguillidae, namely *Anguilla mossambicus* and *A. marmorata* were

not included in the FRAI assessment as they are notoriously very difficult to sample with the techniques used in this project, and have a very high preference for SD habitats.

The data obtained (Table 17) showed that more species were sampled and higher abundances were recorded during LF2010. The LF2009 class was a D (53.9) and the LF2010 class was a C (67.9). As mentioned previously, higher flow and late rains occurred during the 2010 rainy season. However, during sampling in 2010, the flow was lower than during sampling done in 2009. This is slightly ambiguous, as there would be more habitats available for fish when flow volume was higher in 2009, and therefore one would have thought that there would be more fish species sampled. This was not the case, as more species and higher abundances of fish were recorded in 2010, even with lower flow volumes during sampling. What this could point to is that due to high rainfall in the high-flow periods of 2010, the system underwent a flushing of sorts (Dallas & Day, 2004) and the water quality and general habitat of the river was subsequently more suitable for fish species. This is seen in the water quality parameters mentioned in previous chapters, and shows that fish respond to particular drivers within a system. According to FRAI scores, all the metrics involved had a high weighting, except for the introduced species which was scored zero as there are none (Table 18). The physicochemical metric was weighted at 87.5%, and from the water quality parameters tested this is a driving force for lower fish diversity sampled. The velocity-depth and flow modification metrics are at 100%, cover is at 96.88% and migration is at 90.63% meaning that there are other driving forces that are affecting the system and that play a larger role. These all pertain to habitat present, and the amount of flow and cover that is available. Each fish species has a particular preference for habitat and conditions within a river, and FRAI allows for these preferences and scores accordingly. The FRAI classes obtained correspond to the RHP report by Balance et al. (2001) which showed that the section within the park was in a fair class regarding fish assemblages. With a FRAI Class D for LF2009 and Class C for October 2010, these values do fall within a fair to unnatural state. This leads us to the question as to why in terms of fish, the communities' assemblages and fish responses to the drivers are negative, causing a subsequent decrease in the FRAI scores. This may be attributed to the pollution and water abstraction of the Olifants River system due to anthropogenic activities upstream as Venter and Deacon (1995) attributed the loss of five fish species within the Olifants River to a decrease in water quality caused by high salinities, pollution by heavy metals and high silt loads, which is a direct result of the increase in upstream industrial, domestic and agricultural pollution. The RHP (Balance et al., 2001) states that the majority of the upper reaches and tributaries of the Olifants River are extensively mined, and are classified as being in a poor to unacceptable state for water quality, fish and macroinvertebrates. This is attributed to mining effluent, agricultural pollution and domestic waste that enters the system. Furthermore, De Villiers and Mkwelo (2009)

showed that sulphates within the Olifants River are at an elevated level, mainly due to mining effluent and consequent acid mine drainage (AMD). The adverse effects of AMD on biological communities are well known, and in the upper catchments of the Olifants River were seen to cause a decrease in water quality (Bell et al., 2003).

One of the concerns from the results is a lack of sensitive species. Species that require specific habitat types, water quality, flow regimes, etc., or are adapted to only surviving specified conditions, will be the first species to disappear when these conditions are changed. An example would be *Opsaridum peringueyi*, which historically was present, but according to recent studies (Balance et al., 2001; Rashleigh et al., 2009) and this project sampling, is now absent. This species is very intolerant to low flow/no flow, needs a deep water column for habitat, and is very intolerant to modified physicochemical attributes (Kleynhans et al., 2007). The absence of this species can be attributed to disruption in all the above which is corroborated by the FRAI metric weights which indicates the driver metrics responsible for the FRAI score. Another notable absentee is *Labeo congoro.* It is not a common species, and relies on FD and FS habitat types, and substrate for cover, but according to Kleynhans et al. (2007), it should be present in the Olifants River. These habitat types were present at most of the sites sampled, and therefore *L. congoro* should have been recorded. *Labeo rosae* should also be present in higher numbers, but again it relies on SD habitat types. The individuals sampled were collected with a cast net, but they should have been sampled in higher numbers with the sampling techniques used. The *Barbus* spp. mentioned earlier and some of the species omissions could be related to the sampling regime, but more than likely their absence is due to their response to the drivers of the Olifants River itself. This would include physicochemical alterations, flow-regime disruption and consequently habitat loss. It is interesting to note that most *Barbus* spp. expected need SD and SS habitats, but even species such as *B. unitaeniatus* were sampled during the October 2010 sampling period. This indicates that some *Barbus* spp*.* might have been present but were not sampled. However, on the whole the numbers and diversity of the *Barbus* spp. have reduced, as they should have been present in SS habitats as well as in the limited SD habitats sampled. In a study done on the Shingwedzi River in the KNP by Fouche and Vlok (2010), a similar trend was found. Some fish species were absent due to sampling errors mentioned above, but other species that should have been present in particular habitats were absent. The authors attributed this to a decline in water quality and habitat, which are thought to be the same problems affecting the Olifants River. Another study done on the Letaba River by Vlok and Engelbrecht (2000) also showed how species can disappear from systems due to habitat and flow disruption. *Chiloglanis engiops* and *Chiloglanis pretoriae* require FS and FD conditions, rely on substrate and are very intolerant to low-flow conditions (Kleynhans et al., 2007). *Chiloglanis pretoriae* has not been sampled

since 1999 in the Letaba River in the KNP (Vlok & Engelbrecht, 2000; Rashleigh et al., 2009) and it is thought that the populations are severely diminished, if not absent. *Chiloglanis engiops* has not been sampled within the KNP since 1978 (Pienaar, 1978), and this species may be lost from the Letaba River altogether (Vlok & Engelbrecht, 2000). Vlok and Engelbrecht (2000) attribute the demise of these fish populations and the loss of species to flow modifications and habitat loss, specifically caused by the drop in flow caused by upstream abstraction. In the future the Olifants River may face a similar problem to the Letaba River in terms of species loss if it continues to be heavily utilized.

On the whole, the results of this study show some temporal and spatial variation in terms of the fish community structure. Temporally, the number of species and species abundance sampled in LF2009 was lower than the number of species and abundance sampled in LF2010. The total number of species and total abundances for both sampling periods were lower than expected. This is a cause for concern, as the community assemblages are low, and the fact that a number of key species were not sampled and not present emphasizes the importance of management of the river system upstream. What can also be concluded is that the Letaba River plays an important role as a refuge area for fish species after a period of high flow within the Olifants River. This is seen by the increase in the number of species and abundance of species sampled in 2010 after a higher than normal high-flow period within the Olifants River. This emphasizes the potential problem of water abstraction from the Olifants and Letaba Rivers. The lack of flow will diminish the capacity of the Letaba River to be a refuge area, and further abstraction of the Olifants River will compound the effects of pollutants as they will be more concentrated.

Table 15. Fish species expected in the various biotopes with actual fish sampled at each site in the Olifants River for the 2009 low flow survey.

Expected species	Site 1				Site 2				Site 3				Site 4				Site 5				Letaba			
	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD
Amphilius uranoscopus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus afrohamiltoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus annectens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus eutaenia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus lineomaculatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus mattozi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus paludinosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus radiatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus toppini	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus trimaculatus	-	-	7	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Barbus unitaeniatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus viviparus	8	-	20	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-
Brycinus imberi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis engiops	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	13	-	5	-	23	-	-	-	-	-	-	-	7	-	3	-	1	-	-	-	-	-	-	-
Chiloglanis pretoriae	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarias gariepinus	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2	4	-	1	-	-	-	-	2	1
Glossogobius callidus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossogobius giuris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrocynus vittatus	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	120	-	-	2	3
Labeobarbus marequensis	1	15	2	-	9	-	-	1	11	-	11	22	8	8	-	-	-	-	-	-	-	-	1	-
Labeo congoro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo cylindricus	29	-	36	-	41	-	-	3	6	-	9	-	1	-	-	-	2	-	-	-	-	-	6	-
Labeo molybdinus	5	5	4	-	7	-	-	-	2	-	-	-	-	-	-	-	10	3	5	-	-	-	2	2
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marcusenius macrolepidotis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesobola brevinialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Opsaridium peringueyi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oreochromis mossambicus	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	3	-	-	-	-	-	-	5	9
Pseudocrenilabus philander	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Petrocephalus wesselsi	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Synodontis zambezensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Schilbe intermedius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Tilapia rendalli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Tilapia sparmanii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No. of species	**9**				**7**				**6**				**7**				**6**				**8**			
Total:	**57**	**20**	**71**	**0**	**83**	**0**	**0**	**7**	**20**	**0**	**20**	**25**	**16**	**8**	**8**	**7**	**13**	**5**	**5**	**120**	**0**	**0**	**24**	**17**

Table 16. Fish species expected in the various biotopes with actual fish sampled at each site in the Olifants River for the 2010 low flow survey.

Expected species	Site 1				Site 2				Site 3				Site 4				Site 5				Letaba			
	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD
Amphilius uranoscopus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus afrohamiltoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus annectens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus eutaenia	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Barbus lineomaculatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus mattozi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus paludinosus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	31	-
Barbus radiatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus toppini	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus trimaculatus	-	-	-	-	-	-	-	2	1	-	-	-	5	-	10	1	-	-	-	-	-	-	4	-
Barbus unitaeniatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Barbus viviparus	-	-	-	-	-	-	-	4	2	-	-	-	7	-	-	-	-	-	-	-	-	-	15	-
Brycinus imberi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Chiloglanis engiops	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	3	9	-	-	-	-	-	-	7	-	-	-	-	-	-	-	6	1	-	-	-	-	-	-
Chiloglanis pretoriae	1	1	-	-	4	5	-	-	6	-	-	-	-	-	-	-	6	3	-	-	-	-	-	-
Clarias gariepinus	-	-	-	-	-	-	-	1	1	-	-	-	4	-	4	1	-	-	-	-	-	-	1	3
Glossogobius callidus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Glossogobius giuris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	2	-
Hydrocynus vittatus	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	5	-	80	-	-	2	12
Labeobarbus marequensis	5	3	2	3	5	2	-	16	14	6	2	-	5	-	19	2	-	1	-	-	-	-	39	4
Labeo congoro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo cylindricus	1	2	1	-	6	10	-	2	11	3	-	-	-	-	-	-	1	8	-	-	-	-	4	-
Labeo molybdinus	14	7	-	-	6	5	-	4	9	2	2	-	1	-	3	-	33	12	-	-	-	-	7	-
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	18
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marcusenius macrolepidotis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesobola brevinialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Opsaridium peringueyi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oreochromis mossambicus	-	-	3	1	-	-	-	4	4	-	1	-	9	-	5	5	-	2	-	-	-	-	11	7
Pseudocrenilabus philander	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	5	-
Petrocephalus wesselsi	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Synodontis zambezensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Schilbe intermedius	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-
Tilapia rendalli	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	11	3
Tilapia sparmanii	-	-	2	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-	1	7
No. of species	**8**				**10**				**9**				**14**				**10**				**16**			
Total:	**24**	**22**	**8**	**5**	**21**	**22**	**0**	**44**	**55**	**11**	**5**	**0**	**38**	**0**	**47**	**18**	**50**	**34**	**0**	**80**	**0**	**0**	**134**	**57**

Table 17. The Olifants Lowland River FRAI scores obtained over two low-flow sampling periods.

	Automated FRAI	Automated EC	Adjusted FRAI	Adjusted EC
Olifants River 2009	52.2	D	53.9	D
Olifants River 2010	66.1	C	67.9	C

Table 18. Metric groups and weights according to the FRAI scores obtained.

Metric group	Weight (%)
Velocity -depth	100
Cover	96.88
Flow modification	100
Physicochemical	87.5
Migration	90.63
Impact of introduced	0

3.6 Flow requirements for fishes

Habitat modelling

Outcomes of the habitat modelling exercise includes the spatial extent of the 191 habitat units used for this study is graphically presented in Figure 30. The extent of velocity-depth class is presented in Figure 31, surface flow types, habitat unit velocities and substrate types of each habitat unit is graphically presented in Figure 32, Figure 33 and Figure 34 respectively. The three dimensional model of the study area is presented in Figure 35.

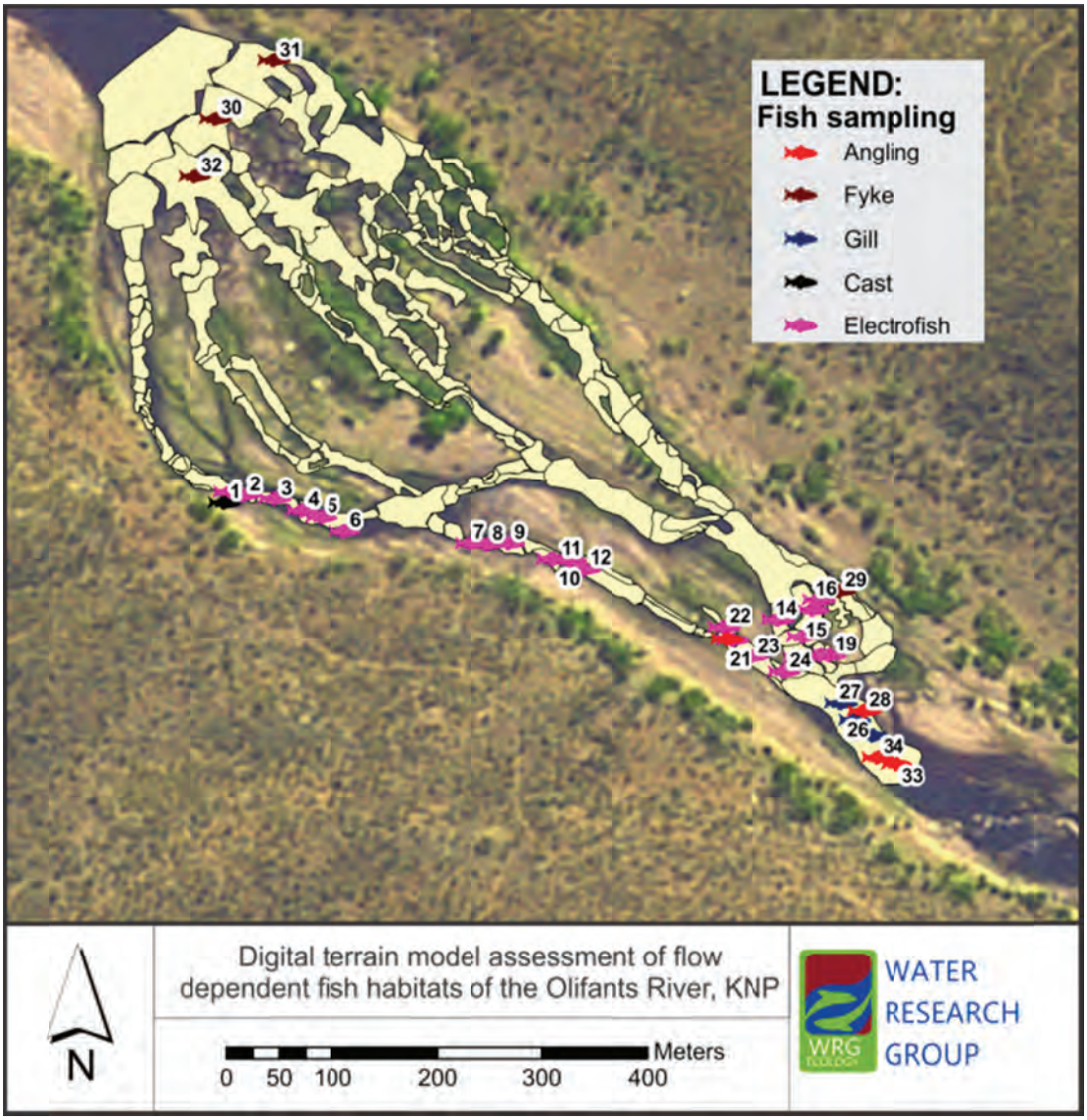

Figure 30: Satellite photograph of the reach of the Olifants River considered in this assessment with the 191 habitat units for the digital terrain model and the 36 fish sampling efforts included.

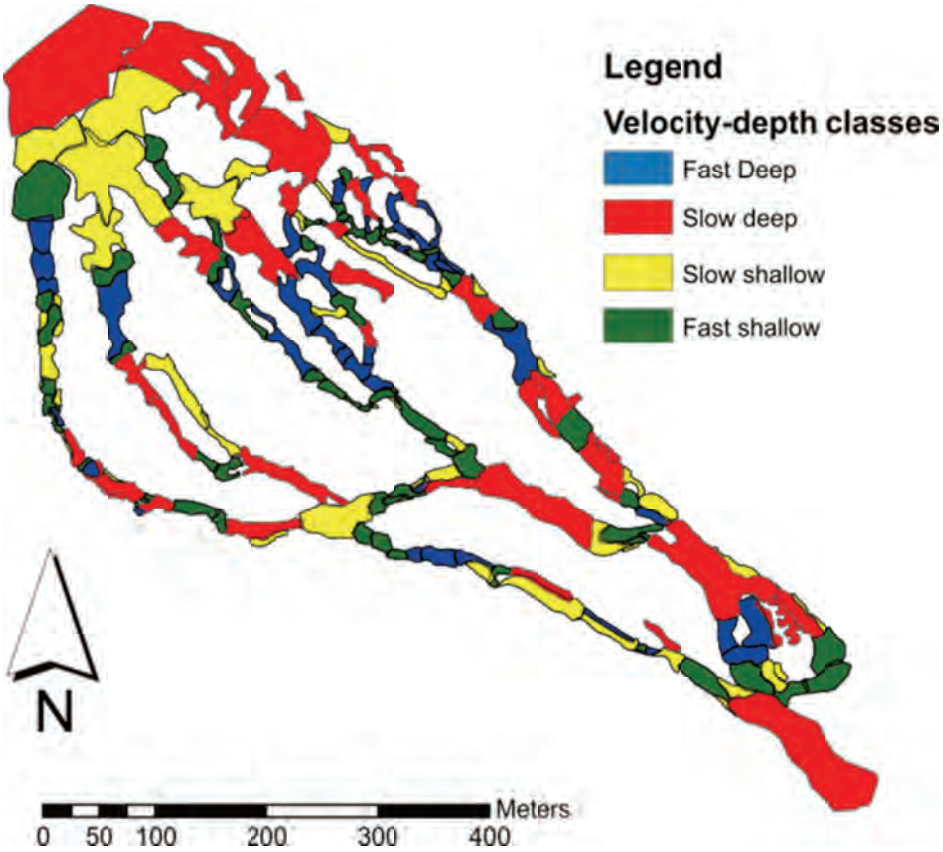

Figure 31: Spatial distribution of the various velocity depth classes observed during the survey.

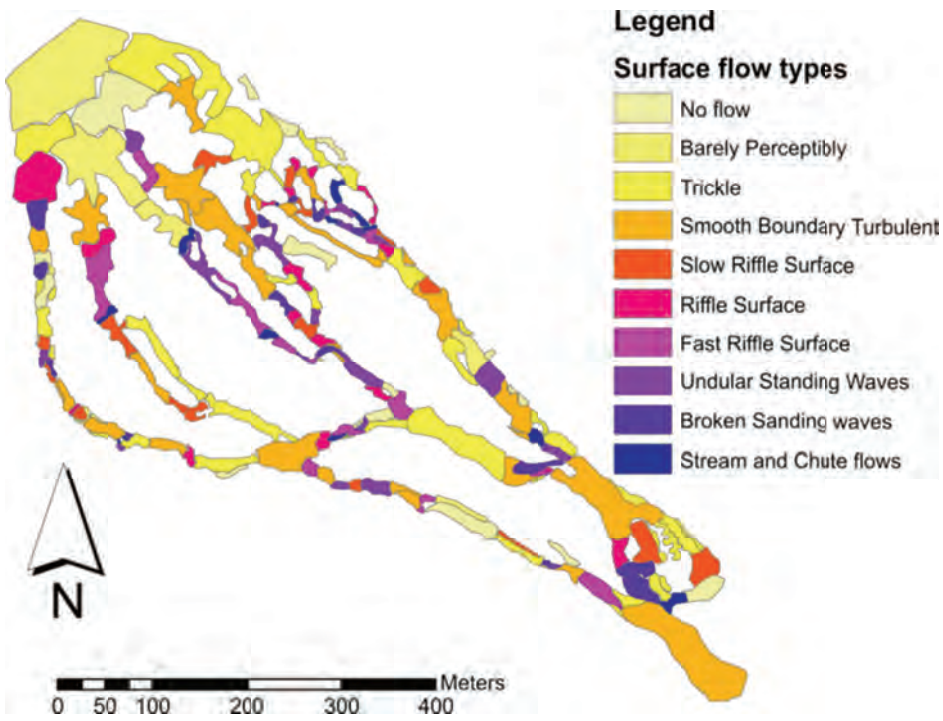

Figure 32: Spatial distribution of the various surface flow types observed during the survey.

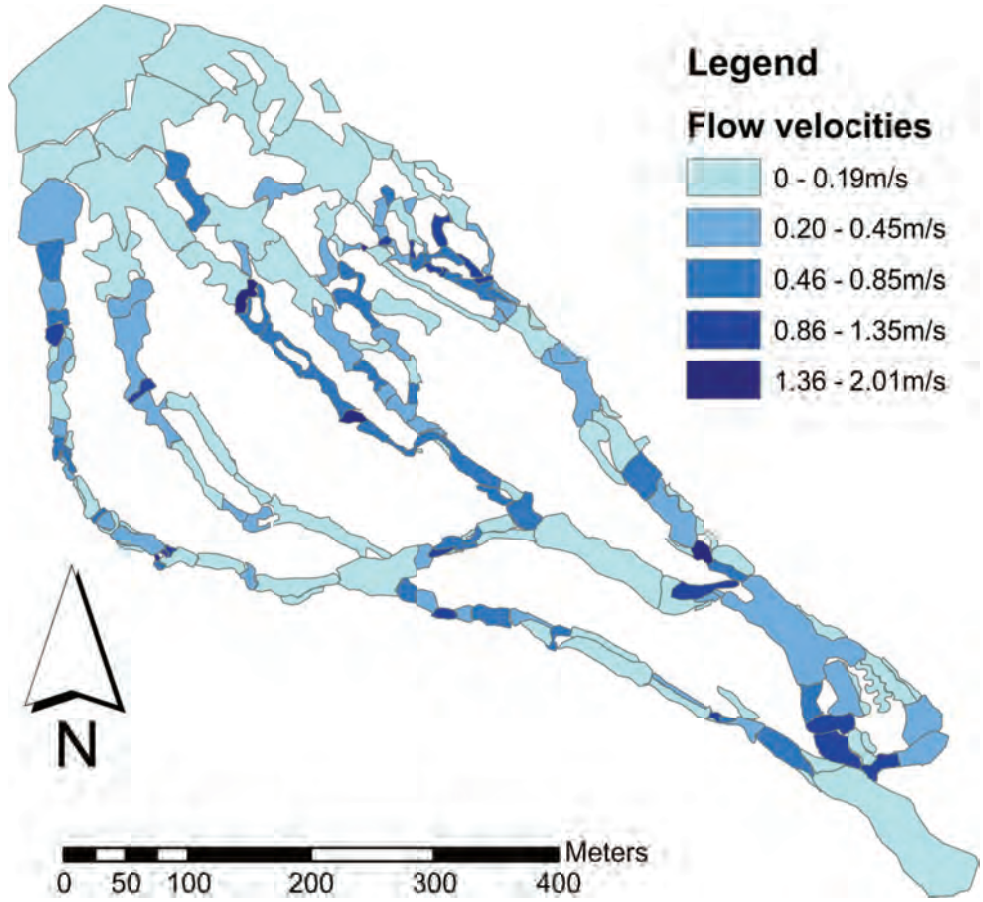

Figure 33: Spatial distribution of the velocities (m/s) of habitats observed during the survey.

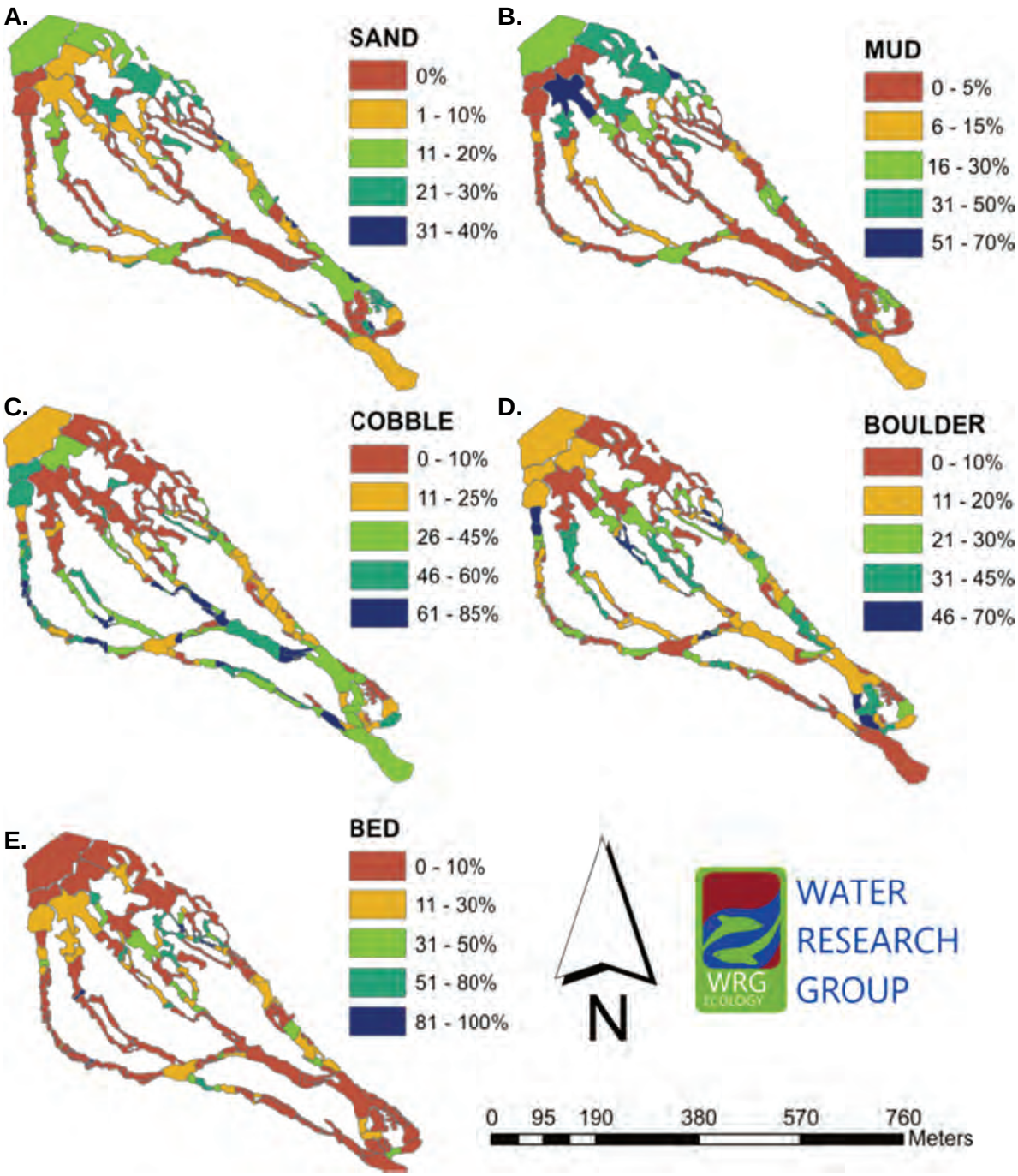

Figure 34: Spatial distribution of the substrate types presented as a percentage of sand (A), mud (B), cobble (C), boulder (D) and bedrock (E) during the survey.

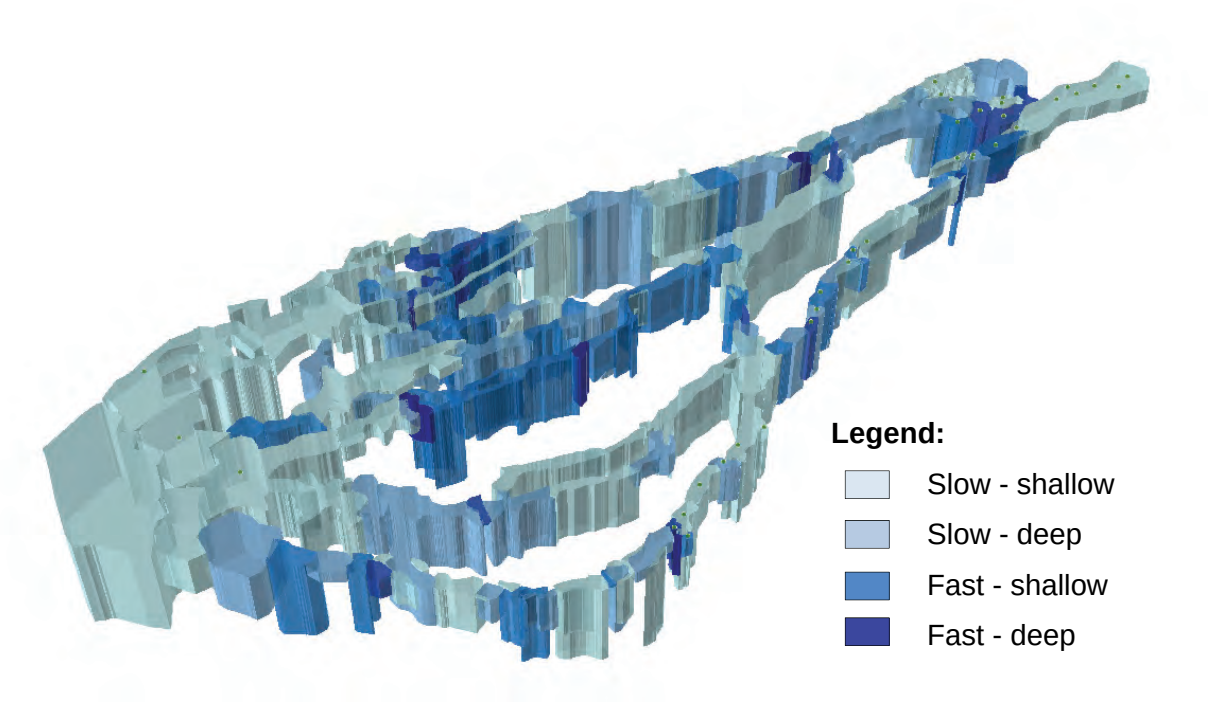

Figure 35: Three dimensional representation of the habitat types [with velocities-depths superimposed (Figure 33)] that were observed during the survey.

Fish community structure

In the study 687 individual fish were collected representing 17 species (Table 19). The most common species obtained included *Chiloglanis paratus* (n = 188) *Labeobarbus marequensis* (n = 110) *Labeo cylindricus* (n = 80) *Labeo molybdinus* (n = 61) and *Synodontis zambezensis* (n = 56). Thereafter moderate abundances (n = 15-32) of *Barbus viviparous*, *Barbus trimaculatus*, *Clarias gariepinus*, *Oreochromis mossambicus* and *Schilbe intermedius* were collected and few *Hydrocynus vittatus* (n = 3), *Labeo congoro* (n = 7), *Marcusenius pongolensis* (n = 2), *Mesobola brevianalis* (n = 3) and *Micralestes acutidens* (n = 1). Fish were collected in all efforts predominantly by electrofishing sampling methods which were suited for sampling most of the habitat types obtained in the study. Other methods were effectively used to sample habitat types that could not be effectively sampled with the electrofisher including the use of gill nets, fyke nets and angling techniques predominantly. The tigerfish *Hydrocynus vittatus* were only collected using angling techniques. Only three individuals were obtained during this assessment.

Table 19. Summary of the diversity and abundance of fishes collected in the study.

	Method						Species															
Effort	ELECTRO	CASTNET	GILL45	GILL90	FYKE	ANGLING	BVIV	BTRI	CPAR	CPRE	CGAR	HVIT	LCON	LCYL	LMOL	LMAR	MPON	MBRE	MACU	OMOS	PWES	SINT	SZAM
#1	1	-	-	-	-	-	-	-	4	3	1	-	-	9	7	15	-	-	-	-	-	-	-
#2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-
#3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
#4	1	-	-	-	-	-	2	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
#5	1	-	-	-	-	-	-	-	6	-	-	-	-	2	2	1	-	-	-	-	-	-	-
#6	1	-	-	-	-	-	1	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-
#7	1	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-
#8	1	-	-	-	-	-	1	-	14	15	-	-	-	12	14	24	-	-	-	-	-	-	-
#9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
#10	1	-	-	-	-	-	1	-	2	1	-	-	-	11	3	6	-	-	-	-	-	-	-
#11	1	-	-	-	-	-	1	-	2	-	-	-	-	2	1	7	-	3	-	-	-	-	-
#12	1	-	-	-	-	-	-	-	4	4	-	-	-	2	3	3	-	-	-	-	-	-	-
#13	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
#14	1	-	-	-	-	-	-	-	34	14	-	-	1	10	16	2	-	-	-	-	1	-	-
#15	1	-	-	-	-	-	-	-	58	17	-	-	-	9	4	-	-	-	-	-	-	-	-
#16	1	-	-	-	-	-	17	13	-	-	1	-	-	9	2	16	-	-	-	1	5	-	1
#17	1	-	-	-	-	-	7	1	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-
#18	1	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-
#19	1	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-	-
#20	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-
#21	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
#22	1	-	-	-	-	-	-	-	1	1	2	-	-	1	-	-	-	-	-	-	-	-	-
#23	1	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-
#24	1	-	-	-	-	-	-	-	27	-	-	-	-	4	-	5	-	-	-	-	-	-	-
#25	1	-	-	-	-	-	-	-	30	9	-	-	-	6	2	16	-	-	1	-	-	-	-
#26	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
#27	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
#28	-	-	-	1	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
#29	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	5
#30	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
#31	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21
#32	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	14	15
#33	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	1	-	-	1	1	3	3
#34	-	-	-	-	-	1	-	-	-	-	11	1	-	-	-	-	-	-	-	-	-	-	-
#35	-	-	-	-	-	1	-	-	-	-	3	-	-	-	-	2	-	-	-	-	-	-	8
TOTALS	23	1	1	2	4	4	32	15	188	65	23	3	7	80	61	110	2	3	1	16	8	17	56

Redundancy Analysis combined with Monte Carlo permutation tests (using the forward selection protocol – CANOCO) were carried out to test the overall natural influences of substrate types, velocity depth types and fish cover features (Figure 36A &Figure 37). Results indicate that all three explanatory variables including substrate types ($p = 0.02$), velocity depth types ($p = 0.02$) and fish cover features ($p = 0.04$) were responsible for significant changes in fish communities. The RDA plot in Figure 36 presents the relationship between the fish communities and substrate variables modelled in the study. Findings show that five groups of fish species were obtained. Four of the groups were closely associated with bedrock and boulder substrate dominated habitats types (Group IV), uncommon mud with bedrock habitat

types (Group I), bedrock dominated habitat types with some mud (Group II) and cobble habitat types (Group V). The remaining group (Group III) was shown to consist of substrate generalist species that was not strongly associated with any substrate type. The RDA plot in Figure 36B presents the relationship between the fish communities and velocity depth classes with measured depth and velocity variables modelled in the study. Four groups of species were closely associated with velocity depth classes including a combination of slow and fast deep habitat types (Group I), a group closely associated with fast deep habitat types (Group II), a group closely associated with fast shallow and deep habitat types (Group III) and a group associated with slow shallow habitat types (Group IV).

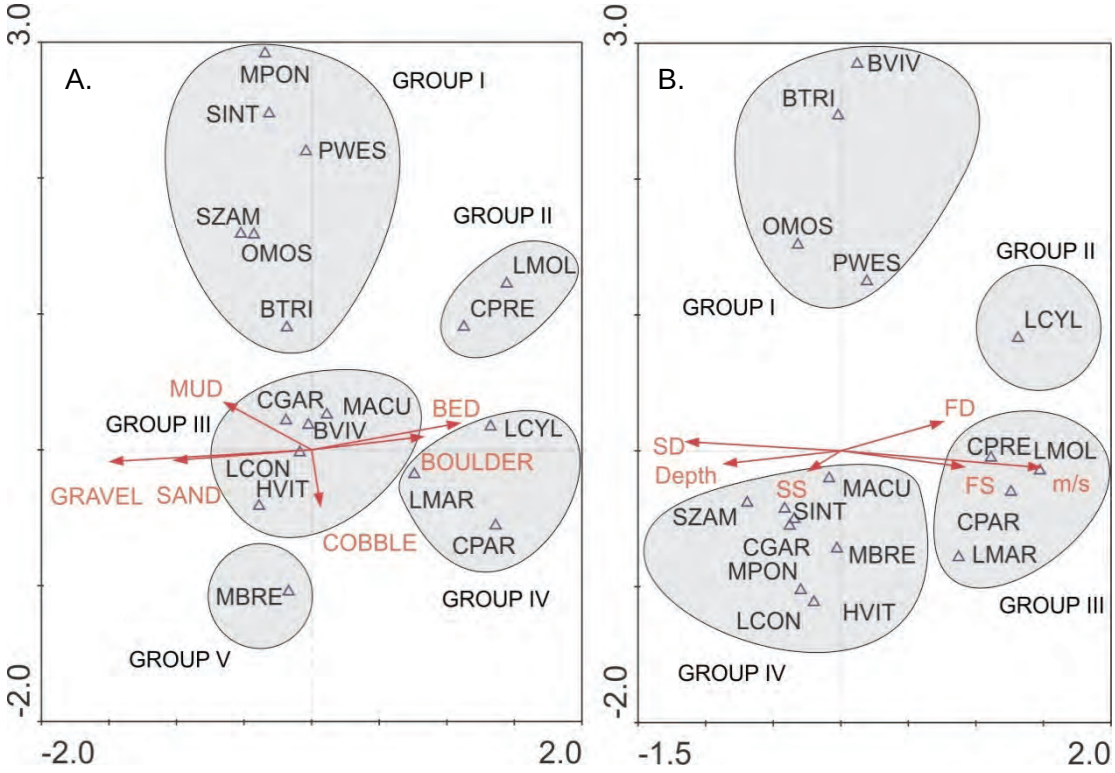

Figure 36. Redundancy analyses plots showing dissimilarity based on the fish communities among efforts included in the study. Graph A presents relationship between fish communities and substrate types where the plot describes 62% of the variation in the data where 72.4% is displayed on the first axis and an additional 21.9% on the second. Graph B presents relationship between fish communities and velocity depth classes with measured velocities and depths where the plot describes 65% of the variation in the data where 83.0% is displayed on the first axis and an additional 10.9% on the second.

The RDA plot in Figure 37 presents the relationship between the fish communities and fish cover features modelled in the study. Results show that four groups were

closely associated with cover features while one group was shown to be cosmopolitan (Group III). Group I and V were shown to be related to substrate types, Group II was determined to be closely associated with undercut banks and root wads as well as overhanging vegetation. Group IV was shown to contain species that are closely associated with water column.

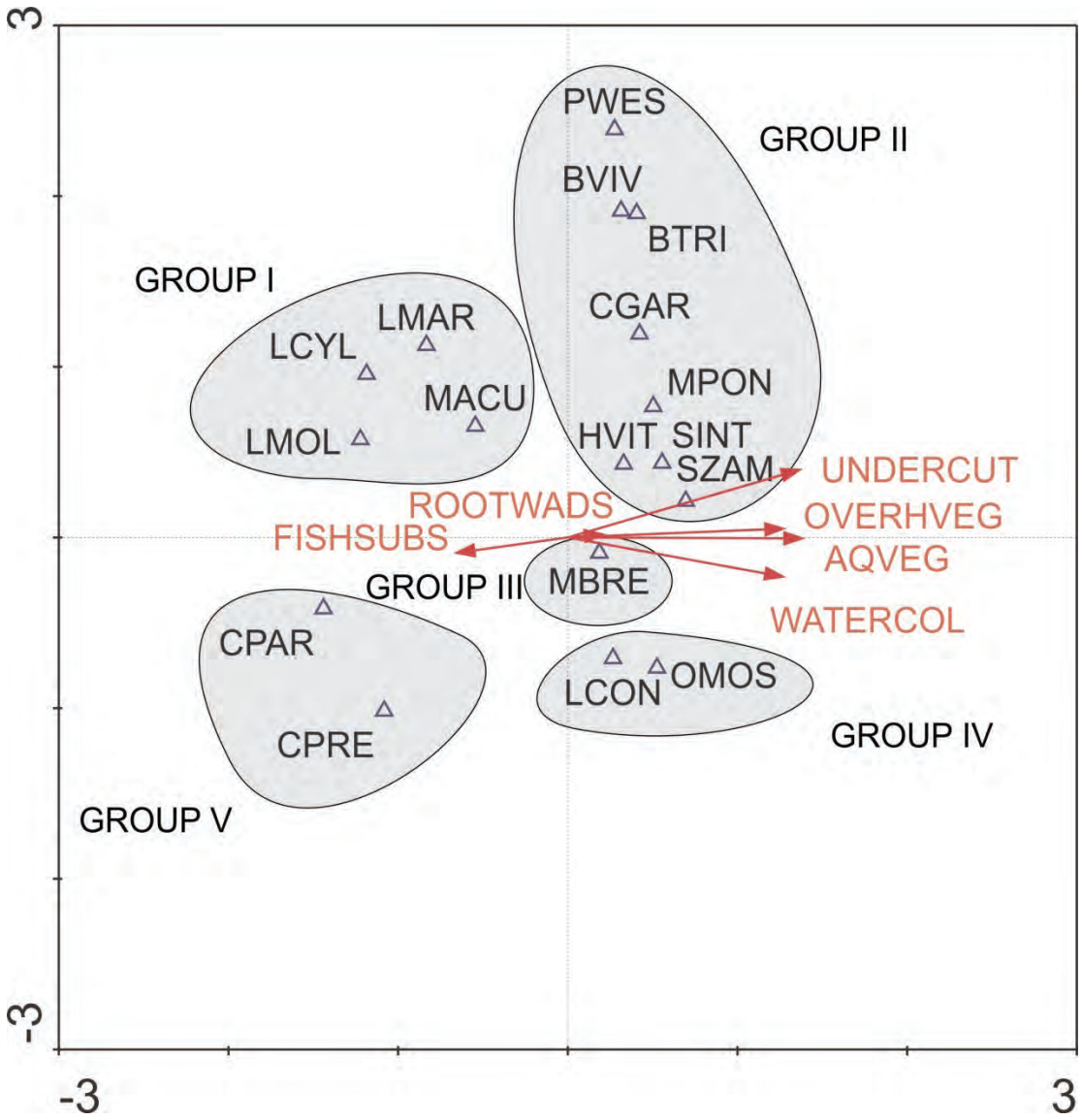

Figure 37. Redundancy analyses plots showing dissimilarity based on the fish communities among efforts included in the study. Graph presents relationship between fish communities and fish cover features where the plot describes 62.4% of the variation in the data where 76.7% is displayed on the first axis and an additional 9.8% on the second.

Fish habitat preference

Only the tigerfish (*Hydrocynus vittatus*) and species that the multivariate statistical assessment could show would be confidently be associated with selected habitat types were included in this assessment (additional data from other expected species are provided in Appendix 2). Combinations of preferred habitat types, velocity depth classes and fish cover features were analysed for species with large abundances which could be considered relatively confidently. These included the *Chiloglanis spp.*, *Labeo cylindricus*, *Labeo molybdinus*, *Labeobarbus marequensis* and *Synodontis zambezensis*. Figure 38 presents the findings of the modelled spatial distribution of preferred habitat units for *C. paratus*, *C. pretoria* and *L. cylindricus* using multivariate statistical assessment (Figure 38A) and using available preferred habitats (Figure 38B) obtained from Kleynhans et al. (2005). Figure 39 presents the findings of the modelled spatial distribution of preferred habitat units for *L. molybdinus*, *L. marequensis* and *S. zambezensis* using multivariate statistical assessment (Figure 39A) and using available preferred habitats (Figure 39B) obtained from Kleynhans et al. (2005). Figure 40 presents the findings of the modelled spatial distribution of preferred habitat units for *H. vittatus* using available preferred habitats obtained from Kleynhans et al. (2005). Findings initially indicate that all species have unique habitat preferences which comprise of unique velocity (m/s), depth, substrate and fish cover features. Although similar trends for preferred habitats by species were obtained using the multivariate statistical assessment and available preferred habitats, the multivariate statistical assessment approach consistently provided more habitat units. Results confirmed that the Chiloglanids; *C. paratus* and *C. pretoria* have high preferences for fast flowing (>0.2 m/s) habitats that are dominated by boulders and bedrock and to a lesser extent cobbles. *Chiloglanis paratus* appears to have a wider habitat preference when compared to *C. pretoria*. The multivariate statistical assessment approach revealed that the Chiloglanids prefer fast deep as well as fast shallow habitat types which are not clearly exhibited when using available preferred habitats method alone. *Labeo cylindricus* and *L. molybdinus* results show that these labeos prefer fast deep habitats (predominantly *L. molybdinus*) but will make use of slower habitat types as long as good substrate types (boulders and bedrock) are available. The *Labeobarbus marequensis* preferred habitat types include fast shallow predominantly by juveniles and deep habitat types predominantly by adults. Substrate types for the yellowfish and *L. cylindricus* appear to be more important than *L. molybdinus* and include boulders and bedrock associated with sufficient water column. The preferred habitat type for *S. zambezensis* includes slower flowing deep habitat types.

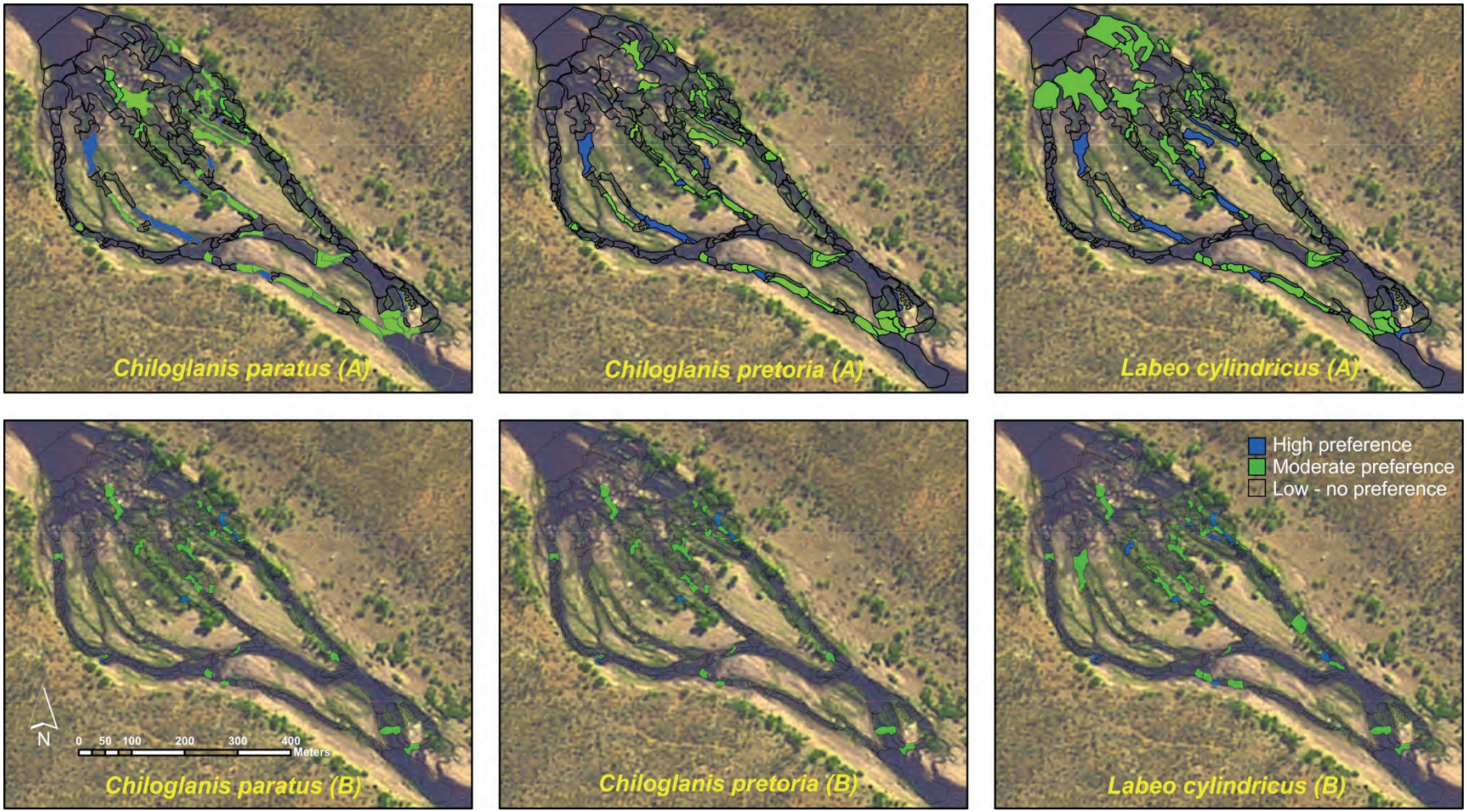

Figure 38. Graphical representation of the modelled spatial distribution of preferred habitat units for selected species sampled in the study area. Graphs of preferred habitats analysed by multivariate statistical assessment (A) and using available preferred habitats (B) (Kleynhans et al., 2005) included.

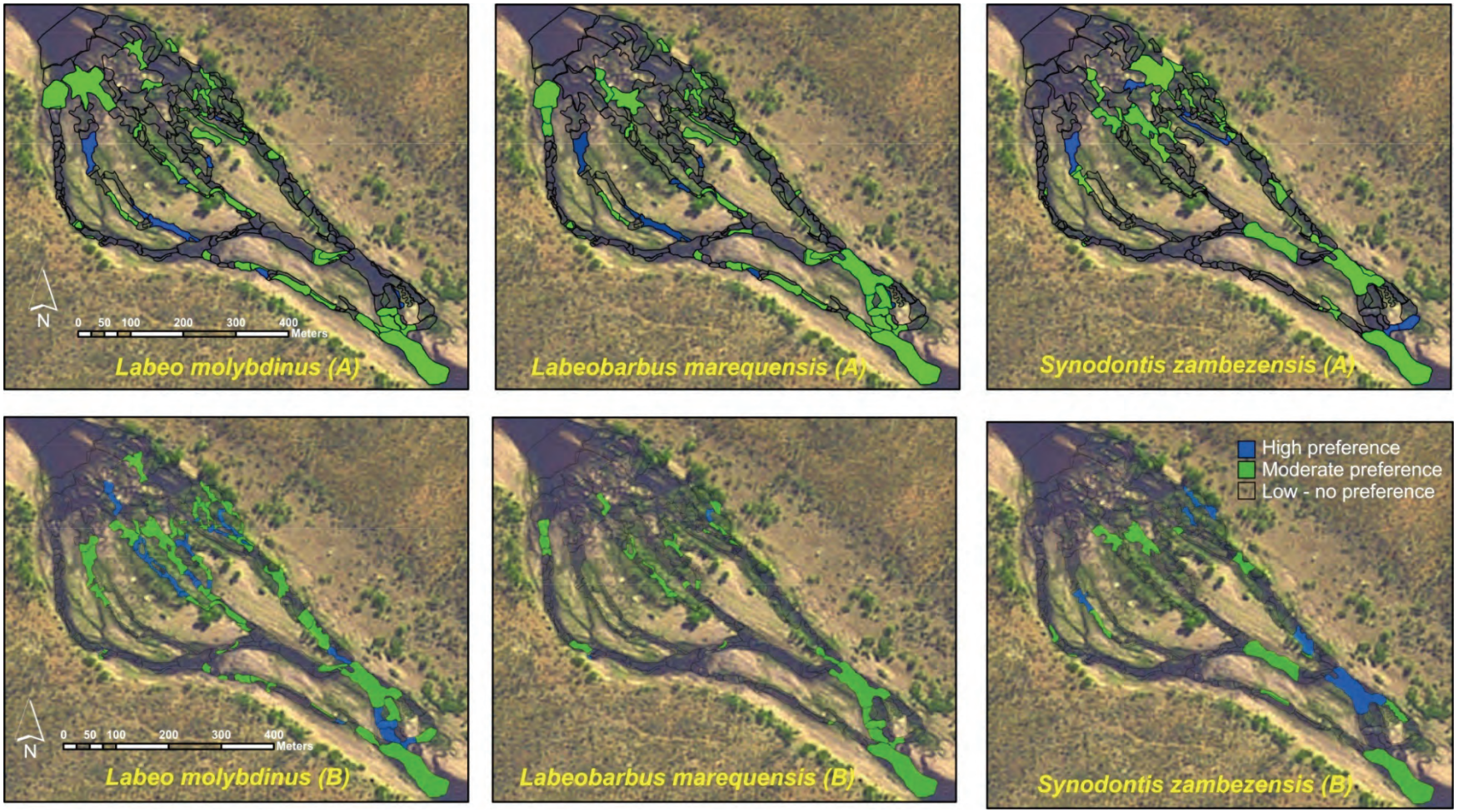

Figure 39. Graphical representation of the modelled spatial distribution of preferred habitat units for selected species sampled in the study area. Graphs of preferred habitats analysed by multivariate statistical assessment (A) and using available preferred habitats (B) (Kleynhans et al., 2005) included

Only available preferred habitat types for the tigerfish *Hydrocynis vittatus* were available for this assessment as only three individuals were collected during the flow dependent habitat type assessment in the Olifants River. Results indicate that the tigerfish has a very high preference for only two habitat types that consist of deep (>1200 mm) fast flowing (>0.8 m/s). There after the species prefers a wider range of relatively deep (>700 mm) no flow to fast flowing (0-1.35 m/s) habitat types. Cover features of importance for the species includes water column and possibly over hanging vegetation.

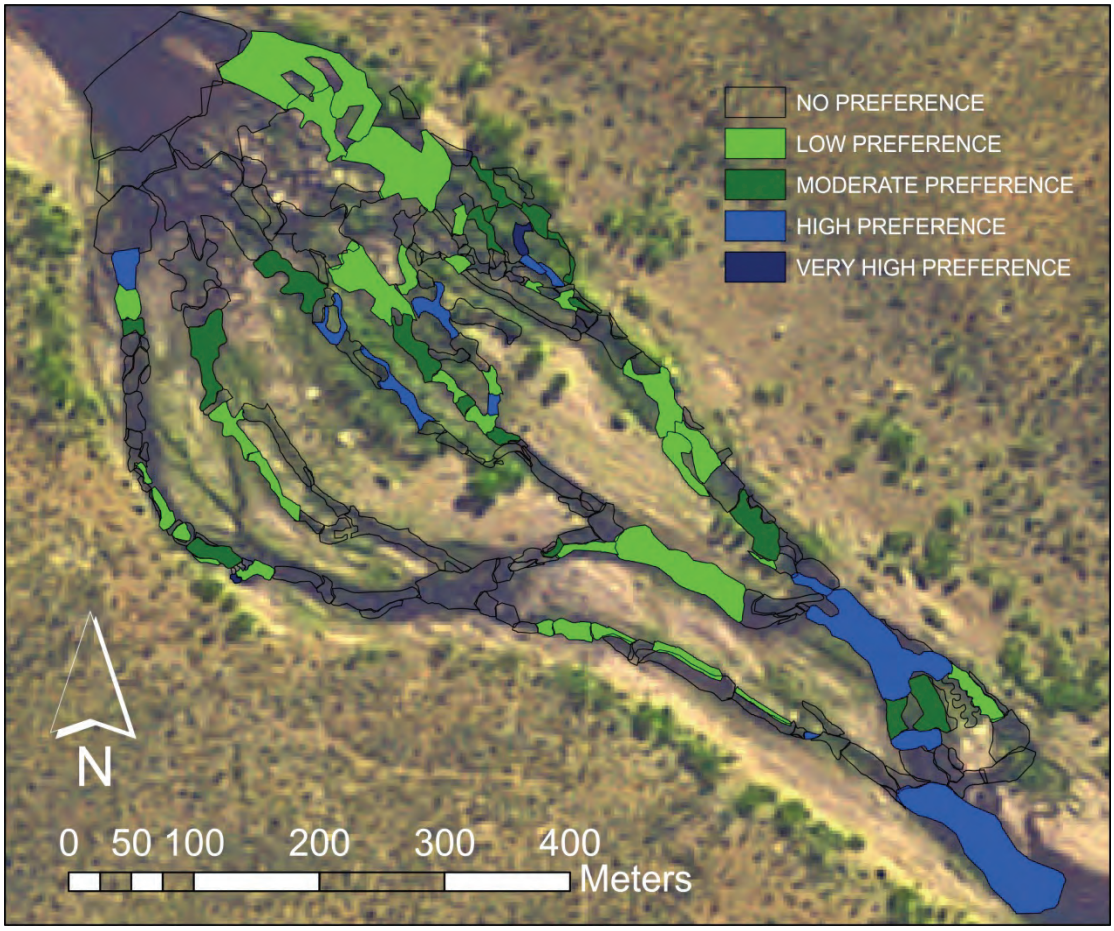

Figure 40. Graphical representation of the modelled spatial distribution of preferred habitat units for *Hydrocynus vittatus* sampled in the study area, using available preferred habitats (B) (Kleynhans et al., 2005).

Flow-stress assessment

Initial findings of the flow stress assessment indicate that the maximum low flow discharge during the wet and dry seasons are 72.716 m^3/s and 17.651 m^3/s respectively. The ecological sub-model uses the fish Flow Classes (FCs) (Figure 41) for the wet and dry months for all possible flow depths lower than the selected maximum low flow discharge. The use of these FCs is largely associated with the requirement for both large and small

rheophillic fish guilds, which are flow sensitive and generally have the highest flow requirements. The FCs are determined using the output from the hydraulic sub-model, to estimate the stress-flow relationships for both seasons. The basis for estimating stress is the reduction in the frequencies of the Fast Shallow (FS), Fast Intermediate (FI) and Fast Deep (FD) FCs coupled with the assumption that a stress of zero is associated with the maximum low flow discharge while zero flow represents a stress of 10. The natural and present baseflow time series are then processed through the stress-flow relationship to generate the natural, present day and several EWR category stress duration curves for the two seasons. Thereafter, FDCs are generated by processing the flow data through a combination of the stress duration curves and the stress-flow relationship. In the ecological sub-model, users are able to specify, for each season, the seasonality of the river system (i.e. perennial or non-perennial), aligning the maximum stress of one EWR category to the present day situation and changing the low stress ends of the frequency curves through the editing 'shift' factors. The defaults options included all EWR categories and all seasons perennial with no alignment. No changes to the ecological sub-model were done. The seven fish flow classes (Figure 41) evaluated for the study area is illustrated as area curves in Figure 42 and Figure 43.

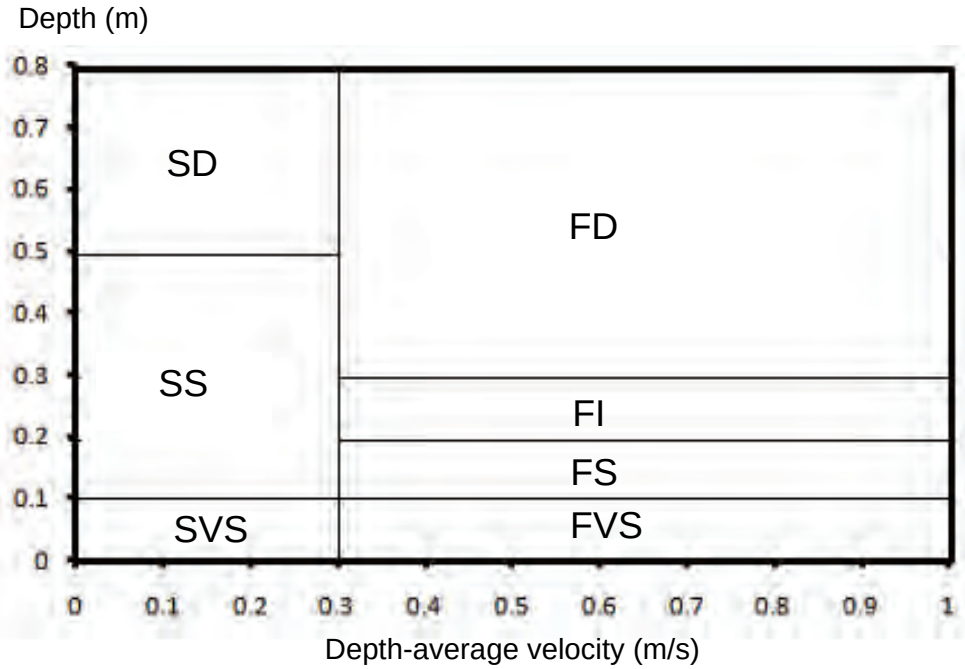

Figure 41. (TOP) Flow classes for fish (or velocity-depth classes), modified from Jordanova et al. (2004). (BOTTOM) (The velocity and depth axes are truncated for plotting purposes). SVS=slow/very shallow; SS=slow/shallow; SD=slow/deep; FVS= fast/very shallow; FS=fast/shallow; FI= fast/intermediate; FD=fast/deep

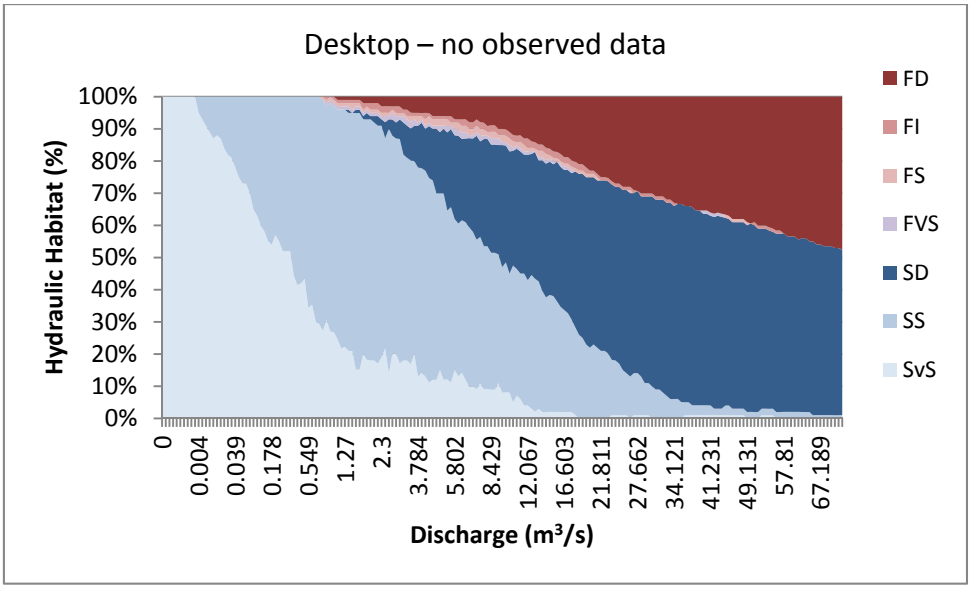

Figure 42. Area curves of availability of fish flow classes for the Olifants River using modelled data.

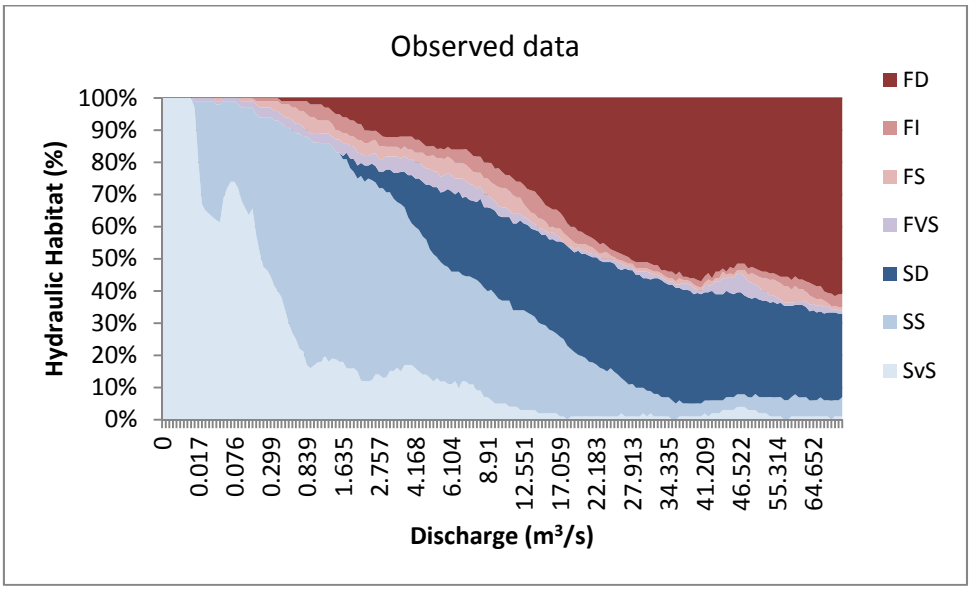

Figure 43. Area curves of availability of fish flow classes for the Olifants River using observed data.

The most important hydraulic habitat characteristics are the frequencies of the fast-deep (FD), fast-intermediate (FI) and fast-shallow (FS) habitats, as these three FCs are used to determine the stress-flow relationships in the ecological sub-model. Given the approach used in the RDRM (and EWR workshops) to estimating the stress-flow relationships, it is therefore the rate at which these FCs decline with discharge, as well as the discharge that they disappear that is important. In this study the habitat preferences that specifically pertain to *Chiloglanis spp.*, *L. cylindricus*, *L. molybdinus*, *L. marequensis*, *S. zambezensis* and *H. vittatus* were related to the changes in distributions of FCs for low flow periods alone.

Available habitat types and associated distributions of preferred habitats for species were related to the velocity depth, depth and velocity maps of the study area (Figure 31 &Figure 33). This was then compared to the FC class distributions and associated threshold categories for the data (Table 20, Appendix 3 and 4).

Findings of the assessment indicate that at base low flows of 17.5 m^3/s for the dry season the availability of fast flowing habitats include 45% (observed data) and 24% (modelled data) (Table 20). These base flows are considered to be suitable and provide sufficient maintenance habitats for all rheophilic species shown in this study to have a high preference for fast deep and shallow velocity depth classes with associated substrate and cover features. This volume of water however is not considered to be sufficient to provide fishes with ecological cues associated to the migration or spawning biology of the species which was not considered in this study. Below a discharge of 4.9 m^3/s the availability of fast flowing categories is considered to reduce to critical levels and for both observed and modelled FS indicating that the indicator rheophilic fishes would be forced to take up refuge in un-preferred habitat types. At <2 m^3/s the fast flowing habitat types for the indicator fishes reduces to unacceptably low availabilities and this represents the worst case scenario for species that occur in this reach of the Olifants River. Species that have been shown in this assessment to respond to reducing flows first includes; *L. cylindricus*, followed by *L. molybdinus* and *L. marequensis*, there after the *Chiloglanis spp*., including *C. pretoriae* initially will be impacted by reduced flows below 8.4 m^3/s.

Table 20. Summary of flow threshold categories obtained in the flow stress assessment. Descriptive data of river cross section and associated distribution of velocity depth classes included.

	Max. depth	Ave. depth	Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
	(m)	(m)	(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
Observed data	0.33	0.12	0.387	27.3	27.41	0.11	0.39	40%	53%	0%	3%	3%	1%	0%
	0.56	0.26	2.174	44.63	44.92	0.19	0.64	12%	63%	4%	3%	4%	5%	8%
	0.75	0.33	4.936	67.5	67.98	0.22	0.75	13%	40%	20%	5%	4%	3%	15%
	0.9	0.4	8.586	85.28	85.91	0.25	0.86	7%	34%	25%	4%	5%	5%	20%
	1.12	0.57	17.547	94.62	95.5	0.33	1.08	1%	24%	30%	0%	3%	5%	37%
Modeled data	0.37	0.12	0.368	47.37	48.58	0.06	0.22	44%	55%	0%	0%	0%	0%	0%
	0.6	0.25	2.193	86.88	89.57	0.1	0.36	17%	74%	3%	1%	1%	2%	2%
	0.77	0.33	4.918	119.74	123.63	0.13	0.44	15%	55%	19%	2%	2%	1%	6%
	0.91	0.4	8.429	143.54	148.07	0.15	0.52	9%	43%	34%	2%	2%	2%	9%
	1.13	0.59 1	17.593	152.78	157.51	0.19	0.69	2%	29%	46%	1%	1%	2%	20%

3.7 Fish health assessment

For comparison to the tigerfish a second species from a different trophic level and feeding guild was selected as part of the health assessment. In the Olifants River this species was the large scale yellowfish, *L. marequensis.*

Labeobarbus marequensis

Necropsy and Condition Indices

The specimen data for *L. marequensis* is presented in Table 21. The somatic index, Condition factor and age data for these specimens are presented in Table 22. The mean HSI values for both sample groups were between 0.5 and 1. The mean GSI values for both the female and male specimens were relatively low. This was not unexpected as the gonadal tissue of most of the sampled fish was observed to be in the immature stages of gametogenesis. The mean SSI values were similar for both sample groups and the mean CF for both groups were close to 1. The mean age of the LF2009 sample group was slightly higher compared to the HF2010 sample group.

Table 21. Specimen data for *Labeobarbus marequensis* from the Olifants River collected during low flow 2009 and high flow 2010. Mean values are presented per sample group.

Sampling period	n	Sex		Body mass	Total length
		♂	♀	g	mm
September 2009	15	9	6	237.33 ± 151.54	272.67 ± 37.22
April 2010	15	10	5	136.00 ± 42.22	247.00 ± 37.22

Table 22. Somatic index, Condition factor and age data for *Labeobarbus marequensis* from the Olifants River collected during low flow 2009 and high flow 2010. Mean values are presented per sample group.

Sampling period	n	HSI	GSI (♂)	GSI (♀)	SSI	CF	Age (Months)
September 2009	15	0.70 ± 0.17	0.62 ± 0.66	0.72 ± 0.70	0.08 ± 0.03	1.08 ± 0.41	82.00 ± 27.65
April 2010	15	0.54 ± 0.16	2.78 ± 3.31	1.53 ± 2.44	0.09 ± 0.02	0.88 ± 0.11	56.20 ± 13.20

HSI = Hepatosomatic Index; GSI = Gonadosomatic Index; SSI = Splenosomatic Index; CF = Condition factor; N/D = Not determined

The necropsy observation revealed a few abnormalities in a number of the *L. marequensis* specimens from the LF2009 survey. These included an inflamed hindgut (n = 2) swollen kidney (n = 3) liver discolouration (n = 4) pale gills (n = 4) and parasitic infections (n = 4). No

macroscopic abnormalities were observed in the 2010 sample group except for parasitic infections within the visceral cavity of 14 specimens.

Histopathological Assessment

The light microscopy analysis showed that the selected target organs of *L. marequensis* from the Olifants River have normal histological structure and seem to be in a normal functional state. Selected histological alterations were identified in liver and kidney samples. These included intracellular deposits, hepatocellular vacuolation and nuclear changes in the liver, as well as vacuolation of the tubular epithelium, hyaline droplet degeneration and eosinophilic degeneration of the tubular epithelium in a number of kidney samples. The percentage prevalence of these alterations for the various sample groups are presented in Table 23.

With regards to the liver alterations, the intracellular deposits were mostly diffuse in nature and were present in most hepatocytes. The hepatocellular vacuolation identified was in most cases characteristic of macrovesicular steatosis, however, the presence of lipid accumulation in hepatocytes was not confirmed through special stains as part of this study. The vacuolated cells were mostly diffuse in nature but a focal area of vacuolated hepatocytes was also identified in one specimen. Nuclear changes identified included mostly pleomorphic nuclei, i.e. nuclei of different sizes within the same tissue region.

Hyaline droplet degeneration and eosinophilic degeneration of the epithelial cells of the renal tubules were only identified in fish from the 2010 survey. Vacuolated tubular epithelial cells were identified in both sample groups, but were more prevalent in the LF2009 survey.

Table 23. Percentage prevalence of histological alterations identified in *Labeobarbus marequensis* from the Olifants River collected during low flow 2009 and high flow 2010.

Organ / alteration	**2009**	**2010**
	%	%
Liver		
Intracellular deposits	0	53
Hepatocellular vacuolation	47	27
Nuclear changes	73	80
Kidney		
Vacuolation of tubular epithelium	81	20
Hyaline droplet degeneration	0	20
Eosinophilic degeneration	0	20

The histological index values for *L. marequensis* from the Olifants River are presented in Table 24. The respective mean Liver and Kidney Index values showed a similar result, i.e. higher Liver Index value compared to a lower Kidney Index value for both the LF2009 and HF2010 sampling surveys. The mean Fish Index was also similar for both sampling surveys (mean index values between 8 and 9) indicative of a similar histological response in fish collected for both surveys. No histological alterations were identified in the gill or gonad samples of any of the collected fish.

Table 24. Mean histological index values for *Labeobarbus marequensis* from the Olifants River collected during low flow 2009 and high flow 2010.

Index	2009	2010
Liver Index	7.7	5.9
Kidney Index	1.0	2.4
Gill Index	0.0	0.0
Testis Index	0.0	0.0
Ovary Index	0.0	0.0
Fish Index	**8.7**	**8.3**

The condition factor has been used extensively in fish health and population assessments and the calculation used for this study, namely Fulton's condition factor described by Carlander (1969) can be indicative of the overall condition and nutritional status of an individual fish (Schmitt and Dethloff, 2000). According to Bolger and Connolly (1989) in studies based on length-weight data, the heavier fish will be in the better condition. There are many factors which affect fish weight including food availability, metabolic rate as dependent on temperature and seasonal changes in terms of breeding activity (Marchand, 2006) and may increase or decrease in response to chemical contaminants (Schmitt and Dethloff, 2000). *Labeobarbus marequensis* values were between 0.88 and 1.08 with the higher values found in the LF2009 samples and it is possible that these differences are also due to seasonality.

The hepatosomatic index (HSI) is a ratio of liver weight to body weight and can be affected by contaminant exposure (Schmitt and Dethloff, 2000). The normal value for HSI ranges from 1-2% for Osteichthyes (Munshi and Dutta, 1996) although the range is species specific. A baseline laboratory-based study of two Southern African fish species showed mean HSI values of 1.08% for *C. gariepinus* specimens and 1.30% for *O. mossambicus* (Van Dyk, 2006). However, a study done in the Okavango panhandle showed HSI values of 0.50% for *C. gariepinus* specimens; 0.60% for *C. ngamensis* specimens; 1.00% for *O. andersonii* specimens; and 0.80% for *S. angusticeps* specimens (Van Dyk *et al.*, 2009a). The HSI values of these specimens from a supposed pristine area were all below the

supposedly normal discussed above. The fish specimens from the Okavango panhandle were affected by parasitic infections and showed moderate histological alterations (Van Dyk *et al*., 2009a) Parasitic infections were also noted in the visceral cavity of 14 of the specimens. The lower than expected HSI value of 0.7 during the LF2009 sampling and the HSI value of 0.54 during the April 2010 sampling trip may be indicative that the fish were under stress.

The gonadosomatic index (GSI) is an indicator of gonadal development and maturity and has been used to assess gonadal changes in response to environmental dynamics (seasonal changes) or exogenous stresses (contaminant exposure) (Schmitt and Dethloff, 2000). The GSI values for the males were 0.62 in LF2009 and 2.78 in HF2010. For the females these values varied between 0.72 (LF2009) and 1.53 (HF2010). There were no histopathological changes found in both the testes and ovaries and therefore the higher values during the April 2010 sampling trip were because of seasonality.

An organ index was calculated for the liver, gills, kidneys and gonad to give an indication of the histological changes in each organ. The liver showed more histopathological changes that the other organs that were assessed, which were expected because the liver is a major detoxification organ and is involved in the metabolism and excretion of heavy metals and xenobiotics. Since the pathway of blood vessels that transport substances from the digestive system, it is the first organ exposed to ingested toxicants (Ross *et al*., 1989). The histopathological changes that were observed were intracellular deposits (only in the 2010 sampling trip), hepatocellular vacuolation and nuclear changes. These changes are all regressive changes. The liver index was 7.7 for the LF2009 and 5.9 for the HF2010 sampling trip. These values show that the liver has a normal structure and the changes that were observed could be due to normal metabolic function of the liver.

Hydrocynus vittatus

Necropsy and Condition Indices

The specimen data for *H. vittatus* is presented in Table 25. The somatic index, Condition factor and age data for these specimens are presented in Table 26. The mean HSI values for the three sample groups were all within the same range of 0.4 to 0.6. The mean GSI values for both the female and male specimens were relatively low. This was not unexpected as the gonadal tissue of most of the sampled fish was observed to be in the immature stages of gametogenesis. The mean SSI values were similar for all three sample groups and the mean CF for all the groups were between 0.7 and 1. The mean age of the LF2009 sample

group was slightly higher compared to the 2010 sample group. Age was not determined for the 2011 sample group.

Table 25. Specimen data for *Hydrocynus vittatus* from the Olifants River collected during low flow 2009, high flow 2010 and high flow 2011. Mean values are presented per sample group.

Sampling period	N	Sex		Body mass	Total length
		♂	♀	(g)	(mm)
September 2009	16	9	7	320.00 ± 211.79	348.63 ± 47.76
April 2010	6	5	1	490.00 ± 194.63	388.63 ± 36.15
June 2011	15	3	12	552.7 ± 465.65	385.3 ± 70.18

Table 26. Somatic index, Condition factor and age data for *Hydrocynus vittatus* from the Olifants River collected during low flow 2009, high flow 2010 and high flow 2011. Mean values are presented per sample group.

Sampling period	n	HSI	GSI (♂)	GSI (♀)	SSI	CF	Age (Months)
September 2009	16	0.54 ± 0.12	0.56 ± 1.02	0.38 ± 0.22	0.06 ± 0.05	0.70 ± 0.27	45.00 ± 21.00
April 2010	6	0.49 ± 0.08	0.83 ± 0.73	1.02	0.06 ± 0.02	0.80 ± 0.09	39.00 ± 11.8
June 2011	15	0.51 ± 0.10	0.31 ± 0.05	0.38 ± 0.40	0.04 ± 0.01	0.81 ± 0.12	N/D

HSI = Hepatosomatic Index; GSI = Gonadosomatic Index; SSI = Splenosomatic Index; CF = Condition factor; N/D = Not determined

The necropsy observations revealed a few abnormalities in a number of the sampled *H. vittatus* specimens. These included liver discolouration (2009: n = 2; 2011: n = 2) parasitic infections (2009: n = 13; 2010: n = 4; 2011: n = 14) nodular spleen (2009: n = 1; 2011: n = 5) and pale gills (2011: n = 1).

Histopathological assessment

The light microscopy analysis showed that the selected target organs of *H. vittatus* from the Olifants River have normal histological structure and seem to be in a normal functional state. Selected histological alterations were identified in liver, kidney and gill samples. These included intracellular deposits, hepatocellular vacuolation and nuclear changes in the liver, vacuolation of the tubular epithelium, nuclear changes and inflammatory responses in the kidney samples, as well as epithelial hyperplasia in selected gill samples. The percentage prevalence of these alterations for the various sample groups are presented in Table 27.

With regards to the liver alterations, the intracellular deposits were mostly diffuse in nature and were present in most hepatocytes. The hepatocellular vacuolation identified was in most cases characteristic of macrovesicular steatosis, however, the presence of lipid

accumulation in hepatocytes was not confirmed through special stains as part of this study. The vacuolated cells were mostly diffuse in nature but focal areas of intracellular lipid accumulation were also identified in three specimens. Nuclear changes identified included mainly pleomorphic nuclei, i.e. nuclei of different sizes within the same tissue regions.

Vacuolated tubular epithelial cells were the most prevalent alteration identified in the kidney samples for all three surveys. An inflammatory response was identified in one kidney sample and was characterized by a focal region of infiltration of inflammatory cells. With the exception of the inflammatory response, the same kidney alterations were identified in *H. vittatus* form all three surveys conducted over the three year period. The histological analysis of the gill samples showed focal gill epithelial hyperplasia in two specimens.

Table 27. Percentage prevalence of histological alterations identified in *Hydrocynus vittatus* from the Olifants River collected during low flow 2009, high flow 2010 and high flow 2011.

Organ / alteration	**2009**	**2010**	**2011**
	%	%	%
Liver			
Intracellular deposits	75	67	40
Hepatocellular vacuolation	69	17	27
Nuclear changes	50	0	6
Kidney			
Vacuolation of tubular epithelium	69	100	53
Nuclear alterations	13	17	0
Inflammation	0	0	7
Gills			
Epithelial hyperplasia	0	0	13

The histological index values for *H. vittatus* from the Olifants River are presented in Table 28. The mean index values for the liver samples indicated a higher mean Liver Index for the 2010 survey compared to the LF2009 and HF2011 surveys respectively. The mean Kidney Index values showed a similar pattern comparing the different sampling surveys. The presence of focal gill epithelial hyperplasia resulted in a Gill Index value of 0.5 for the 2011 sample group. No histological alterations were identified in the gonad samples for any of the fish collected. The mean Fish Index values varied between the three sampling surveys and all fell within the range of 0-15.

Table 28. Mean histological index values for *Hydrocynus vittatus* from the Olifants River collected during low flow 2009, high flow 2010 and high flow 2011.

Index	2009	2010	2011
Liver Index	7.5	10.3	2.9
Kidney Index	2.3	4.0	1.3
Gill Index	0.0	0.0	0.5
Testis Index	0.0	0.0	0.0
Ovary Index	0.0	0.0	0.0
Fish Index	**9.8**	**14.3**	**4.7**

The condition factor for *H. vittatus* varied between 0.70 and 0.81. These values were low because these fish were not in their breeding season. The HSI values for the *H. vittatus* were between 0.49 and 0.54 for the three sampling trips and may indicate that the fish were under stress. The GSI values for the males were low (0.31) in HF2011 and 0.56 in LF2009. The GSI was also low (0.7-0.81) for the females. Since no histopathological alterations were observed in the testes and the ovaries, the low GSI values are most probably due to seasonality because higher GSI values were found for both males and females during the April 2010 sampling trip closer to the summer breeding season.

The liver index values for fish that were bred in toxicant-free water in the laboratory were 9.0 for *C. gariepinus* and 8.2 for *O. mossambicus* specimens. In a study on *C. gariepinus* from the Rietvlei Nature Reserve found mean liver index values of 26.1 in the Marais Dam site and 25.3 in the Rietvlei Dam site (Marchand, 2006). Alterations found in the livers of specimens in this study were lower than in the Rietvlei Nature Reserve 7.5 (2009), 10.3 (2010) and 2.9 (2011). These changes were intracellular deposits, hepatocellular vacuolation and nuclear changes. All of the mean liver index values in this study were lower than those found in the Rietvlei study. It should be noted that liver index values higher that 10 indicated that the liver shows signs of stress.

3.8 Bioaccumulation in *Hydrocynus vittatus*

Metals

Bioaccumulation could be regarded as the resultant of two antagonistic mechanisms. According to Boudou and Ribeyre (1989) bioaccumulation is firstly the result of bio-uptake through adsorption and absorption of exogenous products via the aqueous phase and intermediary ingestion with food, and secondly effluxes that ensure that biotransformation and ultimately excretion of contaminants occurs. Bioaccumulation strategies for metals depend on the mechanisms by which uptake, excretion, and storage or sequestration of individual elements is achieved (Phillips and Rainbow, 1993). Many metals in the

environment are important in animal nutrition, whereas micronutrients, they play an essential role in tissue metabolism and growth. Requirements of different animal species vary substantially (Rand and Petrocelli, 1985), but optimal concentration ranges for micronutrients are frequently narrow. Severe imbalances can result in death, whereas marginal imbalances contribute to poor health and retarded growth. Non-essential trace metals, such as lead, can also be toxic at concentrations commonly observed in sediments and natural waters (Sorensen, 1993).

Metal bioaccumulation in muscle tissue of tigerfish from the Olifants and Letaba Rivers are presented in Figure 44 and Figure 45. For Cd, Co, Cr, Mn, Ni and Zn there is a significant decrease from LF2009 to HF2011. Aluminium, As, and Se concentrations increased whilst Cu, Cd and Fe concentrations remained stable. The Mn and Zn concentrations in tigerfish from the Letaba River were significantly higher than tigerfish from the Olifants River sampled during the corresponding survey. Comparison of metal bioaccumulation during the present study with historical data is difficult since all the previous studies on the Olifants River report metal concentrations on a wet mass basis (Table 29). However if one takes into account a wet mass conversion factor of 60% then Cd, Ni and Pb levels have decreased when compared to tigerfish sampled in the Olifants River in the early 1990s by Du Preez and Steyn (1992). Iron and Zn bioaccumulation has increased whilst Cu and Mn levels have remained stable. What is evident from the historical data (9 studies conducted between 1990 and 1996) and the present study (5 surveys between 2009 and 2011) is that there is considerable variation in the metal bioaccumulation. It is well known that the bioavailability of water and sediment- bound contaminants is greatly influenced by a multitude of variables within the water column and sediment, mainly physical, chemical and biological factors (Wepener et al., 2000). These variables interact in a complex fashion, hindering the prediction of ecological effects of metals (Wepener et al., 2000). This issue was addressed in the following section where the relationship between physico-chemical characteristics of water and sediments were related to the bioaccumulation of metals in fish from the Olifants River.

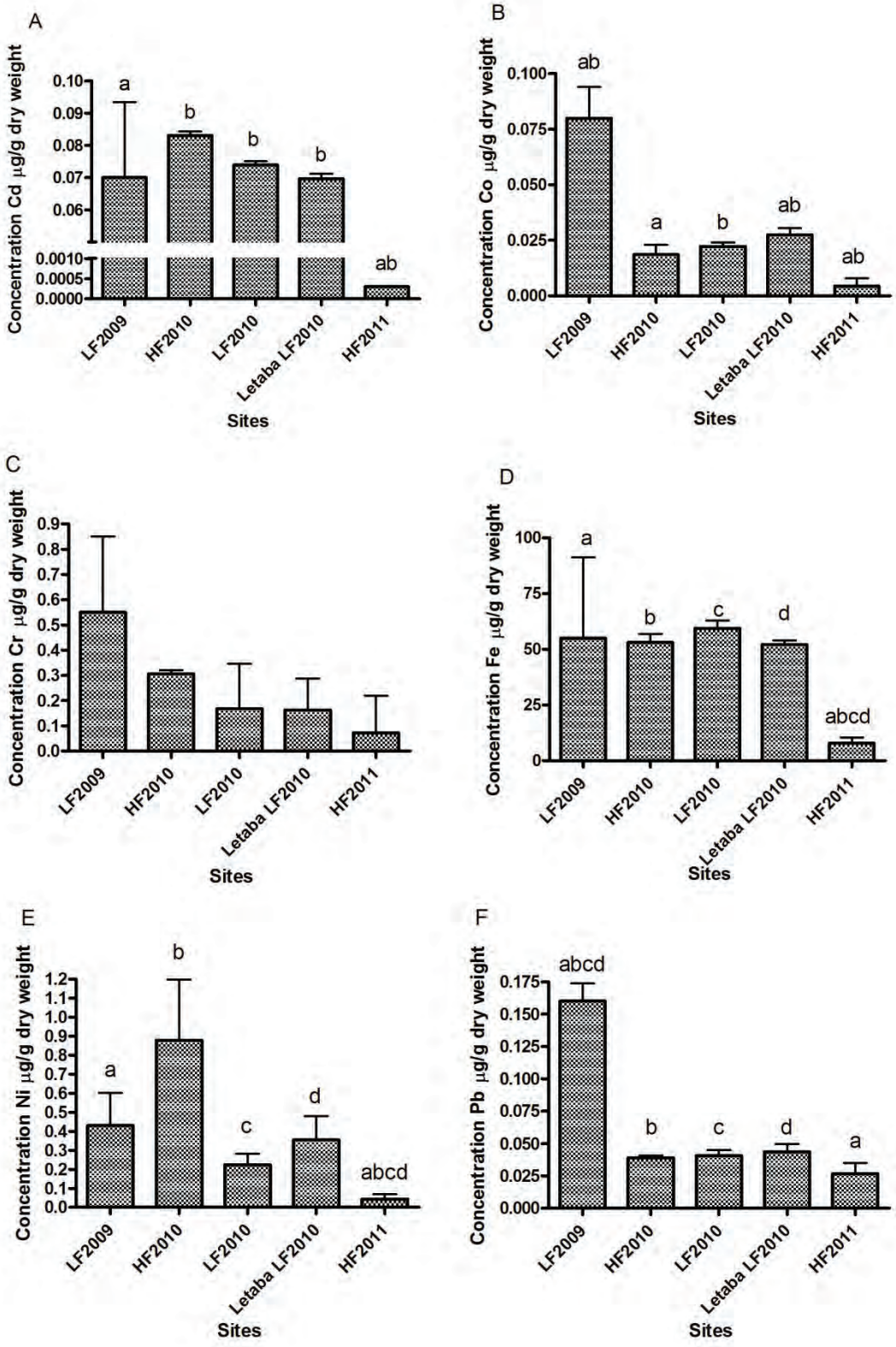

Figure 44. Mean ± standard error concentrations of metals in muscle (µg/g dry mass) in *H. vittatus* muscle tissue from the Olifants and Letaba Rivers. Common superscript within rows indicate significant differences ($p<0.05$).

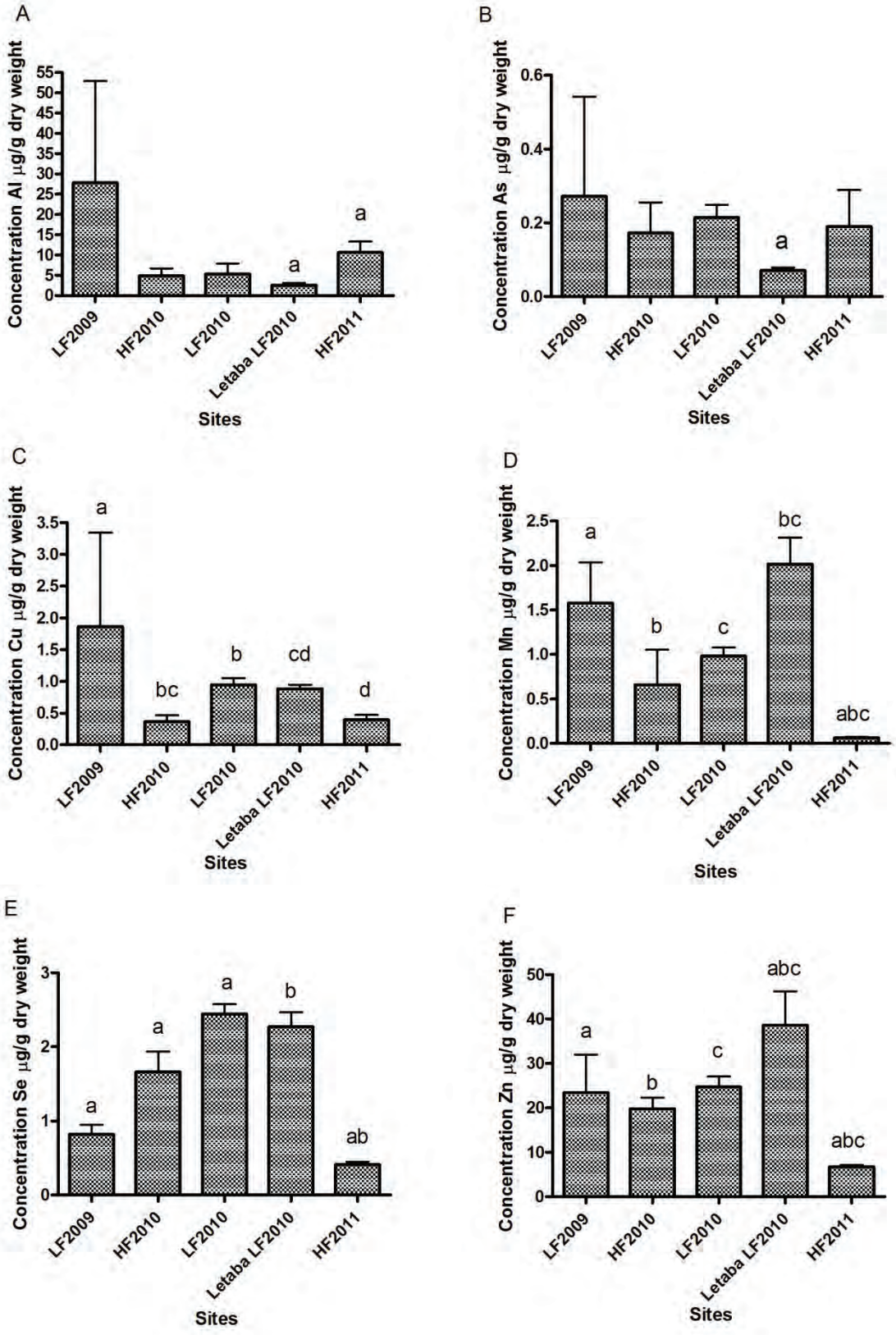

Figure 45. Mean ± standard error concentrations of metals in muscle (µg/g dry mass) in *H. vittatus* muscle tissue from the Olifants and Letaba Rivers. Common superscript within rows indicate significant differences ($p<0.05$).

Table 29. Historical metal bioaccumulation in muscle tissue of different fish species from selected sites in the Olifants River. Metals not measured are represented by NS.

Reference	Study species	Study Area & Month	Concentration metals µg/g muscle tissue							
			Cd	**Cr**	**Ni**	**Pb**	**Fe**	**Cu**	**Mn**	**Zn**
Du Preez and Steyn (1992)*	*H. vittatus*	Balule October 1990	2.1± 0.55	NS	8.7±1.2	10.9±4.8	36.7±12.7	4.25±3.2	1.65±0.8	13.2±6.05
Seymore et al. (1995)*	*B. marequensis*	Whole River November 1992	NS	NS	NS	5±2	NS	NS	2±1	NS
Seymore et al. (1996)*	*B. marequensis*	Whole River October 1991	NS	NS	NS	NS	NS	NS	NS	125±47
Seymore et al. (1996)*	*B. marequensis*	Whole River April 1991-June 1991	NS	6-43	NS	NS	NS	NS	NS	NS
Robinson and Avenant-Oldewage (1997)*	*O. mossambicus*	Mamba & Balule November 1994	NS	15.07±1.365	NS	NS	105.27 ± 19.32	1.84 ± 0.62	3.86 ± 0.75	NS
Du Preez et al. (1997)*	*C. gariepinus*	Mamba & Balule August 1990	NS	20.5 ± 5.6	12.5 ± 2.6	16.5 ± 4.1	NS	4.5 ± 1.25	3.5 ± 1.82	NS
Marx & Avenant-Oldewage (1998)*	*C. gariepinus*	November 1994	NS	NS	NS	8.64 ± 1.57	NS	NS	NS	32.64 ± 9.6
Kotze et al. (1999)*	*C. gariepinus*	Mamba 1994-1995	NS	NS	NS	NS	NS	2 ± 1	NS	31 ± 12
	O. mossambicus		NS	NS	NS	NS	NS	3±3	NS	31 ± 12
Avenant-Oldewage and Marx (2000a, 2000b)*	*C. gariepinus*	November 1994	NS	12.3 ± 3.4	18.1 ± 2.9	NS	196.45 ± 76.2	2.1 ± 0.9	4.7 ± 1.7	NS

*represents studies based on µg/g wet weight

Bioavailability of metals in water and sediments

Aquatic sediments serve as reservoirs for contaminants entering overlying waters from surrounding catchments, including metals and organic contaminants (Zimmerman and Weindorf, 2010). Contaminants such as metals do not remain permanently sequestered within sediments but can be released as a result of changing physico-chemical parameters within the overlying water column. Once released, these metals have the potential to harm aquatic organisms following bioaccumulation within individual species, in addition to biomagnification within the food chain. To understand the potential risk of metals exposure to aquatic organisms requires analysis of various parameters. As metals bind with different affinities to the various phases; e.g. exchangeable, acid-soluble, reducible, oxidizable and residual phases (Maiz et al., 2000), sequential extraction techniques are applied to determine the fractions of particular metals bound to each phase. Elsokkary and Muller (1990) indicate that individual metal species have varying affinities for the various sediment fractions. Metals such as Ni and Pb have high affinities for organic material and sulphides, whilst Cd has a greater binding affinity for the carbonate (acid-soluble) fraction (Elsokkary and Muller, 1990). The AVS concentrations present within aquatic sediment are a function of anaerobic bacterial action, exerting a strong influence on cationic metal activity and toxicity (Di Torro et al., 1990).

Metals such as Cu, Co, Ni, Pb and Zn precipitate out of the water column following reactions with inorganic anions, becoming sequestered within the riverine sediment, and rendering them unavailable for uptake by aquatic biota (Fergusson, 1990). In the water quality assessment we made mention of the potential protective role that the high concentrations of hardness ions (Mg and Ca) can play in reducing metal bioaccumulation specifically in the Olifants River. It is therefore important to assess the role that AVS, organic carbon (expressed through light as OC and the various phases within aquatic sediment through BCR analysis will provide further insight into the bioavailability and thus the potential risk that contaminated sediments may pose to the fish of the Olifants River.

For the purposes of this study we focussed on the leaden labeo (*L. molybdinus*) as it is a benthic dwelling fish that occurs at most of the sampling sites in the Olifants River and it is exposed to both metals in sediments as well as in the water column. Together with the fish samples, water and sediment were collected from all five sites in the Olifants River during the LF2009 survey.

External environmental factors modify the chemical potential to which the organisms are subjected (Di Toro et al., 1990). As a consequence, different sediments will exhibit different degrees of toxicity for the same total quantity of chemical. As such; all environmental factors present need to be considered when assessing metal bioaccumulation

within benthic dwelling aquatic species (Di Toro et al., 1990). Important characteristics which may influence the availability of sediment-bound metals for uptake which were considered as potential contributors within this study included overlying water quality parameters, total suspended and dissolved metal concentrations, and total organic content of the sampled sediment, in conjunction with existing AVS concentrations. The metal concentrations in water and sediment, together with AVS concentrations and physical characteristics of the sediments are presented in Table 30.

The AVS concentrations showed a high variability among the sampling sites and increased from 84.12 µmol/g at site 1, to 548.34 µmol/g at site 5. This is supported by the highly variable results found by De Jonge et al. (2009) for Flemish rivers. The clay content also varied between sites, ranging from 0.04±0.05% at site 4, 8.05±8.03% at site 3, 9.37±19.39% at site 1, 10.92±9.77% at site 5, and 12.59±8.64% at site 2. The organic carbon content (expressed as LOI) was low at all 5 Sites, ranging from 0.41±0.07% at site 2 to 1.18±0.15% at site 3. The spatial bioaccumulation results of Cd, Cu, Ni, Pb and Zn in liver tissue of *L. molybdinus* at sites 1, 2 and 3 are presented in Figure 46. The spatial changes in metal bioaccumulation are presented revealed a significant decrease in Cu, Ni, and Pb from site 1 to 3. Zinc is the only metal that remains high at all three sites.

Di Toro et al. (1990) formulated the SEM-AVS model for sediment toxicology in the early 1990s. This predictive model describes that, when AVS concentrations exceed SEM concentrations on a molar basis within aquatic sediment, i.e. [SEM-AVS] <0, all metals will be bound to the sediment-bound sulphides, and are then unavailable for uptake by aquatic organisms. The AVS-SEM concentrations were <0 for sites 3, 4 and 5 and it could therefore be expected that the bioavailability of metals from the sediments will be lower at these sites. The AVS-SEM>0 at sites 1 and 2 would imply that higher metal bioaccumulation is likely at these sites, which was indeed the case for Zn in *L. molybdinus* liver tissue (Table 30 and Figure 46). The relationship between metal accumulation in *L. molybdinus* and SEM_{Me}–AVS (Figure 47) indicates that for Cd, Pb and Zn AVS does have a protective role in that the bioaccumulation of these metals increased when the AVS-SEM>0. However this was not the case for Cu as the bioaccumulation remained high even when AVS-SEM<0.

The relationship between metal bioaccumulation and other environmental factors in sediments and surface water are presented in Table 31. In most cases, metal bioaccumulation was best correlated with total metal content in the sediment normalised for organic carbon (LOI) and suspended metal concentrations in the surface water. For Cd, Cu, Ni, Pb and Zn SEM_{ME} showed significant correlations with accumulated tissue concentrations even when [SEM-AVS]<< 0. There was a positive relationship between Cu, Ni and Zn bioaccumulation and SEM_{ME}-AVS and Sed_{Me}/LOI, while no relationship was found for Cd and Pb. Positive correlations were present between Ni concentrations of *L. molybdinus* and

SEM-AVS and Zn bioaccumulation and Sed_{Me}/LOI, AVS, SEM, SEM-AVS, SEM-AVS/LOI and $DiSW_{Me}$. The linear regression models that best describe the bioaccumulation processes (Table 32) are for Cu and Zn with AVS-SEM and AVS respectively and Ni bioaccumulation is decreased through increased water hardness.

In this study we found that high levels of Cu, Pb and Zn were accumulated even when AVS concentrations largely exceeded SEM concentrations. These results are in agreement with De Jonge et al. (2009). Recent studies have indicated that the relationship between AVS and metal accumulation in aquatic invertebrates is highly dependent on many variables, including feeding behaviour and ecology (De Jonge et al., 2010) and the results from this study further support these findings.

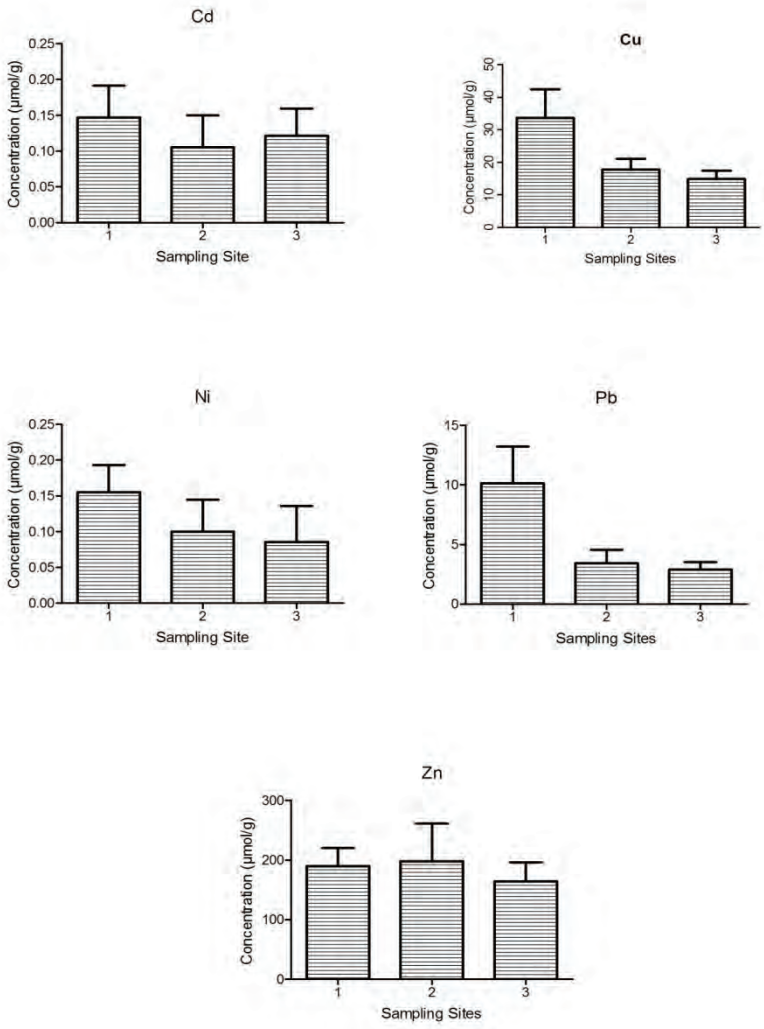

Figure 46. Mean + standard error of metal bioaccumulation in the liver of *Labeo molybdinus* (µmol/g dry weight).

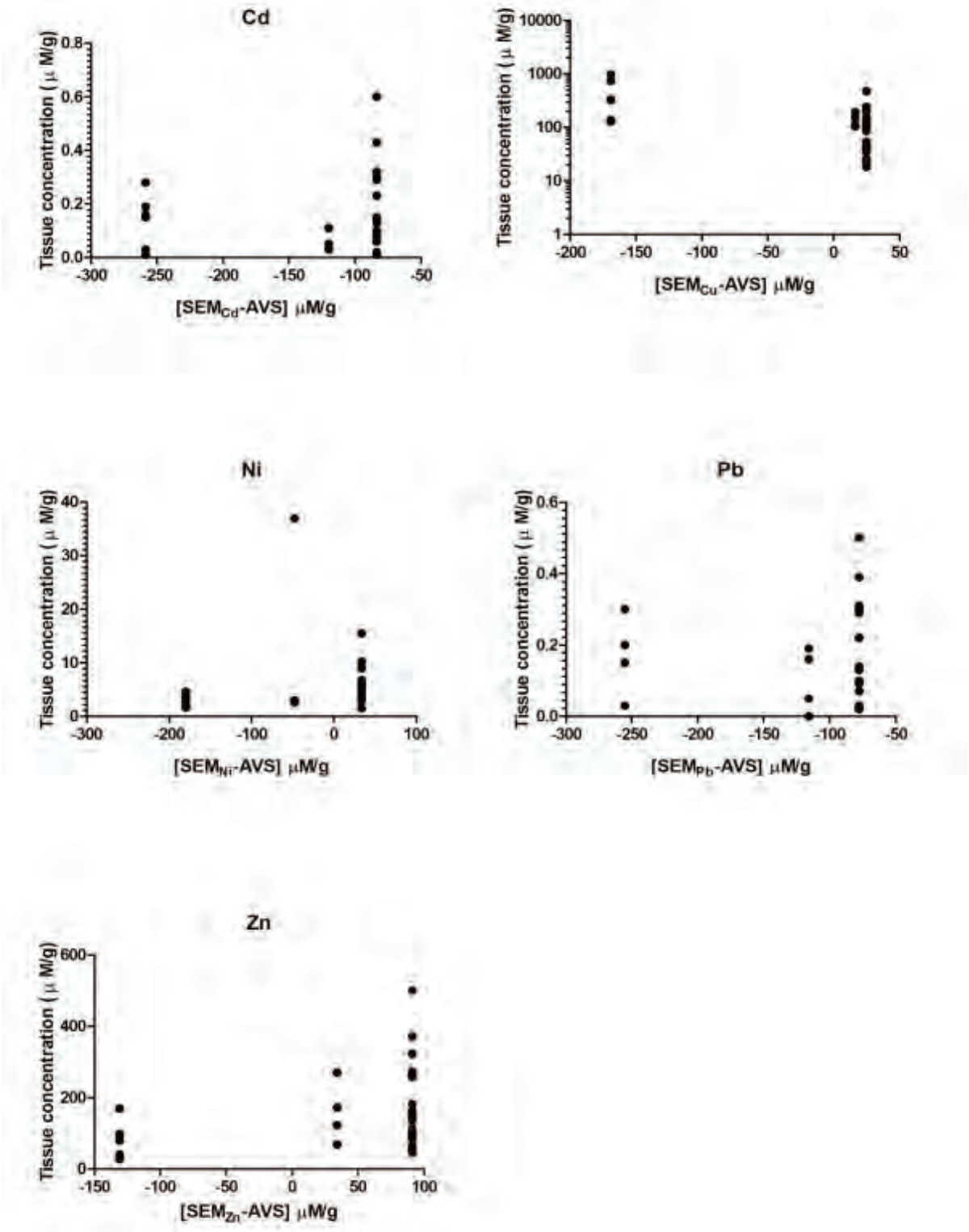

Figure 47. Relationship between metal bioaccumulation in *Labeo molybdinus* liver tissue (µM/g) and [SEM_{Me}-AVS].

Table 30. The AVS, percentage clay particles, SEM and total sediment metal concentrations. Average values (n=3) ± standard deviations are presented for the five sampling sites in the Olifants River. All concentrations are expressed in µmol/g.

	1	2	3	4	5
AVS (µmol/g)	84.12	120.57	259.21	317.79	548.34
Clay Content (%)	9.37 ± 19.39	12.59 ± 8.64	8.05 ± 8.03	0.04 ± 0.05	10.22 ± 9.97
SEM					
Cd (µmol/g)	0.60 ± 0.09	0.58 ± 0.13	0.58 ± 0.33	0.43 ± 0.43	0.37 ± 0.02
Cu (µmol/g)	108.97 ± 12.14	136.95 ± 4.76	90.08 ± 3.65	75.53 ± 2.44	77.27 ± 10.40
Ni (µmol/g)	117.78 ± 6.74	73.01 ± 8.07	79.64 ± 10.61	57.92 ± 22.52	71.74 ± 10.86
Pb (µmol/g)	6.25 ± 1.13	4.61 ± 0.29	3.92 ± 0.38	3.33 ± 0.20	4.40 ± 0.75
Zn (µmol/g)	175.13 ± 55.21	154.71 ± 72.56	128.31 ± 66.23	40.20 ± 29.52	158.69 ± 38.39
Total metal concentrations in sediments					
Cd (µmol/g)	0.74 ± 0.74	0.65 ± 0.53	0.97 ± 0.10	0.13 ± 0.07	0.72 ± 0.07
Cu (µmol/g)	466.61 ± 3.20	587.53 ± 12.74	516.86 ± 17.07	223.62 ± 38.44	297.56 ± 3.46
Ni (µmol/g)	438.35 ± 22.16	252.51 ± 17.84	290.03 ± 5.29	178.73 ± 16.01	271.60 ± 8.65
Pb (µmol/g)	14.99 ± 9.36	9.36 ± 0.84	13.42 ± 1.30	29.57 ± 1.45	16.25 ± 0.37
Zn (µmol/g)	546.49 ± 44.88	714.36 ± 40.56	634.66 ± 21.68	174.54 ± 29.71	397.36 ± 9.12

Table 31. Spearman's rho correlation coefficients among metal concentrations in liver of *L. molybdinus*, sediment fractions and surface water. R-values and significance level are presented. *: $p < 0.05$; **: $p < 0.01$; **. Sed_{Me}: Total metal concentration in the sediment; Sed_{Me}/LOI: Total metal concentration in the sediment normalized for organic matter content; SedMe/clay: Total metal concentration in the sediment normalized for organic matter content; SEM_{Me}–AVS/LOI: Molar difference between SEM and AVS normalized for organic matter content; dissolved metal concentration in the surface water: DiSWMe.

Metal in liver	Sed_{Me}	Sed_{Me}/LOI	Sed_{Me}/Clay	AVS	SEM	SEM_{ME}-AVS	SEM-AVS/LOI	$DiSW_{Me}$
Cd	-	-	-	-	-	-	-	-
Cu	-	-0.478*	-0.451*	0.513**	-	-0.540**	-0.478*	0.438*
Ni	-	-	-	-	-	0.367*	-	-
Pb	-	-	-	-	-	-	-	-
Zn	-	0.495	-	-0.451*	0.493**	0.454*	0.495**	-0.395*

Table 32. Multiple linear regression models for the metal accumulation in liver tissue of *L. molybdinus*. Parameter estimates of the significant variables and the intercept of each model are reported. The significance level is presented as *$p < 0.05$; **$p < 0.01$. Only the significant models are presented ($p < 0.05$). The amount of variation in metal accumulation explained is given by the multiple correlation coefficient (R).

Metal accumulation in liver	Intercept	Parameter estimate	n	R
Cd		No significant models	30	
Cu	+153.79	-1.43 SEM-AVS**	30	0.559
Ni	-138.99	+ 0.02 Mg*	30	0.371
Pb		No significant models	30	
Zn	+238.07	-0.674 AVS*	30	0.423

Organics

The lipids (as % muscle mass) in muscle tissue of tigerfish were significantly lower in the Letaba River during LF2010 (Table 33). The lipids in tigerfish from the Olifants River were also significantly lower during the HF2011 survey than the other surveys. As was found for tigerfish in the Lake Pongolapoort (Wepener et al., 2012) the lower lipids are related to lower metabolic status and reproductive condition of the fish during winter period (Steyn et al. 1996). According to Covaci et al. (2006) the total muscle lipid reserves plays a very important role in bioaccumulation of OCPs in fish. There were distinct flow-related differences in bioaccumulation of OCPs with the low flow periods displaying higher concentrations.

Table 33. Mean ± standard error of organochlorine pesticides (ng/g lipid) in *H. vittatus* muscle tissue from the Olifants and Letaba Rivers. Common superscript within rows indicate significant differences (p<0.05). ND represents OCP not detected.

	LOD ng/g	LF2010 (n=11)	HF2010 (n=6)	Letaba LF2010 (n=16)	HF2011 (n=7)
α-HCH	2	84.64 ± 28.84^{a}	16.97 ± 3.53^{ab}	44.82 ± 16.54^{b}	ND
β -HCH	2	84.42 ± 56.31^{a}	ND	57.17±7.31^{b}	12.86 ± 4.15^{ab}
δ-HCH	2	116.16 ± 89.36^{a}	71.06 ± 12.57^{b}	135.59 ± 11.94^{b}	37.50 ± 14.09^{b}
γ-HCH	2	23.26 ± 11.25^{a}	ND	70.91 ± 13.54^{ab}	13.78 ± 5.18^{b}
ΣHCHs		308.48 ± 106.3	88.03 ± 14.4	308.5 ± 39.1	64.13 ± 15.52
Heptachlor	2	10.98 ± 5.69			2.38 ± 1.11
cis-Nonachlor	2	192.26 ± 84.71	10.40 ± 6.12	41.63 ± 27.94	5.61 ± 3.34
trans-Nonachlor	2	75.47 ± 35.53^{a}	ND	82.17 ± 17.18^{b}	12.90 ± 2.61^{ab}
cis-Heptachlor-epoxide	2	16.60 ± 7.61^{a}	ND	92.74 ± 14.76^{ab}	18.30 ± 4.86^{b}
trans-Heptachlor-epoxide	2	ND	ND	98.75 ± 14.61^{a}	9.52 ± 4.81^{a}
cis-Chlordane	2	ND	15.28 ± 6.80^{a}	123.56 ± 44.18^{ab}	12.51 ± 3.29^{b}
trans-Chlordane	2	124.22 ± 63.23^{a}	ND	59.67 ± 17.31^{b}	8.46 ± 3.39
Oxy-Chlordane	2	58.56 ± 25.05	ND	93.09 ± 12.28	12.14 ± 4.71^{ab}
ΣCHLs		182.78 ± 82.83	15.28 ± 6.8	276.32 ± 54.92	33.11 ± 10.76
Aldrin	2	ND	ND	ND	ND
Dieldrin	2	ND	ND	ND	ND
Endrin	2	29.24 ± 20.85^{a}	ND	39.87 ± 14.59^{b}	1.24 ± 0.69^{ab}
o,p'-DDD	4	362.22 ± 266.16^{a}	50.04 ± 34.94^{b}	512.85 ± 262.07^{bc}	18.71 ± 5.48^{bc}
p,p'-DDD	4	978.02 ± 333.29^{ad}	85.62 ± 28.42^{ab}	1205.75 ± 390.2^{bd}	62.83 ± 13.39^{d}
o,p'-DDE	4	201.97 ± 78.17^{ad}	29.93 ± 6.75^{ab}	125.03 ± 30.15^{bd}	13.63 ± 3.55^{d}
p,p'-DDE	4	4359.55 ± 1923.82^{ad}	181.71 ± 97.99^{ab}	4745.26 ± 1645.47^{bd}	474.89 ± 229.07^{d}
o,p'-DDT	4	971.07 ± 516.47^{ad}	35.52 ± 15.67^{ab}	1465.48 ± 892.38^{bd}	30.05 ± 9.56^{d}
p,p'-DDT	4	2166.83 ± 1128.08^{ad}	116.83 ± 56.35^{ab}	1382.90 ± 248.3^{3bd}	50.42 ± 17.26^{d}
ΣDDTs		9039.66 ± 3221.44	499.64 ± 200.3	9437.27 ± 2395.55	650.53 ± 260.44
***p,p'*-DDE/DDT**		2.01	1.56	3.43	9.42
HCB	4	3.52 ± 1.30^{a}		9.88 ± 1.86^{ab}	3.62 ± 1.02^{b}
Lipid (%)		0.33 ± 0.08^{a}	0.41 ± 0.06^{b}	0.05 ± 0.01^{abc}	0.24 ± 0.03^{bc}

The ΣDDTs (*o,p'*- and *p,p'*-DDE, DDD, DDT) were the most abundant organochlorine pesticides (Table 33) and all of the samples had measurable concentrations of the DDT isomers and were in the order of DDE>DDT>DDD. There were clear flow-related influences on the DDT bioaccumulation and in contrast to findings for Lake Pongolapoort normalisation for lipid content did not influence the DDT levels (data not shown). During the highflow surveys the ΣDDTs concentrations were higher than the 1000 ng/g maximum allowable residue level in edible fat as prescribed by the European Union. The high DDE/DDT ratio

(>3) in the Letaba River and HF2011 survey in the Olifants River is indicative of breakdown processes of DDT in the system and therefore represents historical use (Strandberg and Hites 2001). However the ratios of below 2 recorded during the 2010 surveys could indicate fairly recent input of DDT into the system, in contrast to the Lake Phongola study where the ratios remained above 4 (Wepener et al., 2012). Tigerfish are able to bioaccumulate DDT through their diet and therefore there is a degree of internal biotransformation of DDT to DDE possible (Ssebugere et al. 2009). Comparison with previous studies in the Olifants River shows that levels of total DDTs have increased (Ansara-Ross et al., 2012). Similar to this study the DDE concentrations were also higher than the DDT levels.

The HCHs were next highest with the isomers decreasing in concentration δ>β>α>γ for all surveys except for the Letaba River. In the Letaba there is indication of the use of pure γ-HCH (lindane, the most toxicological active HCH isomer) in the upper catchment. The levels of lindane recorded in the Letaba River are much higher than concentrations in mullet from the Isipingo Estuary (Ansara-Ross et al., 2012). The high concentrations of heptachlor (compared to levels in tigerfish from Lake Pongolapoort) and its breakdown products indicate widespread use in the catchments of the Olifants and Letaba Rivers. Of particular cause for concern hare the levels of the more toxic oxidised form, oxy-chlordane. Levels of this OCP are much higher than reported by Adu-Kumi et al. (2010) in edible fish from three lakes in Ghana. The HCBs were present in the lowest concentrations, with the Letaba River once again having the highest bioaccumulation levels.

3.9 Biomarker response in *H. vittatus*

The results of the biomarkers of exposure and effect measured in *H. vittatus* during the low flow (LF) periods of 2009 and 2010 are presented in Figure 48. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods. Figure 50. There was significant inhibition ($P<0.05$) of AChE activity (Figure 48. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods. A) in the LF2009 samples. The LF2009 survey also had significantly higher CYP450 (Figure 48. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods. B) activity and MT concentrations (Figure 48. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15).

Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods. C) when compared to the LF2010 values. Figure 49 presents the anti-oxidant responses in tigerfish during the two flow periods. The CAT activity (Figure 49A) was slightly lower during the 2010 survey, however both LP (Figure 49C) and PC (Figure 49D) levels were significantly ($P<0.05$) higher than the LF2009 survey. The available energy in muscle tissue of tigerfish (Figure 50F) is represented by the difference between the available energy compounds (Figure 50A-D) and the cellular energy consumption (Figure 50E). All these attributes were significantly higher ($P<0.05$) in the fish sampled during the HF2010 survey.

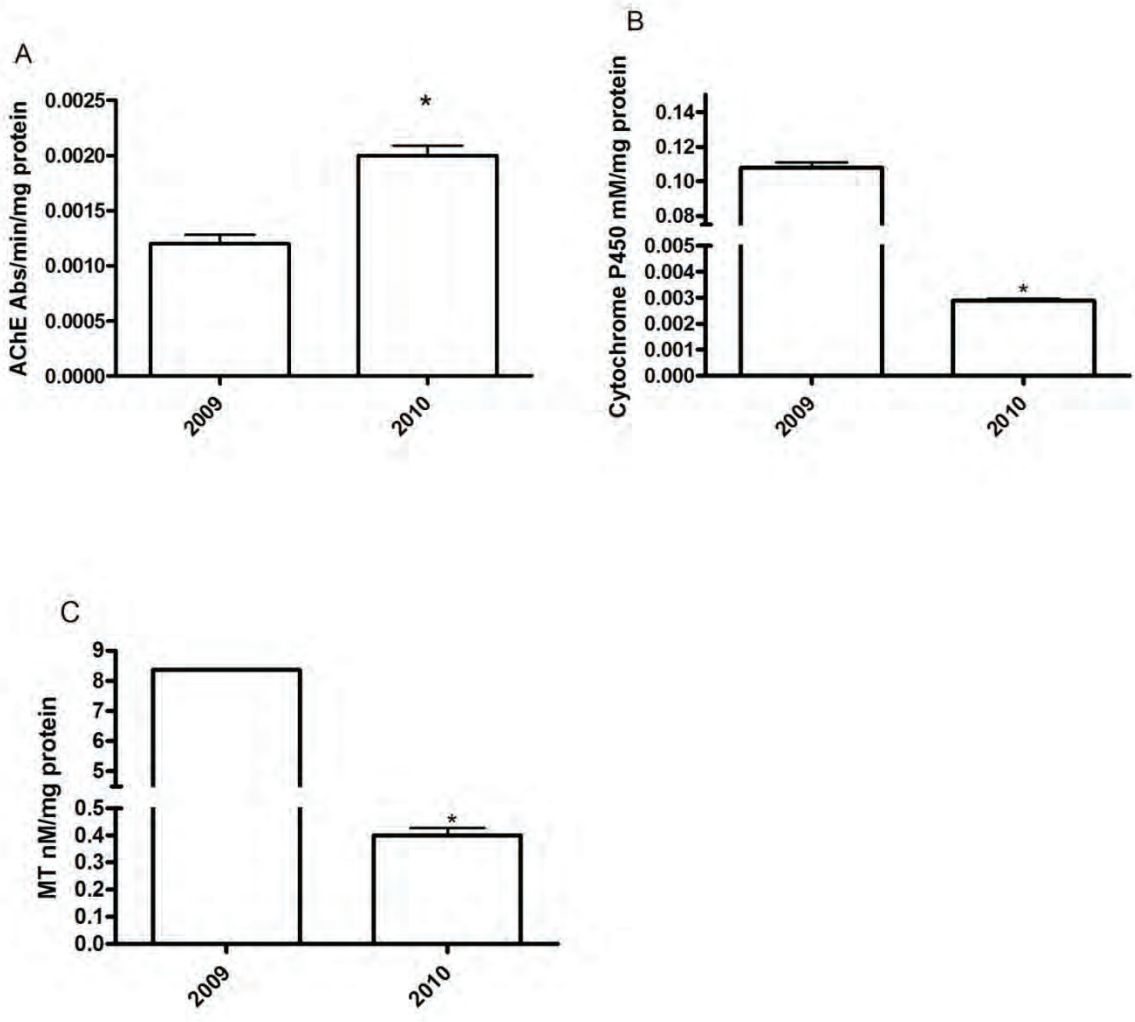

Figure 48. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

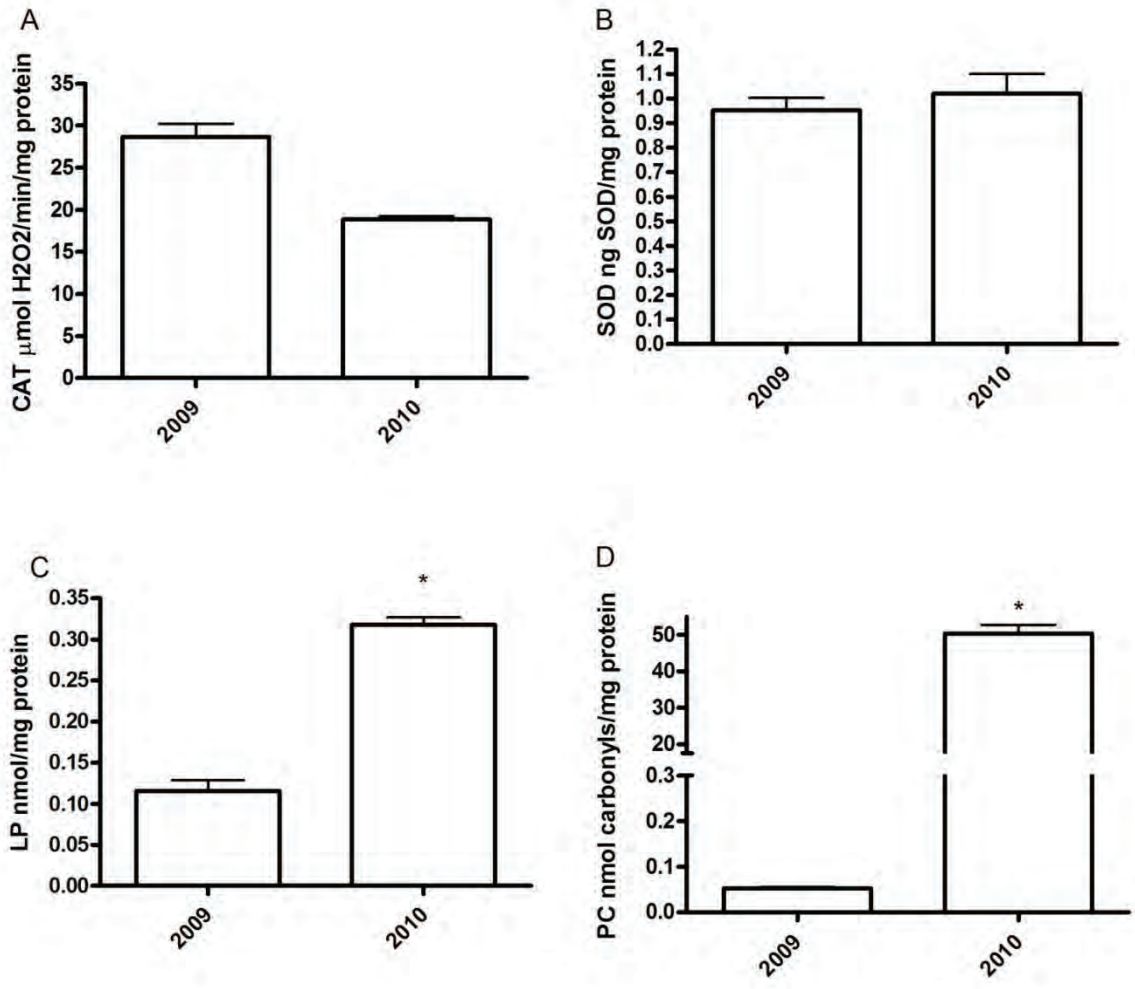

Figure 49. Biomarkers of effect in liver tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

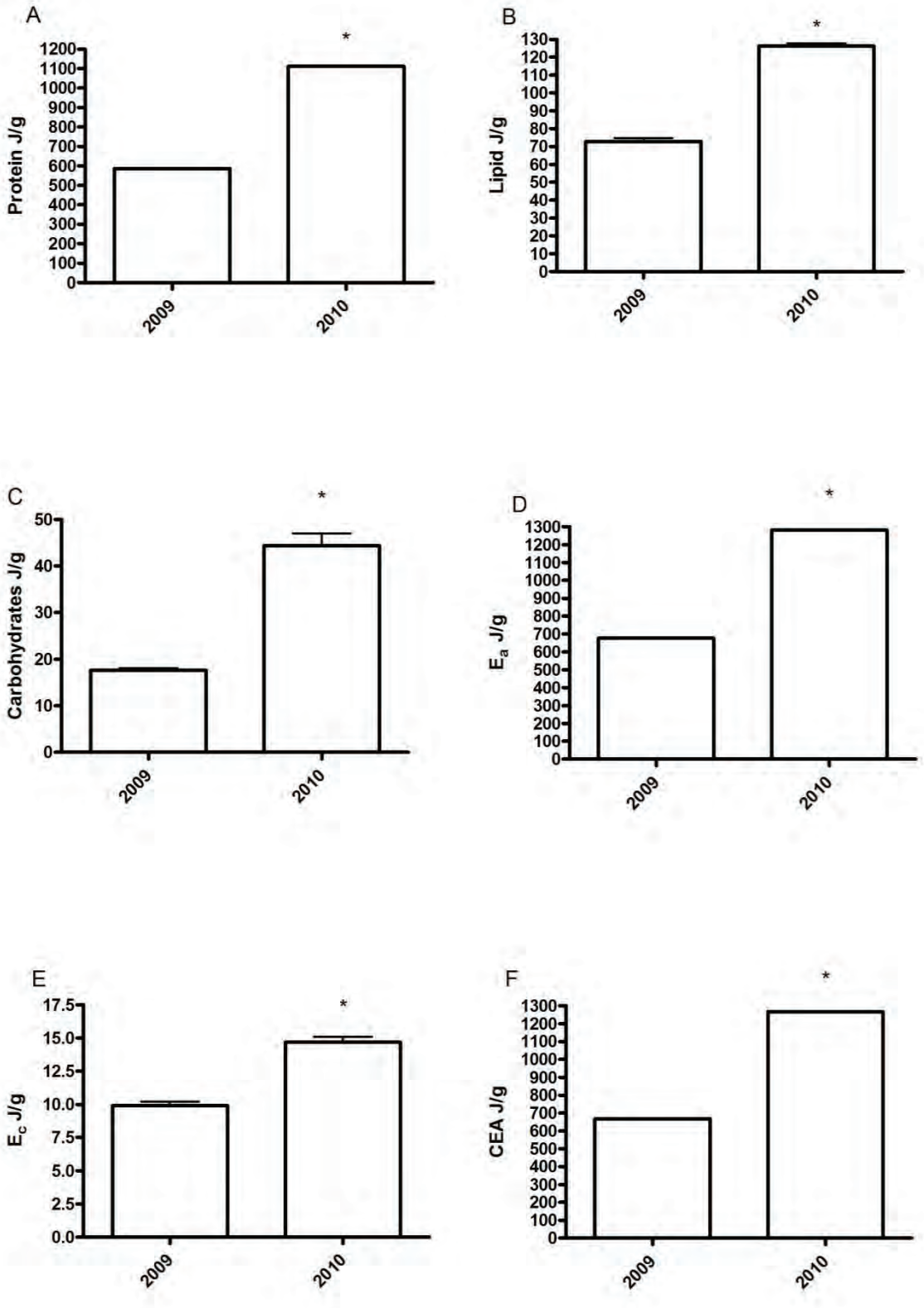

Figure 50. Cellular energy allocation biomarker of effect in muscle tissue of tigerfish collected during the 2009 and 2010 low flow periods in the Olifants River (n=15). Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

Interpretation of biomarker responses

Biomarkers of exposure

Acetylcholine Esterase (AChE): AChE plays an important role in the regulation of nerve impulse transmission at the cholinergic synapses. AChE hydrolyses acetylcholine, a common neurotransmitter, and thereby prevents it from accumulating in and around the synapse (Huggett et al*.,* 1992). Among fish, AChE is predominantly localised in the brain and muscle (Huang *et al.,* 1997). Inhibition of esterases is used as a specific indicator of stress induced by organophosphate and carbamate pesticides (Murphy, 1980). This causes an accumulation of acetylcholine at the nerve synapse resulting in the disruption of nerve function (Peakall, 1992). In addition to organophosphate and carbamate pesticides, a number of other contaminants including mercury and some physiological conditions; i.e. infections, anaemia, malnutrition and liver diseases are known to cause inhibition (Mayer *et al.,* 1992). It is remarkable to what degree AChE in fish can be inhibited, before death occurs. In general, it appears that around a 70 to 80% loss of activity must take place before death occurs (Heath, 1995).

Cytochrome P450-activity (CYP450): Cytochrome P450 refers to a family of enzymes that transform the structure of organic chemicals. The synthesis of CYP1A is induced in a reversible manner in organisms exposed to certain families of contaminants particularly polyaromatic hydrocarbons (PAHs) or polychlorobyphenyls (PCBs), which are wide spread in the aquatic environments. Liver microsomes of animals treated with aromatic or halogenated hydrocarbons show enhanced rates of MFO activity. Many PAHs, which include some, that are potentially hazardous environmental contaminants induce MFO activity. Activities catalysed by P450-EROD activity are largely specific in their response to these compounds (Stegeman and Hahn, 1994). These activities occur at very low; often-undetectable, levels in many control or untreated animals, but are highly induced by treatment with the hydrocarbon compounds.

Metallothioneins (MT): The evaluation of MT induction as a response to metal exposure may be useful as a biomarker of exposure. These low MW, cysteine-rich, heat-stable proteins of a non-enzymatic nature, which are found in most zoological groups, have a high affinity for metal ions (Van der Oost *et al*., 2003). According to Viarengo *et al*. (1997), when heavy metal cations accumulate within an organism's cells, metalloprotein neosynthesis is stimulated, thus leading to an increase in MTs that rapidly react with free metal cations

present in the cytosol. Thus, the quantification of MTs may prove useful in assessing metal exposure and predicting potentially detrimental effects induced by metals.

Biomarkers of effect

Anti-oxidant activity

Changes in antioxidant systems of aquatic organisms, can serve as indicators for a variety of pollutant exposures related to oxidative stress. Thus, it provides sensitive biochemical markers for exposure and toxicity of use in environmental monitoring (Doyotte *et al.*, 1997). It reflects an imbalance between the production and the removal or scavenging of oxidants (Winston and Di Giulio, 1991). Several ROS occur as a result of normal oxygen metabolism, but can be produced in large quantities during toxicant-induced interactions, which can cause oxidative stress. These ROS can cause cytotoxic alterations, including alterations in the redox balance, enzyme inactivation, lipid peroxidation and protein degradation as well as DNA damage and cell death. The extent, to which such biological damage occurs, will depend on the effectiveness of antioxidant defenses to remove ROS (Livingstone, 1993).

Catatalase (CAT) and superoxide dismutase (SOD): The antioxidant, CAT, is a hematin-containing enzyme based in the peroxisomes of cells and is an extremely important component of intracellular and antioxidant defences of aquatic organisms (Jamil, 2002). It reduces the H_2O_2 into water (H_2O) and oxygen (O_2) to prevent oxidative stress and in maintaining cell homeostasis. Catalase is often induced concomitantly with the antioxidant, superoxide dismutase (SOD), as a result of oxidative stress. The decomposition of H_2O_2 is directly proportional to both the concentration of enzyme and the concentration of substrate (Di Giulio *et al*., 1989).

Malondialdehyde (MDA) is a well characterized oxidation product of polyunsaturated fatty acids (PUFAs) in lipoproteins. PUFAs are most sensitive to hydroxyl radicals due to the close proximity of the double carbon bonds, which allows for an easier abstraction of hydrogen atoms from a methylene group (Halliwell and Gutteridge, 1991). The lipid peroxidation process influences membrane fluidity as well as the integrity of biomolecules associated with the membrane (membrane bound proteins or cholesterol). Since these lipids, in fish and other organisms, are in close juxtaposition to electron transport chains and heme iron proteins, which can act as sources of radical oxygen species under normal condition, the lipids may sustain high degrees of damage (Almroth et al., 2005).

Protein carbonyls (PC): Direct damage to proteins or chemical modification of amino acids in proteins during oxidative stress can give rise to protein carbonyls (Zusterzeel *et al*., 2001). The formation of carbonyl derivatives is non-reversible, causing conformational changes, decreased catalytic activity in enzymes and ultimately resulting in breakdown of proteins by proteases due to increased susceptibility (Almroth *et al*., 2005). It has been suggested that induction of protein carbonyl may serve as a surrogate biomarker for general oxidative stress (Reznick *et al*., 1992).

Energy reserves: Some of the best studied effects of pollutants on organisms are those expressed as changes in energetics. Bioenergetics models have been used for many years to study the fate of pollutants in aquatic systems (Rice, 1990). Not only can certain organisms accumulate high metal concentrations, but can also resist and could even adapt to sub-acute toxic stress by elevating their levels of energetics (Sivaramakrishna and Radhakrishnaiah, 2000). Toxic stress induces metabolic changes in organisms, which might lead to a depletion of the energy reserves and therefore, long-term changes in energetics can affect tissue growth, reproduction and the health of an organism (Verslycke et al., 2003). Thus, bioenergetics could link and extrapolate primary toxic effects at the (sub) cellular level to effects at the individual and population level.

The CEA methodology was developed as biomarker technique to assess the effect of toxicants on the energy budget of organisms. This technique provides an integrated quantification of an organism's energy budget. It is based on the biochemical assessment of changes in the energy reserves available (E_a) (total carbohydrate, protein and lipid content) and the energy consumption (E_c), which is estimated by measuring the electron transport activity (ETS) at the mitochondrial level. The ETS system consists of a complex chain of macroenzymes (e.g. cytochromes, flavoproteins and metallic ions) that transport electrons from catabolised foodstuff (sugar, lipid and protein as glucose, fatty acids and amino acids) to oxygen for energy generation. The synthesis and degradation of these enzymes is a function of the respiratory requirements of organisms. Thus, the measurement of the ETS system is directly linked to the cellular respiration rates or oxygen consumption process. The difference between E_a and E_c represents the net energy budget of the organism. The CEA assay allows and evaluation of specific interactions with sub-cellular mechanisms linked with the energy metabolism of an organism. The use of the CEA methodology may be useful to assess the effects of pollutants on the energy metabolism and for predicting long-term effects at higher levels of biological organisation (De Coen and Janssen, 1997; Verslycke *et al*., 2003).

The lower AChE activity and increased MT and CYP1A activities recorded in *H. vittatus* liver tissue during the LF2009 survey indicate that fish are more exposed and responding to metals and organic chemicals during this survey (Figure 48). The biomarkers of anti-oxidant effect indicate lower CAT activity with ensuing lipid and protein breakdown during the LF2010 survey (Figure 49). The lipid and protein catabolism is accompanied by higher energy consumption but also higher energy availability during this period (Figure 50).

4 THE LUVUVHU RIVER

4.1 Water quality

Physico-chemical characteristics

All in situ water quality variables measured in the Luvuvhu River (Table 34) fell within the TWQR for aquatic ecosystems (DWAF, 1996). Spatial trends were observed for temperature pH and conductivity, with an increase in all these variables as the river flows through the park. The DO levels were lowest at Site 4 during all surveys. The ammonium, chloride, COD, nitrate, nitrite and orthophosphate concentrations did not change during the different flow periods (Table 34). Sulphates, however, decreased during HF periods while turbidity increased. Nitrate concentrations increased with successive surveys from an oligotrophic state during LF2009 to a mesotrophic state during HF2010 and hypertrophic during LF2010 (DWAF, 1996).

High sulphate concentrations at Sites 3 and 4 may be attributed to the coal mines in the area (Angliss et al., 2001; EWISA, 2007), and the increase at Site 4 in comparison to the other sites may be due to the confluence of the Luvuvhu and the Mutale Rivers before Site 4, which brings in additional sulphates from coal mining activities in the lower Mutale River (Angliss et al., 2001).

Metal concentrations

Concentrations of dissolved Al exceeded the TWQR and CEV at all sites during all surveys (Table 35). The Al concentrations were highest at Sites 1 and 4 during all surveys, with Site 4 having the highest concentrations during all surveys. On a temporal scale the HF Al concentrations were lower than LF surveys. Dissolved As concentrations decreased over time and were substantially lower than the TWQR (DWAF, 1996). Dissolved Ag concentrations showed no spatial trends but were highest during the HF2010 survey and concentrations exceeded the AEV at Site 1. Dissolved Cd concentrations ranged from below TWQR (LF2009) to below detection limits (LF2010 and HF2011). There were, however, spikes in Cd concentrations resulting in high concentrations above the CEV at Site 1 (LF2010) and Site 4 (HF2011) and above the AEV at all sites during HF2010 survey. Dissolved Cr concentrations were below the TWQR at all sites during all surveys. The 2010 surveys had higher Cr concentrations than the other 2 surveys. Dissolved Co concentrations were similar during the LF2009, LF2010 and HF2011 surveys but much higher during the HF2010. Concentrations of dissolved Cu were all below detection limits except for sites 1 and 4 (LF2009) and Site 1 (HF2011). There were no spatial trends in Fe concentrations, but there was a temporal trend with Fe decreasing from 2009 to 2011. There were no temporal

or spatial patterns in dissolved Pb concentrations. The Pb concentrations were, however, higher during HF2010 and exceeded the TWQR at Sites 1 and 4 during LF2009). The CEV for Pb was exceeded at all sites during HF2010, Site 1 (LF2010) and Site 4 (HF2011). Dissolved Mn concentrations at all sites during all surveys were well below the TWQR, and concentrations were similar throughout all the surveys. During all surveys Mn concentrations showed a slight spatial trend as concentrations decreased downstream. Dissolved Ni concentrations were low and remained constant throughout all surveys at all sites. Dissolved Se concentrations remained low throughout all the surveys and were below the TWQR at all sites during all surveys. The U concentrations were not measured during the first survey, but U concentrations remained constant throughout the other surveys. Dissolved Zn concentrations were below detection limits at many of the sites during the study, particularly in later surveys. The Zn concentrations were above the TWQR at sites 1, 2 and 3 during LF 2009 and site 1 during HF 2011, and exceeded the CEV at site 4 (LF2009), sites 1 and 3 (LF2010) and site 2 (HF 2011). The Ca concentrations were highest during LF2009 compared to the other surveys after which the concentrations remained very similar. The Mg concentrations were substantially higher during LF2009 when compared to the other surveys and also remained constant throughout the remaining surveys. Dissolved Na and K concentrations showed similar trends as the Ca and Mg salts. Calcium concentrations increased down the river gradient, possibly due to calcerous conglomerate (Botha & De Wit, 1996) which forms a major part of the geology of the Luvuvhu River. Concentrations of Mg at Site 1 were higher than all other sites possibly due to the magnesite mine before the entrance of the river to the park (Angliss et al., 2001; EWISA, 2007), and decreased at Site 2. Increasing concentrations of Mg, as the river flows through the park, may be due to natural geological contributions after Site 2. The high concentrations of Ca, As and Zn at Site 4 may be due to the influx of these metals from mining activities in the lower Mutale River (Angliss et al., 2001) which flows into the Luvuvhu River before Site 4.

Metal concentrations in suspended matter

Metal concentrations in the suspended matter (Table 36) were higher for most metals when compared to dissolved metal concentrations. There were notable temporal patterns in metal concentrations with the highest concentrations of Pb and the lowest concentrations of Al, Cd, Fe, Mn and Se during LF2009. HF2010 had the highest concentrations of Al, As, Cr, Co, Cu, Fe, Mn, Ni and Zn and the lowest concentration of U. During the LF2010 the highest concentrations were Cd and Ag and the lowest concentrations Co, Pb, Ni, and U. The HF2011 survey had the highest concentrations of Se and U and the lowest As, Cr, Cu, Ni, Ag and Zn concentrations.

Table 34. Physico-chemical variables measured at four sites in the Luvuvhu and Mutale Rivers during two consecutive high and low flow periods between 2009 and 2011. NS represents no sample available.

Site and survey	Temp. ºC	pH	Oxygen %	Oxygen mg/L	Conductivity µS/cm	TDS mg/L	Ammonium mg/L	Chloride mg/L	COD mg/L	Nitrate mg/L	Nitrite mg/L	Phosphate mg/L	Sulphate mg/L	Turbidity NTU
LV-S1-09LF	28.6	7.22	127.2	8.66	155.6	77.9	0.08	<10	NS	<0.5	<0.01	0.09	130	4
LV-S2-09LF	27.6	7.28	131	12.01	160.7	80.8	0.14	<10	NS	<0.5	<0.01	<0.03	106	1
LV-S3-09LF	27.3	6.64	158.7	10.75	178.1	88	0.09	<10	NS	<0.5	<0.01	0.09	146	1
LV-S4-09LF	30.1	7.58	108	9.4	176	87.6	<0.03	<10	NS	<0.5	<0.01	<0.03	71	2
LV-S1-10HF	NS	NS	NS	NS	NS	NS	0.06 ± 0.01	1.87 ± 0.28	12.73 ± 0.61	0.96 ± 0.31	0.01 ± 0	0.39 ± 0.16	24 ± 5.57	4.33 ± 0.33
LV-S2-10HF	NS	NS	NS	NS	NS	NS	0.05 ± 0	7.37 ± 0.27	12.97 ± 1.24	0.93 ± 0.05	0.01 ± 0	0.36 ± 0.05	97.33 ± 82.83	4 ± 0.58
LV-S3-10HF	NS	NS	NS	NS	NS	NS	0.7 ± 0.13	7.63 ± 0.3	25.63 ± 0.19	2.65 ± 0.9	0.09 ± 0.01	1.44 ± 0.02	96.5 ± 65.5	78 ± 10.12
LV-S4-10HF	NS	NS	NS	NS	NS	NS	1.39 ± 0.11	9.73 ± 0.75	21.4 ± 0.3	2.05 ± 0.21	0.11 ± 0.02	1.24 ± 0.32	115.3 ± 50.99	80.67 ± 6.17
LV-S1-10LF	23.1	8.03	96.5	8.06	129	135	0.07 ± 0	7.57 ± 0.44	17.27 ± 0.2	0.87 ± 0.2	<0.01	0.37 ± 0.09	31.67 ± 7.62	4.67 ± 0.67
LV-S2-10LF	22.1	7.97	94.2	7.94	132.9	139	0.17 ± 0.09	9 ± 0.71	19.63 ± 1.56	11.4 ± 10.3	<0.01	2.88 ± 2.06	26.33 ± 3.71	6.33 ± .033
LV-S3-10LF	25.1	7.88	98.58	7.95	152.9	145	1.03 ± 0.48	8.67 ± 1.04	19.97 ± 1.84	12.41 ± 7.8	<0.01	0.8 ± 0.31	112 ± 63.26	6.33 ± 0.33
LV-S4-10LF	25.4	8.3	87.1	7.02	153.5	145	0.4 ± 0.15	10.03 ± 0.79	16.5 ± 0.5	20.5 ± 4.25	<0.01	1.15 ± 0.65	18.67 ± 2.73	8 ± 0.58
LV-S1-11HF	22.2	7.92	111.1	9.27	112	NS	0.26 ± 0.04	1.17 ± 0.15	14.27 ± 1.76	NS	<0.01	0.03 ± 0.01	73 ± 32.72	8 ± 0.58
LV-S2-11HF	21.9	7.9	119.2	10.06	113	NS	0.25 ± 0.11	1.67 ± 0.32	17.5 ± 0.75	NS	<0.01	0.02	64 ± 19	10.67 ± 0.88
LV-S3-11HF	22.3	7.98	122	10.36	115	NS	2.33 ± 1.09	1.73 ± 0.15	17.87 ± 0.09	NS	<0.01	0.01	85 ± 34.18	8.67 ± 0.33
LV-S4-11HF	22.7	8.27	96.7	8.24	121	NS	0.16 ± 0.04	4.5 ± 1.15	16.83 ± 0.24	NS	<0.01	0.01	92.67 ± 28.83	8 ± 0.1
Mutale 11HF	NS	NS	NS	NS	NS	NS	1.97 ± 0.88	1.7 ± 0.15	17.67 ± 0.35	NS	0.0	0.03 ± 0.01	81.33 ± 23.78	9 ± 0.2

Table 35. Mean ± standard error of the dissolved metal concentrations (µg/L and mg/L) in water from 4 sites in the Luvuvhu River and one site in Mutale River during two consecutive high and low flow periods between 2009 and 2011. BD represents samples below detection limits.

Site and survey	Al µg/L	As µg/L	Cd µg/L	Ca mg/L	Cr µg/L	Co µg/L	Cu µg/L	Fe µg/L	Pb µg/L	Mg mg/L	Mn µg/L	Ni µg/L	K mg/L	Se µg/L	Ag µg/L	Na mg/L	U µg/L	Zn µg/L
LV-S1-09LF	89.87	0.32	0.28	35.27	1.27	0.18	2.43	77.10	1.79	67.60	2.36	0.910	27.97	1.49	0.97	262.18	-	3.18
LV-S2-09LF	25.63	0.34	0.08	39.86	2.06	0.19	1.23	114.90	0.75	51.88	0.55	1.260	18.23	0.96	1.33	182.79	-	2.51
LV-S3-09LF	25.97	0.33	0.03	49.97	0.29	0.14	1.19	15.81	0.82	57.01 ±	0.76	0.890	20.56	1.13	0.86	215.4	-	2.3
LV-S4-09LF	61.93	0.37	0.11	52.01	0.53	0.19	1.86	53.17	1.25 ±	60.70	1.25	0.930	22.12	1.15	1.25	222.83	-	7.39
LV-S1-10HF	45 ± 5.13	0.33 ± 0.05	22.07 ± 0.07	4.56 ± 1.37	8.99 ± 5.61	14.22 ± 0.19	0.46 ± 0.15	43.67 ± 9.53	17.2 ± 0.16	2.06 ± 0.33	5.17 ± 0.31	0.78 ± 0.22	1.16 ± 0.64	0.31 ± 0.09	15.66 ± 0.18	4.12 ±1.32	5.85 ± 0.14	1.25 ± 1.25
LV-S2-10HF	49 ± 5.29	0.31 ± 0.02	21.33 ± 0.41	5.13 ± 0.45	2.08 ± 0.27	12.48 ± 0.88	0.42 ± 0.1	44.33 ± 5.36	15.61 ± 0.83	4.83 ± 0.04	4.47 ± 0.41	0.71 ± 0.07	1.3 ± 0.06	0.57 ± 0.12	13.94 ± 0.86	7.47 ± 0.07	6.91 ± 0.44	BD
LV-S3-10HF	42 ± 21.78	0.31 ± 0.03	19.94 ± 0.22	6.43 ± 0.67	2.04 ± 0.41	9.80 ± 0.35	0.73 ± 0.06	38 ± 19.86	12.98 ± 0.36	3.89 ± 0.14	3.73 ± 0.49	0.83 ± 0.17	1.62 ± 0.13	0.42 ± 0.14	11.19 ± 0.36	7.28 ± 0.42	8.13 ± 0.11	BD
LV-S4-10HF	99.67 ± 26.27	0.29 ± 0	18.45 ± 0.45	7.43 ± 0.62	1.89 ± 0.08	7.84 ± 0.51	0.48 ± 0.06	38.67 ± 1.67	10.84 ± 0.58	3 ± 0.17	3.05 ± 0.03	0.87 ± 0.05	1.66 ± 0.11	0.44 ± 0.16	9.12 ± 0.56	6.68 ± 0.57	8.7 ± 0.13	BD
LV-S1-10LF	64.67 ± 17.07	0.21 ± 0	12.14 ± 2.52	6.06 ± 0.35	4.01 ± 1.29	3.65 ± 1.2	0.54 ± 0.11	43.33 ± 1.2	5.53 ± 1.62	6.19 ± 0.22	4.03 ± 0.46	0.68 ± 0.11	1.15 ± 0.05	0.73 ± 0.06	4.39 ± 1.42	8.8 ± 0.46	9.51 ± 0.19	5.49 ± 5.49
LV-S2-10LF	60.33 ± 13.54	0.17 ± 0.02	BD	7.69 ± 0.83	4.25 ± 2.04	0.2	0.28 ± 0.02	52.33 ± 6.49	0.37 ± 0.01	6.31 ± 0.17	4.04 ± 0.86	0.64 ± 0.06	1.3 ± 0.17	1 ± 0.08	0.21 ± 0.01	9.45 ± 0.24	9 ± 0.04	BD
LV-S3-10LF	44.67 ± 1.67	0.15 ± 0.03	BD	7.94 ± 0.98	6.28 ± 3.62	0.20	0.51 ± 0.07	47.67 ± 7.69	0.52 ± 0.06	5.91 ± 0.17	4.58 ± 1.04	1.79 ± 0.83	1.57 ± 0.33	0.83 ± 0.14	0.19 ± 0.01	9.68 ± 0.71	8.79 ± 0.04	5.99 ± 5.99
LV-S4-10LF	131 ± 43.89	0.1 ± 0.01	BD	7.66 ± 0.47	2.1 ± 0.53	0.19 ± 0.01	0.17 ± 0.14	45.67 ± 3.76	0.44 ± 0.02	5.94 ± 0.46	1.87 ± 0.24	0.7 ± 0.12	0.94 ± 0.08	0.91 ± 0.12	0.22 ± 0.02	9.85 ± 0.74	8.59 ± 0.04	BD
LV-S1-11HF	31.33 ± 13.57	BD	BD	7.51 ± 0.3	0.21 ± 0.01	0.26	2.18 ± 1.21	13.67 ± 2.19	0.63 ± 0.07	5.48 ± 0.13	5.16 ± 0.21	0.76 ± 0.15	1.12 ± 0.18	0.01 ± 0.01	0.17 ± 0	7.82 ± 0.64	6.9 ± 0	2.76 ± 2.76
LV-S2-11HF	30.33 ± 6.49	BD	BD	6.84 ± 0.37	0.18 ± 0.02	0.24	0.67 ± 0.12	10.67 ± 3.28	0.58 ± 0.03	5.54 ± 0.2	2.9 ± 0.23	0.84 ± 0.25	1.41 ± 0.42	0.17 ± 0.09	0.16 ± 0	8.64 ± 0.9	6.92 ± 0	5.1 ± 5.1
LV-S3-11HF	30 ± 2.65	BD	BD	7.12 ± 0.21	0.15 ± 0.02	0.23	0.95 ± 0.04	10.33 ± 0.88	0.54 ± 0.02	5.55 ± 0.1	1.92 ± 0.1	0.65 ± 0.03	1.01 ± 0.06	BD	0.15 ± 0	7.93 ± 0.34	6.93 ± 0	BD
LV-S4-11HF	33 ± 5.69	0.07 ± 0.07	4.92 ± 4.92	6.35 ± 0.11	0.20 ± 0.03	1.69 ± 1.47	0.51 ± 0.15	14.67 ± 2.33	2.7 ± 2.16	4.56 ± 0.01	1.87 ± 0.25	0.63 ± 0.09	0.8 ± 0.1	BD	1.95 ± 1.8	7.63 ± 0.18	7.75 ± 0.81	BD
MUT-S1-11HF	38 ± 5.29	BD	BD	5.76 ± 0.83	0.13 ± 0.01	0.21 ± 0.01	0.81 ± 0.17	12 ± 2.08	0.52 ± 0.01	4.54 ± 0.79	1.76 ± 0.28	0.7 ± 0.03	0.81 ± 0.24	BD	0.13 ± 0	7.39 ± 0.34	6.95 ± 0.01	BD

Table 36. Suspended metal concentrations (µg/g dry mass, mean ± standard error, n=3) from water samples collected from 4 sites in the Luvuvhu River and one site in Mutale River between 2009 and 2011. BD represents samples below detection limits.

Site and survey	Al µg/g	As µg/g	Cd µg/g	Cr µg/g	Co µg/g	Cu µg/g	Fe µg/g	Pb µg/g	Mn µg/g	Ni µg/g	Se µg/g	Ag µg/g	U µg/g	Zn µg/g
LV-S1-09LF	36.15 ± 0.74	2.49 ± 0.03	0.11 ± 0	36.15 ± 0.74	0.55 ± 0.01	13.99 ± 0.15	5561 ± 35.65	14.83 ± 0.64	39.69 ± 0.18	4.93 ± 0.02	0.05 ± 0	2.16 ± 0.02	-	49.35 ± 0.29
LV-S2-09LF	36.4 ± 0.12	1.3 ± 0.2	0.11 ± 0.02	36.4 ± 0.12	0.52 ± 0.06	16.4 ± 0.75	3014 ± 361.6	11.62 ± 5.11	24.75 ± 2.54	5.77 ± 0.65	0.05 ± 0	2.15 ± 0.11	-	51.28 ± 1.28
LV-S3-09LF	39.11 ± 0.36	1.05 ± 0.08	0.11 ± 0.01	39.11 ± 0.36	0.51 ± 0	15.16 ± 0.05	4980 ± 1327	8.04 ± 1.73	15.92 ± 7.12	5.62 ± 0.76	0.05 ± 0	2.49 ± 0.28	-	50.6 ± 1.78
LV-S4-09LF	28.95 ± 0.19	0.91 ± 0.01	0.08 ± 0	28.95 ± 0.19	0.41 ± 0	15.04 ± 0.06	2309 ± 15.26	20.72 ± 5.02	29.99 ± 0.11	7.14 ± 0.03	0.05 ± 0	1.94 ± 0.01	-	54.16 ± 0.09
LV-S1-10HF	21034 ± 7359	5.47 ± 0.83	11.87 ± 0.45	187.9 ± 13.17	2.03 ± 2.03	72.31 ± 4.23	21319 ± 8151	BD	476.8 ± 159.2	1.02 ± 1.02	BD	BD	BD	181.6 ± 60.24
LV-S2-10HF	10712 ± 792	6.14 ± 1.09	12.06 ± 0.6	162.05 ± 7.62	BD	66.35 ± 3.6	9325 ± 555	BD	227.1 ± 17.48	BD	BD	1.21 ± 1.21	BD	181.1 ± 35.54
LV-S3-10HF	74298 ± 3874	4.74 ± 0.27	3.25 ± 0.34	145.34 ± 5.02	4684 ± 538.1	170.3 ± 2.64	84724 ± 2452	4.74 ± 0.4	3760.6 ± 431.9	118.34 ± 2.39	19.42 ± 0.13	BD	BD	224.6 ± 6.23
LV-S4-10HF	69400 ± 33835	3.64 ± 1.78	2.26 ± 1.24	76.66 ± 37.54	1143 ± 1134	74.19 ± 35.63	48471 ± 23386	5.57 ± 2.69	3170.4 ± 1801	31.3 ± 15.12	7.05 ± 3.68	BD	BD	139.7 ± 65.1
LV-S1-10LF	5778 ± 3371	3.41 ± 0.45	7.81 ± 0.33	68.17 ± 10.47	BD	26.12 ± 0.99	1273 ± 497	BD	124.2 ± 11.29	BD	BD	0.4 ± 0.4	BD	81.23 ± 17.51
LV-S2-10LF	7368 ± 1478	5.34 ± 1.68	9.19 ± 1.15	114.64 ± 9.17	0.32 ± 0.32	47.43 ± 7.02	7259 ± 1784	BD	179.9 ± 37.84	BD	BD	0.8 ± 0.8	BD	90.3 ± 22.89
LV-S3-10LF	4740 ± 405	4.32 ± 0.22	7.29 ± 0.21	106.25 ± 5.68	BD	32.83 ± 4.66	3406 ± 341	BD	117.4 ± 5.21	BD	BD	172.6 ± 117.26	BD	69.16 ± 20.52
LV-S4-10LF	8903 ± 218	4.6 ± 0.23	8.25 ± 0.22	113.01 ± 10.15	0.02 ± 0.02	34.67 ± 5.2	7212 ± 112	BD	92.5 ± 1.12	BD	0.9 ± 0.71	101.39 ± 101.39	BD	39.25 ± 5.58
LV-S1-11HF	10873 ± 1623	1.41 ± 0.74	0.02 ± 0.01	7.28 ± 3.64	0.67 ± 0.33	14.53 ± 5.01	11556 ± 154	10.59 ± 1.66	168.5 ± 17.2	BD	23.5 ± 12.24	BD	0.15 ± 0.04	32.81 ± 15.7
LV-S2-11HF	8300 ± 644	1.25 ± 0.21	BD	BD	BD	3.14 ± 1.06	9612 ± 1048	5.36 ± 1.99	110.8 ± 10.29	BD	BD	BD	0.11 ± 0.02	BD
LV-S3-11HF	9251 ± 394	0.55 ± 0.15	0.01 ± 0.01	BD	0.02 ± 0.02	13.71 ± 5.57	10547 ± 401	5.04 ± 0.99	103.8 ± 5.96	BD	7.12 ± 5.59	0.17 ± 0.13	0.11 ± 0.01	4.64 ± 4.64
LV-S4-11HF	12673 ± 972	BD	2.94 ± 2.94	58.53 ± 58.53	0.13 ± 0.13	7.95 ± 4.08	12197 ± 1144	3.05 ± 1.54	93.1 ± 7.24	BD	BD	0.02 ± 0.02	0.12 ± 0.06	19.04 ± 15.37
Mutale-11HF	11554 ± 177	1.29 ± 1.04	BD	BD	0.6 ± 0.51	19.68 ± 3.53	14847 ± 2160	4.08 ± 1.37	133.4 ± 26.75	BD	BD	BD	0.14 ± 0.02	27.42 ± 15

The PCA biplot depicting the spatial and temporal patterns in physico-chemical variables and metals in the Luvuvhu River (Figure 51) did not reveal any spatial patterns within surveys. There was a distinct separation between the different surveys (temporal) with greater spatial variation between sites during HF periods. This is indicated on the PC1 axis that explained 51% of the variation in the data. The HF2010 is more distinct than any of the other survey periods due to higher levels of Co, Cd, Al, Mn, Fe, Cr, Cu, Zn, As and Ni. The sites from the LF2009 period grouped together and separate from other surveys due to higher dissolved salt concentrations (Mg, Na, K and Ca), as well as higher TDS values and higher chloride and sulphate concentrations. Sites from the LF2010 survey are grouped and different to other surveys based on the higher DO concentrations and pH values. The HF2011 survey differed from the other surveys due to higher U concentrations (both dissolved and suspended), higher suspended Al and Fe concentrations, as well as increased ammonium and higher turbidity and COD. There are also notable differences between high flow and low flow periods. Low flow periods are associated with higher temperatures, DO, and salt concentrations, whereas high flow periods are associated with higher dissolved and suspended metal concentrations. Some dissolved metal concentrations (Se, Fe, Cu, Zn and Ni) were also associated with the low flow periods.

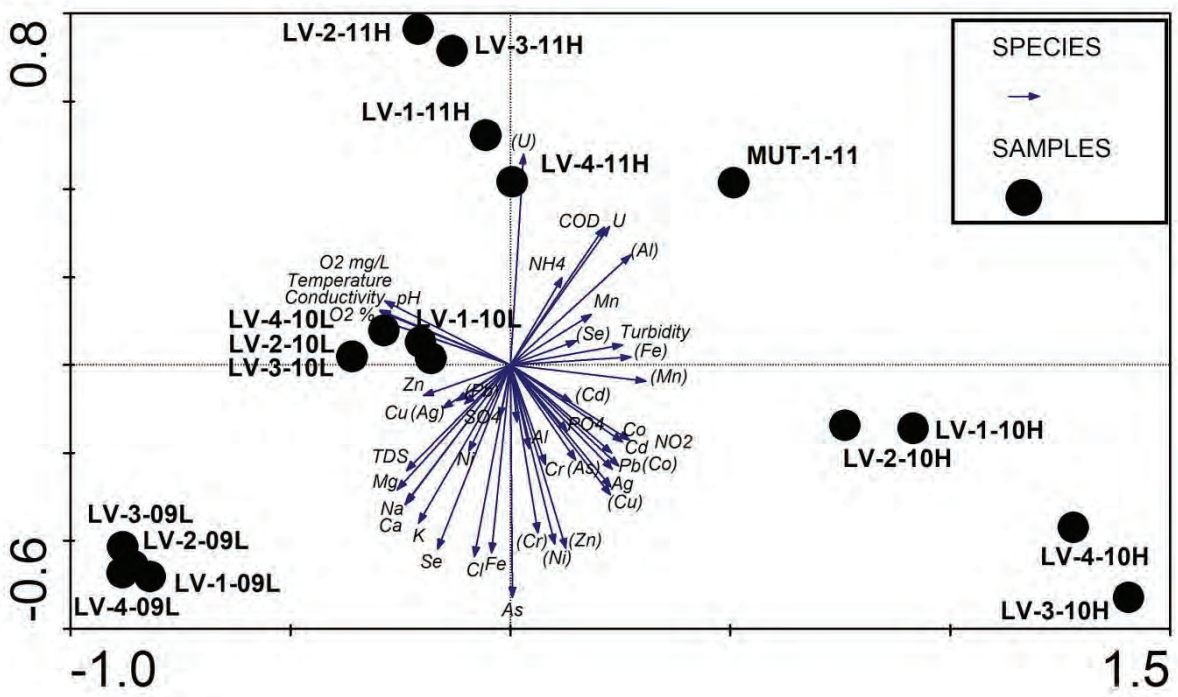

Figure 51. PCA biplot for the Luvuvhu River indicating spatial and temporal patterns of physico-chemical parameters, dissolved and suspended (in parentheses) metal concentrations. The biplot describes 68.3% of the variation in the data, with 50.6% is displayed on the first axis and 17.7% on the second axis.

The ATI scores associated with water quality variables from the Luvuvhu (Table 37) ranged between 55 and 87. There were distinct spatial and temporal variation with the highest ATI scores recorded during the LF2009 survey and an increasing water quality trend was found as the river flows through the park. The HF2010 and LF2010 surveys showed similar trends with high ATI scores at Sites 1 and 2 and then decreasing at Sites 3 and 4 (Figure 52). This was most evident during the HF2010 survey when scores at Sites 3 and 4 dropped to 55 respectively from a high of 78. Based on the classification system developed by Wepener and Vermeulen (1999) the ATI scores at Sites 3 and 4 during the HF2010 survey indicate that there is a moderate risk of fish populations being at risk. As with the Olifants and Letaba Rivers, metal concentrations had no effect on the ATI scores. But unlike these rivers the water quality of the Luvuvhu River was not as affected by high sediment loads, i.e. turbidity. Turbidity gave the lowest scores at Sites 1 and 4 during LF2009 with scores of 68 and 74 respectively, as well as at Sites 2 and 4 during HF2011 with scores of 58 and 60 respectively. These scores reflect water quality ranging between largely to moderately modified with potential risks to sensitive fish species. The ATI scores at sites in the Luvuvhu River were predominantly influenced by high nutrient concentration, specifically increased ammonium and orthophosphates. The combination of these nutrients to deteriorating water quality is especially evident during the 2010 surveys, with increased ammonium concentrations affecting scores during HF2011. Ammonium scores ranged between 24.5 and 59, and orthophosphate scores ranged from 3.1 to 42.17.

Table 37. Individual ATI scores and corresponding lowest rating scores for sites on the Luvuvhu River during all surveys of the study.

Sampling Site	Index score	Lowest Rating
LV-S1-09LF	82.51	Turbidity **(68)**
LV-S2-09LF	87.57	Ammonium **(64.1)**
LV-S3-09LF	81.46	pH **(46.9)**
LV-S4-09LF	94.91	Turbidity **(74)**
LV-S1-10HF	75.79	Orthophosphates **(39.5)**
LV-S2-10HF	77.56	Orthophosphates **(42.17)**
LV-S3-10HF	55.35	Orthophosphates **(3.1),** Ammonium **(28)**
LV-S4-10HF	55	Orthophosphates **(5.1),** Ammonium **(24.5)**
LV-S1-10LF	78.34	Orthophosphates **(39.5)**
LV-S2-10LF	70.37	Orthophosphates **(10),** Ammonium **(59)**
LV-S3-10LF	67.48	Orthophosphates **(14.7),** Ammonium **(26.5)**
LV-S4-10LF	66.8	Orthophosphates **(6.4),** Ammonium **(36)**
LV-S1-11HF	82.43	Ammonium **(48.3)**
LV-S2-11HF	82.98	Ammonium **(49),** Turbidity **(58)**
LV-S3-11HF	78.64	Ammonium **(19)**
LV-S4-11HF	84.5	Turbidity **(60)**
MUT-S1-11HF	73.98	Ammonium **(21.1)**

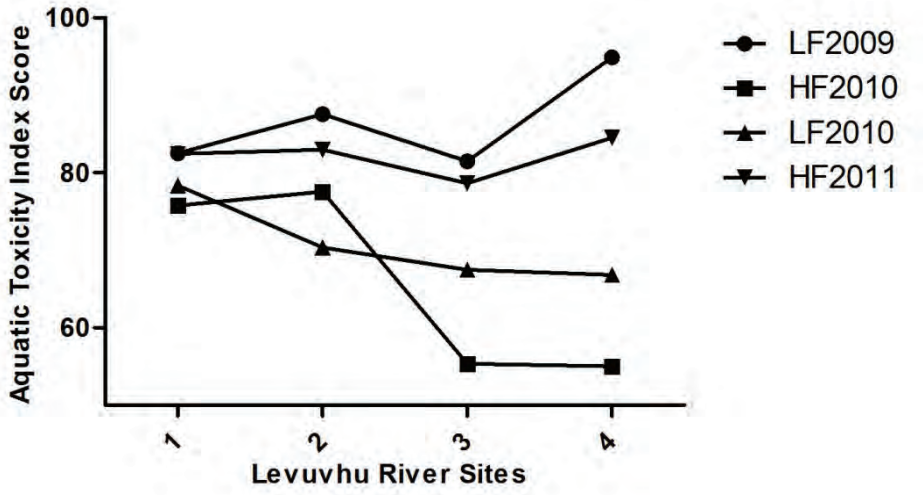

Figure 52. Aquatic toxicity index (ATI) rating scores of water quality at all sites along the Luvuvhu River during all surveys.

4.2 Sediment

Physical characteristics

The moisture content of sediments from the Luvuvhu River sites during all surveys (Table 38) remained constant (20-30%), except for Site 2 during HF2010 (14.71%) and HF2011 (39.39%). The percentage organic matter found at all sites throughout the various surveys (Table 38) ranged between 0.45% and 5.68%. No spatial or temporal trends were observed. During the LF2009 survey Sites 1 to 3 had a low organic content and Site 4 had medium organic content. However during the HF2010 survey only sediment from Site 1 had a low organic content, Site 2 had a moderate to low organic content, whilst Sites 3 and 4 had a medium organic content. Organic content of sediments from the LF2010 survey were low at Sites 2 and 3, moderate to low at Site 1 and high at Site 4. During the HF2011 survey, Sites 1 and 3 had low organic content whilst Site 4 had moderate to low levels and Site 2 had high levels of organic content. The particle size distribution also showed variable spatial and temporal rends (Table 38). During the LF2009 survey Site 2 was dominated by medium sand (< 500 µm) when compared to the other 3 sites which had a predominantly courser sand (> 500 µm). The opposite trend was recorded during the next survey, HF2010, where only Site 2 had a predominantly large grain size. All sites during the last two surveys (LF2010 and HF2011) were dominated by fine sand to mud.

Metal concentrations

No spatial or temporal trends in total metal concentrations were observable for any of the metals tested during the Luvuvhu River surveys (Appendix A5). Total Al concentrations for Sites 1 (LF2009), 2 (LF2009 and HF2011) and 3 (LF and HF2010) were much higher than other sites during any of the surveys. The total As concentration for Site 4 during the LF2010 survey was much higher compared to other site during any of the surveys. Sites 1 and 2 during the LF2009 survey had the highest total Cd concentrations. Total Co concentrations were lowest during the LF2009 survey with concentrations during the other surveys being much higher. Total Cu concentrations were lowest at Site 1 (HF2010) and highest at Sites 3 (LF2009) and 4 (LF2010). Total Pb and Mn concentrations were highest during the LF2009 survey. Total Ni concentrations were highest at Sites 3 (LF2009) and 4 (LF2010). Total Se concentrations remained similar throughout sites and surveys. Total Ag concentrations were highest at Sites 1 and 3 during the LF2009 survey. The highest total Zn concentrations were at all sites during the LF2009 survey and Site 4 during the LF2010 survey.

The spatial sequential extraction results are presented in Figure 53 and Figure 54 and are based on the combined survey data (4 surveys) for each site. Site 1 had the highest bioavailable fractions of Ag, Cd, Cr and Pb, and the lowest for As, Mn, Ni, U, and Zn. Site 2 had the highest bioavailable Fe, Mn, U, and Zn and the lowest bioavailability of Al. Site 3 had the highest bioavailability of Mn while Site 4 had the highest bioavailability of Al, As, Fe and Ni, and the lowest bioavailability of Cd. The bioavailability of Co and Cu, Se, U, was similar throughout sites. The bioavailability of As and Ni increased downstream from Site 1 to 4, whilst the bioavailability of Cd decreased.

Table 38. Percentage moisture, organic content and particle size distribution from selected sites on the Luvuvhu River during 4 separate surveys.

	Moisture content	Organic content	Particle size (µm)					
Sample			>4000	>2000	>500	>212	>50	>0
LV-S1-09LF	-	0.48	25.97	15.58	38.12	19.88	0.21	0.24
LV-S2-09LF	-	0.86	0.19	0.26	5.93	62.34	20.84	10.44
LV-S3-09LF	-	0.64	0.46	1.06	58.69	23.61	15.87	0.31
LV-S4-09LF	-	2.78	22.21	14.74	35.38	14.02	9.10	4.54
LV-S1-10HF	23.41	0.65	10.23	12.02	20.24	44.15	8.26	5.10
LV-S2-10HF	14.71	1.04	37.89	12.52	13.71	16.47	13.64	5.78
LV-S3-10HF	29.11	3.55	4.32	7.93	10.31	22.47	43.78	11.19
LV-S4-10HF	28.38	2.89	6.38	9.02	10.01	18.74	42.06	13.80
LV-S1-10LF	22.75	1.21	11.32	10.52	22.16	44.09	7.27	4.65
LV-S2-10LF	24.66	0.96	7.92	5.90	9.53	35.36	35.74	5.55
LV-S3-10LF	23.27	0.92	3.06	7.04	32.64	41.49	10.45	5.33
LV-S4-10LF	29.25	5.68	15.54	9.43	15.19	14.95	33.96	10.93
LV-S1-11HF	27.99	0.45	1.57	5.68	7.53	64.84	14.21	6.17
LV-S2-11HF	39.39	5.16	6.42	7.88	11.70	28.43	34.77	10.80
LV-S3-11HF	25.83	0.68	1.61	3.97	27.15	49.12	11.84	6.30
LV-S4-11HF	29.08	1.59	1.60	6.51	7.75	33.09	41.10	9.96

The temporal data presented in Figure 55 and Figure 56 are based on combined site data for each survey period. The LF2009 survey had the highest bioavailability of Ag, Al, Cd, Co, Cr, Cu, Mn, Pb and Zn. The LF2010 survey had the highest bioavailability of As while the HF2011 survey had the highest bioavailability of Mn. The bioavailability of U remained similar throughout the surveys. The bioavailability of Fe increased with successive surveys whereas the bioavailability of Cd and Cr decreased. The bioavailability of Al, Ni, Se and Zn were highest during low flow periods. The acid soluble fraction (BCR-A) of Fe and Zn are highest during low flow periods.

The PCA biplot based on total metal concentrations and physical sediment characteristics at the four sites in the Luvuvhu River (Figure 57) revealed temporal differences between the 2009 survey and the 2010 and 2011 surveys. These separate groupings were attributed to Mn, Pb, Ag, Cd, Al and Fe. The percentage coarse sand was also found to be higher during the 2009 survey than the 2010 and 2011 surveys. The 2010 and 2011 surveys were characterised by higher mud and very fine sand fractions. The Co and U concentrations were also higher during the 2010 and 2011 surveys when compared to the 2009 survey. Site 4 during LF2010 was grouped separately while Site 1 during the LF2009 also grouped separately. Apart from these two sites no other spatial differences between the various sites was noted.

Organic contaminant concentrations

During the surveys conducted in the Luvuvhu River, 21 of the 22 organochlorine compounds tested for were present (Table 39). Only o,p'-DDT was not measured in sediments in sediments from any of the sites during both surveys. Only trace amounts of the organic contaminants were found during the surveys. During the LF2010 survey, Site 3 had the least amount of organic contaminants with 13 of the 22 tested for present, Site 1 had 16 and Sites 2 and 4 had the most organic contaminants present (18 out of 22). During HF2011 Site 4 had the least amount of organic contaminants present with 9 of the 22. Site 1 had 15 and Sites 2 and 3 had the highest with 17 and 18 of the 22 respectively.

The PCA ordination of the temporal and spatial distribution of organochlorines in sediments from the Luvuvhu River explained nearly 74% of the variation in the data (Figure 58). The spatial differences between Sites 2 and 4 and Sites 1 and 3 were explained on the PC1 axis (44%) whilst temporal differences between the two flow periods were explained on the PC2 axis (30%). Sites 1 and 3 during the HF2011 period was characterised by medium sand and heptachlor, while Sites 2 and 4 are dominated by very fine sand and mud with high moisture content and high concentrations of o,p'- and p,p'-DDE, breakdown products of chlordane and heptachlor, and endrin.

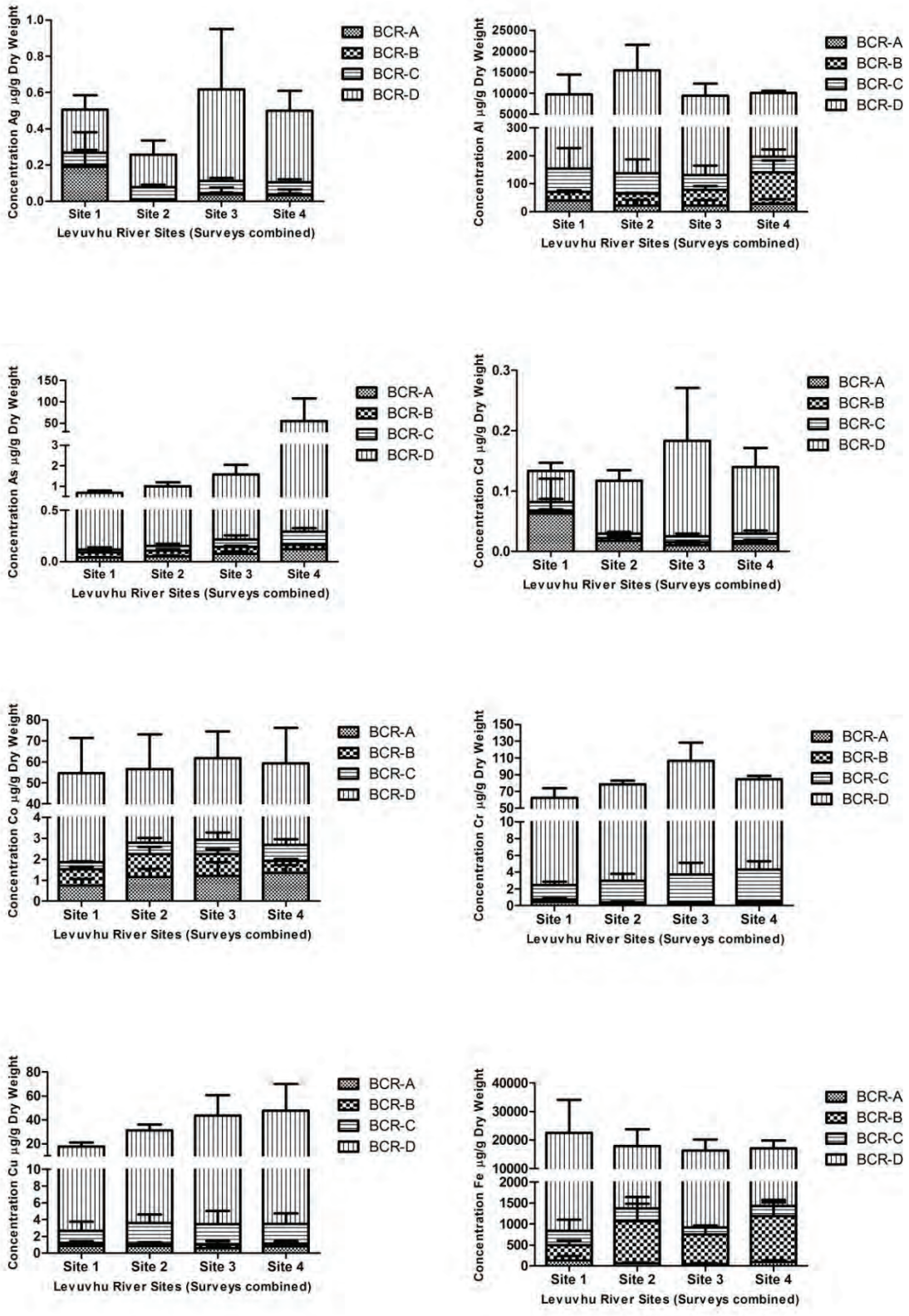

Figure 53. Metal concentrations (µg/g) present in the various fractions of sediment collected from sites on the Luvuvhu River. Data from the various surveys were combined per site. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

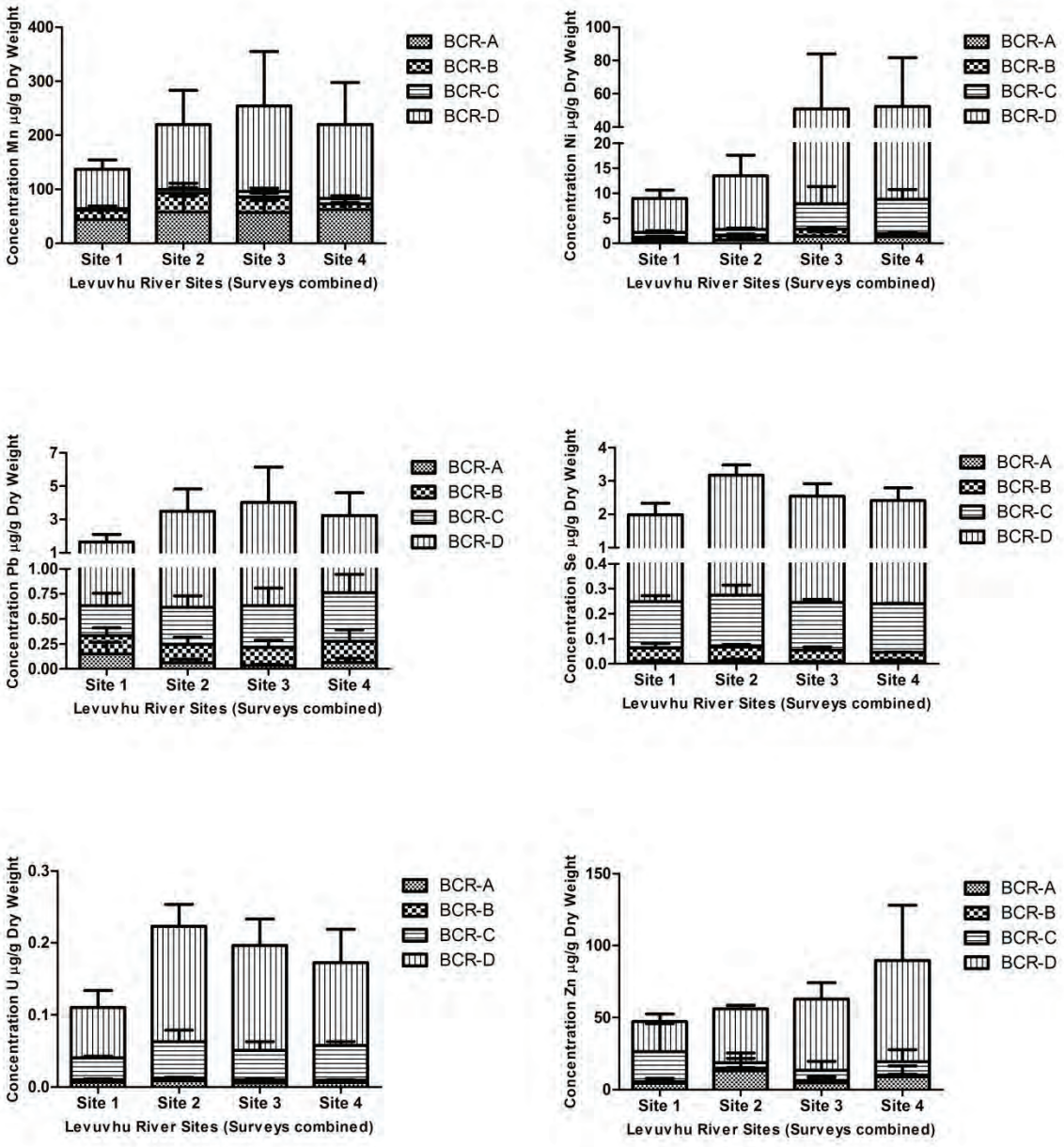

Figure 54. Metal concentrations (µg/g) present in the various fractions of sediment collected from sites on the Luvuvhu River. Data from the various surveys were combined per site. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

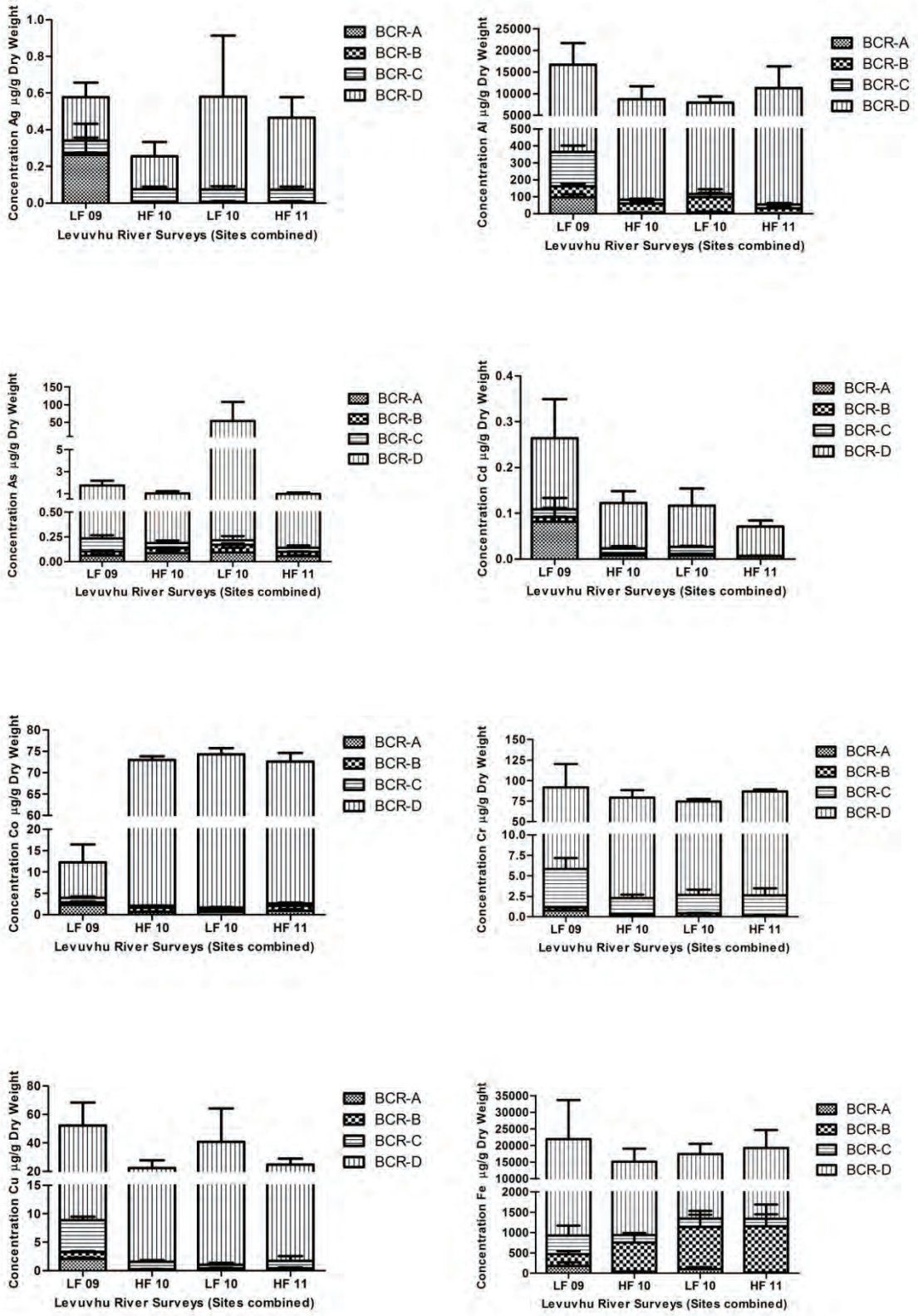

Figure 55. Metal concentrations (µg/g) present in the various fractions of sediment collected from sites on the Luvuvhu River. Data from the various sites were combined per survey.

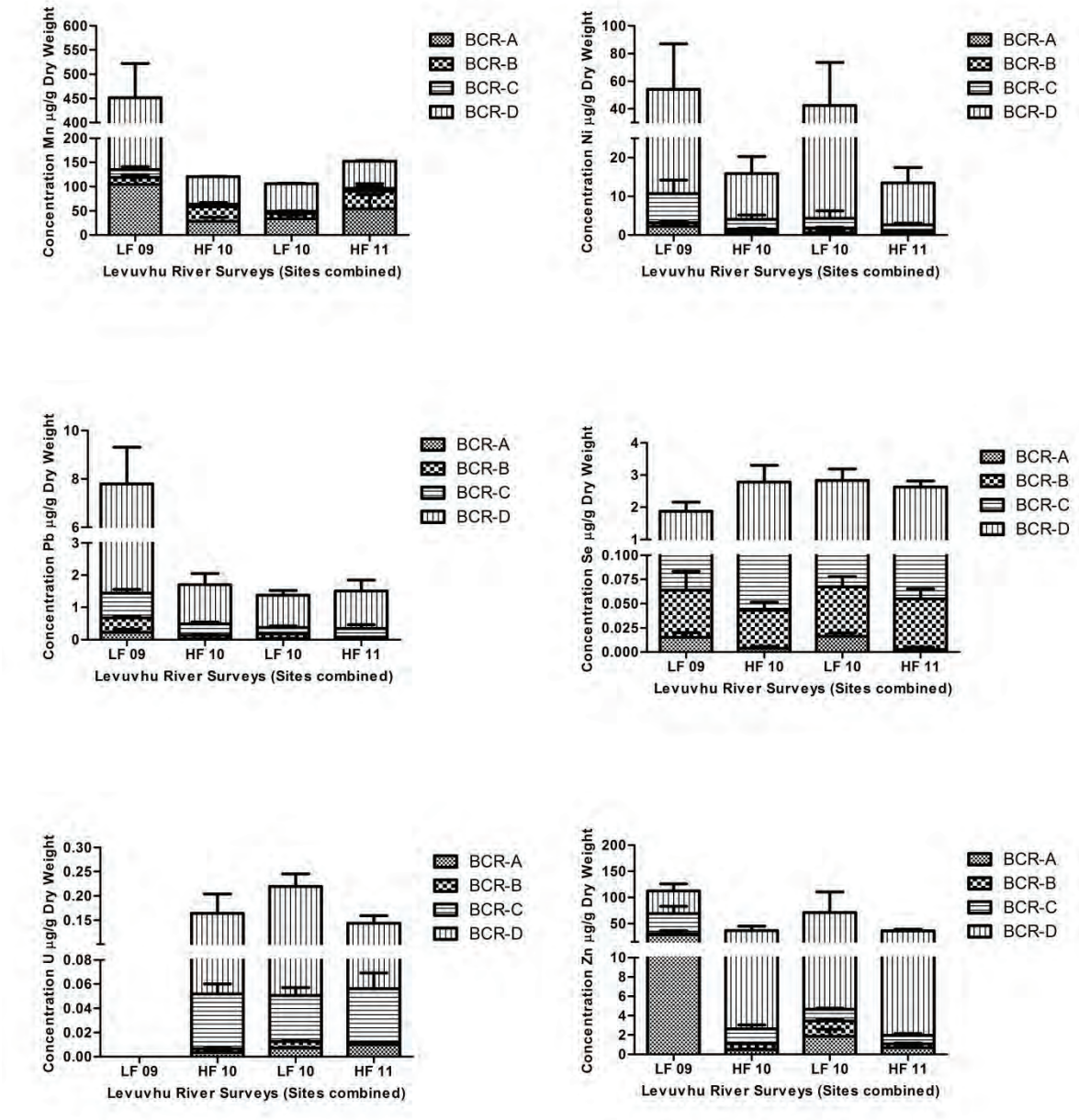

Figure 56. Metal concentrations (µg/g) present in the various fractions of sediment collected from sites on the Luvuvhu River. Data from the various sites were combined per survey. BCR-A – acid soluble fraction, BCR-B – reducible fraction, BCR-C – oxidizable fraction and BCR-D – non-bioavailable fraction.

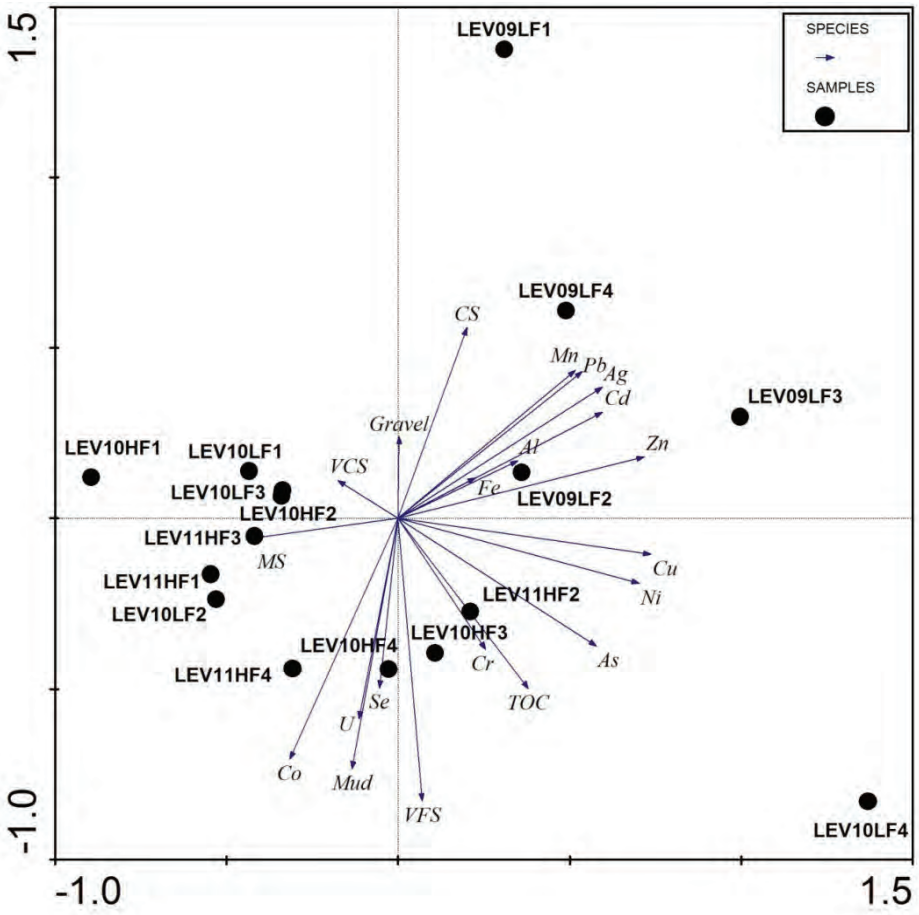

Figure 57. PCA biplot for the Luvuvhu River indicating differences in total metal concentrations and grain size at sites during the various surveys. This biplot describes 57.9% of the variation in the data, where 34.1% is displayed on the first axis, while 23.8% is displayed on the second axis.

Sites 1 and 4 during LF2010 are dominated by very course sand particles and gravel with high percentage organic material (Figure 58). These sediments are characterised by higher concentrations of DDT, DDD, HCHs, HCBs, Aldrin, Dieldrin and cis-Chlordane.

Table 39. Organic contaminant concentrations (ng/g dry weight) in sediments collected from the Luvuvhu River for the LF2010 and HF2011 surveys.

	Organic contaminants ng/g dry weight										
Sample	**a-HCH**	**HCB**	**b-HCH**	**r-HCH**	**d-HCH**	**Heptachlor**	**Aldrine**	**Oxy-Chl**	**cis-Hep-epox**	**trans-Hep-epox**	**OP-DDE**
LV-S1-10LF	0.39	0.01	0.41	0.17	-	-	0.18	-	0.18	-	0.95
LV-S2-10LF	0.31	0.06	0.57	0.13	0.26	-	0.16	0.16	0.24	-	0.68
LV-S3-10LF	0.22	-	0.22	0.08	0.00	0.21	0.13	0.13	0.00	0.18	0.28
LV-S4-10LF	0.21	0.02	0.29	0.10	0.24	-	0.15	0.19	0.19	0.18	0.23
LV-S1-11HF	0.21	-	0.22	0.16	-	0.24	-	0.16	-	-	0.13
LV-S2-11HF	0.21	0.02	0.33	0.11	-	0.23	0.21	0.00	0.21	-	0.27
LV-S3-11HF	0.24	0.01	0.27	0.12	0.24	0.23	0.14	0.17	0.20	0.21	0.28
LV-S4-11HF	-	-	-	1.56	-	-	-	0.62	0.63	1.43	1.25

	Organic contaminants ng/g dry weight										
Sample	**trans-chlordane**	**trans-Nonane**	**cis-Chlordane**	**PP-DDE**	**Dieldrin**	**OP-DDD**	**Endrine**	**OP-DDT**	**cis-Nonach**	**PP-DDD**	**PP-DDT**
LV-S1-10LF	-	0.23	0.18	0.57	0.28	0.50	0.33	-	0.17	0.24	0.72
LV-S2-10LF	-	0.36	0.26	1.31	0.29	0.47	0.37	-	0.17	0.32	0.72
LV-S3-10LF	-	0.14	0.15	0.24	-	0.18	-	-	-	0.08	-
LV-S4-10LF	-	0.19	0.15	0.51	0.28	0.34	-	-	0.54	0.14	0.55
LV-S1-11HF	0.13	0.12	0.18	0.24	-	0.30	0.41	-	0.14	0.11	0.57
LV-S2-11HF	0.21	0.34	0.18	1.16	-	0.53	0.40	-	2.69	0.32	0.60
LV-S3-11HF	-	0.17	0.17	0.35	0.29	0.36	-	-	-	0.10	0.57
LV-S4-11HF	-	0.63	-	1.56	-	-	0.92	-	0.67	-	-

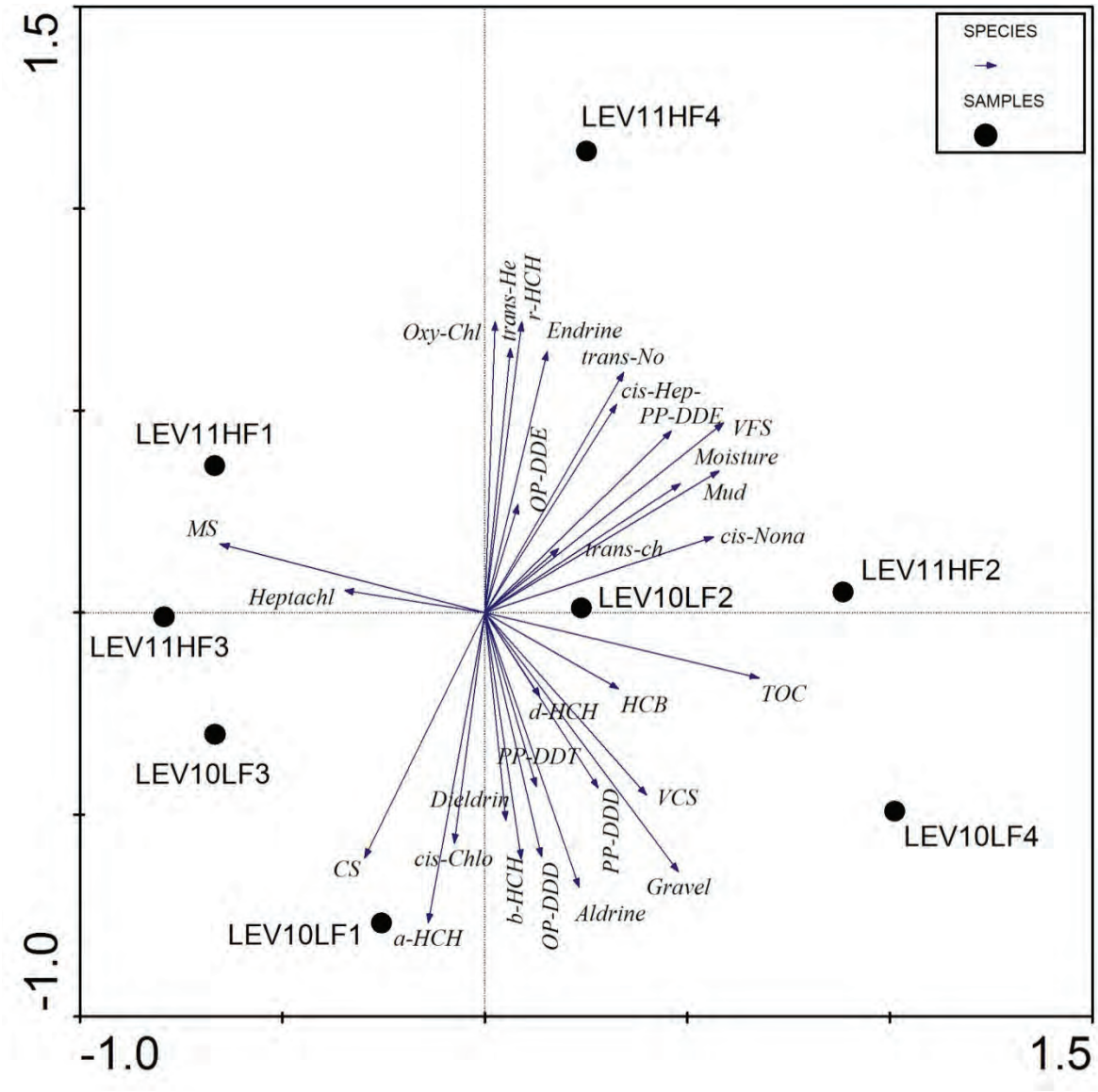

Figure 58. PCA biplot for the Luvuvhu River indicating differences in total organic contaminant concentrations and grain size at sites during the various surveys. This biplot describes 73.4% of the variation in the data, where 43.9% is displayed on the first axis, while 29.5% is displayed on the second axis.

4.3 Habitat

Results from the velocity-depth classes and biotope diversity observed in this study are presented in Table 40 and Table 41.

Table 40. The dominant velocity-depth classes and biotope diversities observed in this study for each site on the Luvuvhu River during the 2009 survey [as determined using method of Dallas (2005)].

	Site 1	Site 2	Site 3	Site 4
Invertebrate habitat				
Stones in current	4	4	4	3
Stones out of current	4	3	3	1
Vegetation	3	3	4	3
GSM	3	3	3	4
Fish habitat				
Slow-deep	4	5	4	3
Fast-deep	3	4	3	0
Slow-shallow	3	2	3	4
Fast-shallow	4	4	4	4
0=absent, 1=rare, 2=sparse, 3=moderate, 4=abundant and 5=very abundant				

Table 41. The dominant velocity-depth classes and biotope diversities observed in this study for each site on the Luvuvhu River during the 2010 survey [as determined using method of Dallas (2005)].

	Site 1	Site 2	Site 3	Site 4
Invertebrate habitat				
Stones in current	3	4	4	3
Stones out of current	3	4	3	1
Vegetation	3	3	3	3
GSM	3	3	3	3
Fish habitat				
Slow-deep	4	5	4	3
Fast-deep	1	3	4	0
Slow-shallow	4	2	2	4
Fast-shallow	4	4	4	4
0=absent, 1=rare, 2=sparse, 3=moderate, 4=abundant and 5=very abundant				

4.4 Macroinvertebrates

According to the State of Rivers Report (2001) from the RHP survey, the macroinvertebrate communities of the Luvuvhu River within the KNP were found to be in a natural state/class. As mentioned previously, the Luvuvhu River sites fall within two ecoregions. Site 1 and Site 2 fall within the Soutpansberg Ecoregion, and Site 3 and Site 4 in the Lowveld Lower Ecoregion (Kleynhans et al., 2005). As a result, two different biological bands were used two ascertain their EC values (Figure 59 &Figure 60).

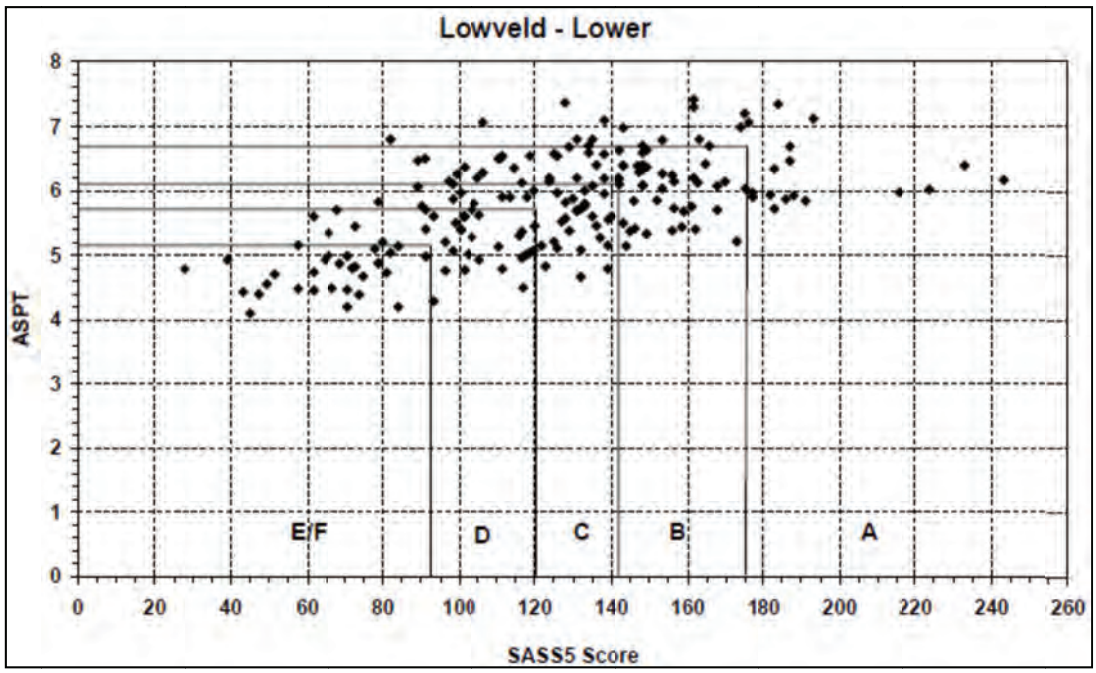

Figure 59. Biological bands for the Lowveld Lower Zone calculated using percentiles from historical data (Dallas, 2007).

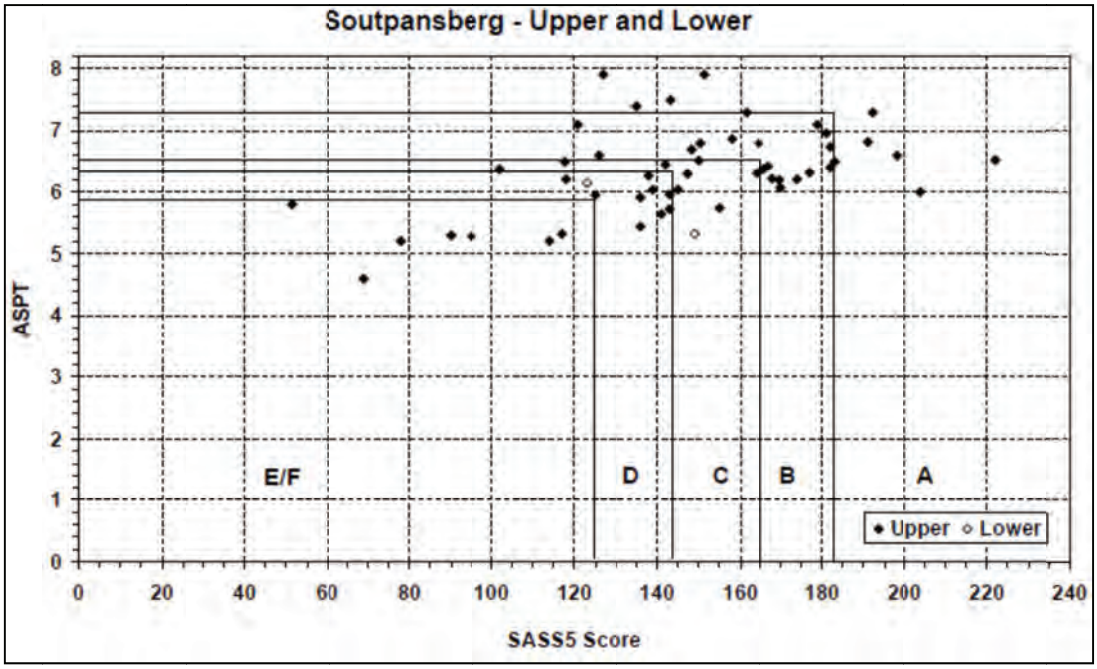

Figure 60. Biological bands for the Soutpansberg Upper and Lower Zones calculated using percentiles from historical data (Dallas, 2007).

The SASS5 results obtained from this study (Table 42) show that the communities were in a seriously modified state for the LF2009 period (Class E/F) and in a fair/good state (Class C/B) for the 2010 period. The highest SASS5 score was 142 at Site 1 during the 2010 survey period and the lowest was 71 for Site 4 during the LF2009 survey period. The highest ASPT was 6.24 at Site 1 for the 2010 survey period, with the lowest being 4.8 at Site 3 for the LF2009 survey period. Looking at these data as a whole, the scores are much lower than expected. Based on the macroinvertebrate communities, the Luvuvhu River was previously thought as a natural system (State of Rivers Report, 2001). The overall decrease in class status from the 2001 State of the Rivers Report for the Luvuvhu, is of concern and may be attributed to an increase in the anthropogenic influence on the Luvuvhu River from upstream activities. The industrial, domestic and agricultural sectors have increased in the catchment since the last comprehensive survey, but the full effects are not yet known (Fouche et al., 2005). However, particular spatial and temporal trends that have developed can be seen. Figure 61 and Figure 62 show these trends for the SASS5 scores and ASPT scores, respectively.

Table 42. SASS5 scores and ASPTs and the consequent ECs for all sites on the Luvuvhu River for both 2009 and 2010 sampling surveys.

	SASS5 score	ASPT	EC
1LUV09	120	5.34	E/F
2LUV09	99	6.19	E/F
3LUV09	72	4.8	E/F
4LUV09	71	4.73	E/F
1LUV10	181	6.24	B
2LUV10	141	5.9	C/D
3LUV10	142	5.91	C/B
4LUV10	141	6.13	C/B

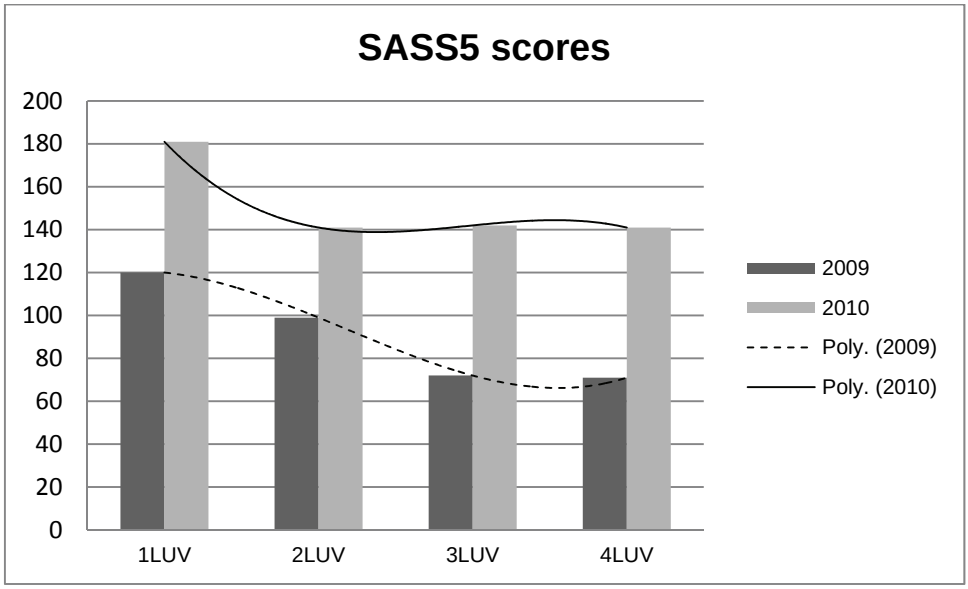

Figure 61. SASS5 scores for all sites on the Luvuvhu River for both survey periods.

The SASS5 scores for the Luvuvhu River (Figure 61) show the same spatial and temporal trends as the Olifants River (Figure 28), this being a spatial decrease of scores downstream along the length of the river, and a temporal trend of scores increasing from the LF2009 sampling period to the LF2010 sampling period. This is of interest as it correlates with the trends of the fish communities within the Luvuvhu River. These overall trends can then be compared to the fish and macroinvertebrate trends seen within the Olifants River. The trends are similar and show the same temporal and spatial variations for both the LF2009 survey and the LF2010 survey.

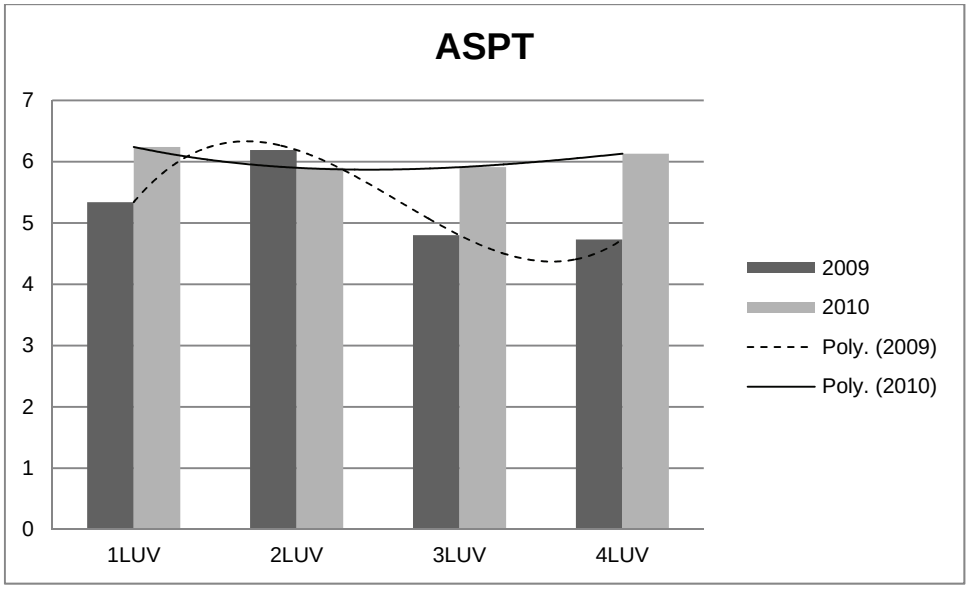

Figure 62. ASPT scores for all sites on the Luvuvhu River for both survey periods.

As mentioned previously, the ASPT is more accurate in determining trend patterns. Figure 62 shows that the trends seen using the ASPT scores are not as pronounced as those using the SASS5 scores (Figure 61). There is still a spatial trend with the ASPT decreasing along the length of the river for the 2009 period, but this is not evident during the 2010 period, with the ASPT averaging at around 6. There is, however, a temporal trend, with the LF2009 period having lower ASPT scores than the LF2010 period. This is important as it corroborates the trends of the fish communities within the Luvuvhu River. These overall trends can then be compared to the fish and macroinvertebrate trends seen within the Olifants River. It is interesting to note that the Luvuvhu River does not have a salinisation problem like the Olifants River has. Despite salinisation, the Luvuvhu River still exhibits the same trend of decreasing SASS5 scores when compared to previously published literature. It can be attributed to the increased abstraction of the Luvuvhu River and the consequent effects it has on available habitat and habitat biotopes essential for the survival of macroinvertebrate communities. Vlok and Engelbrecht (2000) showed the adverse effects of abstraction on the fish communities of the Letaba River and Fouche and Vlok (2010) showed the adverse effects of abstraction and adverse water quality on biological communities in the Shingwedzi River. It can thus be assumed that the macroinvertebrate communities of the Luvuvhu River would suffer a similar fate, and this can be seen with the SASS5 score that the Letaba River obtained in this study. According to Angliss et al. (2001), the macroinvertebrate community of the Letaba River was in a fair to natural state. If we use the techniques described by Dallas (2007), the communities can now be said to be in a seriously modified Class E for LF2009 and a poor Class D for LF2010. This shows that abstraction and the consequent effects it has regarding habitat and water quality have adverse effects

on macroinvertebrate communities within the Letaba River, and the same reasoning could be applied to the reduction in macroinvertebrate communities within the Luvuvhu River.

4.5 Fish Response Assemblage Index

Fish sampled in the Luvuvhu River for both the sampling periods showed a similar trend to those sampled in the Olifants River. A large number of species were absent, and species sampled are in low abundance (Table 43 & Table 44). The fish communities have temporal trends similar to those observed for the Olifants River, namely the number of species sampled is similar for the LF2009 and LF2010 periods, but the abundances in LF2010 are higher. Similar to the Olifants River, some habitats were not sampled, especially SD habitats. The remaining habitats were sampled as comprehensively as possible, but as with the Olifants River some species may not have been sampled or were missed because of this. The Luvuvhu River differs from the Olifants River in that the water quality parameters did not indicate that there was much physicochemical pressure on the Luvuvhu River and the water quality was at a level that would suit the fish species expected. With this in mind, the absence of species such as the *Barbus* spp. could be attributed to sampling errors as well as habitat loss through water abstraction and low-flow volumes. For the LF2009 survey, *B. annectens, B. lineomaculatus, and B. trimaculatus* were sampled in SS and SD habitats. In the LF2010 survey, *B. trimaculatu*s and *B. viviparus* were sampled on SD and SS habitats. The absence of the other species could mean that because of the lower flow during sampling in LF2010, habitat biotopes needed (SD and SS) were not readily available, and species diversity therefore decreased. What could be more applicable is that these species were there, but not in high enough abundance to be sampled in the limited SD preferred habitats that were sampled. As with the Olifants River the *Anguillidae* were not included in the FRAI for the same reasons mentioned. It must, however, be mentioned that a single *A. mossambicus* was collected at Site 2 on the Luvuvhu River in the LF2009 survey in the SS habitat biotope.

The FRAI ecological class and scores for the LF2009 lower foothills section were a B/C (77.7) and for the LF2010 survey a C (67.6). For the LF2009 lowland river, the class and scores were a D (56.9) and for LF 2010 the class and scores were C/D (60.7) (Table 45). These results are slightly ambiguous. This is because the upper section (lower foothill) scores dropped temporally, whereas the lower sections (lowland river) increased temporally. But, looking at these results on a spatial scale, it shows that for the Luvuvhu River, for both sampling periods the FRAI scores decreased from the upper section (lower foothill) to the lower section (lowland river). In LF2009, the class and score dropped form a B/C (77.7) to a D (56.9). In LF 2010, the class and score dropped from a C (67.6) to a C/D (60.7). This indicates that the sampling period as a whole, and for the LF2009 survey, the FRAI scores

were higher than the LF2010 scores. This is interesting as it contrasts to what was seen on the Olifants River, where there was a distinct increase in FRAI scores from the different sampling seasons and an increase in species abundance and diversity. Comparing the individual sections to each other on a temporal scale, it is seen that regarding the lower foothills section, the FRAI scores drop. Looking at the lowland river sections, the scores increase. The fluctuations are primarily based on which fish species were sampled, and which were absent. But, it also based on the response the fish had to certain drivers and metrics (Table 46 & Table 47). For the lower foothills section, fish responding to the velocity – depth and cover metrics have the highest weight when the score is calculated. This means that fish relying on these metrics have the greatest response as these metrics are most important for their survival. For the lowland river section, the same metrics are responsible for the scores obtained. What this does mean is that the Luvuvhu River, especially the section within the KNP, is very susceptible to flow volume changes. Over the years, the increased abstraction and utilization of the river for agricultural and domestic use, has resulted in a general trend of lower flow volumes, especially in low-flow periods (Fouche et al., 2005). When flow is reduced, habitat biotopes are affected, and species reliant on those habitats can diminish in number, and become absent from the river. This has been previously documented on the Olifants River (Venter & Deacon, 1995) and in the Letaba River (Vlok & Engelbrecht, 2000). Fish that rely on cover from overhanging vegetation, velocity and depth substrate all come under stress. This is because as the flow reduces, so does the available habitat in which to feed and hide from predators. With the newly completed Nandoni Dam, and the existing Albasini Dam, the 2010 season was the first season during which the combined effects of these impoundments were observed. Changes in the fish communities in the Luvuvhu River will follow, as without the suitable habitat and living conditions, most species of fish will start disappearing from sections of the river, especially from the lower sections within the KNP. Water quality problems will be compounded in the lower sections within the KNP as parameters will be concentrated by lower water volumes and high evaporation rates. For now, Luvuvhu River water quality seems to be of an acceptable standard, but reduction in flow and consequent habitat loss are the driving forces causing negative impacts, and continued development upstream will negatively affect the quality of water entering the river and will exacerbate the situation. The general reduction in flow must not be confused with the higher than average high flow experienced in the high-flow period of 2010. This then indicates a general trend that is now developing regarding flow and abstraction for the Luvuvhu River. This explains why certain sensitive species are not present. The absence of some of the *Barbus* spp. has been explained previously, but there are other species that are worth mentioning. *Labeo congoro* and *L. ruddi* are two species that were absent, with *L. rosae* being sampled in low

abundances. All of these species need SD habitats and rely on substrate for cover (Kleynhans et al., 2007). For these species, their absence can be attributed to sampling errors previously mentioned, but also to the problem described about decreasing flow volumes and habitat loss. An example of habitat loss is *Brycinus imberi* which was absent due to habitat loss during the low-flow period. However, in a survey done in April 2010 (not included in this study) when habitat was present, they were found in abundance showing the difference habitat availability can have with a species. The loss of species due to a drop in flow regime has previously been described by Vlok and Engelbrecht (2000) in another of the KNP rivers, the Letaba River. It showed how species such as *Chiloglanis engiops* and *Opsaridum peringueyi* have not been sampled in the Letaba River since the early nineties. It is attributed to a drop in flow due to abstraction, and the consequent loss in habitat is thought to be the driving force of the species loss (Vlok and Engelbrecht, 2000).

In summary, in comparison to the previous comprehensive survey reported by the State of Rivers Report (2001) as part the RHP, this section of the Luvuvhu River is no longer in a natural state regarding fish communities and assemblages. Certain species might not have been sampled due to not being able to sample SD habitats comprehensively, but sections that were sampled should have at least yielded one or two of these species, which it did not. These results can be attributed to disruptions in the natural state of the river, caused by water abstraction, leading to flow modifications which in turn will lead to habitat modifications.

Table 43. Fish species expected in the various habitat biotopes with actual fish sampled at each site on the Luvuvhu River for the low flow 2009 survey.

Expected species	Site 1				Site 2				Site 3				Site 4			
	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD
Amphilius uranoscopus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus afrohamiltoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus annectens	-	-	-	1	1	-	2	-	-	-	-	-	-	-	-	-
Barbus lineomaculatus	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Barbus radiatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus toppini	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus trimaculatus	-	-	-	1	-	-	-	-	-	-	2	-	-	-	-	-
Barbus unitaeniatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus viviparus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis pretoriae	10	-	-	-	18	-	-	-	-	-	-	-	7	-	-	-
Clarias gariepinus	-	-	1	-	-	-	1	-	-	-	1	1	-	-	1	-
Glossogobius callidus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Glossogobius giuris	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-
Hydrocynus vittatus	-	-	-	5	-	-	-	5	-	-	-	8	-	-	-	1
Labeobarbus marequensis	-	-	-	1	16	-	-	-	-	-	-	-	5	-	-	-
Labeo congoro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo cylindricus	8	-	-	-	14	-	-	-	1	-	-	-	10	-	-	-
Labeo molybdinus	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marcusenius macrolepidotis	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesobola brevinialis	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens	2	-	-	-	-	-	-	-	2	-	3	-	-	-	-	-
Oreochromis mossambicus	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-
Pseudocrenilabus philander	-	-	3	4	-	-	-	-	-	-	-	-	-	-	-	-
Petrocephalus wesselsi	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Synodontis zambezensis	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Schilbe intermedius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tilapia rendalli	9	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Tilapia sparmanii	-	-	-	-	-	-	-	-	4	-	-	2	-	-	10	-
No. of species	**16**				**10**				**7**				**7**			
Total:	**37**	**0**	**5**	**14**	**51**	**0**	**8**	**5**	**7**	**0**	**7**	**13**	**23**	**0**	**11**	**1**

Table 44. Fish species expected in the various habitat biotopes with actual fish sampled at each site on the Luvuvhu River for the low flow 2010 survey.

Expected species	Site 1				Site 2				Site 3				Site 4			
	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD	FS	FD	SS	SD
Amphilius uranoscopus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus afrohamiltoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus annectens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus paludinosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus radiatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus toppini	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus trimaculatus	2	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-
Barbus unitaeniatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus viviparus	-	-	-	-	-	-	10	-	-	-	-	-	3	-	-	-
Brycinus imberi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis engiops	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-
Chiloglanis pretoriae	34	-	-	-	18	-	-	-	34	-	1	-	43	-	-	-
Clarias gariepinus	-	-	-	-	-	-	5	-	-	-	1	4	-	-	1	10
Glossogobius callidus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossogobius giuris	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
Hydrocynus vittatus	-	-	-	3	-	-	-	3	-	-	-	6	-	-	-	10
Labeobarbus marequensis	22	-	2	-	16	-	-	-	3	-	-	-	1	-	2	-
Labeo congoro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo cylindricus	6	-	3	-	4	-	4	-	6	-	9	-	42	-	3	-
Labeo molybdinus	16	-	2	-	23	-	10	-	2	-	11	-	10	-	-	-
Labeo rosae	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marcusenius macrolepidotis	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-
Mesobola brevinialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens	-	-	-	-	2	-	-	-	-	-	-	23	-	-	-	-
Oreochromis mossambicus	-	-	-	-	-	-	-	-	-	-	6	-	-	-	1	-
Pseudocrenilabus philander	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Petrocephalus wesselsi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Synodontis zambezensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Schilbe intermedius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tilapia rendalli	-	-	4	9	-	-	10	-	-	-	6	-	-	-	2	-
Tilapia sparmanii	5	-	4	4	-	-	10	-	-	-	-	-	-	-	-	-
No. of species	**8**				**10**				**11**				**12**			
Total:	**85**	**0**	**17**	**18**	**63**	**0**	**55**	**3**	**45**	**0**	**35**	**34**	**109**	**0**	**10**	**23**

Table 45. The Luvuvhu River FRAI scores obtained over two low-flow sampling periods.

	Automated FRAI	Automated EC	Adjusted FRAI	Adjusted EC
Lower foothills 2009	72.5	C	77.7	B/C
Lowland river 2009	53.4	D	56.9	D
Lower foothills 2010	65.3	C	67.6	C
Lowland river 2010	61.2	C/D	60.7	C/D

Table 46. Metric groups and weights according to the FRAI scores obtained for the Luvuvhu lower foothill river for the low flows of 2009 and 2010.

Metric group	Weight (%)
Velocity – depth	100
Cover	97.22
Flow modification	94.44
Physicochemical	72.22
Migration	52.77
Impact of introduced	0

Table 47. Metric groups and weights according to the FRAI scores obtained for the Luvuvhu lowland river for the low flows of 2009 and 2010.

Metric group	Weight (%)
Velocity – depth	94.4
Cover	100
Flow modification	91.6
Physicochemical	77.7
Migration	58.5
Impact of introduced	0

4.6 Fish Health Assessment

Similar to the Olifants River an additional species representing a different trophic level and feeding guild as the tigerfish were assess as part of the Fish Health Assessment. In the Luvuvhu *Labeo cylindricus* were used as the comparative species.

Labeo cylindricus

Necropsy and Condition Indices

The specimen data for *L. cylindricus* is presented in Table 48. The somatic index, Condition factor and age data for these specimens are presented in Table 49. The mean values of the different indices fell within the normal ranges for each of the respective indices.

Table 48. Specimen data for *Labeo cylindricus* from the Luvuvhu River collected during low flow 2009. Mean values are presented per sample group.

Species	Sampling period	n	Sex		Body mass	Total length
			Male	Female	g	mm
L. cylindricus	Nov 2009	10	5	5	104.22 ± 86.88	208.70 ± 39.55

Table 49. Somatic index, Condition factor and age data for *Labeo cylindricus* from the Luvuvhu River collected during low flow 2009. Mean values are presented per sample group.

Sampling period	N	HSI	GSI (Male)	GSI (Female)	SSI	CF	Age (Months)
Nov 2009	10	0.65 ± 0.34	2.15 ± 0.70	6.58 ± 9.06	0.17 ± 0.11	1.14 ± 0.90	N/D

HSI = Hepatosomatic Index; GSI = Gonadosomatic Index; SSI = Splenosomatic Index; CF = Condition factor; N/D = Not determined

The necropsy observation revealed no macroscopic abnormalities for any of the sampled *L. cylindricus* specimens.

Histopathological assessment

The light microscopy analysis showed that the selected target organs of *L. cylindricus* from the Luvuvhu River have normal histological structure and seem to be in a normal functional state. Selected histological alterations were identified in liver and kidney samples (Figure 63). These included intracellular deposits, hepatocellular vacuolation and nuclear changes in the liver and vacuolation of the tubular epithelium and nuclear alterations in the kidney samples. The percentage prevalence of these alterations for the specific sample group is presented in Table 50.

With regards to the liver alterations, the intracellular deposits were mostly diffused in nature and were present in most hepatocytes of affected fish. The hepatocellular vacuolation identified was in most cases characteristic of

macrovesicular steatosis. However, the presence of lipid accumulation in hepatocytes was not confirmed through special stains as part of this study. The vacuolated cells were mostly diffuse in nature. Nuclear changes identified included mainly pleomorphic nuclei, i.e. nuclei of different sizes within the same tissue region. Kidney alterations included vacuolation of tubular epithelium and nuclear alterations of the tubular epithelial cells.

Table 50. Percentage prevalence of histological alterations identified in *Labeo cylindricus* from the Luvuvhu River collected during low flow 2009.

Organ / alteration	**2009**
	%
Liver	
Intracellular deposits	30
Hepatocellular vacuolation	90
Nuclear changes	80
Kidney	
Vacuolation of tubular epithelium	30
Nuclear alterations	20

As was the case with the fish from the Olifants River, the mean Liver Index value was higher compared to the Kidney Index, mainly as a result of either a higher number of alterations identified, or, as a result of a higher severity of occurrence of specific alterations within the tissue samples assessed (Table 51). No histological alterations were identified in the gill and gonad samples of any of the fish collected. A final Fish Index value of 10 was calculated. The profile of the histological index results of *L. cylindricus* was similar to the profiles calculated for the *L. marequensis* sample groups, i.e. higher Liver Index values compared to Kidney Index values as well as Fish Index values within the range of 8-10.

Table 51. Mean histological index values for *Labeo cylindricus* from the Luvuvhu River collected during low flow 2009.

Index	**2009**
Liver Index	8.2
Kidney Index	1.8
Gill Index	0.0
Testis Index	0.0
Ovary Index	0.0
Fish Index	**10.0**

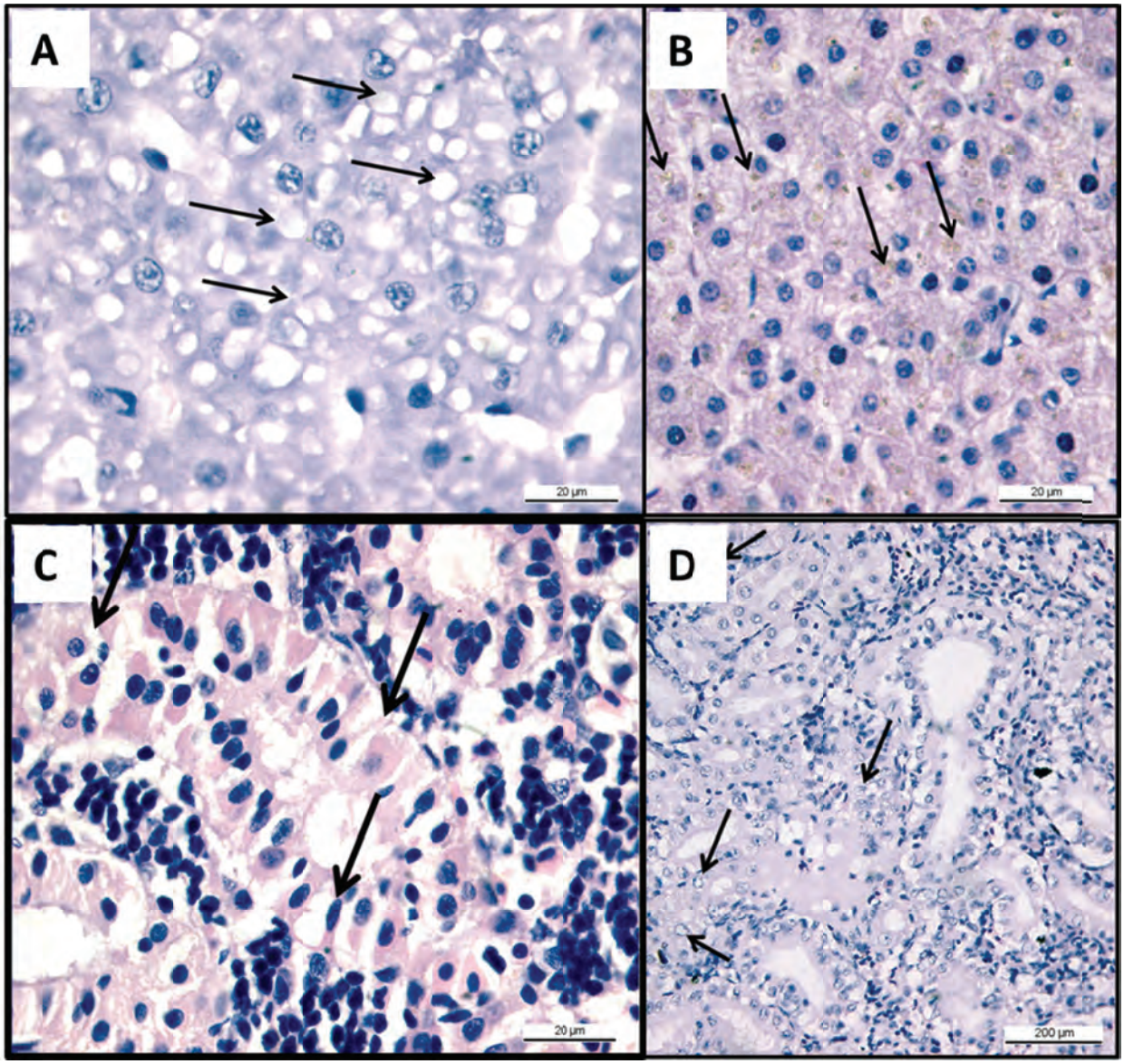

Figure 63. Micrograph representing histopathological changes in the liver (A & B) and kidney (C & D) of *Labeo cylindricus*. A. Hepatocellular vacuolation (100X) B. Intracellular deposits (100X) C. Vacuolation of tubular epithelium (100X) D. Nuclear alterations (10X).

Discussion

The sampling size for the *L. cylindricus* (n=10) were lower that for the *H. vittatus* (n=34), but an adequate ratio between males and females were present. The necropsy observation revealed no macroscopic abnormalities for any of the sampled *L. cylindricus.* The mean HSI were 0.65 and the GSI values were 2.15 for the males and 6.58 for the females. The mean values of the different indices fell within the normal ranges for each of the respective indices. The liver index value (8.2) was higher that the kidney index value (1.8). These values were all below 10 which indicate that the histological structure of the liver is normal.

Hydrocynus vittatus

Necropsy and Condition Indices

The specimen data for *H. vittatus* is presented in Table 52. The somatic index, Condition factor and age data for these specimens are presented in Table 53. The mean HSI value for the HF2010 sample group was lower compared to the LF2009 and HF2011 sample groups. However, sample size should be considered in this case. The mean GSI values for the male specimens of the 2009 sample group was higher compared to the other two sample groups, as well as compared to the fish from the Olifants River. This was not unexpected as the gonadal tissue of most of the 2009 sampled male fish was observed to be in the mature stages of spermatogenesis. The mean SSI values were similar for all three sample groups and the mean CF for all groups were between 0.6 and 1. The mean age of the HF2010 sample group was slightly higher compared to the LF2009 sample group. Age was not determined for the HF2011 sample group.

Table 52. Specimen data for *Hydrocynus vittatus* from the Luvuvhu River. Mean values are presented per sample group.

Sampling period	n	Sex		Body mass	Total length
		Male	Female	g	Mm
November 2009	16	9	7	708.28 ± 866.70	362.06 ± 144.60
May 2010	2	1	1	830.00 ± 692.96	474.50 ± 130.81
May 2011	16	8	8	697.5 ± 561.50	413.75 ± 102.76

Table 53. Somatic index, Condition factor and age data for *Hydrocynus vittatus* from the Luvuvhu River. Mean values are presented per sample group.

Sampling period	n	HSI	GSI (Male)	GSI (Female)	SSI	CF	Age (Months)
November 2009	16	0.46 ± 0.19	4.28 ± 1.32	4.41 ± 2.70	0.03 ± 0.01	0.95 ± 0.23	60.88 ± 20.28
May 2010	2	0.27 ± 0.29	1.02	0.54	0.06 ± 0.01	0.68 ± 0.08	70.00 ± 8.49
May 2011	16	0.67 ± 0.19	0.59 ± 0.35	0.86 ± 0.65	0.04 ± 0.02	0.80 ± 0.11	N/D

HSI = Hepatosomatic Index; GSI = Gonadosomatic Index; SSI = Splenosomatic Index; CF = Condition factor; N/D = Not determined

The necropsy observation revealed a few abnormalities in a number of the sampled *H. vittatus* specimens. These included liver discolouration (2009: n = 2) and parasitic infections (2009: n = 8; 2010: n = 2; 2011: n = 9).

Histopathological assessment

The light microscopy analysis showed that the selected target organs of *H. vittatus* from the Luvuvhu River have normal histological structure and seem to be in a normal functional state. Selected histological alterations were identified in liver and kidney samples. These included intracellular deposits, hepatocellular vacuolation and nuclear changes in the liver samples. The kidney samples showed vacuolation of the tubular epithelium, hyaline droplet degeneration and eosinophilic degeneration of the tubular epithelium. The percentage prevalence of these alterations for the various sample groups are presented in Table 54.

With regards to the liver alterations, the intracellular deposits were mostly diffuse in nature and were present in most hepatocytes of affected fish. The hepatocellular vacuolation identified was in most cases characteristic of macrovesicular steatosis. However, the presence of lipid accumulation in hepatocytes was not confirmed through special as part of this study. The vacuolated cells were mostly diffuse in nature but focal areas of intracellular lipid accumulation were also identified in one specimen. Nuclear changes identified included mainly pleomorphic nuclei, i.e. nuclei of different sizes within the same tissue region.

The histological results for the kidney samples showed a high prevalence of vacuolated tubular epithelium in the 2009 and 2010 sample groups, hyaline droplet degeneration only in the 2009 sample group, and eosinophilic degeneration only in the 2011 sample group.

Table 54. Percentage prevalence of histological alterations identified in *Hydrocynus vittatus* from the Luvuvhu River.

Organ / alteration	2009	2010	2011
	%	%	%
Liver			
Intracellular deposits	81	50	20
Hepatocellular vacuolation	75	50	40
Nuclear changes	25	50	0
Kidney			
Vacuolation of tubular epithelium	69	100	0
Hyaline droplet degeneration	25	0	0
Eosinophilic degeneration	0	0	13

As was the case with the fish from the Olifants River, the 2011 sample group had a lower Liver and Kidney Index, and subsequently a lower Fish Index value compared to the 2009 and 2011 sample groups (Table 55). The Liver Index values were also higher compared to the Kidney Index values for all three sampling surveys. No histological alterations were identified for the gill and gonad samples collected. The mean Fish Index values fell within the same range of 0-15 as was the case for the fish from the Olifants River.

Table 55. Mean histological index values for *Hydrocynus vittatus* from the Luvuvhu River.

Index	2009	2010	2011
Liver Index	8.0	8.0	2.4
Kidney Index	5.0	3.0	0.5
Gill Index	0.0	0.0	0.0
Testis Index	0.0	0.0	0.0
Ovary Index	0.0	0.0	0.0
Fish Index	**13.0**	**11.0**	**2.9**

Discussion

The condition factor varied between 0.68 and 0.80 with the highest mean value being from the November 2009 sampling trip. Since this trip was taken in November it is possible that these higher values are because of seasonality, where the higher Cf results are because of fish that are closer to breeding and thus their body mass is increased as a result of increased gonad mass, these results are reflected in the GSI values (4.28).

The liver index values were higher than the kidney index values for all three the sampling trips, but all these values were still within the normal range.

4.7 Bioaccumulation in *H. vittatus*

Measurement of metal and organic chemicals through direct chemical analysis in water and sediment are limited in reliability (Smolders et al., 2004) and this has led to the application of living organisms as indicators of environmental exposure through the process of bioaccumulation. However, cautioned should be practised when interpreting the results of bioaccumulation monitoring studies. According to Chapman (1997) and Rainbow (2007) bioaccumulation studies can provide information on contaminant-specific bioavailability, assist in identifying possible causative agent(s) of toxicity, and relate body burdens to food chain accumulation values relative to secondary poisoning or biomagnification. Too often residue levels in tissues of aquatic organisms are used to make comments on potential toxicity due to the presence of the toxicants. Bioaccumulation results that are presented should be seen as a biological measure of metal and organic chemical bioavailability within the study area.

Metals

Metal bioaccumulation in muscle tissue of *H. vittatus* from the Luvhuvu River is presented in Figure 64 and Figure 65. The concentrations of Cd, Cu, Mn and Zn have decreased from 2009 to 2011, whereas the rest of the metals studied have remained constant over the three year sampling period. There were no significant temporal changes in bioaccumulation of individual metals. With the exception of Al, all metals were lower in tigerfish from the Luvuvhu when compared to the Olifants River bioaccumulation results.

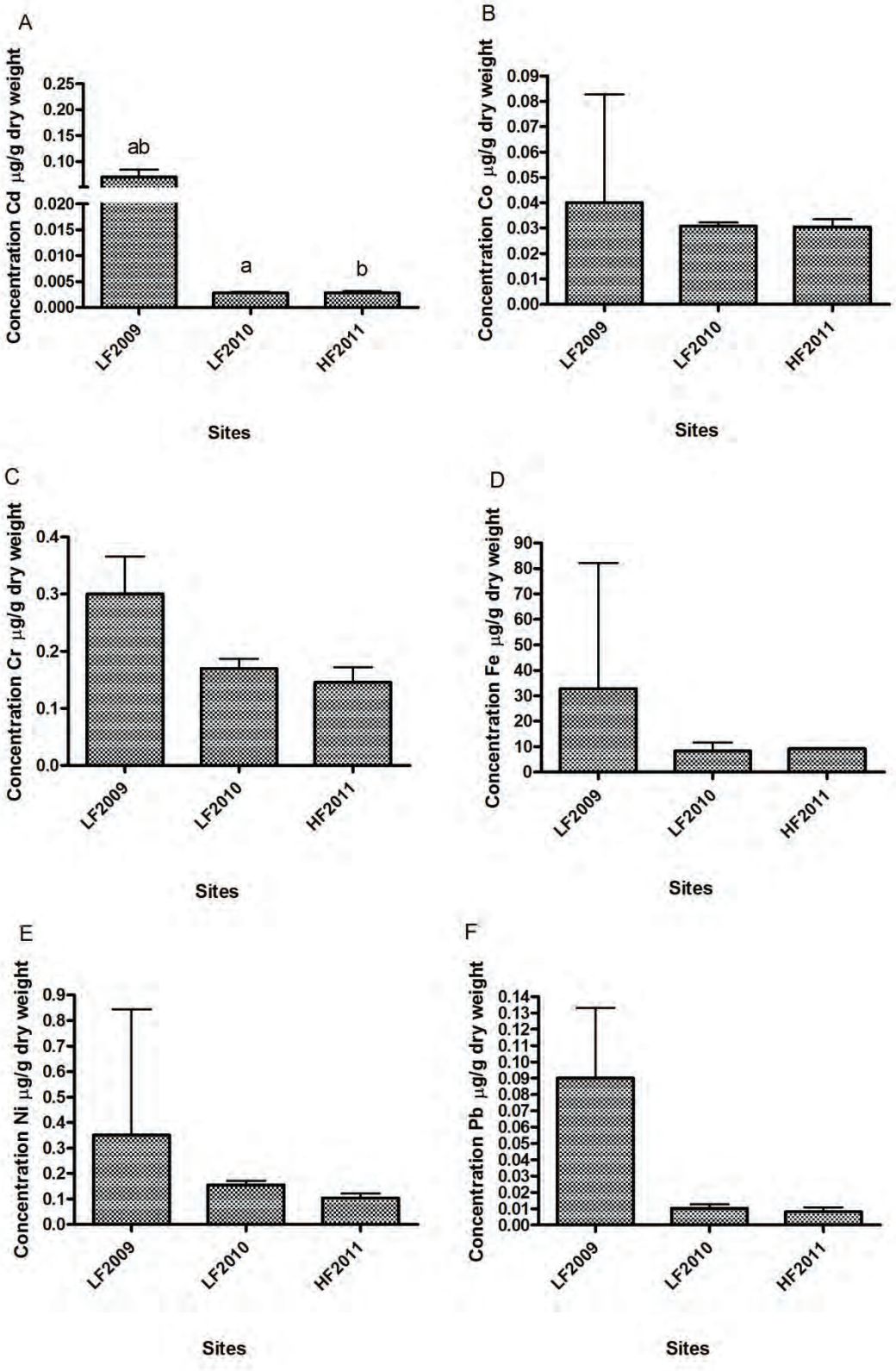

Figure 64. Mean ± standard error concentrations of metals in muscle (µg/g dry mass) in *H. vittatus* muscle tissue from the Luvuvhu River. Common superscript within rows indicate significant differences ($p<0.05$).

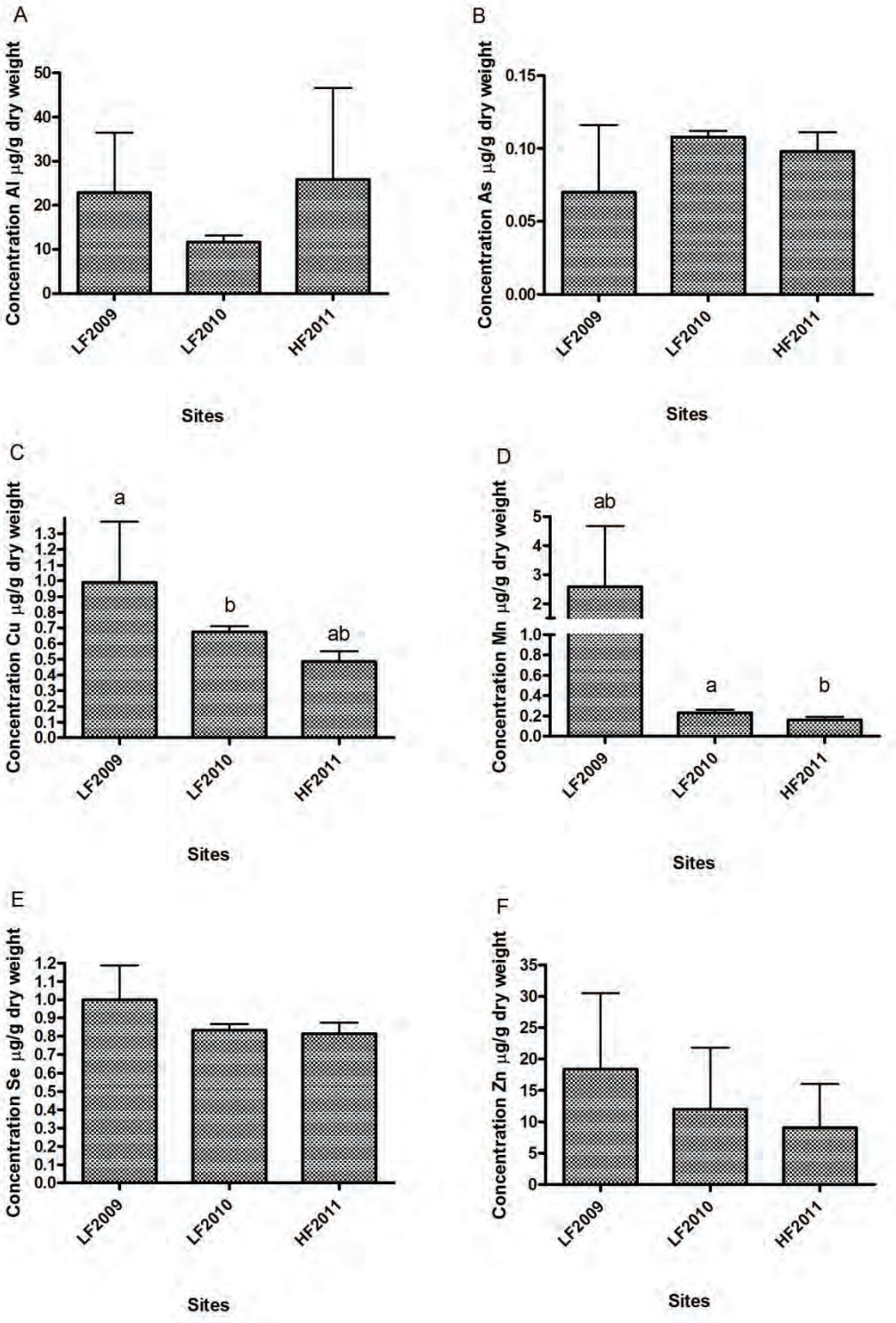

Figure 65. Mean ± standard error concentrations of metals in muscle (µg/g dry mass) in *H. vittatus* muscle tissue from the Luvuvhu River. Common superscript within rows indicate significant differences ($p<0.05$).

Organics

There were no significant differences on the lipid content of the muscle tissue between the two flow periods. Therefore the temporal OCP bioaccumulation patterns (see Table 56) reflect the OCP usage and run-off patterns. All the measured OCPs are significantly higher during the lowflow period, which would suggest that input from diffuse sources has a longer residence time in the environment (i.e. reduced sediment transport) with ensuing bioaccumulation.

Table 56. Mean ± standard error of organochlorine pesticides (ng/g lipid) in tigerfish muscle from the Luvuvhu River. Common superscript within rows indicate significant differences ($p<0.05$). ND represents OCP not detected.

	LOD ng/g	LF2010 (n=16)	HF2010 (n=16)
α-HCH	2	101.24 ± 53.43	36.17 ± 6.42
β -HCH	2	120.38 ± 45.21	ND
δ-HCH	2	156.85 ± 80.82	370.87 ± 62.96
γ-HCH	2	122.86 ± 46.23	18.76 ± 6.38
ΣHCHs		501.34 ± 221.97	425.8 ± 70.64
Heptachlor	2	43.18 ± 34.32	54.27 ± 10.94
cis-Nonach	2	72.60 ± 33.76	7.19 ±2.43
trans-Nonane	2	119.61 ± 29.26	4.45 ± 3.61
cis-Hep-epox	2	112.26 ± 34.53	ND
trans-Hep-epox	2	121.77 ± 35.38	ND
cis-Chlordane	2	76.66 ± 36.23	15.57 ± 4.70
trans-Chlordane	2	152.43 ± 33.71	28.72 ± 10.69
Oxy-Chlordane	2	94.48 ± 24.67	3.83±2.08
ΣCHLs		323.57 ± 85.39	48.12 ± 11.17
Aldrin	2	63.62 ± 23.86	10.99 ± 4.09
Dieldrin	2	ND	ND
Endrin	2	109.51 ± 61.05	8.43 ± 2.88
o,p'-DDD	4	258.89 ± 68.30	64.47± 11.33
p,p'-DDD	4	3411.15 ± 1106.75	451.45 ± 171.08
o,p'-DDE	4	103.06 ± 34.85	78.64 ± 15.83
p,p'-DDE	4	16184.23 ± 5026.47	2342.58 ± 945.66
o,p'-DDT	4	479.23 ± 134.79	122.63 ± 31.38
p,p'-DDT	4	11934.22 ± 2860.89	1189.45 ± 554.89
ΣDDTs		32370.78 ± 8031.94	4249.22 ± 1679.98
***p,p'*-DDE/DDT**		1.36	1.97
HCB	4	26.93 ± 11.08	7.81 ± 1.54
Lipid (%)		0.10 ± 0.03	0.15 ± 0.06

The ΣDDTs measured during the LF2010 survey are the highest levels recorded in fish from South African freshwater systems (see review by Ansara-Ross et al., 2012).

The high levels can be attributed to the application of DDT for malaria vector control in the upper catchment of the Luvuvhu River (Van Dyk et al., 2010). The low DDE:DDT ratio indicates that the DDT exposure is a mixture of recent DDT application and historical levels. The wide-scale application of OCPs in the catchment of the study area is evident from the high chlordane, lindane, Endrin and Aldrin. It was interesting to note that although there were measurable levels of Dieldrin in sediment samples from both surveys, this highly persistent and toxic pesticide did not bioaccumulate in tigerfish muscle. Bornman et al. (2010) also recorded the presence of dieldrin in water samples from the Luvuvhu system.

4.8 Biomarker response in *H. vittatus*

The biomarkers of exposure (Figure 66) indicate that AChE activity was significantly lower during the 2010 survey (Figure 66A), whilst both CYP450 (Figure 66B) and MT (Figure 66C) were significantly ($P<0.05$) during the LF2009 survey. The anti-oxidative stress biomarkers (Figure 67) show that activity of both CAT (Figure 67A) and SOD (Figure 67B) are significantly higher ($P<0.05$) during the LF2010 survey. The LP levels were however significantly lower than the LF2009 survey (Figure 67C), while the PC levels were significantly higher. The energy compounds (Figure 68A-C) making up the available energy (Figure 68D) were significantly higher during the KF2010 survey. Although the energy consumption (Figure 68E) was also significantly higher ($P<0.05$) than the LF2009 survey the total available CEA was still significantly higher in the 2010 survey period.

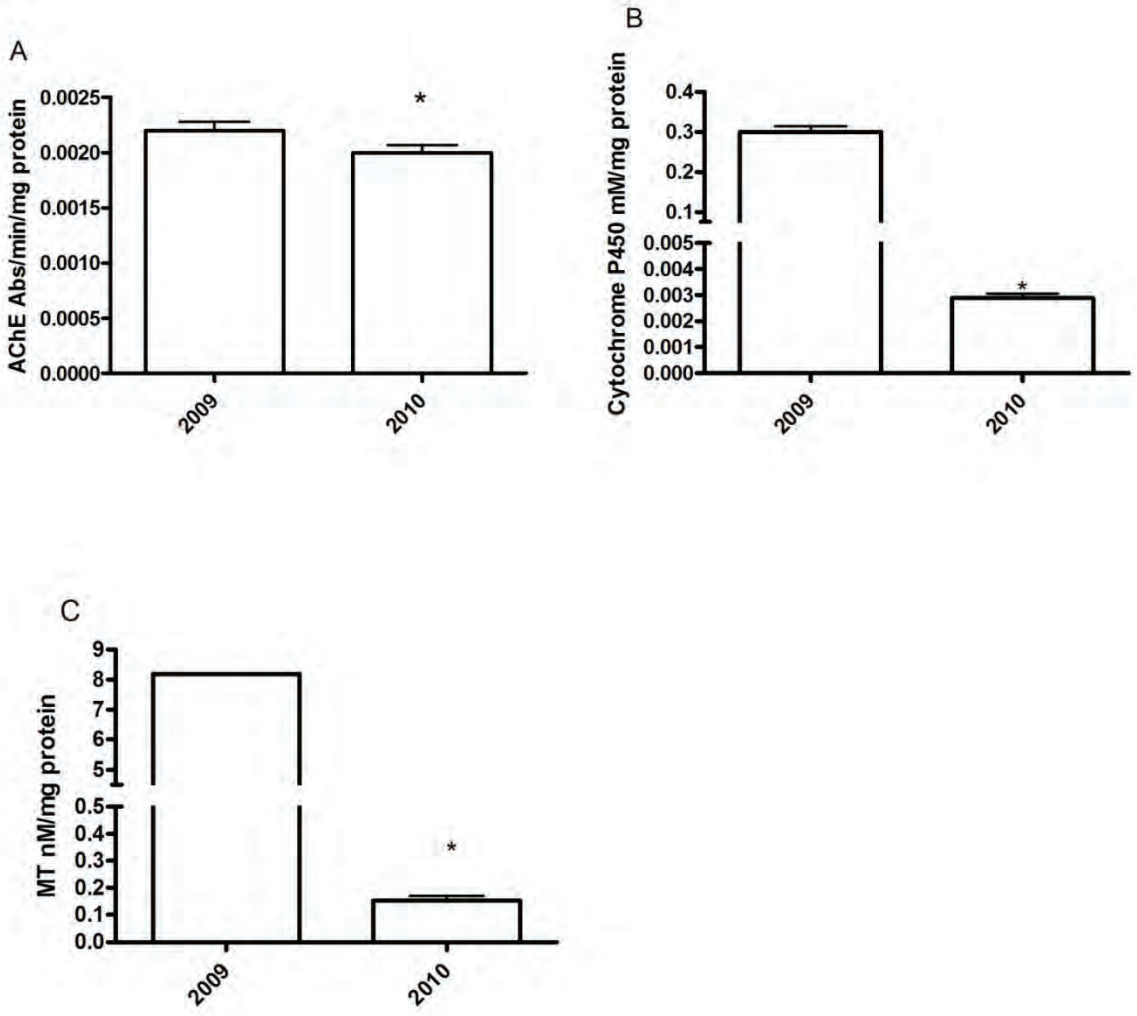

Figure 66. Biomarkers of exposure in liver tissue of tigerfish collected during the 2009 (n=8) and 2010 (n=15) low flow periods in the Luvuvhu River. Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

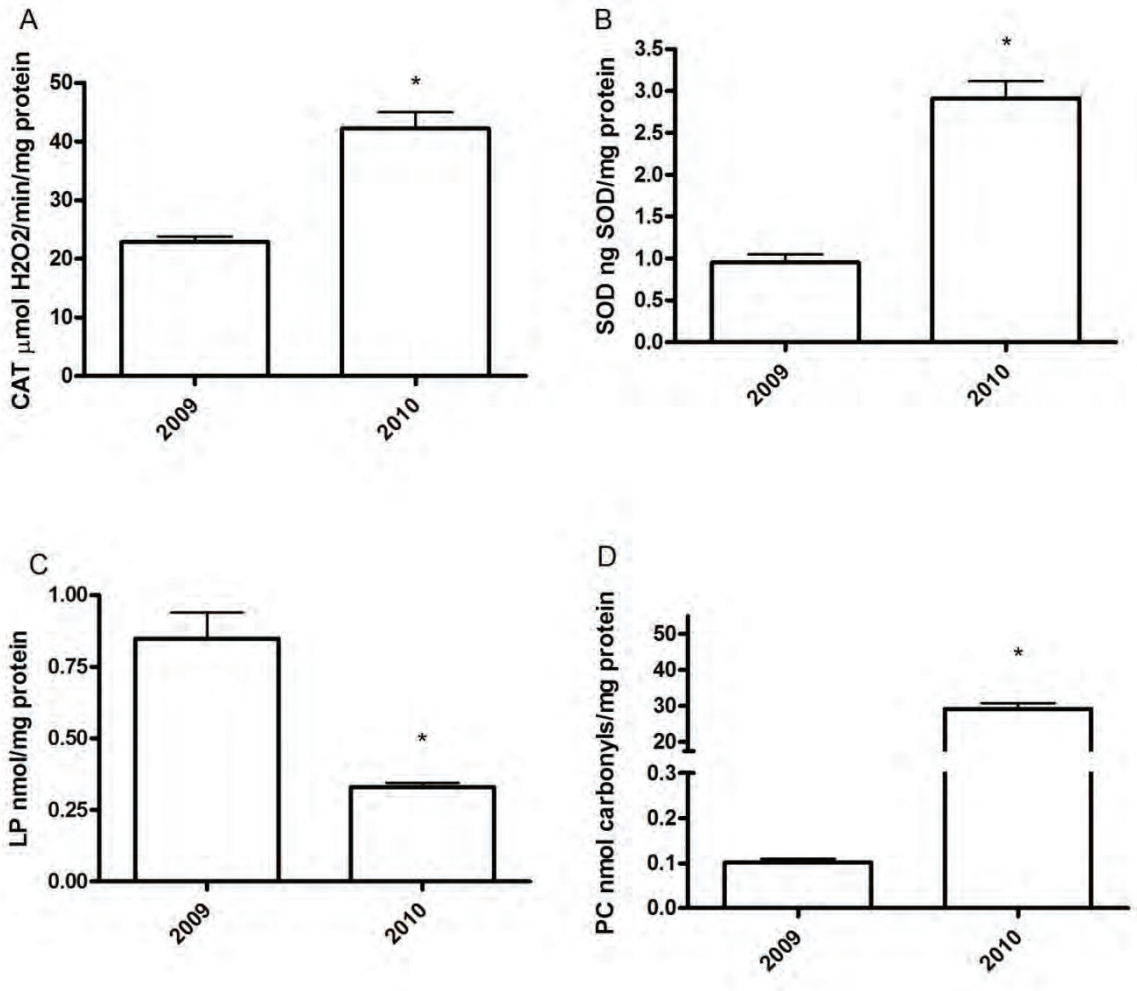

Figure 67. Biomarkers of effect in liver tissue of tigerfish collected during the 2009 (n=8) and 2010 (n=15) low flow periods in the Luvuvhu River. Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

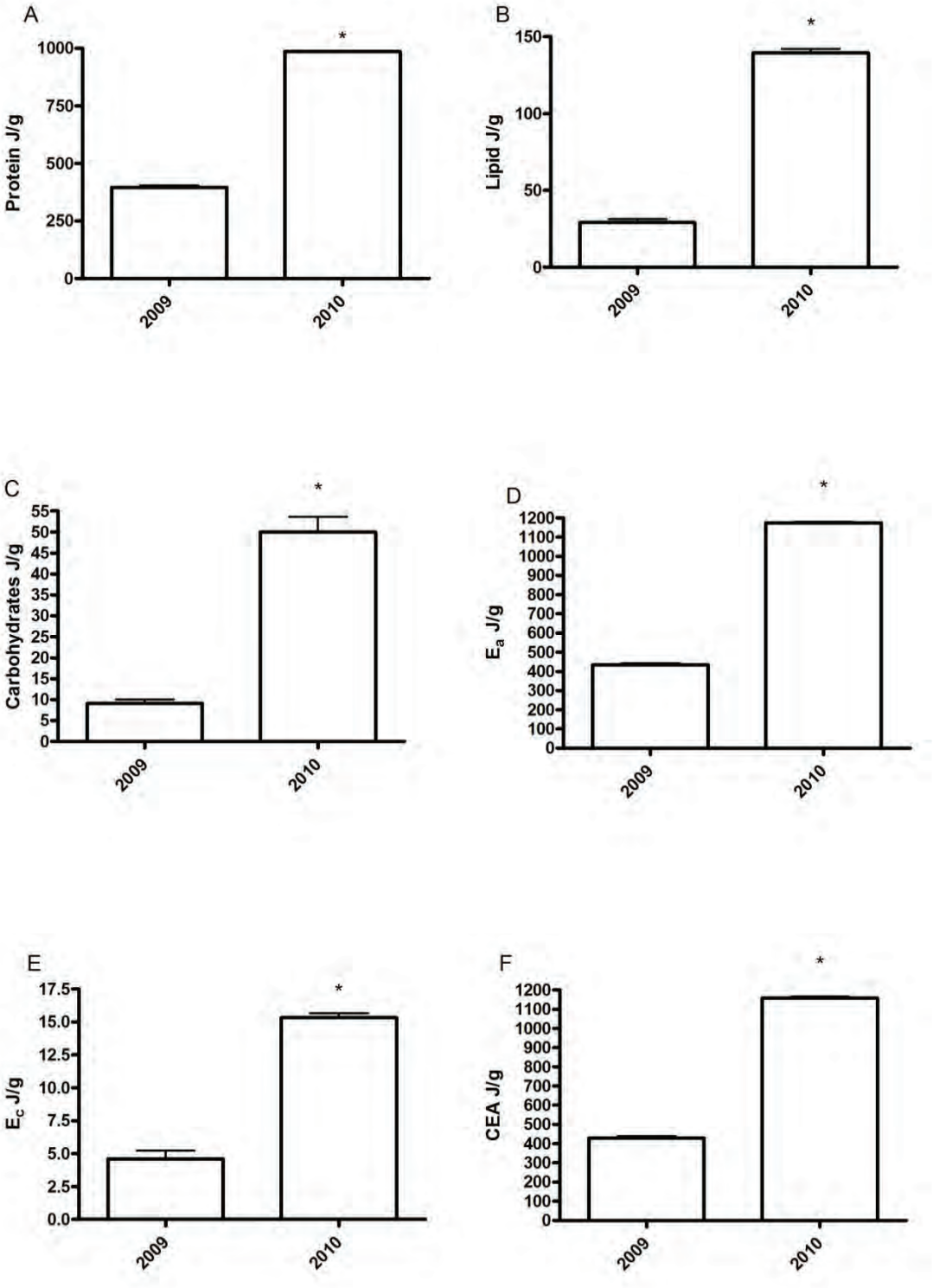

Figure 68. Cellular energy allocation biomarker of effect in muscle tissue of tigerfish collected during the 2009 (n=8) and 2010 (n=15) low flow periods in the Luvuvhu River. Bars represent mean + standard error and an asterisk indicates a significant difference between the two survey periods.

Interpretation of biomarker responses

The interpretation of the increasing or decreasing nature of the biomarker responses are presented in Table 57 and is based on the biomarker background provided in the Olifants River biomarker section (Section 3.9).

Table 57. Summary of the diagnostic nature of the biomarker responses and their interpretation.

Biomarker	Increase/ decrease	Exposure or effect interpretation
Acetylcholine esterase (AChE)	↓	Inhibition due to pesticide exposure
Cytochrome P450 (CYP1A)	↑	Stimulation in the presence of organics
Metallothionein (MT)	↑	Stimulation in the presence of metals
Catalase (CAT)	↑	Produced in response to ROS formation
Superoxide dismutase (SOD)	↑	Produced in response to ROS formation
Malondialdehyde (MDA)	↑	Indicative of liver peroxidation due to ROS
Protein carbonyl (PC)	↑	Damage to proteins due to ROS
Cellular energy allocation (CEA)	↓ and ↑	Decrease due to stress compensation requiring additional energy sources. Increases associated with additional energy sources.

The biomarker responses in liver tissue of *H. vittatus* from the Luvuvhu River indicate that there are responses to metal (increased MT) and organochlorine (increased CYTP450) during the LF2009 survey (Figure 66). The stimulated CAT and SOD activity (Figure 67) is indicative of activated ROS protective mechanisms and this is reflected in the lower lipid break down products (i.e. MDA) that are formed. These are energy consuming processes as displayed in the significant increase in energy consumption (Figure 68). The energy consumption is also associated with increases in all energy reserves.

5 GENERAL DISCUSSION AND CONCLUSION

5.1 Abiotic assessments of the Olifants and Luvuvhu Rivers

Water quality

The combined properties of the physical qualities and the chemical constituents of an aquatic ecosystem can be termed environmental water quality (Palmer et al., 1996). Water quality is used to describe the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses, and for the protection of the health and integrity of aquatic ecosystems. Many of these properties are controlled or influenced by components that are either dissolved or suspended in water as a result of either natural or anthropogenic input, or both (DWAF 1996). All biotic communities living within the aquatic ecosystem are reliant upon water quality, as this is the environment to which they are limited. As such these communities may be influenced negatively if water quality decreases. Water quality can not only be negatively affected by sources of pollution but also by changes in flow regimes (Malan et al., 2003). Aquatic biota already stressed by changed flow and flow regulation of rivers are likely to be more susceptible to changes in the quality of the water in which they live (DWAF, 1996). Pollution of waterways and the human demand for freshwater affect both aquatic biodiversity and ecosystem functioning (Naiman & Turner, 2000). As human population pressures and economic development activities increase so will the demand for water. Unless managed in a sustainable manner water quality in our rivers will deteriorate, particularly in downstream reaches (Deksissa et al., 2003). Decreased water quantity can negatively affect the water quality in lower river reaches due to diminished dilution capabilities (Deksissa et al., 2003).

The water quality of a system can be assessed by various means. These include in situ variables, chemical analyses and dissolved and suspended metal concentrations. In situ water quality variables give an indication as to the availability of contaminants present in the aquatic environment. Through chemical analyses nutrient levels can be assessed and anthropogenic inputs can be determined. Whereas by determining the dissolved and suspended metal concentrations present in the water, one can assess the amount of metal pollutants an aquatic organism is directly exposed to.

The physico-chemical quality and metal concentrations in the Olifants, Letaba and Luvuvhu Rivers are influenced greatly by flow conditions with more than 50% of the variation in the water quality data demonstrating these influences (Figure

69 – PC axis 1). Only 16% of the variation in the data can be explained by river specific factors influencing the water quality of the three rivers studied. Low flow conditions are characterised by increased DO, pH and electrical conductivity (as witnessed in high anion and cation concentrations). The majority of metals (both dissolved and suspended) are associated with high flow conditions together with increased turbidity and nutrient levels.

Dissolved Cu, Se and Zn were notably higher in the Olifants River when compared to the Luvuvhu River and these levels were elevated during both flow periods. The Luvuvhu River had higher U and suspended Al and Fe compared to the Olifants River, while Mn was elevated in both systems.

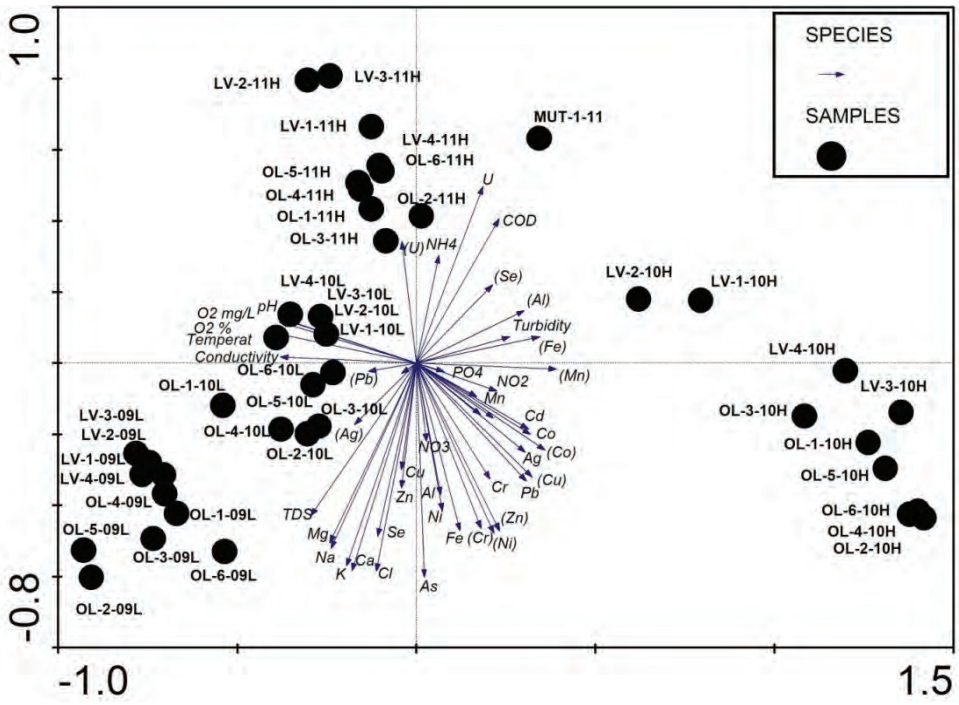

Figure 69. PCA biplot for the Olifants, Letaba and Luvuvhu Rivers based on physico-chemical parameters and dissolved and uspended (in parentheses) metal concentrations, at sites during four surveys. This biplot describes 69.1% of the variation in the data, where 53.3% is displayed on the first axis, while 15.8% is displayed on the second axis.

Historically the Olifants River has been regarded as a system of which the water quality is influenced more by anthropogenic activities within the catchment (e.g. mining and agricultural practices) than by geogenic factors (Seymore et al., 1994; Wepener et al., 1999; De Villiers & Mkwelo, 2009). However, this study has demonstrated that these water quality modifying influences are present to a similar

extent in the Luvuvhu system. Elevated metals in both the Olifants and Luvuvhu Rivers are likely to be due to mining activities in the Bushveld complex and erosion of land respectively (Coetzee et al., 2002). Significantly higher concentrations of Mn in the Letaba and Luvuvhu Rivers were likely to be due to erosion of Yugawaralite in the Letaba formation and runoff from the magnesite mine before the Luvuvhu River enters the KNP (Angliss et al., 2001) respectively. Higher Mg concentrations in the Olifants River water than in the Luvuvhu River water was attributed to the presence of local mining and sewage treatment works as discussed by Coetzee et al. (2002). Magnesium and Ca are important factors in determining water hardness. Increased water hardness is known to decrease the availability and toxicity of many heavy metals (DWAF, 1996; Seymore et al., 1996a) and thus high concentrations of these ions may lead to increased buffering of waters in the Olifants River and a subsequent decrease in metal toxicity. Water hardness along the Olifants River in this study was much greater as reported by Seymore et al. (1994), and this could result in lower concentrations of many metals in water when compared to past studies.

Sediment quality

Contaminants such as metals and organochlorides can take various pathways once they have entered the aquatic environment. These pathways include the adsorption of metals to the surfaces of sediments and colloids and deposition into organic debris contained in silts. The availability of the chemical for uptake by biota is determined by the strength of bond found between the solid and the chemical, and as a pollutant degrades it may either become less toxic or more toxic (Sandoval et al., 2001). Environmental factors such as temperature, pH, sunlight and the properties of the adsorbing surfaces will determine the rate at which a contaminant degrades (Walker et al., 2006).

According to Sandoval et al. (2001) the determination of the bioavailability of heavy metals depends on the understanding of the physico-chemical properties of the receiving environment. Heavy metals are generally subject to immobilisation and deposition, and changes in properties such as pH, conductivity, temperature, dissolved oxygen and turbidity affect the speciation and distribution of many heavy metals. The solubility of metals is found to increase under changing pH and as a result this increases their potential to become bioavailable as they move from sediments into the water column.

Sediments act as the main sinks for pollutants, and processes such as dissolution, desorption, complexation, precipitation and absorption affect the mobility

of these pollutants (Amiard et al., 2007). Individual sediment particles possess large surface areas allowing for the attachment of many molecules such as metals and organic contaminants (Kwon & Lee, 2001). Therefore sediments containing a high organic content and small grain size will commonly contain elevated concentrations of contaminants. Metals trapped in sediments tend to have long residence times and these sediments may serve as a constant supply of contaminants (Filgueiras et al., 2004).

In contrast to the water quality, the spatial characteristics were more important in explaining the variation in the data (Figure 70, PC1 – 33%). The Olifants River sediments were dominated by fine, organic rich sediments with high metal concentrations, while the Luvuvhu system sediments consisted mainly of course sand and gravel. The influence of flow attributed to 20% in the variation of the data with sediments during high flow periods in the Olifants River consisting of high percentages of mud and fine sands.

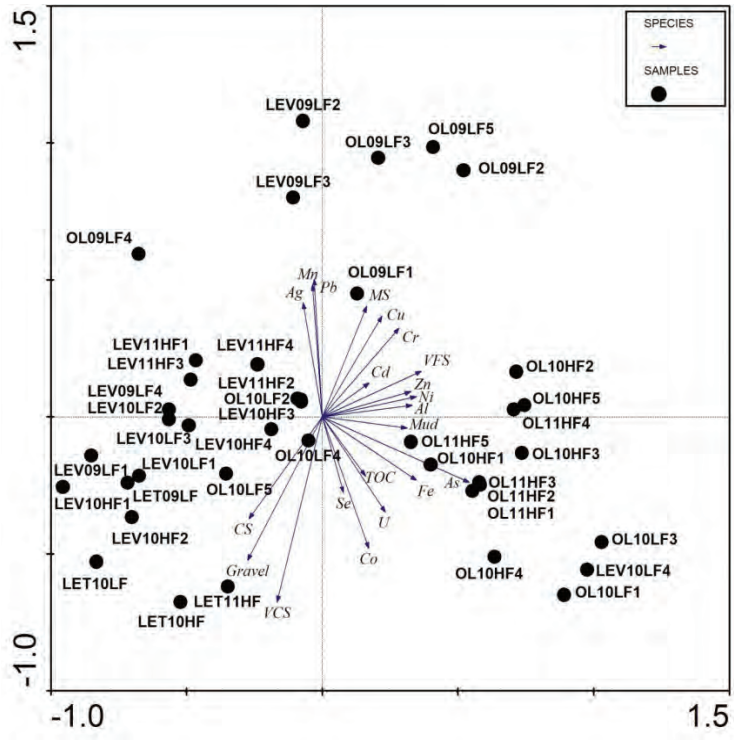

Figure 70. PCA biplot for Olifants, Letaba and Luvuvhu Rivers based on physical sediment characteristics and total metal concentrations. The biplot describes 53.6% of the variation in the data, where 33.2% is displayed on the first axis, while 20.4% is displayed on the second axis.

In comparison with total metal concentrations measured in the Olifants River during the 1990s there appears to be fluctuations with Cu and Zn appearing to have

increased but other metals such as Pb and Cr having decreased. Although the majority of metals were in the inert residual fraction of the sediment, there were some metals that occurred in high proportions in the bioavailable acid-soluble and reducible fractions. These metals therefore could pose a risk to aquatic biota due to their increased potential for biological uptake (Baeyens et al., 2003). In the Olifants River the bioavailable fraction of Mn was high at all sites, while Zn was highest at Site 1. The LF2009 survey had the highest bioavailable fractions for Cu, Mn and Zn compared to the other surveys. Bioavailable Mn and Zn fractions were also highest during the LF2009 survey in the Luvuvhu system. Similar to the Olifants system Mn bioavailability was also high at all sites in the Luvuvhu, while Cd was high in the bioavailable sediment fractions at Site 1. The relationship between sediment characteristics and metal bioaccumulation in fish were discussed in detail in section 3.8.

Organochlorine pesticide concentrations in sediments of the Olifants and Luvuvhu Rivers were dependent on the flow conditions and associated physical characteristics of the sediments. The PCA biplot (Figure 71) indicates that flow describes 45% of the variation in data, with the highest OCPs in sediments during the high flow periods. The majority of the OCPs were present during the high flows in sediments characterised by fine, organic rich particles. Those sites with medium sand composition contained the highest cis-chlordane and heptachlor concentrations. Dieldrin was only recorded in sediments at all sites in the Luvuvhu River during LF2010 and Site 3 during HF2011. Concentrations are very similar to OCP concentrations measured in sediments from selected industrial sites in the Vaal triangle (Quinn et al., 2009) and much lower than known contaminated sites in South Africa (Ansara-Ross et al., 2012), e.g. the ΣDDTs were lower than those recorded in sediments from the Pongola floodplain during the early 2000s where concentrations were as high as 13 ng/g compared to the maximum of 3 ng/g measured at site 1 in the Luvuvhu River during the LF2010 survey.

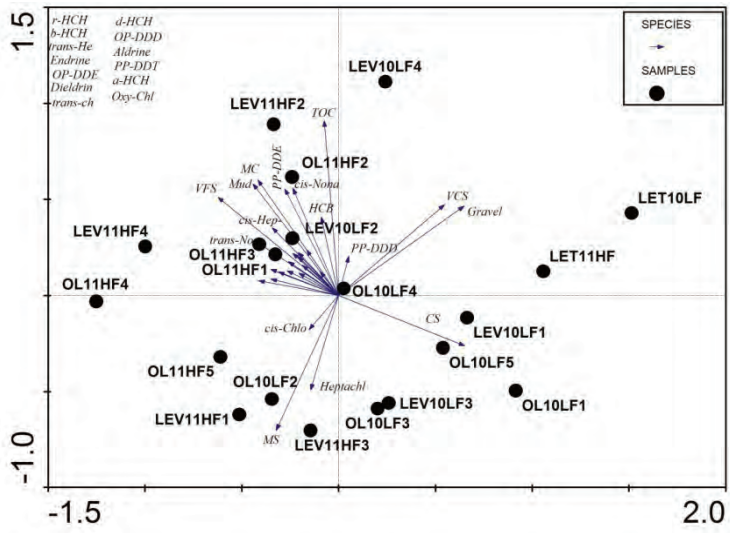

Figure 71. PCA biplot for the Olifants, Letaba and Luvuvhu Rivers based on physical sediment characteristics and organochlorine concentrations. This biplot describes 70.7% of the variation in the data, where 44.9% is displayed on the first axis, while 25.8% is displayed on the second axis.

5.2 Biological assessment of the Olifants and Luvuvhu Rivers

Invertebrates

At the start of this study, in terms of the biological component of the two rivers studied it was hypothesised that although the Luvuvhu itself is being put under anthropogenic pressure, its biological communities should have a greater diversity than those of the Olifants River. The second hypothesis was that the ecological state of the biological communities has improved at the point where both the rivers leave the park compared to where they enter. To test these hypotheses and in order to obtain an indication of temporal and spatial trends among the macroinvertebrate communities, the data were transformed to presence/absence data and Bray-Curtis similarity-based cluster analysis and NMDS were performed (Figure 72 and Figure 73). The ANOSIM test revealed these groupings were significant with a *R* value of 0.705. There is a comprehensive grouping of the rivers on both temporal and spatial levels. This finding is corroborated by the SASS5 scores and ASPT temporal and spatial trends. The groupings show the temporal variation mentioned above, and consequently the LF2009 Olifants River sites group together and the LF2010 Olifants River sites group together. The Luvuvhu River communities are grouped separately from both Olifants River communities, but both Luvuvhu flow periods are grouped together. There are small dissimilarities, but not enough to group the two Luvuvhu flow periods separately. It can then be said that the Olifants River macroinvertebrate

communities differ in terms of the two flow periods and in terms of the Luvuvhu River communities. This variance further explains what was previously mentioned regarding the trends seen with the SASS5 score and ASPT, and the possible cause for this temporal variation. On the whole, statistically the macroinvertebrate communities differed temporally between the LF2009 to LF2010 survey periods. These data are corroborated by the SASS5 data previously explained, which is that there was a very clear temporal, and a small spatial variation in both the rivers sampled. The driving forces and causes for these groupings can basically be attributed to the effects of the high-rainfall and high-flow period during the 2010 rainy season on the Olifants and Luvuvhu Rivers. It caused the system to be flushed resulting in more favourable conditions being created for the macroinvertebrate community. The result was that communities recovered and reproduced sufficiently to produce higher SASS5 results during the low-flow survey of 2010. The spatial trends seen were not as conclusive, but also show the decrease in scores and community structure along the length of both rivers. If these data are studied in conjunction with the fish results in the previous section, the overall temporal and spatial trends for the biological communities are similar, and show a significant variation between the two survey periods. An overall decrease in the community structure, abundance and diversity has occurred when the results of this study are compared to various historical data published in the literature.

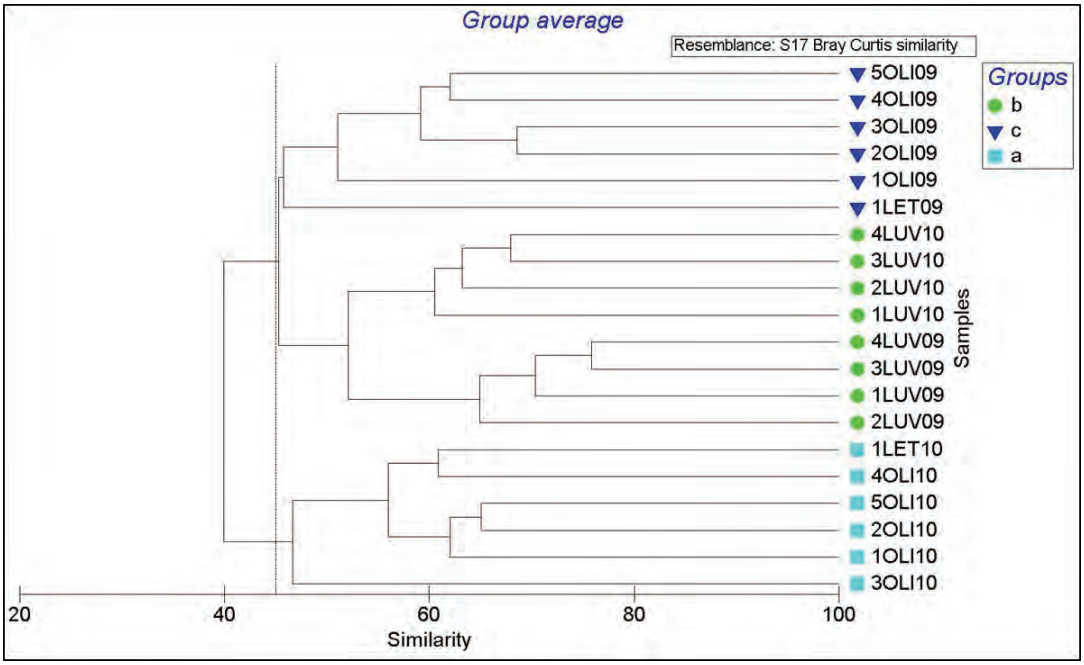

Figure 72. Bray-Curtis similarity matrix-based cluster analysis for all macroinvertebrate taxa sampled at all sites on the Olifants and Luvuvhu Rivers for both low-flow periods.

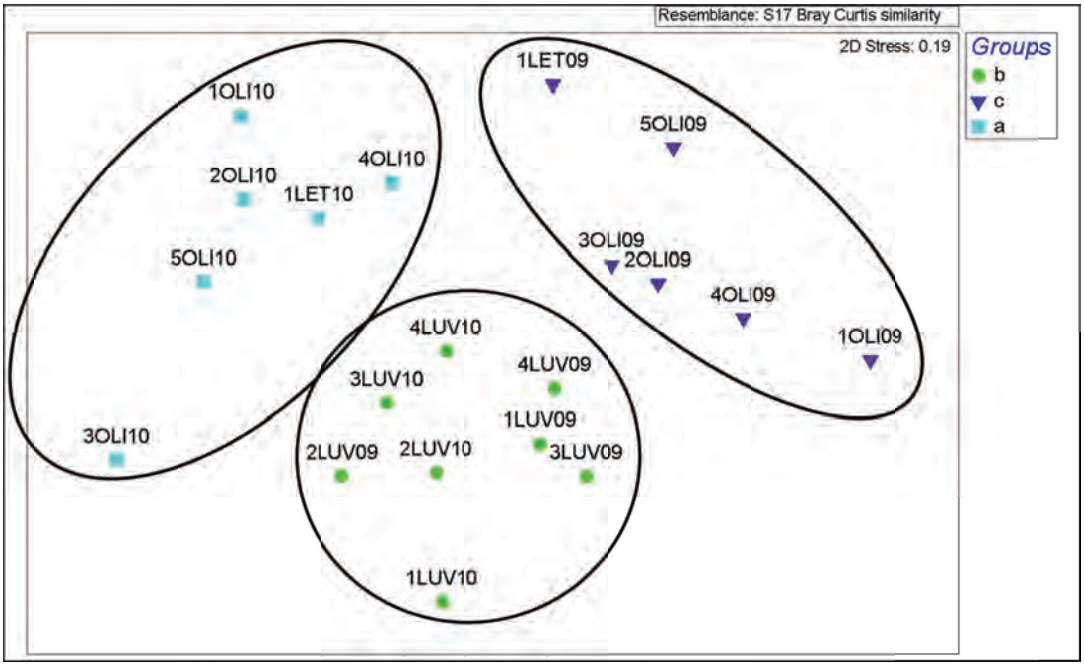

Figure 73. Two-dimensional representation of the NMDS ordination of all macroinvertebrate taxa sampled at all sites on the Olifants and Luvuvhu River for both low-flow periods.

Fishes

With reference to the total abundances of fish species sampled, the LF2010 sampling period for both rivers yielded the most fish (Figure 74). There was large temporal variation between the LF2009 and LF2010 periods within the Luvuvhu River. This can be attributed to factors previously mentioned regarding flow, habitat and water quality. Site 1 for the LF2009 period had a lower abundance of fish when compared to LF2010, but had a higher number of species (Figure 75). This can be attributed to habitat availability, as the majority of fish sampled favour overhanging vegetation which was not present in LF2009 to the extent that it was in LF2010. The Olifants River did show some temporal variation, with the 2010 period yielding more fish. Both rivers showed spatial variation, indicating an increasing trend in abundance downstream. This was also evident in the Luvuvhu River, but it did not match the degree of variation in the Olifants River.

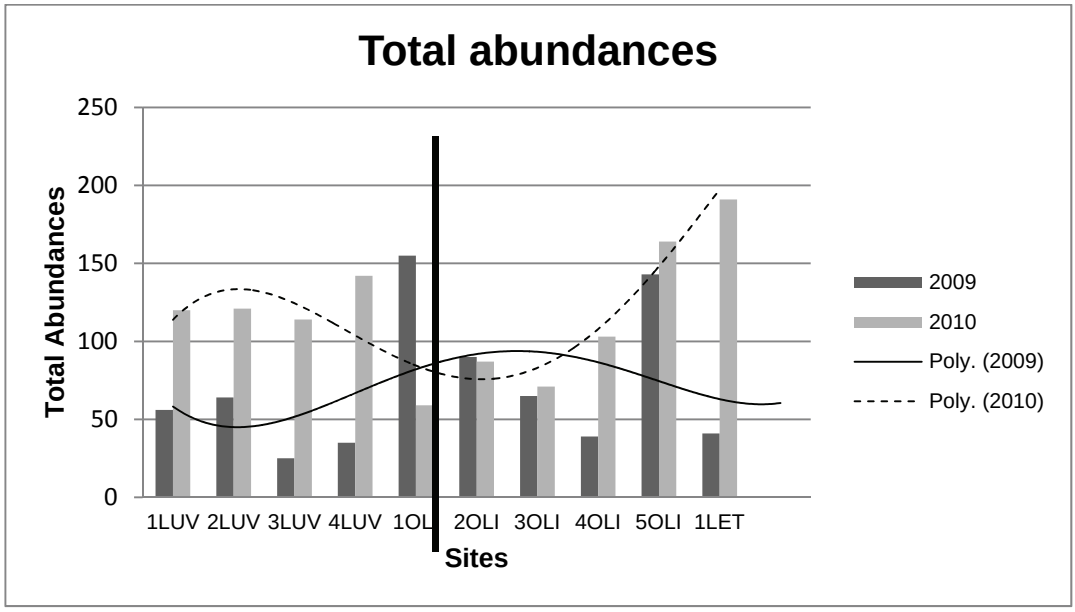

Figure 74. Total abundances of fish species sampled at all sites on the Olifants and Luvuvhu Rivers for both survey periods

A temporal trend is evident in the number of species sampled per site, as there are generally more species present in the LF2010 period for both rivers (Figure 75). The LF2009 period yielded far fewer species, with the exception of Site 1 on the Luvuvhu River. This can also be attributed to habitat, habitat preferences, and flow and water quality variables. In general, the number of species is lower than expected for both river systems and is thought to be caused by upstream anthropogenic impacts (State of Rivers Report, 2001).

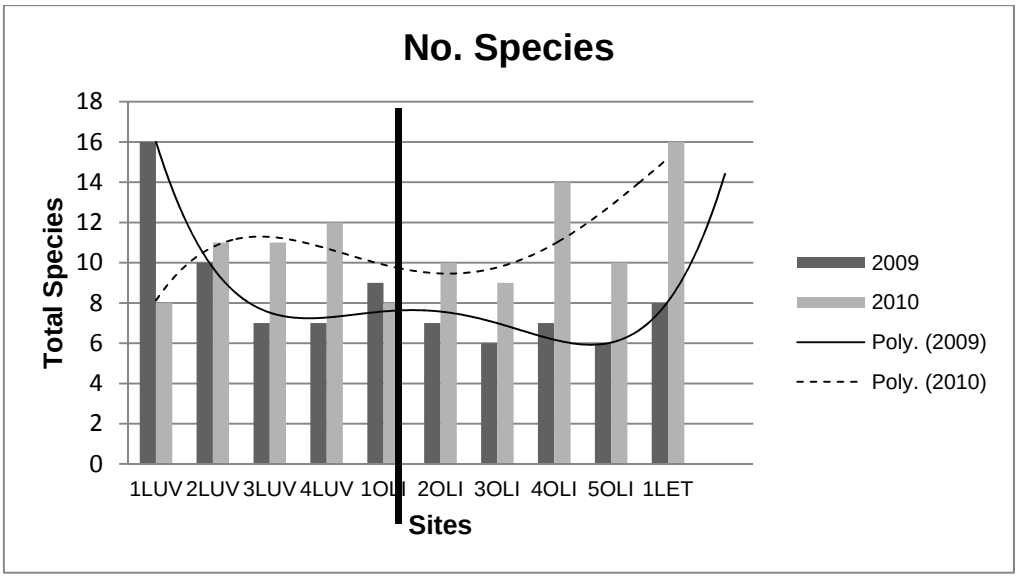

Figure 75. Total number of fish species sampled at all sites on the Olifants and Luvuvhu Rivers for both survey periods.

Margalef's index indicates the level of species richness and the higher the value obtained, the higher the level of species richness (Figure 76). A similar trend for both flow periods for the Olifants and Luvuvhu Rivers was observed, with higher species richness during the 2010 period, with the exception of Site 1 on the Luvuvhu River. The largest variation was found at Site 1 during the period LF2009 to LF2010. This is not in line with the general trend, and has been explained above.

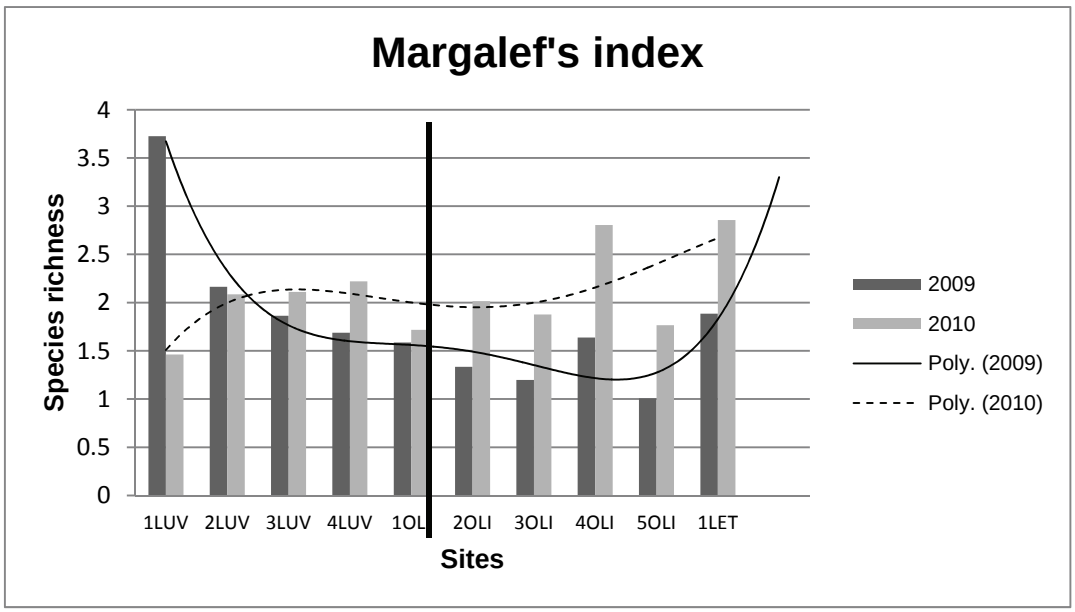

Figure 76. Margalef's index showing a level of species richness at all sites on the Olifants and Luvuvhu Rivers for both survey periods.

The evenness of species distribution (Figure 77) allows a measure of how species were distributed per site, and shows possible variations and dominance of species. Even though it was mentioned previously that species richness differed between rivers and sites, the evenness of the distribution of species is at an acceptable level. The Luvuvhu River has the highest level of species evenness for both flow periods, with communities showing a high level of stability. However, the Olifants River shows a temporal variation in species evenness, as in LF2009 the level of evenness is lower than for the LF2010 period. Spatial variation was also observed during the LF2009 period. This can once again be attributed to factors previously mentioned, as the fish communities in the Olifants River in LF2010 were found to be in a more natural state than in LF2009.

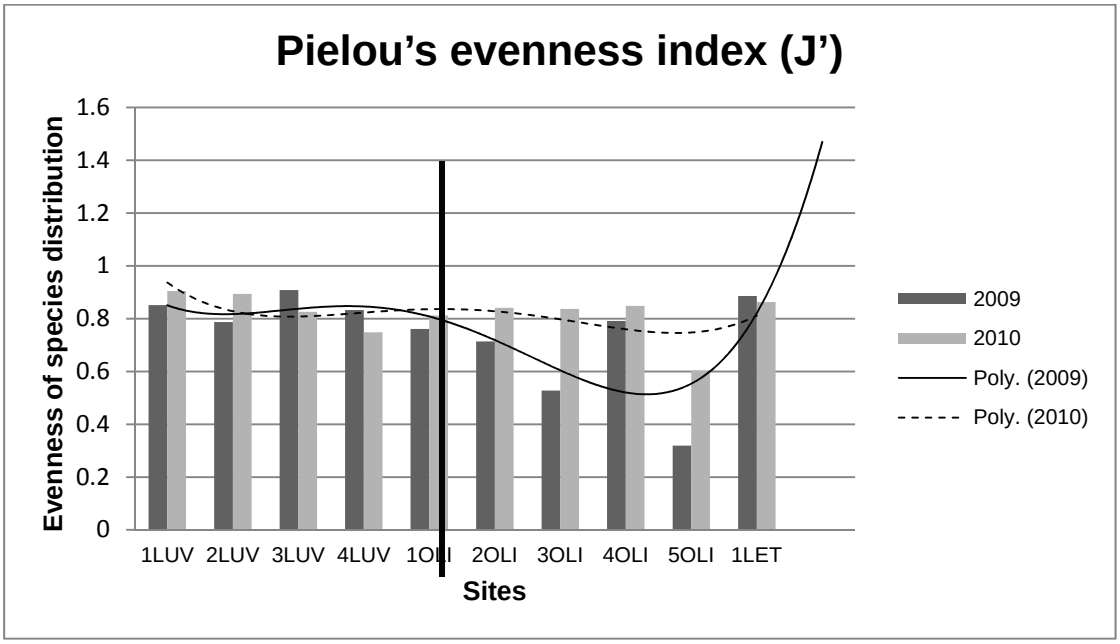

Figure 77. Pielou's evenness index (J') showing an evenness of species distribution at all sites on the Olifants and Luvuvhu Rivers for both survey periods.

A very similar trend was observed for temporal and spatial variations in the number of species present (Figure 75) and in species diversity, as the level of species diversity (Figure 78) is a function of the number of species present. The LF2009 survey period showed a general trend in decreasing diversity along the length of the river, with the exception of the Letaba Comparative Site. In LF2010, this trend seemed to stabilize, and species diversity was similar for the entire length of the river, with the exception of the last site, Site 5 (Gorge). This shows that regarding the hypothesis of the fish communities improving along the length of the river, the opposite seems to be occurring as species richness decreases along the length of the Olifants River, and remains stable to an extent along the Luvuvhu River.

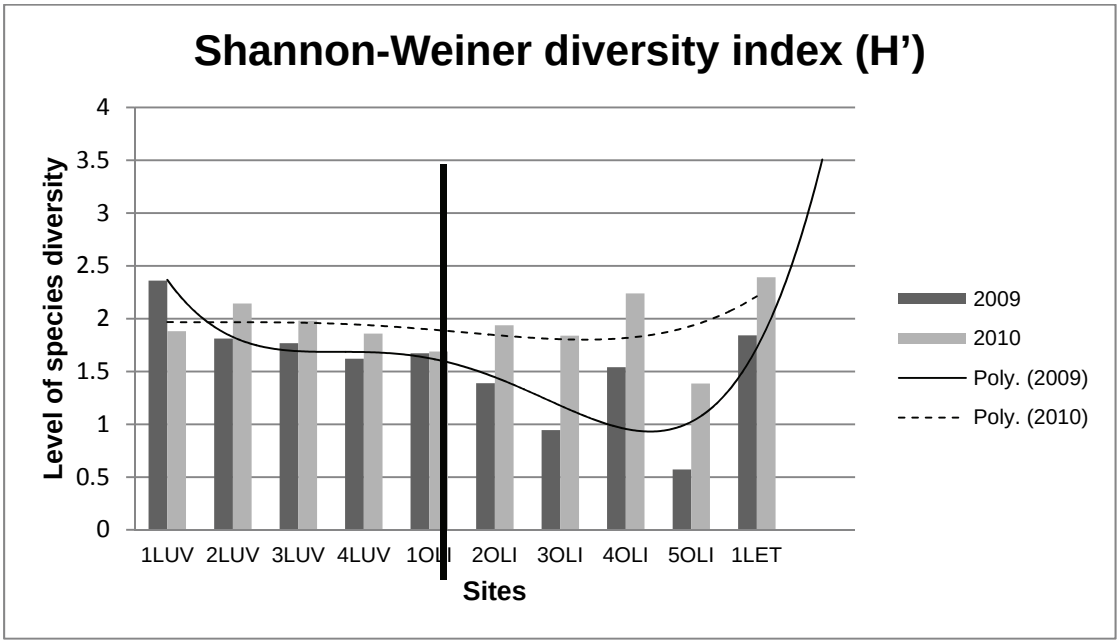

Figure 78. Shannon-Weiner diversity index showing a level of species diversity at all sites on the Olifants and Luvuvhu Rivers for both survey periods.

As previously mentioned, our first hypothesis is that although the Luvuvhu River itself is under pressure, its biological communities are stronger in diversity and structure than those in the Olifants River and the second hypothesis is whether or not the ecological state of the biological communities has improved where both the rivers leave the park compared to where they enter. To test these hypotheses and in order to obtain an indication of temporal and spatial trends among the fish communities, Bray-Curtis similarity-based cluster analysis and NMDS were performed (Figure 79 to Figure 83). The data were transformed to presence/absence data and then converted to a logarithmic scale. By comparing the data for all the sites for both rivers and survey periods, very few trends can be identified, the exception being the Luvuvhu River's fish communities for the LF 2010 sampling period (Figure 79). They are clustered together at a 60% similarity. In addition, a few Olifants River sites, namely Site 1 and Site 2 for both sampling periods form a cluster at 63% similarity, showing temporal similarities. In general, when comparing the two rivers, there is little spatial and temporal variation in the fish communities for both rivers. The Luvuvhu River fish communities do, however, show some similarities for the LF 2010 sampling period, and as such are grouped together. The NMDS ordination for both rivers and all the sites shows the above groupings in a different manner (Figure 80). Site 5 (Gorge) on the Olifants River for both surveys groups together, due to a similar number of species and abundances, but mainly attributed to the presence of *H. vittatus*. The Letaba Site for both survey periods is clustered with Site 4 (Balule) on the Olifants

River due to similar species diversity and abundances, and similar species found. The grouping seen for the LF2009 survey on the Luvuvhu River also corroborates what Figure 80 indicates, in that these sites and the river itself for this period had similar fish communities and abundances. When comparing the fish communities on a temporal and spatial basis for both rivers and flow periods, the general conclusion is that not many visible or clearly evident trends could be identified. This is not uncommon when comparing similar fish communities to each other, as fish are long lived and it is difficult to pick up trends between river systems. It should be noted though that the rivers are in different state regarding their fish communities, and this was explained in detail previously.

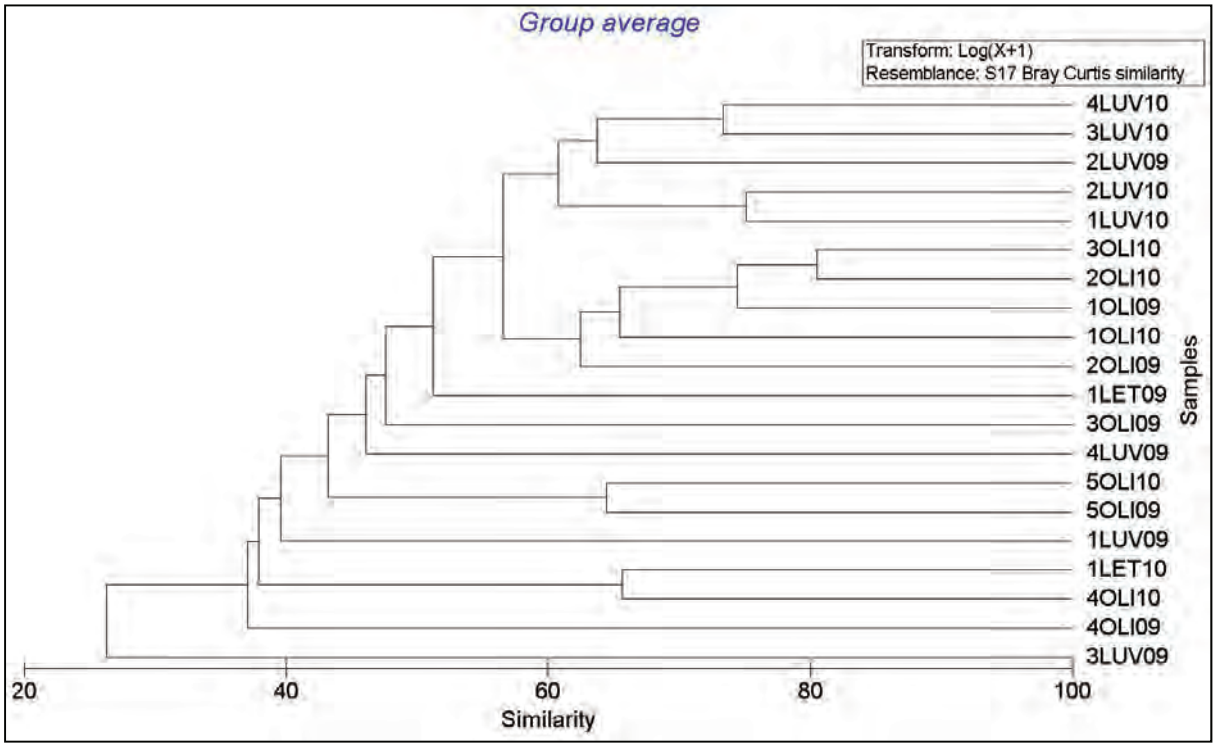

Figure 79. Bray-Curtis similarity matrix-based cluster analysis for all fish species sampled at all sites on the Olifants and Luvuvhu Rivers for both low-flow periods.

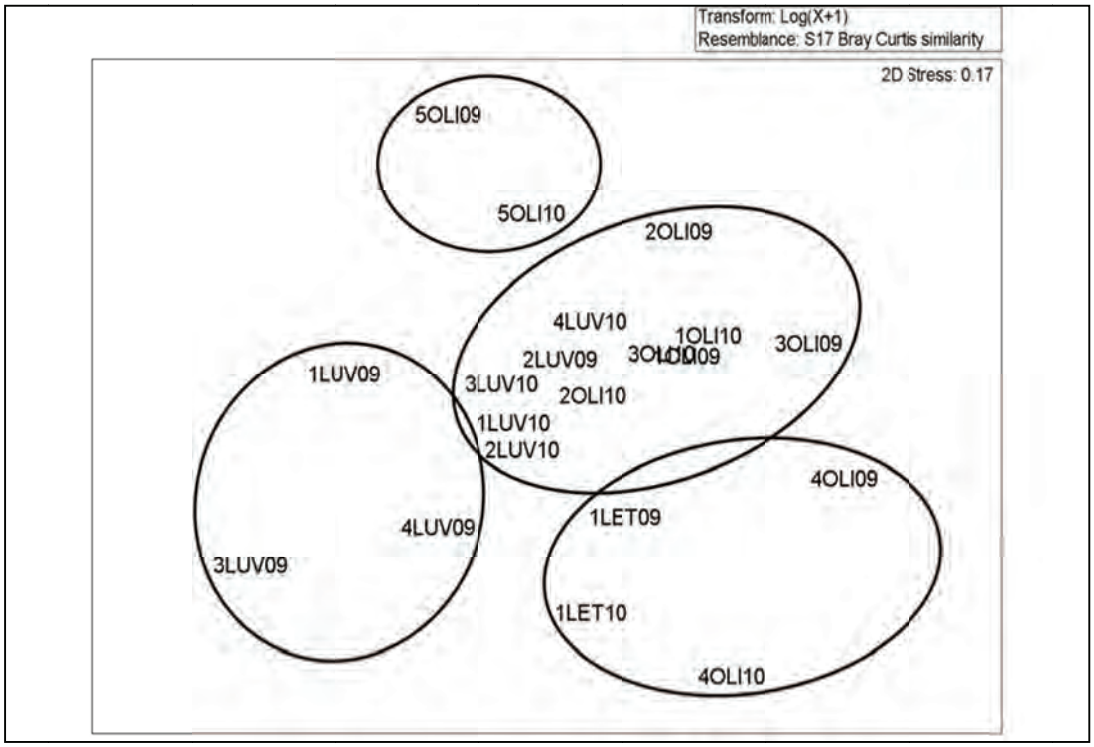

Figure 80. Two-dimensional representation of the NMDS ordination of all fish species sampled at all sites on the Olifants and Luvuvhu Rivers for both low-flow periods.

As no clear or evident trends could be identified when comparing the rivers as a whole, each river system was then compared to itself on an individual basis in order to identify any spatial and temporal trends. The Olifants River fish communities for all sites and survey periods were then compared (Figure 81 & Figure 82). The ANOSIM test revealed these groupings to be significant with a *R* value of 0.801. At a 45% similarity, very few temporal differences are seen between the two low-flow periods (Figure 81). However, spatial trends were observed. The Letaba Comparative Site is grouped together for both periods, as well as with Site 4 for the LF2010 sampling period. This is expected as the Letaba Site has a high diversity and abundance of fish, as did Site 4 on the Olifants for the LF2010 period. The Gorge sites (Site 5) for both surveys are grouped together and are separate from all other sites. This is mainly due to the presence of tigerfish *(H. vittatus)*, as the balance of the fish assemblage at Site 5 was similar to other sites. All the other sites are grouped together, because they did not exhibit sufficient temporal trends for the two flow periods to be regarded different enough to form separate groupings. The species sampled and the abundances present were similar. Regarding the hypothesis previously mentioned there is not sufficient evidence to show that the fish communities are stronger in diversity and structure from where the river enters the

KNP to where it leaves the KNP; rather, fish communities seem to decrease in diversity as the river flows through the KNP.

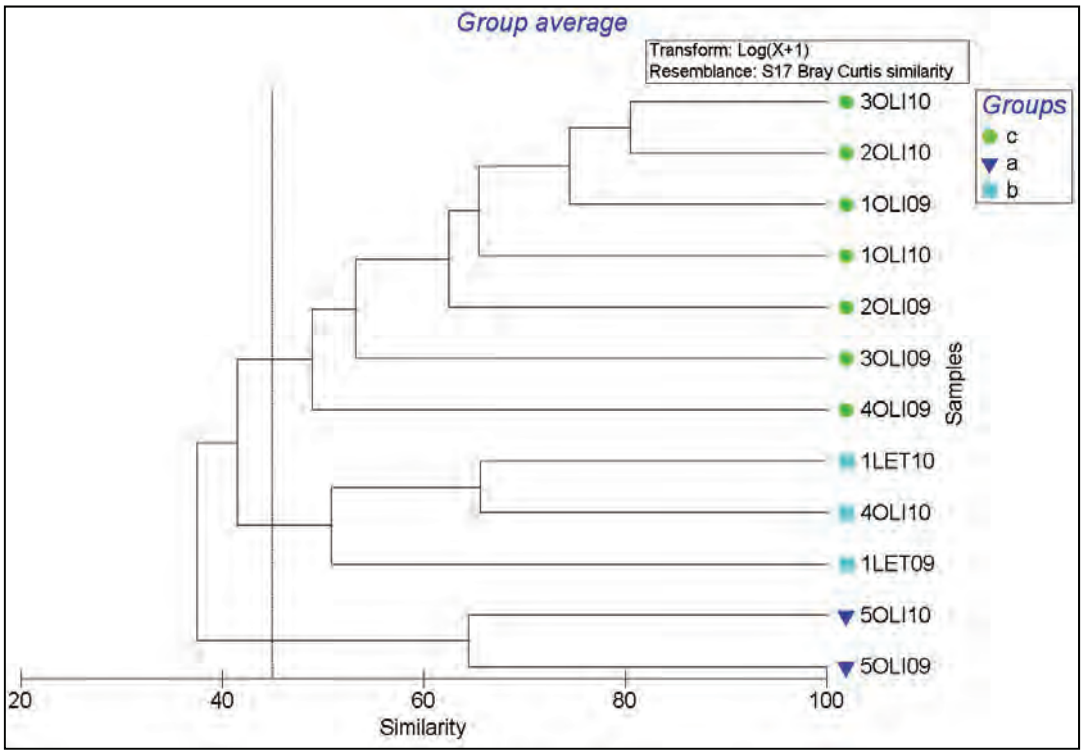

Figure 81. Bray-Curtis similarity matrix-based cluster analysis for all fish sampled at all sites on the Olifants River for both low-flow periods.

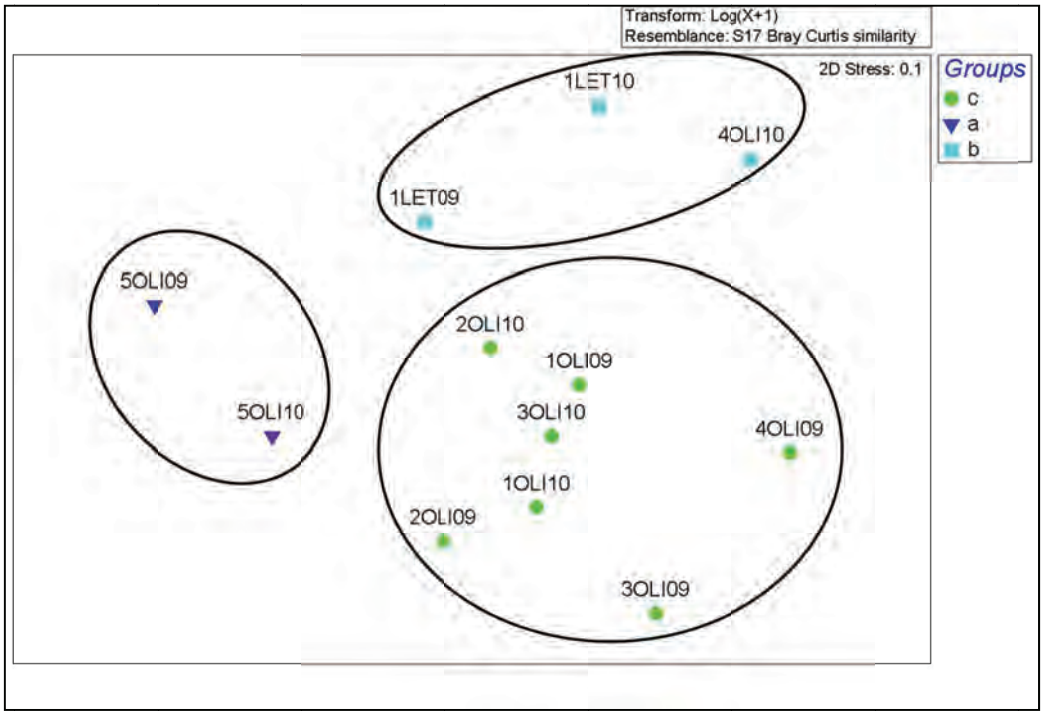

Figure 82. Two-dimensional representation of the NMDS ordination of fish species sampled on the Olifants River for both low-flow periods.

In terms of the Luvuvhu fish communities, the groupings obtained are very clear (Figure 83). The ANOSIM test revealed these groupings to be significant with a *R* value of 0.897. Both figures show that all sites, with one exception (3LUV09), are very similar and group together. There is no spatial and temporal variation within the river and for both survey periods, and Site 3 for the LF2009 period is the only outlier. This site had the lowest diversity and abundance for the Luvuvhu River for both survey periods, and as such is grouped accordingly (Figure 83). According to the *in situ* water quality variables, there was no particular reason to attribute this to a drop in water quality. What could be the reason is that the habitat availability at the site was not adequate and diverse, and as such, species preferring certain habitat types were not sampled. The FRAI scoring done for this section of the river (Table 45) resulted in low scores and the metrics responsible indicated a high correlation with habitat availability, cover and velocity depth preferences. This could be a reason but it is more likely that sampling errors or site-specific conditions contributed to this variation in grouping.

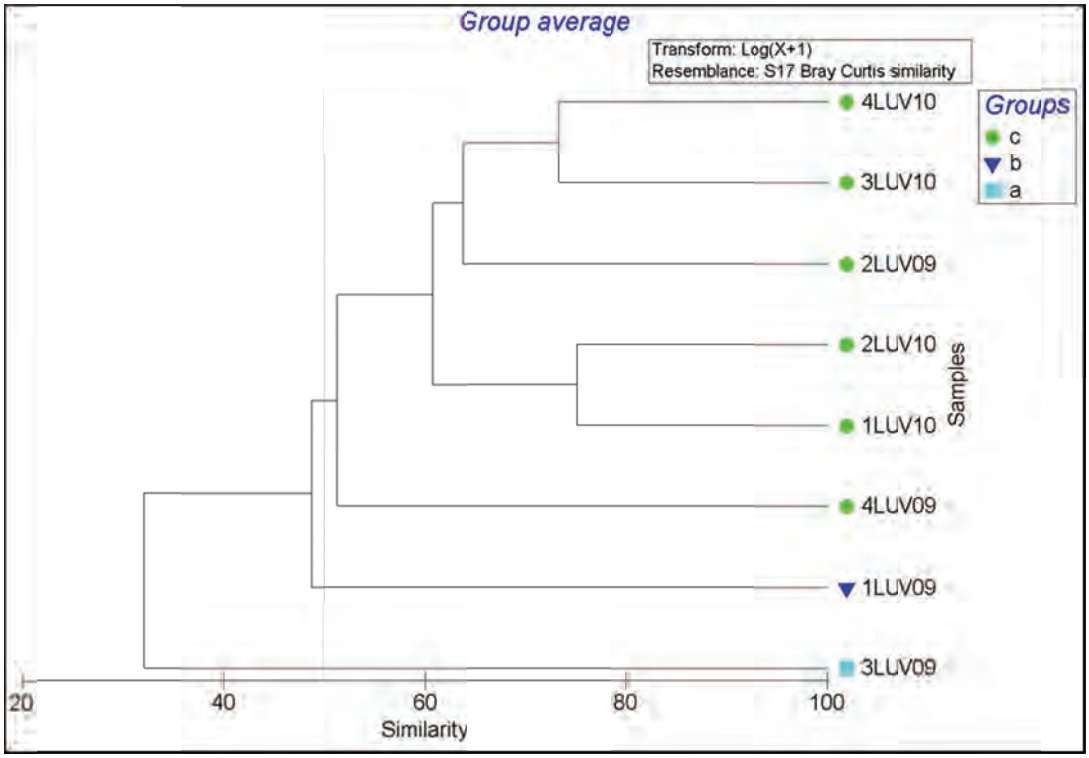

Figure 83. Bray-Curtis similarity matrix-based cluster analysis for all fish sampled at all sites on the Luvuvhu River for both low-flow periods.

5.3 Histology-based fish health assessment of *H. vittatus* populations from the Olifants and Luvuvhu Rivers

Although both the Olifants and Luvuvhu Rivers are known to be polluted by anthropogenic activities, the semi-quantitative histological assessment results indicate that the fish sampled in this study were in good health based on macroscopic and microscopic observations respectively. The *H. vittatus* specimens did have higher histopathological organ and fish index values when compared to *L. marequensis* and *L. cylindricus*. However, these values were within a normal range and were lower than values found in polluted systems where the fish were affected by heavy metals and EDC pollution. The age of fish did not have an effect on histopathological alterations in the fish sampled in this study but this may be because the fish sampled in this study were all relatively young. The mean liver index values of *H. vittatus* from the Olifants River were already in Class 2 (moderate histological alterations) which indicates that the livers of those fish were affected. Another consideration is that histological alterations serve as an early warning system. Although the alterations observed in this study were mild in terms of severity, they were nevertheless present in Olifants River *H. vittatus* during the LF2010 sampling trip.

5.4 Metal and organic bioaccumulation in *H. vittatus* in the Olifants and Luvuvhu Rivers

Bioaccumulation of metals and organic compounds in the muscle tissue of fish was used as an indication of contaminant-specific bioavailability and therefore possible causative agent(s) of toxicity (Chapman, 1997; Rainbow, 2007). The PCA biplot based on temporal and spatial metal bioaccumulation in muscle tissue of tigerfish (Figure 84) indicates a high degree of spatial and temporal variation in the data (67.5%). The metal bioaccumulation patterns of tigerfish from the two flow surveys in the Olifants/Letaba Rivers during 2010 are distinguished from the LF2009 Olifants and Luvuvhu River bioaccumulation patterns based on elevated Se and lower Co, Cu, Cr and Pb concentrations. The Luvuvhu 2010 and HF2011 survey in the Olifants River was characterised by lower metal bioaccumulation.

The addition of the OCP data to the dataset identified flow-dependent patterns in metal and OCP bioaccumulation data with 90.3% of the variation explained by the ordination in Figure 85.

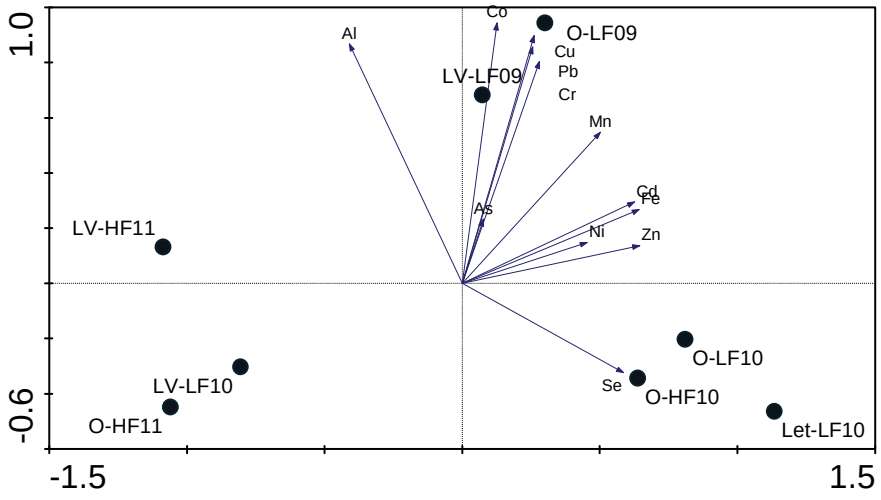

Figure 84. PCA biplot of metal bioaccumulation in muscle tissue of *H. vittatus* from the Olifants, Letaba and Luvuvhu Rivers during different flow periods. The ordination describes 93% of the variation in the data, with 67.5% displayed on the first axis, while 25.5% is displayed on the second axis.

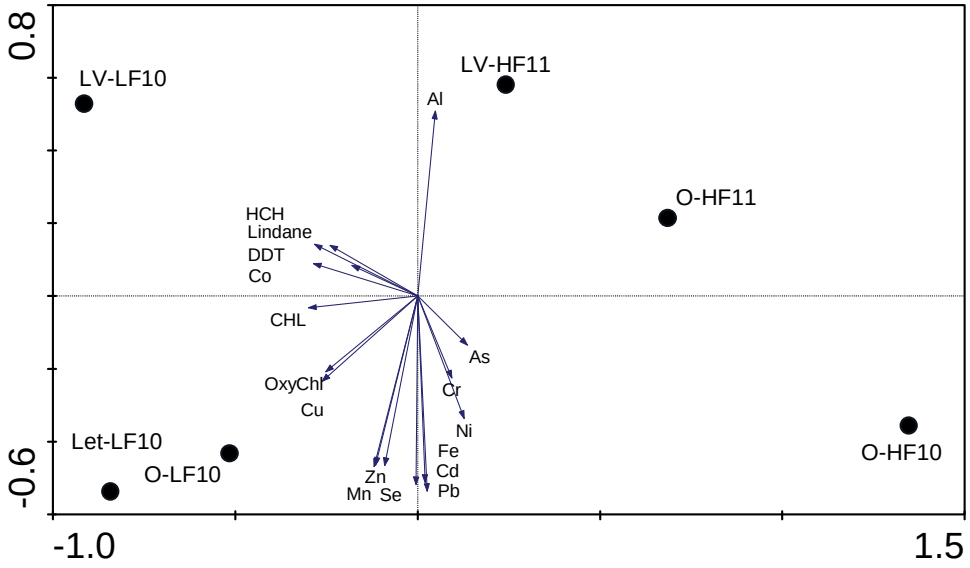

Figure 85. PCA biplot of metal and organohlorine pesticide bioaccumulation in muscle tissue of *H. vittatus* from the Olifants, Letaba and Luvuvhu Rivers during different flow periods. The ordination describes 90.3% of the variation in the data, with 69.2% displayed on the first axis, while 21.1% is displayed on the second axis.

The tigerfish bioaccumulation patterns in the Letaba and Olifants LF2010 survey were characterised by elevated Cu and oxy-Chlordane concentrations, while the Luvuvhu LF2010 fish had high concentrations of DDTs, HCHs, Lindane and Co. The Luvuvhu HF2011 tigerfish had distinctively high Al concentrations. It was therefore clear that site and survey specific conditions were responsible for the metal and organic bioaccumulation patterns observed.

The influence of physico-chemical characteristics on the bioaccumulation of dissolved and sediment-bound metals revealed that particulate metals are not permanently sequestered in aquatic sediment due to consistently fluctuating variables within aquatic systems. They thus remain environmentally significant due to their potential for future toxicity, mobility and availability for uptake by aquatic biota. Acid volatile sulphides played an important role in influencing the availability of sediment- bound metals within aquatic systems. Due to sulphur's affinity for binding with a number of divalent metals to form insoluble metal sulphides, AVS is able to control metal concentrations in the sediments. Where Zn, Ni and Cu SEM concentrations exceeded AVS concentrations (SEM-AVS >0) sediment-bound metals were available for biological uptake. This was demonstrated by increased Zn and Ni

bioaccumulation. The results also indicated that Cu bioaccumulation was not dependent on the sediment characteristics but was a function of the dissolved Cu concentrations. These results underline the importance of understanding (and elucidating) the underlying mechanisms responsible for metal and organic chemical uptake before interpreting the biological consequences of exposure to these substances.

5.5 Biomarker response of *H. vittatus* in the Olifants and Luvuvhu Rivers

When toxicants such as metals cross the cell membrane, they react with the cytosolic components and are usually complexed in different ways (e.g. chelation) to cytosolic compounds, such as high affinity, specific ligands (metallothioneins – MTs), substrates, products of enzymatic activity and/or enzymes themselves (Viarengo et al., 1997). The measurement of biomarker responses offer to demonstrate that toxicants have entered an organism, been distributed within the tissue, and are eliciting a toxicological effect on biological structures and functions (McCarthy and Shugart, 1990). Organisms' responses are measurements of cellular and physiological processes or biomarkers that are normal components of an organism's attempt to deal with metabolic processes and to maintain a constant internal balance.

The main purpose for the use of biomarkers is to give evidence of exposure to pollutants and consequent toxic effects (Walker, 1998). Biomarkers represent an organism's attempt to compensate for or tolerate stress effects (Cormier and Daniel, 1994). Thus, biomarkers also examine whether normal detoxification or repair capacities have been exceeded (Martin and Black, 1998). Effects of pollutants on aquatic organisms may be manifested at all levels of biological organization (Wepener, 2008). Under most circumstances, stressors, like pollutants indirectly affect higher levels of the ecosystem hierarchy (populations/communities), but directly affect molecular and cellular (sub-organism) level processes (Downs et al., 2001). For the purpose of this study, the definition for a biomarker refers to a change in cellular or biochemical components or in processes, structures or functions that are measurable in a biological system or sample. A biomarker is considered as any biological response to a pollutant or toxicant measured at the sub-individual level, indicating a deviation from the normal status that cannot be detected in the intact organism (Van der Oost *et al*., 2003).

For this study two types of biomarkers were selected, i.e. biomarkers of exposure and effect. The exposure biomarkers were AChE (pesticide exposure), MT (metal exposure) and CYP1A (chlorinated organic compounds, e.g. OCPs). The effect-biomarkers primarily reflected the oxidative status of cells through the use of

enzymes such as CAT SOD, MDA and PC. The CEA biomarker is an indication of cellular energy utilization during stress conditions.

Principal component analysis (PCA) was completed on the biomarker results obtained for *H. vittatus* in the two systems during LF2009 and LF2010. The ordinations represent the (dis)similarity between sites based on the biomarker responses. The resulting biplot (Figure 86) represents 65.4% of the variation in the data. The first PC axis represents temporal differences (50. 4% of the variation) between the LF2009 and LF2010 surveys. The higher metal and OCP exposures in tigerfish from the Olifants and Luvuvhu Rivers, respectively were alluded to in the sections on the individual rivers. These exposures resulted in increases oxidative stress as demonstrated by the elevated CAT and SOD activities. The LF2010 survey biomarker responses in tigerfish did not differ much between the two river systems. In both systems this survey period was characterised by higher available energy reserves.

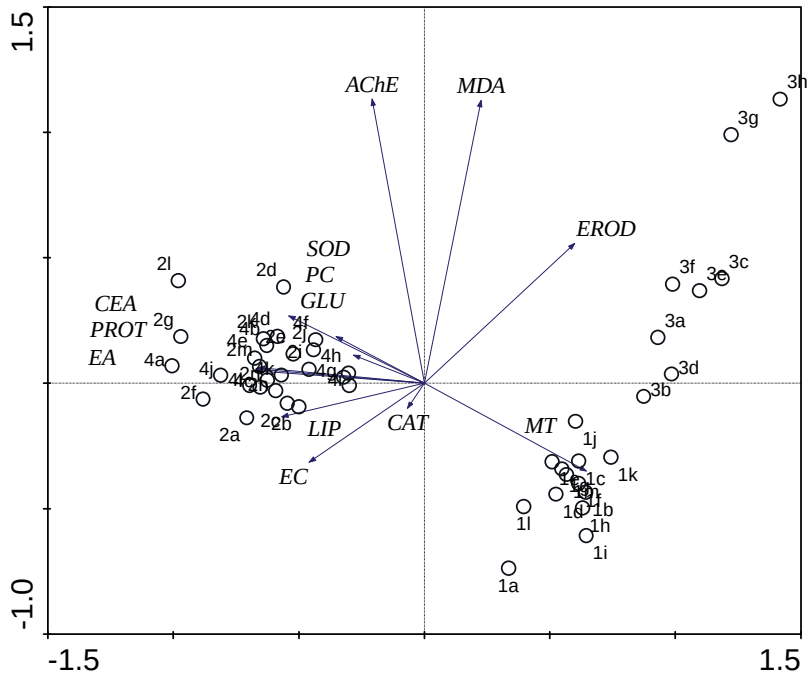

Figure 86. PCA ordination of spatial and temporal biomarker responses in *H. vittatus*. The two axes represent 65.4% of the variation in the data. The individual biomarker values were normalised prior to statistical analyses. Data points 1 and 2 represent Olifants River LF2009 and LF2010 respectively, while 3 and 4 represent Luvuvhu River LF2009 and LF2010 biomarker data.

5.6 Factors that might possibly limit the distribution of *H. vittatus* in the Olifants River

The first aim of this project was to establish the current distribution of tigerfish in the Luvuvhu and Olifants Rivers and the fourth aim to determine the factors that might possibly limit the distribution of *H. vittatus* in the Olifants River. As expected tigerfish were collected from all 4 sites in the Luvuvhu and thus confirming that the Luvuvhu is currently a good reference site for tigerfish. Surprisingly we also managed to collect tigerfish at all 5 sites in the Olifants River, even above Mamba Wier (Site 1). These records of tigerfish in the Olifants River on the western border of the KNP are the first in more than 20 years. Important to note is that all the tigerfish collected at Sites 1-4 in the Olifants River were young fish of less than 350 mm TL and probably not more than 2 years old (none of these fish were sacrificed for research, but released after capture). The abundance of these tigerfish at Sites 1 to 4 was also very low. At all these sites the number of tigerfish caught varied from 0 per survey to a maximum of 4. When comparing that to the very high density of tigerfish at Site 5 (confluence of the Olifants and Letaba Rivers at the start of Olifants Gorge), where the 15 tigerfish permitted were caught by six anglers within a maximum 5 minutes. This clearly indicates that although widely distributed in the Olifants River, upstream to and even above Mamba Wier, the population above Olifants Gorge consist of young fish and in very low numbers. This probably indicates that the upstream migration of tigerfish in the Olifants River are ad hoc occurrences that take place after good rainfalls that provide sufficiently high flows, especially during the low flow season.

The histological fish health assessment clearly showed that the tigerfish in the Olifants River is in a healthy state (section 3.7), despite some metals exceeding the Target Water Quality Guidelines (see section 3.1). Furthermore it appears that the tigerfish is also currently not affected by the pansteatitis that is implicated as the cause of recent crocodile and catfish deaths in the Olifants Gorge (Huchzermeyer et al., 2011). It is thus clear that the main factor influencing the limited distribution of tigerfish is water quantity and the resulting availability, or the lack of, suitable habitat.

5.7 Biological requirements of *H. vittatus* in the Olifants Rivers

The second aim of this project was to determine the biological requirements of tigerfish followed by the third aim of identifying whether the current environmental water allocation for the Olifants and Luvuvhu Rivers is sufficient to sustain a healthy tigerfish population. The findings of the study show that although tigerfish are not uniquely rheophilic specialists and be maintained in slow flowing habitat types, the

species does make extensive use of habitats that contain moderate to fast velocities as long as a sufficient water column is available.

The presence of a healthy tigerfish population along the length of the Luvuvhu River in KNP, as found in this study, clearly indicates that the current environmental water allocation is suitable for this species. However, this study also showed that there are a few points of concern regarding the water quality of the Luvuvhu that might influence the health of the tigerfish in this system in the near future. These are issues related to metal and OCP exposure (see section 4.7 on the bioaccumulation of metals and organic pollutants in Luvuvhu tigerfish as well as section 4.8 on the biomarker response to the presence of pollutants).

Although present throughout the Olifants River, and with individuals in a relatively healthy condition, the tigerfish populations above the Olifants Gorge (Site 5) are in a fragile state. The tigerfish seem to have recently returned to upstream areas (see Section 5.6) possibly due to consecutive years of consistent high rainfall that increased the flow, even in low flow seasons, and improved the water and sediment quality of the Olifants River (see results on water quality, section 3.1 and sediment, section 3.2). However, in order to sustain a healthy tigerfish population in the Olifants River the current ecological water allocation of the Olifants needs to improve in terms of quantity and quality. The bioaccumulation results indicated that there are changes in pollutant uptake and are manifested in changes in biological responses (as witnessed in the biomarker results). The good rainfalls during past two years have been particularly beneficial not for only just allowing the expansion of the tigerfish population range in the Olifants River but also for reducing pollutant exposure. However based on the initial results from the “back-end” of a particularly poor period in terms of water quantity and quality (the LF2009 survey), water quality issues are likely to remain biochemical cause for concern when considering that the newly established upstream tigerfish populations are already stressed populations. The histological fish health results also indicated that the livers of these fishes possess histological alterations that must serve as an early warning of detoriation in their health.

The main factor, however influencing the ability to sustain a healthy tigerfish population in the Olifants River remains water quantity. The outcomes of this assessment indicate that below a discharge of approximately 6 m^3/s the availability of fast deep (FD) habitat types would reduce to such low levels that this habitat type in the Olifants River would not be utilized by tigerfish Thereafter the tigerfish will be confined to slow deep flowing habitat types in pools, etc. in the system. Discharges below 4.9 m^3/s will reduce the availability of slow deep habitat types and may result

in the removal of suitable habitat types for the tigerfish and thus the collapse of the tigerfish population above the Olifants Gorge (Site 5).

6 RECOMMENDATIONS

The fifth and final aim of this project focused on management strategy for the conservation of tigerfish in the KNP with emphasis on mitigating measures to stimulate tigerfish populations to return to their original natural habitats. It also aims to validate and consolidate the use of tigerfish as indicator species of quality and quantity related Threshold of Potential Concern (TPC) in the Olifants and Luvuvhu Rivers.

6.1 The use of tigerfish as an indicator species for water quality and quantity in the KNP

The individual tigerfish studied from 2009 to 2011 in both the Olifants and Luvuvhu Rivers were in a healthy state. This was despite the fact that biochemically these fishes showed various levels and types of stress responses to the bioaccumulation of metal and organic pollutants. It is therefore clear that tigerfish do respond to the presence of low levels of pollutants. However, due to their highly mobile nature they may be able to avoid exposure to debilitating stressors and since one of the key criteria for the choice of a bioindicator is that they should represent the ambient conditions, the tigerfish may not be an ideal indicator species for water quality. However, results from the flow assessment done as part of this study clearly showed that tigerfish have very specific flow and habitat requirements, thus making them an excellent species to use as indicator of water quantity.

6.2 Recommendations on the environmental water allocation for the Olifants River

This study has shown that the fishes from the Olifants River have identifiable habitat preferences which were successfully used to evaluate the effects of reduced flows. Below modelled natural base low flow discharges of approximately 17 m^3/s the fishes in the Olifants River may begin to show heightened levels of stress due to reductions in habitat diversity and abundances. If the discharge of the Olifants River in the Kruger National Park reduces to below 4.9 m^3/s the resulting reduction in flow dependent habitat types would become severe. If maintained for extensive periods these reduced flows may become detrimental to the conservation of rheophilic fishes in particular and ultimately negatively impact on the structure and function of the system. For a discharge of 4.9 m^3/s to 6 m^3/s the tigerfish in the Olifants River would

be obligated to migrate into slow deep refuge areas. If these low flows are sustained it would become detrimental for the survival and conservation of this population. If the flow velocity drops below 4.9 m^3/s the habitat availability for the local tigerfish population would become unsuitable and result in the systematic reduction of the population in the Olifants River. The methodology used in the present study to determine flow and habitat preference for fishes (see section 2.7) is easily implemented and extremely informative and indicates that the available habitat preference information for the species considered here is limited and potentially not a true reflection of the life-cycle habitat preferences of the fishes in the Olifants River. In particular, the outcomes of the study suggest that the habitat preferences of fishes are dynamic and potentially change in response to habitat accessibility and other environmental factors such as water physico-chemistry. In addition, very little of the maximum stress levels and ability of fishes to survive in refuge areas in the Olifants River is known. The conservation and management of the fishes in the system should be considered holistically which includes the management of other populations that have access to each other in the catchment, and ability of fishes from refuge areas to populate impacted areas during periods of heightened stress, which includes reduced flows in the Olifants River.

Monitoring protocols and programs should also be implemented to observe and evaluate the impact of reduced flows in the Olifants River after events of extreme low flow. Finally the synergistic effects of heightened stress levels of populations in the Olifants River, due to other impacts including water quality stressors for example, during extreme low flow periods is unknown and should be evaluated.

6.3 Proposed management strategy for the conservation of *H. vittatus* in the KNP

This study showed that many fishes occurring in the Olifants River including the tigerfish have specific flow-dependent habitat requirements that are impacted by reduced flows in the system. These reduced flows initially causes rheophilic species to compete for limited suitable habitats potentially resulting in increased stress levels of populations on a reach scale. Thereafter if flow reduction continues, the total removal of fast and deep habitats will occur and which for would force those species have a high preference for these habitats into refuge areas where they may be able to maintain populations for a limited period. In the Olifants River, *L. cylindricus*, *L. molybdinus, L. marequensis* and the *Chiloglanis spp*. were all important indicators of flow stress for the system. Although these species would respond to, and possibly be negatively impacted on by reduced flows in the system before tigerfish, tigerfish will

also be negatively impacted on by reduced flows in the Olifants River. Flows of approximately 17.5 m^3/s have been shown to be suitable low flows for the Olifants River during which period sufficient habitat diversities should exist to allow all species considered to maintain their population structure. If the discharge of the Olifants River in the Kruger National Park reduces to below 4.9 m^3/s reduction in habitat availability and diversity is considered to become unacceptable for rheophilic species, which would then force them to occupy refuge areas for a limited period. From a discharge of 6 m^3/s to 4.9 m^3/s the tigerfish population in the Olifants River will be forced into slow deep refuge areas that are totally unsuitable habitats and may be detrimental for the maintenance and conservation of the population.

A study was carried out to evaluate the instream flow requirements (IFRs) of the Olifants River including the Kruger National Park (DWAF, 2000). Findings of this study obtained for IFR Site 17, located at Balule Bridge, showed that in September during a typically dry month, the fishes are most stressed due to low flows and higher water temperatures under natural conditions. By compounding the low flows during this period in particular, stress levels of fishes may rise to unacceptable levels influencing the stability of local populations. The recommended management category established in 2000 for the Olifants River was a “B” or largely natural category from the existing “C” modified state category. This resulted in the establishment of desired minimum IFRs for the river of 7.0-20 m^3/s during maintenance low flow periods and between 2.0-5.0 m^3/s during drought periods. The findings of the current study indicate that although these minimum flows fall into the minimum flow ranges for the Olifants River the threshold for the drought flows may be too low and should be increased to a minimum of 5.0 m^3/s. During these low flow periods the local tigerfish populations would be maintained for limited periods for a few months in slow-deep refuge areas. It is recommended that the population health be monitored during and after such events to ensure survivability of the population.

6.4 Recommendations for the Thresholds for Potential Concern (TPCs) for river health in the KNP

The Kruger National Park managers have created Thresholds of Potential Concern (TPCs) for fish and water quality as part of their management strategy. TPCs comprise a set of operational goals that together define the spatiotemporal heterogeneity conditions in terms of which the Kruger ecosystem is managed (Biggs and Rodgers, 2003). TPCs are essentially upper and lower limits along a continuum of change in selected environmental indicators (Biggs and Rodgers, 2003). When the upper or lower TPC levels are reached, or when modelling predicts that they will

soon be reached, this prompts an assessment of the cause of the extent of change (Biggs and Rodgers, 2003).

Electrical conductivity (EC) and Total Dissolved solids (TDS)

Olifants River

Electrical conductivity (EC) is a measure of the ability of water to conduct an electrical current (DWA, 1996) as a result of the presence of ions in water which carry an electrical charge. These ions include carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium (DWA, 1996). During this study values between 135 and 655 µS/cm were recorded for LF 2010, but also went as high as 2000 µS/cm in LF2009. The current KNP TPCs for EC values are set at 1200 µS/cm and TDS values of 800 mg/ℓ. These are extreme ranges, and are thought to be too high. TWQR for freshwater ecosystems states that the EC and TDS should not deviate more than 15% from natural cyclic and reference conditions (DWA, 1996). The Olifants River is naturally high in salts (Balance *et al*., 2001) and it is proposed that values of between 250 µS/cm and 500 µS/cm are set as the TPC values. It is thus recommended that the current TPC for EC and TDS be lowered to 1000 µS/cm and TDS values of 700 mg/ℓ respectively.

Luvuvhu River

The EC TPC value for the Luvuvhu River as set by the KNP is 800 µS/cm, with a TDS of 520 mg/ℓ. These values are thought to be high, as Barker (2006) showed that from 1984 to 2004 the conductivity value of the Luvuvhu River rarely exceeded 200 µS/cm. When compared to the Olifants River the EC values found in this study were much lower and fall within expected ranges for the Luvuvhu River and follow the same trend that Barker (2006) found. However, the EC value for LF2009 was higher than for LF2010. This shows a temporal difference, and can be attributed to higher flows and later rains for the high-flow season of 2010. The increase in flow during the high-flow period of 2010 points towards a degree of ‘flushing’ of the system, leading to lower EC values in the low-flow sampling period. As expected, spatial trends develop for both sample periods with a slow increase of EC values downstream. This is to be expected as there will generally be an increase in dissolved salts downstream in most rivers, as evaporation increases and flow decreases. It is thus recommended that, similar to the Olifants River, the EC TPC for the Luvuvhu River be lowered to 600 µS/cm, with a TDS of 420 mg/ℓ.

Fish communities

The current TPC for fish communities is described as follows: “the fish present ecological state (PES) per river reach should not drop one biological condition class (A-F) or show a continuous negative trend in the biological integrity categories (metrics) established for each river”. These TPCs (fish EC) are outdated and are based on the Fish Assessment Integrity Index (FAII) (Kleynhans, 1999). FRAI is now the accepted index regarding the RHP, and as such the FAII has now been replaced (Kleynhans *et al*., 2007). This index is based on fish responses to drivers as opposed to the FAII which was based on assemblages, but FRAI has the same scoring classes (A-F). It is thus proposed that the current Fish community TPC for KNP be amended to include the use of FRAI rather than FAII. The threshold lowering of a biological condition class is proposed to be suitable to act as a TPC and should thus be retained. Based on the findings from the present study, the Luvuvhu River has dropped one biological condition class and this is a matter of concern and should receive urgent attention from KNP managers.

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8 APPENDIX

Appendix 1. Selected metal concentrations (mean ± standard error µg/g dry mass) in sediments from selected sites in the Olifants and Letaba Rivers, during 4 separate surveys. BCR-A (acid soluble) and B (reducible) and BCR-C (oxidizable) and D (non-bioavailable), derived from a sequential extraction procedure.

	Aluminium (Al) µg/L					Arsenic (As) µg/L					Cadmium (Cd) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
OLI-S1-09LF	3.87	83.71	131.9	9905	10124 ± 6112	0.05	0.06	0.11	6.98	7.19 ± 1.48	0.03	0.03	0.02	0.09	0.16 ± 0.01
OLI-S2-09LF	2.94	106.7	74.00	24554	24737 ± 3698	0.11	0.05	0.12	8.45	8.74 ± 2.04	0.03	0.02	0.02	0.09	0.17 ± 0.02
OLI-S3-09LF	4.60	386.0	36.49	9185	9612 ± 9128	0.07	0.06	0.07	7.73	7.93 ± 1.27	0.03	0.02	0.01	0.09	0.15 ± 0.01
OLI-S4-09LF	1.64	74.71	142.2	3016	3234 ± 39.28	0.03	0.02	0.08	3.02	3.15 ± 0.27	0.01	0.03	0.02	0.05	0.11 ± 0.01
OLI-S5-09LF	30.58	410.2	2872	10650	13962 ± 4750	0.07	0.05	0.09	8.49	8.69 ± 1.58	0.01	0.02	0.01	0.08	0.12 ± 0
OLI-S6-09LF	10.66	235.5	20551	25282	46079 ± 15810	0.13	0.06	0.09	2.14	2.42 ± 0.18	0.02	0.04	0.02	0.09	0.17 ± 0.01
OLI-S1-10HF	14.88	149.6	28.57	19922	20115 ± 8193	0.23	0.18	0.06	26.25	26.72 ± 0.79	0.03	0.22	0.02	0.02	0.28 ± 0.22
OLI-S2-10HF	6.84	75.88	12.01	23409	23503 ± 1194	0.14	0.13	0.03	51.43	51.74 ± 0.29	0.02	0.01	0.01	0.03	0.07 ± 0
OLI-S3-10HF	4.07	39.50	8.62	16485	16537 ± 4188	0.12	0.25	0.02	61.17	61.57 ± 0.64	0.01	0.50	BD	0.06	0.58 ± 0.49
OLI-S4-10HF	28.25	283	105.7	41738	42155 ± 10669	0.39	0.15	0.19	48.89	49.62 ± 0.37	0.04	0.01	0.02	0.07	0.14 ± 0.01
OLI-S5-10HF	5.03	66.31	9.72	28848	28929 ± 9575	0.11	0.35	0.02	49.89	50.38 ± 0.5	0.01	0.81	0.01	0.06	0.89 ± 0.82
OLI-S6-10HF	4.71	40.94	8.27	2810	2864 ± 578.2	0.06	0.09	0.00	12.81	12.96 ± 0.05	0.01	BD	0.01	0.03	0.05 ± 0.01
OLI-S1-10LF	3.30	24.82	5.78	11642	11676 ± 4596	0.15	0.07	0.01	208.82	209.1 ± 207	0.01	BD	BD	0.07	0.09 ± 0.02
OLI-S2-10LF	4.83	38.87	9.00	21310	21363 ± 3679	0.13	0.07	0.02	2.65	2.87 ± 0.34	0.01	BD	BD	0.05	0.07 ± 0.01
OLI-S3-10LF	2.59	30.32	6.98	11256	11296 ± 2126	0.14	0.07	0.02	247.28	247.5 ± 245.6	0.01	BD	BD	0.06	0.07 ± 0.01
OLI-S4-10LF	4.14	40.91	11.17	15923	15979 ± 8283	0.15	0.09	0.03	2.53	2.78 ± 0.78	0.01	BD	BD	0.06	0.07 ± 0.01
OLI-S5-10LF	3.65	42.25	9.42	9867	9923 ± 3082	0.15	0.10	0.03	1.43	1.71 ± 0.28	0.01	BD	BD	0.05	0.06 ± 0.01
OLI-S6-10LF	4.31	42.15	15.83	6262	6324 ± 1737	0.12	0.04	0.04	0.81	1.02 ± 0.16	0.01	BD	BD	0.05	0.06 ± 0.01
OLI-S1-11HF	6.11	30.30	45.10	22852	22933 ± 7267	0.25	0.12	0.09	45.81	46.26 ± 1.35	0.02	BD	BD	0.08	0.1 ± 0.03
OLI-S2-11HF	9.18	42.98	42.52	36912	37006 ± 252.9	0.33	0.11	0.09	38.74	39.26 ± 0.1	0.03	BD	BD	0.06	0.09 ± 0
OLI-S3-11HF	7.92	38.47	34.34	30275	30356 ± 2270	0.27	0.08	0.08	43.04	43.48 ± 0.3	0.02	BD	BD	0.05	0.08 ± 0.01
OLI-S4-11HF	4.92	33.60	25.66	31534	31598 ± 1695	0.23	0.08	0.10	44.44	44.86 ± 0.23	0.02	BD	0.01	0.06	0.09 ± 0.01
OLI-S5-11HF	2.91	28.93	16.32	19691	19739 ± 819	0.16	0.07	0.10	28.23	28.56 ± 0.14	0.01	BD	0.01	0.03	0.06 ± 0.01
OLI-S6-11HF	1.61	25.54	16.96	4121	4165 ± 342	0.08	0.04	0.07	14.82	15 ± 0.03	0.01	BD	BD	0.03	0.04 ± 0

Appendix 1. continued

	Chromium (Cr) µg/L					Cobalt (Co) µg/L					Copper (Cu) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
OLI-S1-09LF	0.59	0.83	3.52	211.51	216.5 ± 25.67	2.67	1.09	0.38	7.47	11.61 ± 0.36	1.77	1.09	4.55	44.27	51.67 ± 2.3
OLI-S2-09LF	0.24	0.57	45.35	171.27	217.4 ± 80.05	2.39	1.83	0.62	11.70	16.53 ± 2	0.87	2.44	5.18	58.08	66.58 ± 8.74
OLI-S3-09LF	0.26	0.65	1.39	116.40	118.7 ± 21.45	1.05	1.31	0.47	10.87	13.71 ± 1.17	0.49	0.68	4.47	54.11	59.75 ± 11.55
OLI-S4-09LF	0.14	1.73	0.70	37.05	39.62 ± 3.41	0.64	0.94	1.96	2.87	6.4 ± 1.29	0.45	0.33	4.28	11.26	16.31 ± 0.74
OLI-S5-09LF	0.16	0.81	1.95	162.06	165 ± 38.71	1.20	1.26	0.47	6.43	9.35 ± 0.41	0.26	0.76	3.64	26.38	31.04 ± 2.11
OLI-S6-09LF	0.09	0.35	1.50	53.87	55.81 ± 11.48	1.00	0.79	0.59	4.68	7.07 ± 1.19	0.55	0.52	6.37	34.06	41.51 ± 10
OLI-S1-10HF	0.18	0.18	4.51	104.06	108.9 ± 28.45	0.37	0.43	0.24	101.08	102.1 ± 14.44	0.06	BD	0.30	14.00	14.37 ± 2.73
OLI-S2-10HF	0.06	0.84	1.70	104.18	106.8 ± 29.6	0.37	2.29	0.18	100.72	103.6 ± 18.42	0.07	0.16	0.21	22.01	22.45 ± 5.3
OLI-S3-10HF	0.02	0.13	1.24	88.44	89.83 ± 3.94	0.32	0.28	0.22	98.64	99.47 ± 4.31	0.08	BD	0.09	24.14	24.32 ± 5.83
OLI-S4-10HF	0.29	0.83	16.61	141.24	159 ± 54.1	1.76	1.90	0.85	133.17	137.7 ± 36.16	0.18	0.03	0.65	31.54	32.39 ± 1.21
OLI-S5-10HF	0.03	0.26	1.29	81.49	83.08 ± 1.98	0.41	0.50	0.21	90.95	92.07 ± 2.16	0.09	BD	0.16	28.30	28.55 ± 0.26
OLI-S6-10HF	BD	0.46	0.88	6.99	8.34 ± 1.3	0.18	0.95	0.11	97.69	98.92 ± 2.11	0.14	BD	0.28	8.23	8.65 ± 3.52
OLI-S1-10LF	0.03	0.24	1.03	78.40	79.7 ± 2.77	0.37	0.58	0.26	65.29	66.5 ± 2.1	0.22	BD	0.17	16.51	16.89 ± 5.51
OLI-S2-10LF	0.06	0.29	1.38	92.56	94.28 ± 2.54	0.37	0.56	0.21	76.98	78.13 ± 2.17	0.09	BD	0.09	20.40	20.58 ± 5.82
OLI-S3-10LF	0.18	0.32	1.13	92.67	94.29 ± 3	0.37	0.59	0.26	77.11	78.32 ± 2.54	0.12	BD	0.09	27.49	27.71 ± 2.11
OLI-S4-10LF	0.13	0.32	1.68	87.52	89.65 ± 2.35	0.46	0.79	0.22	72.85	74.32 ± 1.92	0.21	BD	0.16	20.46	20.83 ± 1.91
OLI-S5-10LF	0.19	0.46	1.59	87.32	89.55 ± 1.15	0.69	0.53	0.38	72.69	74.29 ± 1.15	0.35	BD	0.22	22.02	22.59 ± 7.25
OLI-S6-10LF	0.13	0.25	1.59	58.03	60.01 ± 25.38	0.56	0.30	0.24	67.84	68.94 ± 1.44	0.41	BD	0.45	13.39	14.26 ± 3.03
OLI-S1-11HF	0.13	0.28	1.09	85.04	86.55 ± 2.1	1.36	1.72	0.48	70.65	74.21 ± 2.03	0.20	BD	0.35	24.58	25.12 ± 8.24
OLI-S2-11HF	0.11	0.25	4.94	92.80	98.09 ± 3.67	0.72	1.97	0.43	77.09	80.21 ± 2.78	0.10	BD	0.30	24.96	25.35 ± 1.39
OLI-S3-11HF	0.08	0.20	3.87	96.36	100.5 ± 4.2	0.61	1.22	0.34	80.06	82.23 ± 3.17	0.09	BD	0.25	24.44	24.78 ± 7.71
OLI-S4-11HF	0.05	0.18	2.59	91.23	94.04 ± 4.2	0.66	0.88	0.25	75.89	77.69 ± 3.02	0.14	BD	0.97	23.23	24.33 ± 1.08
OLI-S5-11HF	0.03	0.16	1.58	91.24	93.01 ± 1.25	0.43	0.75	0.21	75.91	77.3 ± 0.93	0.12	BD	0.29	14.18	14.58 ± 4.7
OLI-S6-11HF	BD	0.10	1.64	91.04	92.78 ± 30.66	0.45	0.32	0.18	72.91	73.87 ± 3.75	0.36	BD	0.61	9.95	10.93 ± 0.49

Appendix 1. continued

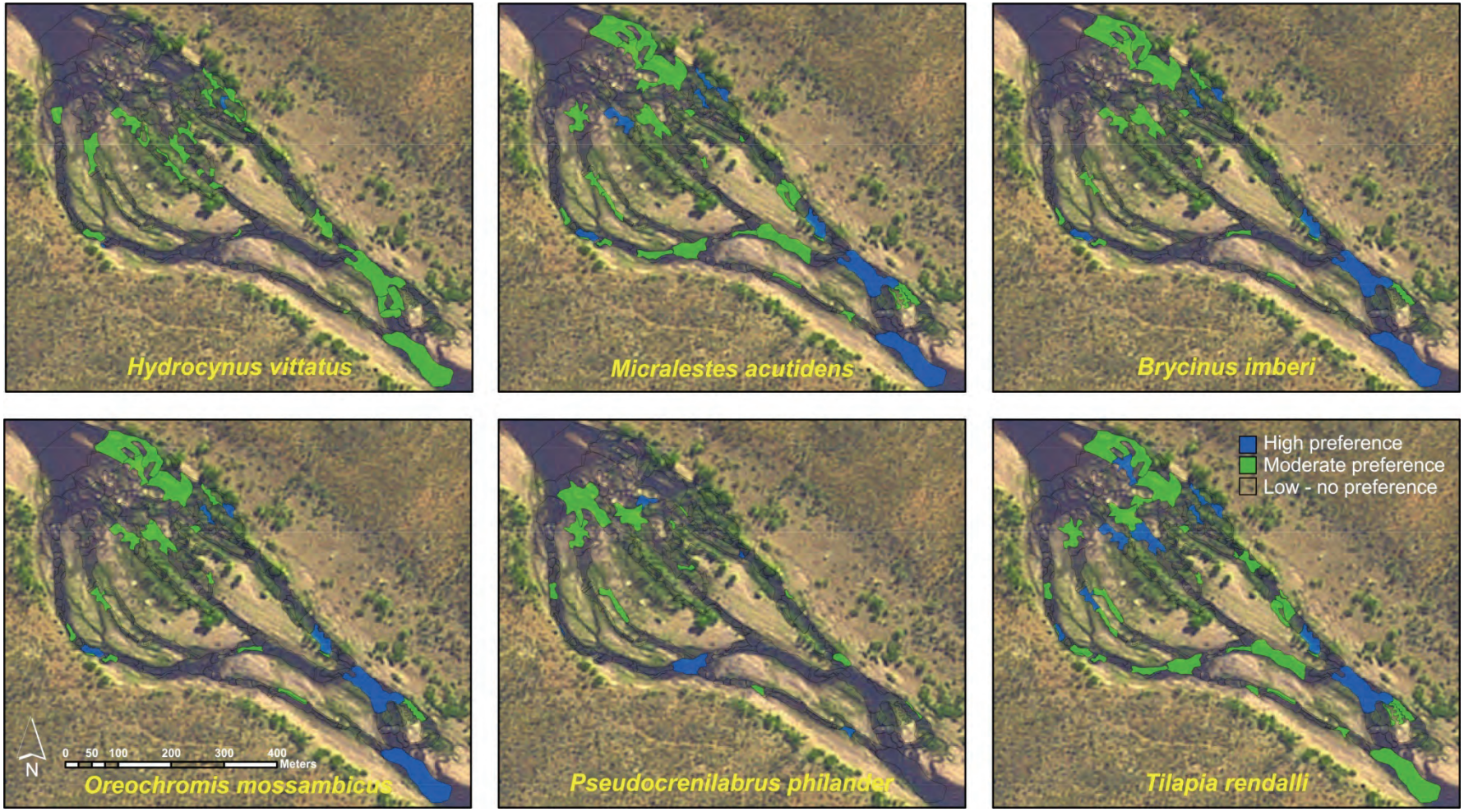
	Lead (Pb) µg/L					Iron (Fe) µg/L					Manganese (Mn) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
OLI-S1-09LF	0.09	0.58	0.60	4.75	6.02 ± 0.49	1.30	262	71.94	15688	16023 ± 9158	113.82	20.40	5.55	269.57	409.3 ± 48.94
OLI-S2-09LF	0.08	0.36	0.66	3.98	5.08 ± 0.5	1.67	315.6	138.9	31393	31849 ± 7664	98.47	23.63	10.27	384.49	516.8 ± 79.03
OLI-S3-09LF	0.05	0.32	0.42	3.69	4.48 ± 0.32	1.36	269	64.56	4986	5321 ± 4924	81.65	31.04	5.64	351.61	469.9 ± 90.82
OLI-S4-09LF	0.06	0.32	0.30	2.50	3.18 ± 0.06	1.63	161.3	61.64	6457	6681 ± 1599	41.18	32.07	171.09	65.04	309.4 ± 161
OLI-S5-09LF	0.02	0.35	0.44	4.33	5.14 ± 0.15	64.37	524.3	8375	18669	27632 ± 8092	97.76	23.06	6.53	196.85	324.2 ± 24.04
OLI-S6-09LF	0.06	0.34	0.56	3.59	4.55 ± 0.71	29.17	270	17985	17131	35416 ± 18650	135.40	73.55	10.57	157.17	376.7 ± 149.5
OLI-S1-10HF	0.01	0.14	0.22	0.85	1.22 ± 0.11	16.25	1279	145.7	14645	16086 ± 2952	27.58	9.24	5.69	77.73	120.2 ± 19.66
OLI-S2-10HF	0.02	0.24	0.11	0.87	1.23 ± 0.2	31.48	876	95.76	27271	28274 ± 5249	16.26	54.91	2.63	77.55	151.3 ± 53.77
OLI-S3-10HF	0.02	0.20	0.12	1.14	1.47 ± 0.07	21.13	470	60.25	68244	68795 ± 27260	12.65	9.24	2.63	76.82	101.4 ± 0.81
OLI-S4-10HF	0.02	0.24	0.84	2.12	3.21 ± 0.72	92.07	2674	337.3	39310	42413 ± 2099	146.07	37.51	21.30	102.28	307.2 ± 20.82
OLI-S5-10HF	0.02	0.09	0.10	1.43	1.63 ± 0.44	30.40	1036	90.64	38075	39232 ± 416.2	14.01	9.50	2.50	70.82	96.84 ± 4.86
OLI-S6-10HF	0.02	0.16	0.10	0.79	1.06 ± 0.16	12.22	321.9	73.61	7045	7453 ± 2029	8.59	24.42	1.36	75.89	110.3 ± 9.24
OLI-S1-10LF	0.03	0.06	0.11	0.79	1 ± 0.32	84.59	436.4	60.81	98738	99319 ± 50695	18.52	16.75	3.49	52.47	91.23 ± 4.82
OLI-S2-10LF	0.02	0.06	0.13	0.65	0.86 ± 0.05	40.22	533.8	69.80	22070	22714 ± 7780	17.36	12.61	3.01	61.93	94.91 ± 4.8
OLI-S3-10LF	0.02	0.05	0.10	0.49	0.66 ± 0.01	35.56	563.8	54.59	63560	64214 ± 27537	22.19	8.58	3.45	62.04	96.26 ± 4.05
OLI-S4-10LF	0.02	0.06	0.12	0.53	0.74 ± 0.15	99.33	713.3	74.74	21377	22264 ± 1739	22.12	26.62	2.98	58.61	110.3 ± 15.49
OLI-S5-10LF	0.04	0.10	0.10	0.47	0.71 ± 0.09	210.67	661.8	80.70	30769	31723 ± 15307	23.72	20.88	16.14	58.43	119.2 ± 10.83
OLI-S6-10LF	0.04	0.06	0.14	0.48	0.72 ± 0.11	151.96	866.6	98.19	9602	10719 ± 1726	101.78	11.58	7.33	54.49	175.2 ± 61.05
OLI-S1-11HF	0.01	0.02	0.42	1.32	1.77 ± 0.91	12.46	559	144.3	45904	46619 ± 15736	21.89	37.96	7.19	56.85	123.9 ± 10.86
OLI-S2-11HF	0.01	0.02	0.32	1.14	1.48 ± 0.04	6.02	946.6	108.6	23098	24159 ± 622.8	58.60	47.38	7.87	62.01	175.9 ± 5.02
OLI-S3-11HF	BD	0.02	0.24	0.94	1.2 ± 0.26	7.26	814.9	85.60	35981	36889 ± 12243	39.43	27.24	5.85	64.39	136.9 ± 15.81
OLI-S4-11HF	0.01	0.03	0.15	0.85	1.04 ± 0.08	17.34	703.7	85.29	22949	23756 ± 2586	37.67	14.53	2.82	61.07	116.1 ± 7.63
OLI-S5-11HF	0.01	0.03	0.10	0.61	0.75 ± 0.06	16.91	611	59.24	10237	10924 ± 3449	21.58	12.30	2.26	61.05	97.18 ± 5.61
OLI-S6-11HF	0.02	0.06	0.12	0.44	0.64 ± 0.04	38.57	540	104.70	7653	8336 ± 582.9	24.27	7.19	1.84	58.49	91.8 ± 6.61

Appendix 1. continued

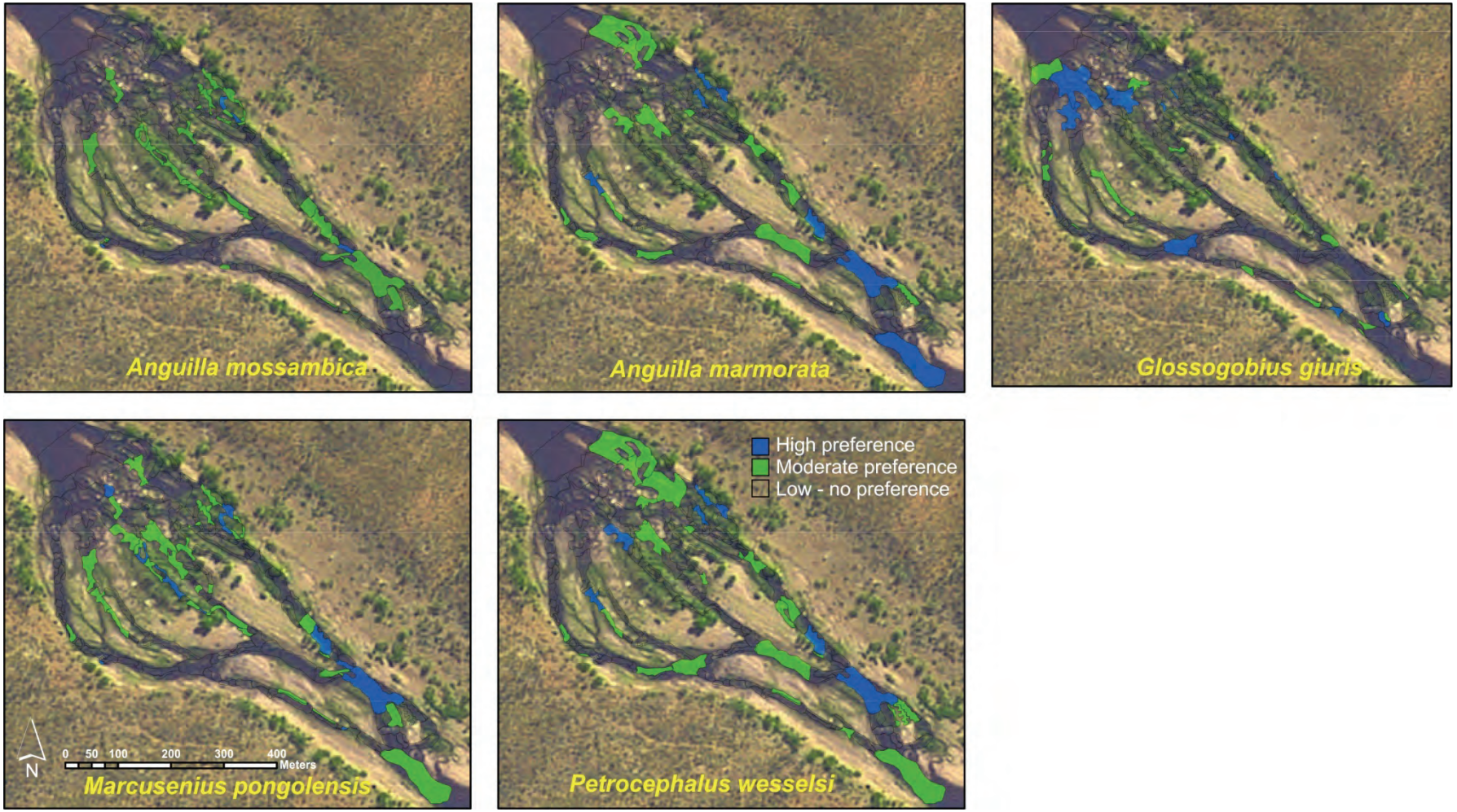
	Nickel (Ni) µg/L					Selenium (Se) µg/L					Silver (Ag) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
OLI-S1-09LF	3.94	1.77	1.70	37.17	44.57 ± 6.97	0.01	0.08	0.05	1.28	1.41 ± 0.37	0.03	0.11	0.02	0.63	0.79 ± 0.09
OLI-S2-09LF	2.69	1.85	19.21	31.88	55.64 ± 19.08	0.01	0.07	0.03	1.30	1.4 ± 0.57	0.05	0.12	0.02	0.63	0.82 ± 0.07
OLI-S3-09LF	1.96	1.74	1.12	25.05	29.87 ± 0.83	0.01	0.08	0.02	0.93	1.03 ± 0.18	0.05	0.23	0.02	0.67	0.97 ± 0.06
OLI-S4-09LF	1.55	1.69	4.36	11.42	19.02 ± 3.19	0.01	0.05	0.02	0.79	0.87 ± 0.02	0.05	0.20	0.01	0.54	0.81 ± 0.04
OLI-S5-09LF	1.47	1.72	1.36	21.06	25.61 ± 0.43	0.11	0.06	0.05	0.42	0.65 ± 0.09	0.03	0.10	0.03	0.45	0.6 ± 0.07
OLI-S6-09LF	1.06	0.79	1.09	18.48	21.42 ± 4.56	0.02	0.14	0.04	1.64	1.84 ± 0.05	0.04	0.15	0.04	0.80	1.02 ± 0.16
OLI-S1-10HF	0.92	0.48	2.43	9.02	12.85 ± 5.55	0.00	0.04	0.20	1.87	2.12 ± 0.41	BD	0.05	0.08	0.09	0.22 ± 0.04
OLI-S2-10HF	0.55	2.96	1.23	5.74	10.48 ± 2.56	0.01	0.07	0.19	2.47	2.73 ± 0.89	BD	0.00	0.09	0.12	0.21 ± 0.07
OLI-S3-10HF	0.51	0.29	1.01	10.13	11.94 ± 2.11	0.03	0.03	0.14	2.07	2.27 ± 0.4	BD	0.10	0.10	0.09	0.28 ± 0.08
OLI-S4-10HF	3.10	3.06	7.60	28.69	42.44 ± 12.5	0.01	0.07	0.27	2.07	2.42 ± 0.27	BD	BD	0.09	0.20	0.3 ± 0.06
OLI-S5-10HF	0.61	0.67	1.11	15.75	18.14 ± 8.6	0.02	0.03	0.17	2.11	2.34 ± 0.3	BD	0.16	0.09	0.11	0.36 ± 0.19
OLI-S6-10HF	0.23	1.17	0.76	3.32	5.48 ± 1.46	0.01	0.04	0.23	1.11	1.4 ± 0.37	BD	BD	0.09	0.09	0.18 ± 0.03
OLI-S1-10LF	0.50	0.66	0.86	220.64	222.7 ± 106.7	BD	0.01	0.11	2.59	2.71 ± 1.04	BD	0.01	0.10	0.06	0.16 ± 0.01
OLI-S2-10LF	0.56	0.71	1.06	7.86	10.18 ± 0.38	BD	0.01	0.12	2.40	2.53 ± 0.5	BD	0.01	0.09	0.06	0.16 ± 0.02
OLI-S3-10LF	0.51	0.85	1.01	135.32	137.69 ± 126.8	0.01	0.01	0.11	3.41	3.55 ± 0.07	BD	BD	0.09	0.03	0.13 ± 0.01
OLI-S4-10LF	0.63	0.98	1.23	52.05	54.89 ± 44.26	0.01	0.03	0.14	2.55	2.72 ± 0.22	BD	BD	0.09	0.07	0.17 ± 0.03
OLI-S5-10LF	0.87	0.68	1.37	8.56	11.48 ± 1.97	0.04	0.03	0.17	2.63	2.87 ± 0.73	BD	BD	0.10	0.05	0.15 ± 0.02
OLI-S6-10LF	0.48	0.24	1.15	7.80	9.67 ± 2.49	0.01	0.03	0.23	1.56	1.83 ± 0.37	BD	BD	0.10	0.10	0.2 ± 0.03
OLI-S1-11HF	1.81	2.53	4.05	9.18	17.57 ± 2.26	0.01	0.07	0.18	1.88	2.14 ± 0.13	BD	BD	0.07	0.12	0.19 ± 0.06
OLI-S2-11HF	1.50	2.11	3.72	18.61	25.94 ± 1.27	BD	0.04	0.21	1.34	1.6 ± 0.05	BD	BD	0.07	0.09	0.16 ± 0.01
OLI-S3-11HF	1.17	1.48	2.65	15.21	20.51 ± 5.34	BD	0.04	0.19	1.40	1.63 ± 0.54	BD	BD	0.07	0.08	0.15 ± 0.01
OLI-S4-11HF	0.89	1.19	2.05	11.82	15.95 ± 2.4	BD	0.05	BD	1.56	1.61 ± 1.13	BD	BD	0.03	0.08	0.11 ± 0.03
OLI-S5-11HF	0.64	1.02	1.43	8.09	11.18 ± 1.5	0.01	0.05	BD	1.16	1.21 ± 0.65	BD	BD	0.00	0.02	0.03 ± 0
OLI-S6-11HF	0.45	0.36	1.19	5.20	7.19 ± 0.76	0.01	0.04	0.18	1.00	1.22 ± 0.08	BD	BD	0.01	0.05	0.05 ± 0.01

Appendix 1. continued

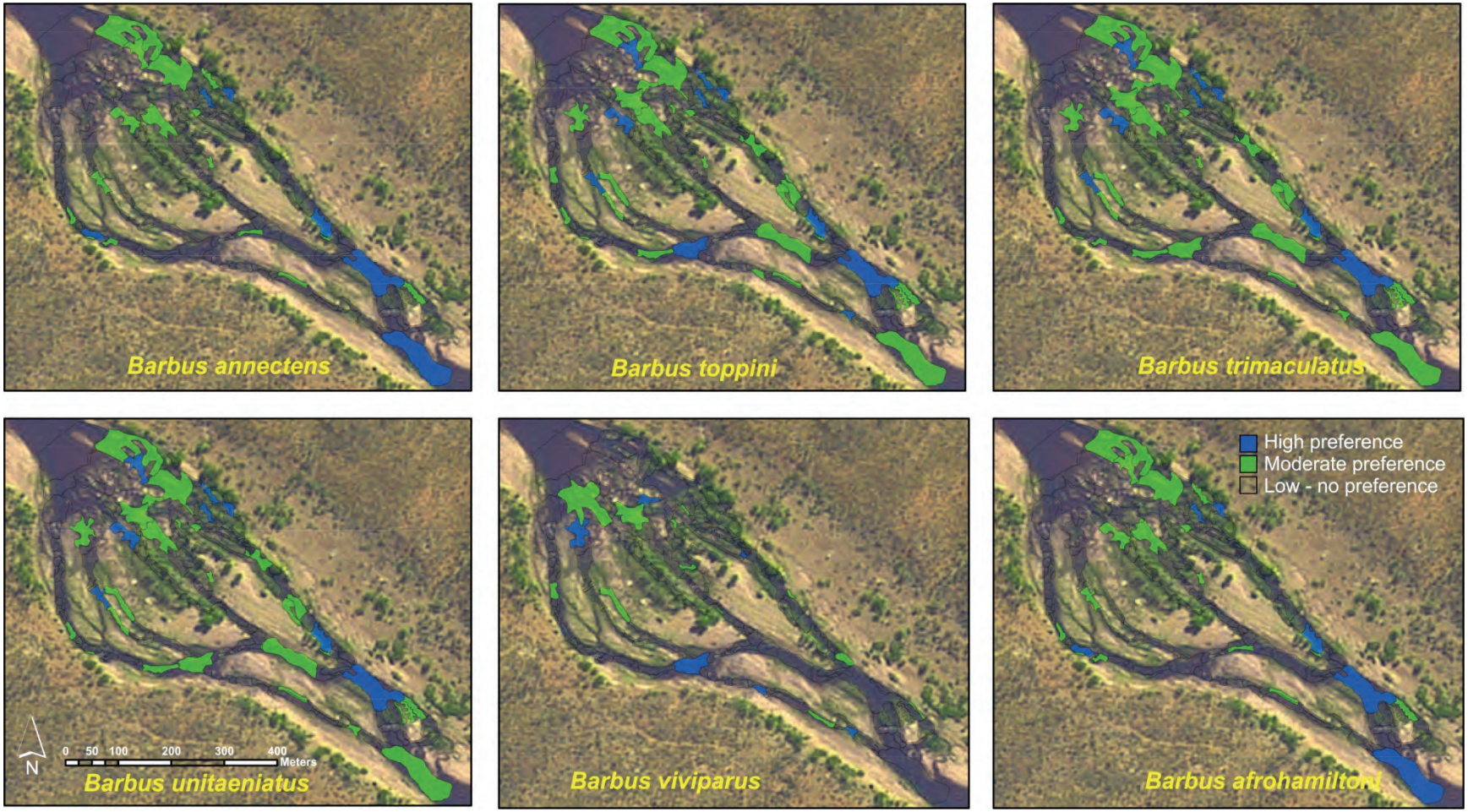
	Uranium (U) µg/L					Zinc (Zn) µg/L				
Sample	**BCR-A**	**BCR-B**	**BCR-C**	**BCR-D**	**Total**	**BCR-A**	**BCR-B**	**BCR-C**	**BCR-D**	**Total**
OLI-S1-09LF	-	-	-	-	-	17.29	3.24	8.49	47.21	76.23 ± 7.37
OLI-S2-09LF	-	-	-	-	-	1.86	5.78	6.92	68.31	82.86 ± 13.21
OLI-S3-09LF	-	-	-	-	-	1.10	3.59	4.57	66.04	75.3 ± 15.86
OLI-S4-09LF	-	-	-	-	-	2.08	2.23	4.70	13.83	22.83 ± 2.63
OLI-S5-09LF	-	-	-	-	-	0.86	5.07	3.90	29.40	39.23 ± 3.79
OLI-S6-09LF	-	-	-	-	-	2.55	3.29	6.99	33.59	46.43 ± 10.39
OLI-S1-10HF	0.01	0.05	0.04	0.26	0.36 ± 0.06	1.18	1.65	2.12	26.25	31.21 ± 6.08
OLI-S2-10HF	0.01	0.01	0.03	0.35	0.39 ± 0.07	0.61	1.70	1.97	51.43	55.71 ± 17.48
OLI-S3-10HF	0.01	0.08	0.03	0.30	0.41 ± 0.06	3.79	1.73	1.32	61.17	68.02 ± 20.98
OLI-S4-10HF	0.03	0.01	0.14	0.40	0.59 ± 0.09	2.91	2.11	1.34	48.89	55.24 ± 5.2
OLI-S5-10HF	0.01	0.08	0.03	0.39	0.5 ± 0.11	3.89	1.57	1.10	49.89	56.44 ± 10.72
OLI-S6-10HF	0.00	0.01	0.03	0.20	0.24 ± 0.01	1.26	2.64	2.46	12.81	19.17 ± 3.25
OLI-S1-10LF	0.01	BD	0.03	0.07	0.11 ± 0.02	2.20	0.85	1.15	315.0	319.2 ± 151.8
OLI-S2-10LF	0.01	BD	0.03	0.07	0.11 ± 0.01	1.04	0.40	0.31	43.90	45.65 ± 14.95
OLI-S3-10LF	0.01	0.01	0.02	0.07	0.1 ± 0.01	1.18	2.88	0.34	225.3	229.7 ± 168.1
OLI-S4-10LF	0.01	0.01	0.03	0.08	0.13 ± 0.02	2.59	0.91	2.13	41.73	47.36 ± 4.58
OLI-S5-10LF	0.01	0.01	0.03	0.05	0.1 ± 0.01	3.75	0.23	0.28	48.27	52.54 ± 15.61
OLI-S6-10LF	0.01	BD	0.03	0.04	0.08 ± 0.01	1.36	0.89	0.56	18.57	21.37 ± 4.72
OLI-S1-11HF	0.02	BD	0.07	0.10	0.19 ± 0.09	0.33	0.60	0.58	45.81	47.33 ± 15.33
OLI-S2-11HF	0.02	BD	0.05	0.10	0.16 ± 0.01	0.22	0.55	0.48	38.74	40 ± 2.36
OLI-S3-11HF	0.01	BD	0.04	0.08	0.13 ± 0.02	0.17	0.41	0.48	43.04	44.1 ± 13.6
OLI-S4-11HF	0.01	BD	0.02	0.08	0.11 ± 0	0.41	0.55	0.62	44.44	46.03 ± 4.49
OLI-S5-11HF	0.01	BD	0.01	0.05	0.07 ± 0.01	0.28	0.36	0.69	28.23	29.55 ± 11.4
OLI-S6-11HF	0.01	BD	0.01	0.03	0.06 ± 0	0.52	0.27	0.92	14.82	16.52 ± 2.02



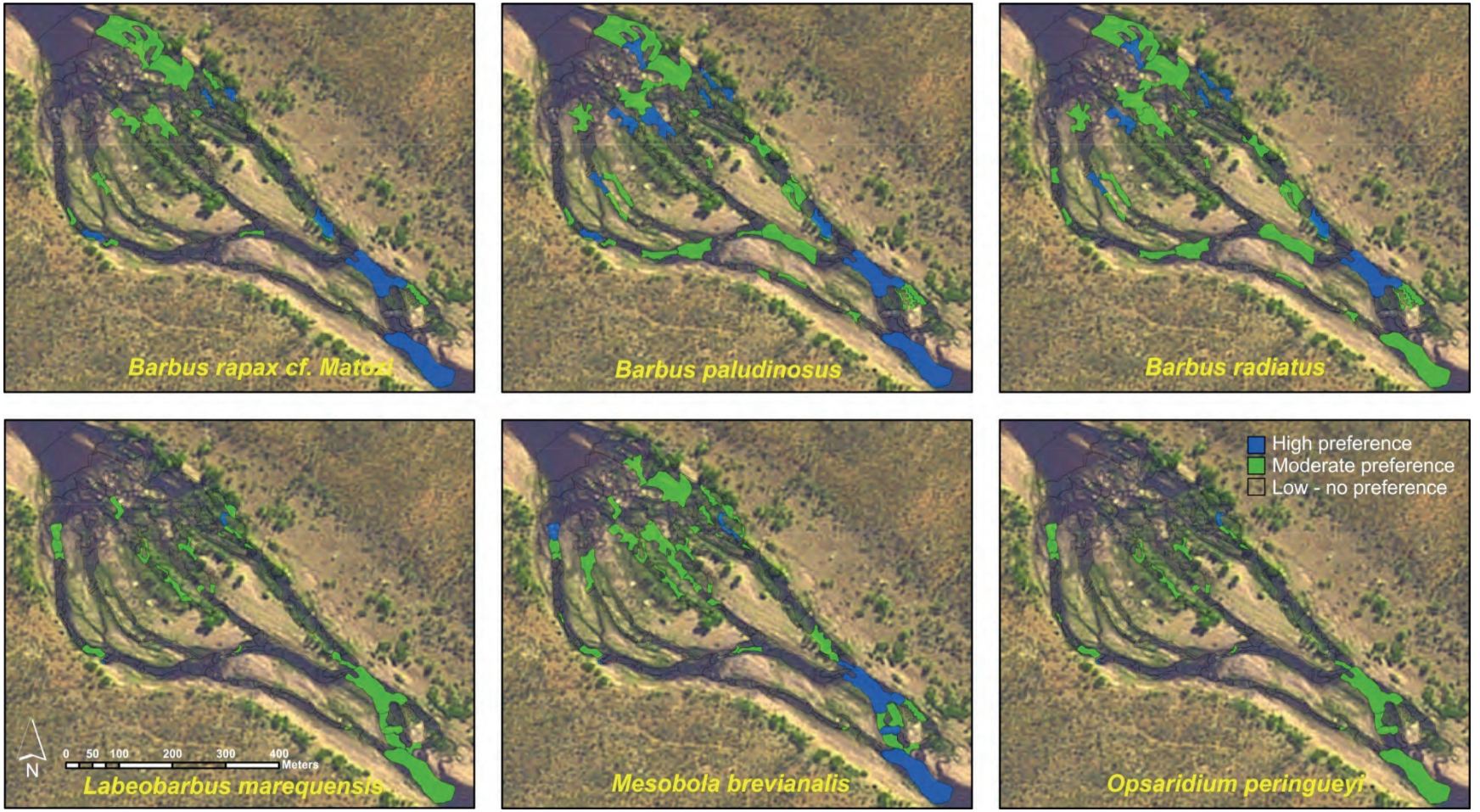
Appendix 2. Spatial distribution of high, moderate and low to no preference habitat units for fishes expected to occur in the Olifants River.



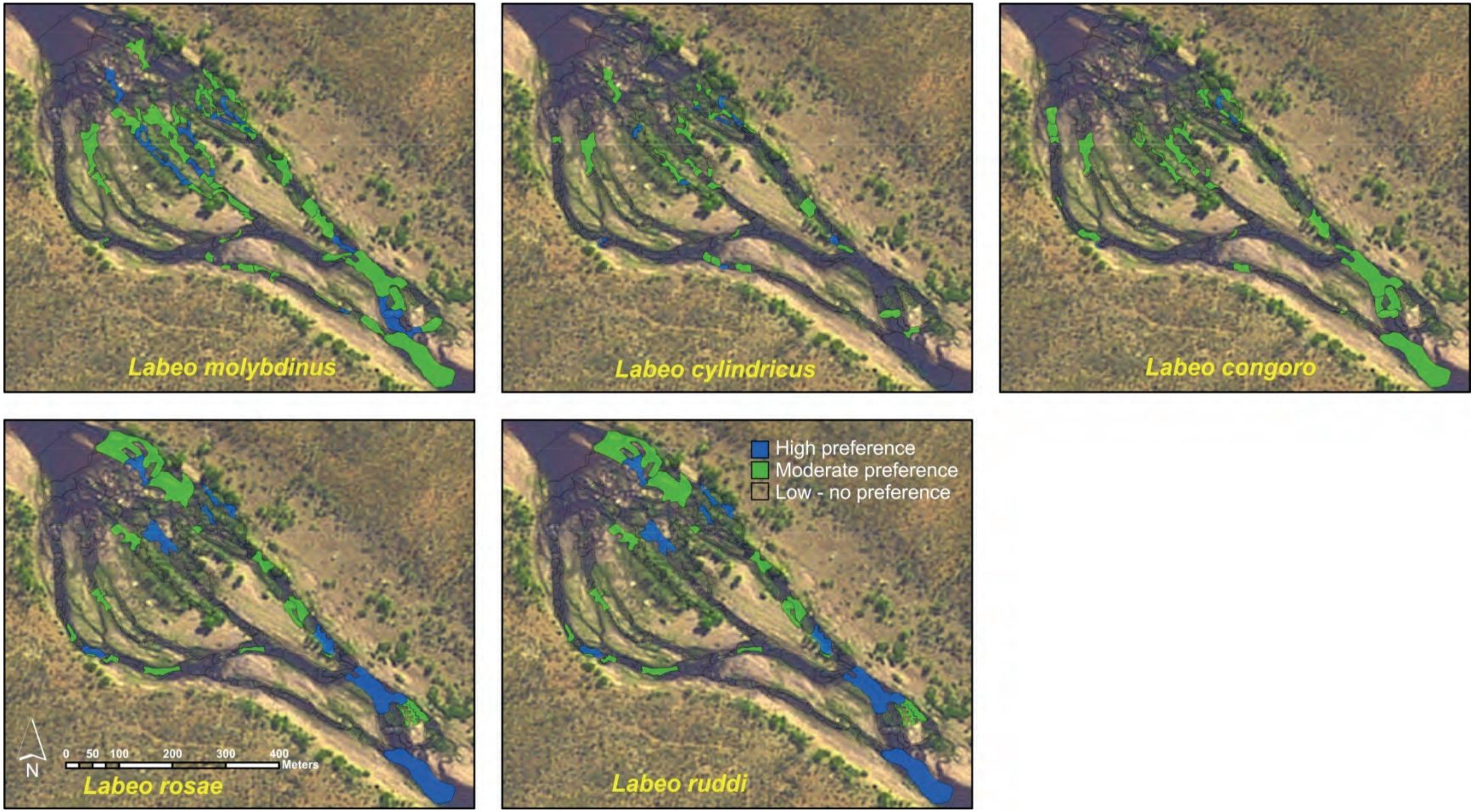
Appendix 2. continued



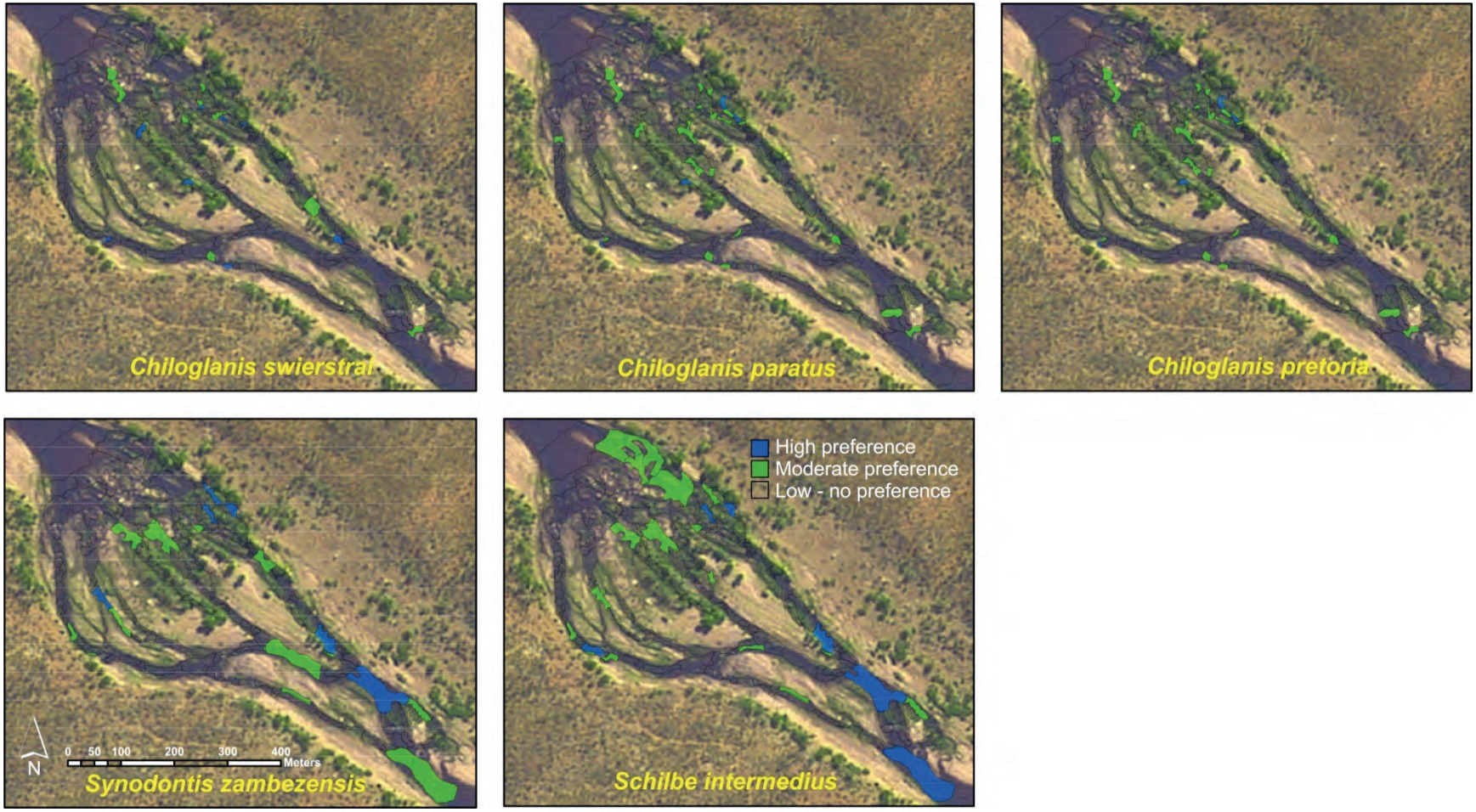
Appendix 2. continued



Appendix 2. continued



Appendix 2. continued



Appendix 2. continued

Appendix 3. Descriptive data associated with flow classes for observed modeling data.

Max. depth (m)	Ave. depth (m)	Discharge (m3/s)	Width (m)	Wet perrimeter (m)	Ave. velocity (m/s)	Velocity 98% (m/s)	Distribution (%) of VD. classes						
							SvS	SS	SD	FVS	FS	FI	FD
0.01	0	0	0.61	0.61	0.01	0.05	100	0	0	0	0	0	0
0.02	0.01	0	1.22	1.22	0.02	0.07	100	0	0	0	0	0	0
0.03	0.02	0.001	1.51	1.52	0.03	0.11	100	0	0	0	0	0	0
0.04	0.02	0.002	1.81	1.81	0.04	0.13	100	0	0	0	0	0	0
0.05	0.03	0.003	2.1	2.1	0.04	0.15	100	0	0	0	0	0	0
0.06	0.04	0.004	2.39	2.4	0.05	0.17	100	0	0	0	0	0	0
0.07	0.04	0.006	2.69	2.69	0.05	0.19	100	0	0	0	0	0	0
0.08	0.05	0.008	2.85	2.86	0.06	0.21	100	0	0	0	0	0	0
0.09	0.06	0.011	3.01	3.02	0.07	0.23	100	0	0	0	0	0	0
0.1	0.06	0.014	3.18	3.19	0.07	0.25	98	2	0	1	0	0	0
0.11	0.07	0.017	3.34	3.35	0.08	0.26	81	18	0	1	0	0	0
0.12	0.07	0.021	3.65	3.67	0.08	0.27	67	32	0	1	0	0	0
0.13	0.07	0.023	4.37	4.39	0.08	0.27	65	34	0	1	0	0	0
0.14	0.07	0.027	5.1	5.12	0.08	0.27	64	35	0	1	0	0	0
0.15	0.07	0.031	5.82	5.84	0.08	0.27	63	36	0	1	0	0	0
0.16	0.07	0.036	6.54	6.57	0.08	0.27	62	36	0	1	1	0	0
0.17	0.07	0.043	7.26	7.29	0.08	0.28	62	37	0	1	1	0	0
0.18	0.07	0.046	9.35	9.38	0.07	0.26	69	30	0	1	0	0	0
0.19	0.07	0.053	11.1	11.14	0.07	0.26	72	27	0	1	0	0	0
0.2	0.07	0.062	12.86	12.91	0.07	0.26	74	25	0	1	0	0	0
0.21	0.07	0.076	13.57	13.62	0.08	0.27	74	25	0	1	0	0	0
0.22	0.08	0.092	14.28	14.33	0.08	0.29	72	26	0	2	0	0	0
0.23	0.08	0.109	14.99	15.05	0.09	0.3	68	29	0	2	1	0	0
0.24	0.09	0.127	15.99	16.05	0.09	0.31	65	31	0	2	1	0	0
0.25	0.09	0.145	17.31	17.38	0.09	0.32	63	33	0	2	1	0	0
0.26	0.09	0.16	19.54	19.61	0.09	0.32	65	31	0	2	1	0	0
0.27	0.1	0.188	20.14	20.22	0.1	0.33	58	38	0	3	1	1	0
0.28	0.1	0.217	20.75	20.83	0.1	0.35	52	42	0	3	2	1	0
0.29	0.11	0.241	22.52	22.61	0.1	0.35	48	47	0	3	2	1	0
0.3	0.11	0.265	24.82	24.9	0.1	0.36	47	48	0	3	2	1	0
0.31	0.11	0.299	26.22	26.32	0.1	0.36	45	50	0	3	2	1	0
0.32	0.12	0.342	26.73	26.83	0.11	0.37	42	52	0	3	3	1	0
0.33	0.12	0.387	27.3	27.41	0.11	0.39	40	53	0	3	3	1	0
0.34	0.13	0.435	27.87	27.98	0.12	0.41	38	54	0	3	3	1	1
0.35	0.14	0.485	28.44	28.55	0.12	0.42	34	57	0	3	4	1	1
0.36	0.15	0.538	29.01	29.13	0.13	0.44	30	61	0	3	4	1	1
0.37	0.15	0.595	29.53	29.66	0.13	0.45	27	63	0	3	4	2	1
0.38	0.16	0.655	29.94	30.07	0.14	0.47	25	64	0	3	5	2	1
0.39	0.17	0.718	30.36	30.49	0.14	0.48	22	67	0	3	4	3	1
0.4	0.18	0.781	30.98	31.12	0.14	0.51	21	66	0	3	5	3	1
0.41	0.18	0.839	32.04	32.19	0.15	0.5	17	71	0	2	5	4	1
0.42	0.18	0.9	33.09	33.26	0.15	0.5	16	71	0	2	5	4	2
0.43	0.19	0.964	34.15	34.32	0.15	0.52	17	70	0	3	5	4	2
0.44	0.19	1.031	35.21	35.39	0.15	0.53	18	69	0	3	4	5	2
0.45	0.2	1.106	36.05	36.25	0.16	0.54	18	68	0	3	4	5	2
0.46	0.2	1.163	37.96	38.16	0.16	0.53	20	66	0	3	4	4	3
0.47	0.2	1.252	38.51	38.72	0.16	0.54	18	68	0	3	4	4	3
0.48	0.21	1.345	39.06	39.28	0.16	0.56	19	66	0	3	4	4	4
0.49	0.22	1.44	39.61	39.84	0.17	0.58	19	65	0	4	3	5	5
0.5	0.23	1.539	40.16	40.4	0.17	0.58	18	65	0	4	3	5	5
0.51	0.23	1.635	40.92	41.16	0.17	0.6	18	63	1	4	3	5	6
0.52	0.24	1.734	41.73	41.99	0.18	0.6	16	65	2	3	3	5	6
0.53	0.24	1.835	42.56	42.82	0.18	0.62	16	63	3	4	3	5	7
0.54	0.25	1.941	43.38	43.65	0.18	0.62	16	61	4	4	3	5	7
0.55	0.25	2.055	44.01	44.29	0.18	0.65	15	60	4	4	4	5	8
0.56	0.26	2.174	44.63	44.92	0.19	0.64	12	63	4	3	4	5	8
0.57	0.27	2.295	45.26	45.55	0.19	0.65	12	62	5	3	4	4	10
0.58	0.27	2.394	46.73	47.04	0.19	0.65	12	63	4	3	4	4	10
0.59	0.27	2.518	47.56	47.88	0.19	0.66	12	61	5	3	4	3	10
0.6	0.27	2.613	49.33	49.66	0.19	0.67	14	59	5	4	4	3	10
0.61	0.28	2.757	49.86	50.19	0.2	0.69	14	58	5	4	4	4	11
0.62	0.28	2.885	50.93	51.28	0.2	0.68	13	59	5	4	4	3	12
0.63	0.29	3.017	52.01	52.36	0.2	0.69	13	57	7	4	3	3	12
0.64	0.29	3.112	54.2	54.57	0.2	0.69	15	55	7	4	3	3	12
0.65	0.29	3.245	55.57	55.94	0.2	0.69	15	53	8	5	3	3	12

Appendix 3. continued

Max. depth (m)	Ave. depth (m)	Discharge (m3/s)	Width (m)	Wet perrimeter (m)	Ave. velocity (m/s)	Velocity 98%	ibution (%) of velocity depth cla						
						(m/s)	SvS	SS	SD	FVS	FS	FI	FD
0.71	0.31	4.168	63.46	63.9	0.21	0.73	16	44	15	5	3	4	13
0.72	0.32	4.359	64.32	64.77	0.21	0.74	15	44	16	5	3	4	13
0.73	0.32	4.548	65.35	65.81	0.22	0.75	14	43	17	5	4	3	14
0.74	0.33	4.739	66.43	66.9	0.22	0.75	14	42	17	5	4	3	15
0.75	0.33	4.936	67.5	67.98	0.22	0.75	13	40	20	5	4	3	15
0.76	0.33	5.138	68.58	69.07	0.22	0.76	12	40	21	5	5	3	15
0.77	0.34	5.345	69.66	70.16	0.23	0.77	13	38	22	5	4	3	16
0.78	0.34	5.528	71.35	71.86	0.23	0.79	12	38	22	5	6	3	16
0.79	0.34	5.711	73.16	73.68	0.23	0.79	12	36	23	5	5	3	16
0.8	0.34	5.904	74.92	75.45	0.23	0.78	11	36	24	5	5	3	15
0.81	0.35	6.104	76.68	77.22	0.23	0.79	11	35	25	5	5	3	16
0.82	0.35	6.332	78.03	78.58	0.23	0.8	12	34	24	5	5	4	16
0.83	0.35	6.567	79.37	79.94	0.23	0.78	10	36	25	4	5	4	16
0.84	0.36	6.808	80.72	81.3	0.24	0.82	12	33	24	6	5	4	16
0.85	0.36	7.056	82.07	82.65	0.24	0.82	12	33	25	5	5	5	16
0.86	0.37	7.353	82.67	83.26	0.24	0.83	11	34	24	5	5	5	17
0.87	0.38	7.652	83.32	83.92	0.24	0.84	11	33	24	5	4	5	18
0.88	0.38	7.958	83.97	84.59	0.25	0.85	9	34	24	4	5	5	18
0.89	0.39	8.269	84.63	85.25	0.25	0.84	9	33	26	4	4	5	18
0.9	0.4	8.586	85.28	85.91	0.25	0.86	7	34	25	4	5	5	20
0.91	0.4	8.91	85.93	86.57	0.26	0.88	7	33	26	4	5	5	20
0.92	0.41	9.239	86.58	87.24	0.26	0.89	6	34	25	3	5	6	20
0.93	0.42	9.574	87.23	87.9	0.26	0.88	5	34	26	3	5	5	22
0.94	0.43	9.917	87.87	88.54	0.27	0.9	5	33	26	3	6	6	22
0.95	0.43	10.266	88.5	89.19	0.27	0.92	5	32	26	3	6	5	23
0.96	0.44	10.621	89.14	89.83	0.27	0.93	5	32	26	3	6	4	24
0.97	0.45	10.982	89.77	90.48	0.27	0.94	4	33	26	2	6	5	24
0.98	0.45	11.35	90.41	91.12	0.28	0.95	4	30	27	3	6	5	25
0.99	0.46	11.744	90.8	91.52	0.28	0.96	4	30	27	3	5	5	26
1	0.47	12.144	91.19	91.92	0.28	0.96	3	31	28	2	5	5	26
1.01	0.48	12.551	91.58	92.32	0.29	0.97	3	31	27	2	4	6	27
1.02	0.49	12.965	91.95	92.69	0.29	0.98	3	30	27	2	3	6	28
1.03	0.49	13.386	92.32	93.07	0.29	1	3	30	27	2	3	7	29
1.04	0.5	13.812	92.69	93.45	0.3	1.02	3	29	27	2	3	7	29
1.05	0.51	14.257	92.93	93.71	0.3	1.02	2	28	28	1	3	7	30
1.06	0.52	14.709	93.17	93.96	0.3	1.02	2	28	28	2	3	6	32
1.07	0.53	15.166	93.41	94.22	0.31	1.03	2	27	28	1	3	5	33
1.08	0.54	15.63	93.65	94.48	0.31	1.06	2	26	28	2	2	6	34
1.09	0.54	16.1	93.89	94.73	0.31	1.07	2	25	28	2	2	5	34
1.1	0.55	16.576	94.14	94.99	0.32	1.08	2	25	29	2	2	6	35
1.11	0.56	17.059	94.38	95.25	0.32	1.08	1	25	30	1	3	5	36
1.12	0.57	17.547	94.62	95.5	0.33	1.08	1	24	30	0	3	5	37
1.13	0.58	18.042	94.86	95.76	0.33	1.09	0	23	31	0	3	4	39
1.14	0.59	18.538	95.14	96.06	0.33	1.1	1	21	31	1	2	4	40
1.15	0.6	19.04	95.42	96.35	0.34	1.13	1	20	31	1	2	5	40
1.16	0.6	19.549	95.7	96.65	0.34	1.13	1	19	32	1	1	4	41
1.17	0.61	20.063	95.99	96.95	0.34	1.14	1	19	32	1	2	4	42
1.18	0.62	20.584	96.27	97.24	0.34	1.15	1	18	32	1	2	3	42
1.19	0.63	21.111	96.55	97.54	0.35	1.15	1	17	33	1	2	3	43
1.2	0.64	21.644	96.83	97.83	0.35	1.16	1	17	33	1	2	3	44
1.21	0.64	22.183	97.11	98.13	0.35	1.17	1	16	33	1	1	3	44
1.22	0.65	22.728	97.39	98.43	0.36	1.18	1	15	33	1	1	3	45
1.23	0.66	23.279	97.67	98.72	0.36	1.18	1	15	34	1	2	3	46
1.24	0.67	23.837	97.95	99.02	0.36	1.19	1	14	34	1	2	2	46
1.25	0.68	24.401	98.23	99.31	0.37	1.2	1	15	33	1	1	2	47
1.26	0.68	24.97	98.52	99.61	0.37	1.21	1	14	34	1	1	2	48
1.27	0.69	25.547	98.8	99.91	0.37	1.23	1	13	33	2	1	2	48
1.28	0.7	26.129	99.08	100.2	0.38	1.22	2	11	34	2	1	2	49
1.29	0.71	26.717	99.36	100.5	0.38	1.23	1	11	35	1	1	2	49
1.3	0.72	27.312	99.64	100.8	0.38	1.24	1	10	36	1	1	2	50
1.31	0.72	27.913	99.92	101.09	0.39	1.25	1	10	35	1	1	1	50
1.32	0.73	28.52	100.2	101.39	0.39	1.26	1	9	35	1	1	2	51

Appendix 3. continued

Max. depth (m)	Ave. depth (m)	Discharge (m3/s)	Width (m)	Wet perrimeter (m)	Ave. velocity (m/s)	Velocity 98%	ibution (%) of velocity depth cla						
						(m/s)	SvS	SS	SD	FVS	FS	FI	FD
1.38	0.78	32.305	101.82	103.1	0.41	1.3	1	7	36	1	1	2	52
1.39	0.79	32.976	102.01	103.3	0.41	1.31	1	6	36	1	1	1	53
1.4	0.8	33.652	102.2	103.5	0.41	1.32	1	6	36	1	1	2	54
1.41	0.81	34.335	102.39	103.7	0.42	1.33	1	6	35	1	1	2	54
1.42	0.81	35.024	102.57	103.9	0.42	1.33	0	6	36	1	1	2	54
1.43	0.82	35.72	102.76	104.1	0.42	1.34	0	5	36	1	1	2	55
1.44	0.83	36.422	102.95	104.3	0.43	1.37	1	5	35	1	2	2	54
1.45	0.84	37.13	103.14	104.5	0.43	1.38	1	4	35	2	1	1	55
1.46	0.85	37.845	103.33	104.7	0.43	1.39	1	4	35	2	1	1	55
1.47	0.86	38.565	103.52	104.9	0.43	1.4	1	4	35	2	1	1	56
1.48	0.87	39.293	103.71	105.1	0.44	1.41	1	4	34	2	1	2	56
1.49	0.87	40.026	103.9	105.3	0.44	1.42	1	4	34	1	1	2	56
1.5	0.88	40.766	104.09	105.51	0.44	1.42	1	4	34	1	1	2	57
1.51	0.88	41.209	105.46	106.89	0.44	1.43	2	4	34	2	1	2	56
1.52	0.88	41.662	106.84	108.28	0.44	1.41	1	5	34	2	2	2	55
1.53	0.88	42.126	108.22	109.67	0.44	1.4	2	4	34	2	1	1	56
1.54	0.88	42.601	109.59	111.06	0.44	1.42	2	4	33	4	1	2	54
1.55	0.88	43.087	110.97	112.45	0.44	1.4	2	4	33	4	1	2	54
1.56	0.88	43.584	112.35	113.84	0.44	1.41	3	3	33	5	2	1	53
1.57	0.88	44.214	113.24	114.74	0.44	1.39	3	4	33	4	1	1	54
1.58	0.88	44.777	114.43	115.94	0.45	1.41	3	4	32	5	1	2	52
1.59	0.88	45.349	115.61	117.14	0.45	1.41	3	4	32	5	1	2	53
1.6	0.88	45.93	116.8	118.34	0.45	1.41	4	4	32	6	1	2	52
1.61	0.88	46.522	117.99	119.54	0.45	1.41	4	4	32	6	1	2	52
1.62	0.89	47.38	118.18	119.74	0.45	1.42	4	4	31	6	2	2	52
1.63	0.9	48.246	118.37	119.94	0.45	1.42	3	4	31	5	2	2	53
1.64	0.91	49.119	118.56	120.15	0.46	1.45	3	4	31	5	3	2	53
1.65	0.92	49.999	118.75	120.35	0.46	1.46	3	5	30	4	3	2	53
1.66	0.92	50.887	118.95	120.55	0.46	1.47	2	5	30	4	3	2	53
1.67	0.93	51.782	119.14	120.76	0.47	1.48	2	5	30	3	4	2	54
1.68	0.94	52.654	119.43	121.06	0.47	1.47	2	5	30	3	4	2	54
1.69	0.95	53.533	119.73	121.37	0.47	1.49	1	6	29	2	5	2	54
1.7	0.96	54.42	120.02	121.68	0.47	1.48	1	6	30	2	5	2	55
1.71	0.96	55.314	120.32	121.99	0.48	1.5	1	6	29	2	5	2	55
1.72	0.97	56.215	120.61	122.3	0.48	1.5	1	6	29	1	5	2	55
1.73	0.98	57.123	120.9	122.61	0.48	1.5	0	6	29	1	5	3	55
1.74	0.99	58.038	121.2	122.91	0.49	1.52	0	6	29	1	5	3	55
1.75	0.99	58.961	121.49	123.22	0.49	1.53	1	6	28	1	4	3	56
1.76	1	59.891	121.79	123.53	0.49	1.54	1	7	28	1	5	3	56
1.77	1.01	60.829	122.08	123.84	0.49	1.53	1	6	29	1	4	3	57
1.78	1.02	61.774	122.38	124.15	0.5	1.54	1	6	29	1	4	3	57
1.79	1.02	62.726	122.67	124.46	0.5	1.56	1	6	28	2	3	3	57
1.8	1.03	63.685	122.97	124.77	0.5	1.56	1	5	28	2	3	4	58
1.81	1.04	64.652	123.26	125.07	0.5	1.57	1	5	28	2	2	4	58
1.82	1.05	65.634	123.53	125.36	0.51	1.59	1	5	27	2	2	4	58
1.83	1.06	66.624	123.8	125.64	0.51	1.6	1	6	27	2	2	4	59
1.84	1.06	67.622	124.08	125.93	0.51	1.58	1	5	27	2	2	3	60
1.85	1.07	68.626	124.35	126.21	0.52	1.59	1	5	27	2	1	3	60
1.86	1.08	69.638	124.62	126.5	0.52	1.58	0	6	28	1	1	4	62
1.87	1.09	70.658	124.89	126.78	0.52	1.6	1	5	27	1	1	3	61
1.88	1.09	71.685	125.16	127.07	0.52	1.6	1	5	27	1	1	4	61
1.89	1.1	72.72	125.43	127.35	0.53	1.62	1	6	26	1	1	4	61
1.9	1.11	73.762	125.7	127.63	0.53	1.63	1	6	26	1	1	3	62
1.91	1.12	74.811	125.97	127.92	0.53	1.64	1	6	26	2	1	3	62
1.92	1.12	75.868	126.24	128.2	0.53	1.64	0	5	26	1	1	3	63
1.93	1.13	76.933	126.51	128.49	0.54	1.65	0	5	26	1	1	3	63
1.94	1.14	78.005	126.78	128.77	0.54	1.65	0	5	26	1	2	3	64
1.95	1.15	79.085	127.05	129.06	0.54	1.66	0	5	26	1	2	3	64
1.96	1.15	80.172	127.33	129.34	0.55	1.69	1	4	26	2	1	2	64
1.97	1.16	81.267	127.6	129.63	0.55	1.7	1	5	25	2	2	2	64
1.98	1.17	82.37	127.87	129.91	0.55	1.72	1	5	25	2	2	2	64
1.99	1.18	83.48	128.14	130.2	0.55	1.7	1	4	25	2	1	1	65

Appendix 3. continued

Max. depth	Ave. depth	Discharge	Width	Wet perrimeter	Ave. velocity	Velocity 98%	ibution (%) of velocity depth cla						
(m)	(m)	(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
2.05	1.22	90.301	129.76	131.9	0.57	1.73	1	4	25	2	1	1	66
2.06	1.23	91.465	130.03	132.19	0.57	1.74	0	4	25	1	2	2	66
2.07	1.24	92.637	130.31	132.47	0.57	1.73	0	4	25	1	1	2	67
2.08	1.24	93.816	130.58	132.76	0.58	1.74	0	4	25	1	1	2	67
2.09	1.25	95.004	130.85	133.04	0.58	1.75	0	4	25	1	1	2	67
2.1	1.26	96.199	131.12	133.33	0.58	1.75	0	3	25	1	1	2	67
2.11	1.27	97.401	131.39	133.61	0.59	1.76	0	3	25	1	1	2	67
2.12	1.27	98.612	131.66	133.9	0.59	1.76	0	3	26	1	1	2	67
2.13	1.28	99.83	131.93	134.18	0.59	1.79	1	3	25	1	1	1	67
2.14	1.25	99.217	136	138.26	0.58	1.73	1	3	25	2	2	2	66
2.15	1.26	100.492	136.22	138.5	0.59	1.74	1	3	25	2	2	2	66
2.16	1.27	101.775	136.45	138.74	0.59	1.75	1	3	25	2	2	1	66
2.17	1.28	103.066	136.67	138.97	0.59	1.79	1	3	25	2	2	2	64
2.18	1.28	104.366	136.89	139.21	0.59	1.79	1	3	25	2	2	3	64
2.19	1.29	105.674	137.12	139.45	0.6	1.79	1	3	25	2	2	2	65
2.2	1.3	106.99	137.34	139.69	0.6	1.8	1	3	25	2	2	2	66
2.21	1.31	108.315	137.56	139.92	0.6	1.81	1	2	25	2	2	1	66
2.22	1.32	109.647	137.78	140.16	0.6	1.83	1	2	24	2	2	2	66
2.23	1.32	110.547	138.86	141.25	0.61	1.82	1	3	24	2	2	2	66
2.24	1.32	111.459	139.93	142.33	0.61	1.81	1	2	24	3	2	1	66
2.25	1.32	112.428	140.92	143.33	0.61	1.8	1	3	24	3	2	1	66
2.26	1.32	113.793	141.17	143.59	0.61	1.81	1	2	24	3	2	1	67
2.27	1.33	115.168	141.42	143.85	0.61	1.81	1	3	24	2	2	2	66
2.28	1.34	116.551	141.67	144.11	0.61	1.82	1	3	24	2	2	2	66
2.29	1.35	117.942	141.92	144.37	0.62	1.82	1	3	24	1	2	3	66
2.3	1.35	119.342	142.17	144.63	0.62	1.85	1	3	23	2	2	2	66
2.31	1.36	120.751	142.42	144.89	0.62	1.87	1	3	23	2	2	2	66
2.32	1.37	122.168	142.67	145.15	0.63	1.87	1	3	23	2	2	3	66
2.33	1.38	123.594	142.92	145.41	0.63	1.86	1	3	23	2	2	2	67
2.34	1.38	125.029	143.17	145.67	0.63	1.87	1	3	23	2	2	2	68
2.35	1.39	126.472	143.42	145.93	0.63	1.86	1	3	23	1	2	2	68
2.36	1.4	127.924	143.67	146.19	0.64	1.87	1	3	23	1	2	2	68
2.37	1.41	129.384	143.92	146.45	0.64	1.88	0	3	23	1	2	2	68
2.38	1.42	130.854	144.17	146.71	0.64	1.88	0	2	23	1	2	2	69
2.39	1.42	132.332	144.41	146.97	0.64	1.91	1	3	22	2	2	3	67
2.4	1.43	133.819	144.66	147.23	0.65	1.91	0	3	22	1	2	3	68
2.41	1.44	135.314	144.91	147.49	0.65	1.9	0	3	22	1	2	4	68
2.42	1.45	136.924	144.99	147.57	0.65	1.92	0	3	22	1	2	4	68
2.43	1.46	138.544	145.07	147.66	0.66	1.91	0	3	22	0	2	4	68
2.44	1.47	140.173	145.15	147.75	0.66	1.94	0	3	22	1	2	4	69
2.45	1.47	141.81	145.23	147.83	0.66	1.95	0	3	22	1	2	4	69
2.46	1.48	143.457	145.31	147.92	0.67	1.94	0	3	22	0	2	4	70
2.47	1.49	145.114	145.39	148.01	0.67	1.96	0	3	21	1	1	2	71
2.48	1.5	146.779	145.47	148.09	0.67	1.95	0	3	22	0	1	2	72
2.49	1.51	148.453	145.55	148.18	0.67	1.98	0	3	21	1	1	2	72
2.5	1.52	150.137	145.63	148.27	0.68	1.99	0	3	21	1	1	2	72
2.51	1.53	151.83	145.7	148.35	0.68	1.99	0	3	21	0	1	2	72
2.52	1.54	153.532	145.78	148.44	0.68	2	0	3	21	0	1	2	72
2.53	1.55	155.243	145.86	148.53	0.69	1.99	0	3	21	0	1	2	73
2.54	1.56	156.963	145.94	148.61	0.69	1.99	0	3	21	0	1	2	73
2.55	1.57	158.693	146.02	148.7	0.69	2	0	3	21	0	1	2	73
2.56	1.58	160.432	146.1	148.78	0.7	2.01	0	2	21	0	1	2	74
2.57	1.58	162.18	146.18	148.87	0.7	2.03	0	2	20	0	1	2	73
2.58	1.59	163.937	146.26	148.96	0.7	2.03	0	2	20	0	1	2	74
2.59	1.6	165.703	146.34	149.04	0.71	2.04	0	2	20	0	1	2	74
2.6	1.61	167.479	146.42	149.13	0.71	2.02	0	2	21	0	0	1	75
2.61	1.62	169.264	146.5	149.22	0.71	2.03	0	2	21	0	0	1	76
2.62	1.63	170.93	146.75	149.47	0.72	2.06	0	2	20	1	1	1	75
2.63	1.64	172.605	146.99	149.73	0.72	2.07	0	2	20	1	1	1	74
2.64	1.64	174.289	147.24	149.98	0.72	2.06	0	2	20	0	1	1	75
2.65	1.65	175.982	147.49	150.24	0.72	2.09	0	2	20	1	1	1	75
2.66	1.66	177.684	147.74	150.5	0.73	2.07	0	2	20	0	1	1	75

Appendix 3. continued

Max. depth (m)	Ave. depth (m)	Discharge (m3/s)	Width (m)	Wet perrimeter (m)	Ave. velocity (m/s)	Velocity 98%	ibution (%) of velocity depth cla						
						(m/s)	SvS	SS	SD	FVS	FS	FI	FD
2.72	1.7	188.087	149.24	152.03	0.74	2.1	0	1	20	1	1	1	76
2.73	1.71	189.852	149.49	152.29	0.74	2.11	0	1	20	1	1	1	76
2.74	1.71	191.627	149.74	152.55	0.75	2.13	0	1	20	1	1	1	75
2.75	1.72	193.412	149.99	152.8	0.75	2.11	0	1	20	1	1	1	76
2.76	1.73	195.189	150.25	153.08	0.75	2.14	0	1	19	1	1	1	76
2.77	1.73	196.92	150.59	153.42	0.75	2.13	0	1	20	1	1	1	76
2.78	1.74	198.66	150.94	153.76	0.76	2.15	0	1	19	1	1	0	76
2.79	1.75	200.409	151.28	154.1	0.76	2.15	0	1	19	2	1	0	76
2.8	1.75	202.168	151.62	154.44	0.76	2.14	0	1	20	1	1	0	76
2.81	1.76	203.936	151.96	154.78	0.76	2.15	0	1	19	1	1	0	76
2.82	1.77	205.714	152.3	155.13	0.77	2.18	0	1	19	2	2	0	76
2.83	1.77	207.501	152.64	155.47	0.77	2.16	0	1	19	1	1	0	77
2.84	1.78	209.298	152.98	155.81	0.77	2.16	0	1	19	1	1	0	77
2.85	1.78	211.105	153.32	156.15	0.77	2.18	0	1	19	2	2	0	76
2.86	1.79	212.921	153.66	156.49	0.77	2.18	0	1	19	2	2	0	76
2.87	1.8	214.747	154	156.83	0.78	2.16	0	1	19	1	1	0	77
2.88	1.8	216.583	154.34	157.18	0.78	2.17	0	1	19	1	1	1	76
2.89	1.81	218.428	154.68	157.52	0.78	2.21	0	1	19	2	2	1	76
2.9	1.81	220.283	155.02	157.86	0.78	2.18	0	1	19	1	1	1	77
2.91	1.82	222.352	155.14	157.98	0.79	2.2	0	1	19	1	1	1	77
2.92	1.83	224.431	155.27	158.1	0.79	2.2	0	1	19	1	1	1	77
2.93	1.84	226.52	155.39	158.23	0.79	2.21	0	1	19	1	1	1	77
2.94	1.85	228.619	155.51	158.35	0.8	2.22	0	1	19	1	1	1	77
2.95	1.86	230.728	155.63	158.47	0.8	2.22	0	1	18	1	1	1	77
2.96	1.86	232.847	155.75	158.59	0.8	2.23	0	1	18	1	1	2	77
2.97	1.87	234.976	155.87	158.72	0.8	2.25	0	1	18	1	1	2	77
2.98	1.88	237.115	155.99	158.84	0.81	2.27	0	1	18	1	1	2	77
2.99	1.89	239.265	156.11	158.96	0.81	2.27	0	1	18	1	1	2	77
3	1.9	241.424	156.23	159.08	0.81	2.27	0	1	18	1	1	2	77

Appendix 4: Descriptive data associated with flow classes for no observed modeling data.

Max. depth	Ave. depth	Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
(m)	(m)	(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
0.01	0	0	0.15	0.15	0.01	0.03	100	0	0	0	0	0	0
0.02	0.01	0	0.31	0.31	0.01	0.04	100	0	0	0	0	0	0
0.03	0.01	0	0.46	0.46	0.02	0.06	100	0	0	0	0	0	0
0.04	0.02	0	0.65	0.66	0.02	0.06	100	0	0	0	0	0	0
0.05	0.02	0	0.84	0.86	0.02	0.07	100	0	0	0	0	0	0
0.06	0.03	0.001	0.98	1	0.02	0.09	100	0	0	0	0	0	0
0.07	0.04	0.001	1.12	1.14	0.03	0.1	100	0	0	0	0	0	0
0.08	0.04	0.001	1.46	1.5	0.03	0.1	100	0	0	0	0	0	0
0.09	0.03	0.002	2.19	2.24	0.03	0.09	100	0	0	0	0	0	0
0.1	0.03	0.003	2.71	2.77	0.03	0.1	100	0	0	0	0	0	0
0.11	0.04	0.004	3.28	3.36	0.03	0.1	95	5	0	0	0	0	0
0.12	0.04	0.005	4.1	4.19	0.03	0.11	93	7	0	0	0	0	0
0.13	0.04	0.006	4.94	5.05	0.03	0.11	91	9	0	0	0	0	0
0.14	0.05	0.008	5.79	5.92	0.03	0.11	89	11	0	0	0	0	0
0.15	0.05	0.011	6.48	6.63	0.03	0.12	87	13	0	0	0	0	0
0.16	0.05	0.014	7.83	8.01	0.03	0.12	88	12	0	0	0	0	0
0.17	0.05	0.017	8.87	9.08	0.04	0.13	87	13	0	0	0	0	0
0.18	0.06	0.021	10.2	10.45	0.04	0.13	84	16	0	0	0	0	0
0.19	0.06	0.025	12.44	12.72	0.04	0.14	82	18	0	0	0	0	0
0.2	0.06	0.031	13.92	14.24	0.04	0.14	81	19	0	0	0	0	0
0.21	0.06	0.039	14.98	15.34	0.04	0.15	78	22	0	0	0	0	0
0.22	0.07	0.048	16.22	16.62	0.04	0.16	75	25	0	0	0	0	0
0.23	0.07	0.057	17.68	18.13	0.04	0.16	73	27	0	0	0	0	0
0.24	0.08	0.067	19.64	20.13	0.05	0.16	73	27	0	0	0	0	0
0.25	0.08	0.078	21.5	22.04	0.05	0.16	70	30	0	0	0	0	0
0.26	0.08	0.092	23.16	23.75	0.05	0.17	65	35	0	0	0	0	0
0.27	0.09	0.106	25.16	25.8	0.05	0.17	63	37	0	0	0	0	0
0.28	0.09	0.122	27.08	27.77	0.05	0.18	60	40	0	0	0	0	0
0.29	0.09	0.14	29	29.75	0.05	0.19	58	42	0	0	0	0	0
0.3	0.1	0.16	31.16	31.97	0.05	0.19	55	45	0	0	0	0	0
0.31	0.1	0.178	34.26	35.13	0.05	0.19	54	46	0	0	0	0	0
0.32	0.1	0.199	37.7	38.63	0.05	0.19	57	43	0	0	0	0	0
0.33	0.1	0.226	40.15	41.14	0.06	0.2	55	45	0	0	0	0	0
0.34	0.11	0.258	41.92	42.96	0.06	0.2	52	48	0	0	0	0	0
0.35	0.11	0.293	43.57	44.67	0.06	0.22	52	48	0	0	0	0	0
0.36	0.12	0.329	45.44	46.6	0.06	0.22	52	48	0	0	0	0	0
0.37	0.12	0.368	47.37	48.58	0.06	0.22	44	55	0	0	0	0	0
0.38	0.13	0.41	49.29	50.57	0.07	0.22	41	58	0	0	0	0	0
0.39	0.13	0.453	51.54	52.88	0.07	0.24	42	58	0	0	0	0	0
0.4	0.14	0.499	53.81	55.23	0.07	0.25	43	56	0	0	0	0	0
0.41	0.14	0.549	55.9	57.39	0.07	0.24	34	65	0	0	0	0	0
0.42	0.15	0.604	57.73	59.29	0.07	0.26	35	64	0	0	0	0	0
0.43	0.15	0.662	59.47	61.09	0.07	0.26	30	69	0	0	0	0	0
0.44	0.16	0.725	61.13	62.81	0.08	0.26	29	70	0	0	0	0	0
0.45	0.16	0.79	62.91	64.66	0.08	0.27	27	71	0	0	1	0	0
0.46	0.17	0.859	64.58	66.39	0.08	0.28	31	67	0	1	1	1	0
0.47	0.17	0.929	66.57	68.44	0.08	0.28	27	71	0	1	1	0	0
0.48	0.18	1.006	68.13	70.05	0.08	0.3	27	71	0	1	1	1	0
0.49	0.19	1.09	69.29	71.27	0.08	0.3	25	73	0	1	1	1	1
0.5	0.19	1.178	70.42	72.46	0.09	0.31	22	75	0	1	1	1	1
0.51	0.2	1.27	71.6	73.7	0.09	0.32	22	73	0	1	1	1	1
0.52	0.21	1.364	72.79	74.95	0.09	0.33	21	74	1	1	1	1	1
0.53	0.21	1.461	74.04	76.25	0.09	0.34	21	74	0	1	2	1	1
0.54	0.22	1.559	75.49	77.77	0.09	0.33	15	79	1	1	1	1	1
0.55	0.22	1.659	77.06	79.42	0.1	0.34	15	79	1	1	1	1	1
0.56	0.23	1.761	78.81	81.23	0.1	0.36	20	73	1	1	1	2	2
0.57	0.23	1.861	80.97	83.46	0.1	0.36	18	74	2	1	1	1	2
0.58	0.24	1.972	82.63	85.18	0.1	0.36	18	75	1	1	1	2	2
0.59	0.24	2.082	84.62	87.24	0.1	0.36	18	74	2	1	1	2	2
0.6	0.25	2.193	86.88	89.57	0.1	0.36	17	74	3	1	1	2	2
0.61	0.25	2.3	89.58	92.35	0.1	0.37	19	72	2	1	1	2	3

Appendix 4. continued

Max. depth	Ave. depth		Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
(m)	(m)		(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
0.62	0.25		2.414	92.29	95.13	0.1	0.38	22	66	5	2	1	2	3
0.63	0.25		2.538	94.6	97.53	0.11	0.36	14	76	3	1	1	2	3
0.64	0.26		2.673	96.59	99.59	0.11	0.38	20	67	5	2	1	1	3
0.65	0.26		2.818	98.27	101.35	0.11	0.39	20	67	5	2	1	2	3
0.66	0.27		2.971	99.8	102.95	0.11	0.39	17	69	6	1	1	2	3
0.67	0.28		3.128	101.37	104.59	0.11	0.39	18	64	10	2	1	1	4
0.68	0.28		3.288	102.98	106.27	0.11	0.41	18	63	10	2	1	2	4
0.69	0.29		3.451	104.69	108.05	0.11	0.41	17	64	10	2	2	1	5
0.7	0.29		3.616	106.57	110	0.12	0.43	20	60	11	2	1	1	5
0.71	0.3		3.784	108.53	112.03	0.12	0.41	13	65	13	1	2	1	5
0.72	0.3		3.957	110.47	114.03	0.12	0.41	14	63	14	1	1	1	5
0.73	0.31		4.133	112.55	116.18	0.12	0.43	13	64	13	1	2	2	5
0.74	0.31		4.314	114.64	118.34	0.12	0.42	11	63	16	1	2	1	5
0.75	0.32		4.504	116.55	120.32	0.12	0.43	12	62	16	1	2	1	6
0.76	0.32		4.707	118.21	122.04	0.12	0.43	12	58	20	1	2	1	6
0.77	0.33		4.918	119.74	123.63	0.13	0.44	15	55	19	2	2	1	6
0.78	0.33		5.131	121.39	125.35	0.13	0.44	12	58	20	1	2	1	6
0.79	0.34		5.348	123.1	127.11	0.13	0.45	12	52	24	2	2	1	6
0.8	0.34		5.572	124.77	128.84	0.13	0.45	11	54	24	1	1	2	6
0.81	0.35		5.802	126.44	130.56	0.13	0.47	15	47	26	2	2	1	7
0.82	0.35		6.037	128.1	132.28	0.13	0.49	13	47	27	2	2	1	7
0.83	0.36		6.283	129.64	133.87	0.13	0.49	14	47	25	2	2	2	7
0.84	0.36		6.525	131.46	135.74	0.14	0.49	12	49	26	2	2	2	7
0.85	0.37		6.765	133.54	137.87	0.14	0.49	10	50	28	1	2	2	8
0.86	0.37		6.982	136.52	140.89	0.14	0.48	10	48	29	1	2	2	8
0.87	0.38		7.25	138.2	142.61	0.14	0.48	9	46	32	1	2	2	7
0.88	0.38		7.528	139.74	144.18	0.14	0.5	11	46	30	2	2	2	8
0.89	0.39		7.832	140.73	145.2	0.14	0.5	9	44	33	1	2	2	8
0.9	0.39		8.126	142.17	146.68	0.14	0.51	9	44	33	1	1	2	9
0.91	0.4		8.429	143.54	148.07	0.15	0.52	9	43	34	2	2	2	9
0.92	0.41		8.76	144.33	148.88	0.15	0.53	9	43	34	2	2	2	9
0.93	0.42		9.086	145.42	149.98	0.15	0.54	11	40	34	2	1	2	10
0.94	0.42		9.437	146.04	150.62	0.15	0.53	8	39	38	1	2	2	10
0.95	0.43		9.793	146.68	151.27	0.15	0.55	8	40	36	1	2	2	10
0.96	0.44	1	0.156	147.31	151.92	0.16	0.56	8	37	38	2	2	2	11
0.97	0.45	1	0.504	148.44	153.06	0.16	0.56	5	43	36	1	2	2	12
0.98	0.45	1	0.881	149.05	153.68	0.16	0.58	7	39	37	1	1	2	12
0.99	0.46	1	1.265	149.64	154.27	0.16	0.57	6	39	38	1	2	2	12
1	0.47	1	1.661	150.14	154.78	0.16	0.58	4	41	37	1	2	2	13
1.01	0.48	1	2.067	150.55	155.21	0.17	0.59	4	39	39	1	1	3	13
1.02	0.49	1	2.487	150.81	155.48	0.17	0.59	3	42	38	1	1	2	14
1.03	0.50	1	2.914	151.08	155.75	0.17	0.6	2	41	39	0	1	2	14
1.04	0.51	1	3.347	151.34	156.02	0.17	0.61	3	40	38	1	2	2	15
1.05	0.52	1	3.788	151.58	156.26	0.18	0.62	2	38	41	1	2	2	15
1.06	0.53	1	4.241	151.73	156.42	0.18	0.63	2	36	42	1	2	2	16
1.07	0.54	1	4.7	151.88	156.57	0.18	0.64	2	36	41	1	1	2	16
1.08	0.55	1	5.166	152.03	156.73	0.18	0.65	2	36	41	1	1	2	17
1.09	0.55	1	5.638	152.18	156.88	0.19	0.65	2	34	43	0	1	2	17
1.1	0.56	1	6.117	152.33	157.04	0.19	0.66	2	33	44	1	1	2	18
1.11	0.57	1	6.603	152.48	157.2	0.19	0.67	2	32	44	1	1	2	19
1.12	0.58	1	7.094	152.63	157.35	0.19	0.68	2	31	45	1	1	2	19
1.13	0.59	1	7.593	152.78	157.51	0.19	0.69	2	29	46	1	1	2	20
1.14	0.60	1	8.097	152.93	157.66	0.2	0.69	1	27	48	0	1	2	20
1.15	0.61	1	8.609	153.08	157.82	0.2	0.7	0	26	50	0	1	2	21
1.16	0.62	1	9.126	153.23	157.97	0.2	0.71	0	25	51	0	1	2	21
1.17	0.63	1	9.65	153.38	158.13	0.2	0.72	0	23	52	0	1	2	22
1.18	0.64	2	0.181	153.53	158.28	0.21	0.72	0	22	53	0	1	1	23
1.19	0.65	2	0.718	153.68	158.44	0.21	0.73	0	23	52	0	1	1	23
1.2	0.66	2	1.262	153.83	158.59	0.21	0.74	0	22	52	0	1	1	24
1.21	0.67	2	1.811	153.98	158.75	0.21	0.75	0	21	53	0	0	1	25
1.22	0.68	2	2.368	154.13	158.91	0.21	0.75	0	21	53	0	0	1	25
1.23	0.69	2	2.931	154.28	159.06	0.22	0.76	0	20	53	0	0	1	25
1.24	0.70	2	3.5	154.43	159.22	0.22	0.78	1	17	54	0	0	1	26

Appendix 4. continued

Max. depth	Ave. depth		Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
(m)	(m)		(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
1.25	0.70	2	4.076	154.58	159.37	0.22	0.79	1	17	54	0	0	1	27
1.26	0.71	2	4.658	154.73	159.53	0.22	0.8	1	16	55	0	0	1	27
1.27	0.72	2	5.246	154.88	159.68	0.23	0.8	1	14	56	0	0	1	28
1.28	0.73	2	5.842	155.03	159.84	0.23	0.81	1	13	57	0	0	1	28
1.29	0.74	2	6.443	155.18	159.99	0.23	0.82	0	13	57	0	1	1	28
1.3	0.75	2	7.051	155.33	160.15	0.23	0.83	1	13	56	0	0	1	29
1.31	0.76	2	7.662	155.51	160.33	0.23	0.83	1	13	56	0	0	0	29
1.32	0.77	2	8.279	155.69	160.52	0.24	0.84	1	12	56	0	0	1	30
1.33	0.78	2	8.902	155.88	160.72	0.24	0.84	1	10	58	0	0	1	30
1.34	0.79	2	9.532	156.07	160.91	0.24	0.85	1	10	58	0	0	1	30
1.35	0.80	3	0.168	156.25	161.1	0.24	0.84	0	10	59	0	0	1	30
1.36	0.81	3	0.811	156.44	161.29	0.24	0.85	0	9	59	0	0	1	31
1.37	0.81	3	1.46	156.62	161.48	0.25	0.86	0	9	59	0	0	1	31
1.38	0.82	3	2.115	156.81	161.67	0.25	0.87	0	8	60	0	0	1	31
1.39	0.83	3	2.777	157	161.86	0.25	0.88	0	7	60	0	0	1	32
1.4	0.84	3	3.446	157.18	162.05	0.25	0.88	0	6	61	0	0	1	32
1.41	0.85	3	4.121	157.37	162.25	0.25	0.89	0	6	60	0	0	1	33
1.42	0.86	3	4.803	157.56	162.44	0.26	0.9	0	6	60	0	0	0	33
1.43	0.87	3	5.491	157.74	162.63	0.26	0.9	0	5	61	0	0	0	33
1.44	0.88	3	6.185	157.93	162.82	0.26	0.91	1	4	61	0	0	0	34
1.45	0.89	3	6.886	158.11	163.01	0.26	0.92	1	4	61	0	0	0	34
1.46	0.90	3	7.594	158.3	163.2	0.27	0.93	1	3	61	0	0	0	34
1.47	0.90	3	8.308	158.48	163.39	0.27	0.94	1	3	60	0	0	0	35
1.48	0.91	3	9.029	158.67	163.58	0.27	0.95	1	3	60	0	0	0	35
1.49	0.92	3	9.757	158.86	163.77	0.27	0.96	1	3	59	1	0	0	35
1.5	0.93	4	0.491	159.04	163.96	0.27	0.96	1	3	59	1	0	0	35
1.51	0.94	4	1.231	159.23	164.15	0.28	0.96	1	3	59	1	0	0	36
1.52	0.95	4	1.979	159.41	164.34	0.28	0.98	1	2	59	1	0	0	36
1.53	0.96	4	2.732	159.6	164.54	0.28	0.98	1	2	60	1	0	0	36
1.54	0.97	4	3.493	159.78	164.73	0.28	0.99	1	2	59	1	0	0	36
1.55	0.98	4	4.269	159.92	164.87	0.28	0.99	1	3	59	0	1	0	37
1.56	0.99	4	5.057	160.03	164.98	0.29	1.01	1	3	58	0	1	0	37
1.57	0.99	4	5.852	160.13	165.09	0.29	1.02	1	2	58	0	1	0	38
1.58	1.00	4	6.662	160.2	165.15	0.29	1.01	1	2	58	0	1	0	38
1.59	1.01	4	7.478	160.27	165.22	0.29	1.02	1	2	58	0	1	0	38
1.6	1.02	4	8.301	160.33	165.29	0.29	1.02	1	2	58	0	1	0	38
1.61	1.03	4	9.131	160.4	165.36	0.3	1.02	0	2	58	0	1	0	39
1.62	1.04	4	9.968	160.46	165.43	0.3	1.03	0	2	58	0	0	0	39
1.63	1.05	5	0.811	160.53	165.5	0.3	1.04	0	2	58	0	0	1	39
1.64	1.06	5	1.662	160.59	165.57	0.3	1.04	0	2	57	0	0	1	40
1.65	1.07	5	2.519	160.66	165.64	0.31	1.06	1	2	56	0	0	1	40
1.66	1.08	5	3.384	160.73	165.71	0.31	1.06	1	2	56	0	0	1	40
1.67	1.09	5	4.255	160.79	165.77	0.31	1.07	1	2	56	0	0	1	41
1.68	1.10	5	5.134	160.86	165.84	0.31	1.07	1	2	55	0	0	1	41
1.69	1.11	5	6.019	160.92	165.91	0.31	1.07	0	2	56	0	0	1	42
1.7	1.12	5	6.911	160.99	165.98	0.32	1.08	0	2	56	0	0	1	42
1.71	1.13	5	7.81	161.05	166.05	0.32	1.09	0	2	55	0	0	0	42
1.72	1.14	5	8.716	161.12	166.12	0.32	1.11	0	2	54	0	0	0	43
1.73	1.15	5	9.63	161.19	166.19	0.32	1.11	0	2	54	0	0	0	43
1.74	1.16	6	0.55	161.25	166.26	0.32	1.12	0	2	54	0	0	0	43
1.75	1.17	6	1.477	161.32	166.33	0.33	1.13	0	2	53	0	0	0	44
1.76	1.18	6	2.411	161.38	166.4	0.33	1.13	0	2	54	0	0	0	44
1.77	1.19	6	3.353	161.45	166.46	0.33	1.13	0	2	54	0	0	0	44
1.78	1.20	6	4.301	161.52	166.53	0.33	1.13	0	2	53	0	0	0	45
1.79	1.20	6	5.257	161.58	166.6	0.34	1.13	0	1	54	0	0	0	45
1.8	1.21	6	6.219	161.65	166.67	0.34	1.15	0	1	52	0	0	0	45
1.81	1.22	6	7.189	161.71	166.74	0.34	1.16	0	1	52	0	0	0	45
1.82	1.23	6	8.166	161.78	166.81	0.34	1.17	0	1	52	0	0	0	46
1.83	1.24	6	9.15	161.84	166.88	0.34	1.18	0	1	52	0	0	0	46
1.84	1.25	7	0.141	161.91	166.95	0.35	1.18	0	1	52	0	0	0	46
1.85	1.26	7	1.139	161.98	167.02	0.35	1.18	0	1	52	0	0	0	47
1.86	1.27	7	2.145	162.04	167.08	0.35	1.19	0	1	52	0	0	0	47
1.87	1.28	7	3.157	162.11	167.15	0.35	1.2	0	1	51	0	0	0	47

Appendix 4. continued

Max. depth	Ave. depth		Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
(m)	(m)		(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
1.88	1.29	7	4.177	162.17	167.22	0.35	1.2	0	1	51	0	0	0	48
1.89	1.30	7	5.205	162.24	167.29	0.36	1.19	0	1	51	0	0	0	48
1.9	1.31	7	6.239	162.31	167.36	0.36	1.2	0	1	51	0	0	0	48
1.91	1.32	7	7.278	162.38	167.44	0.36	1.21	0	1	50	0	0	0	48
1.92	1.33	7	8.325	162.45	167.51	0.36	1.22	0	1	50	0	0	0	49
1.93	1.34	7	9.379	162.53	167.59	0.37	1.24	0	1	49	0	0	0	49
1.94	1.35	8	0.44	162.6	167.66	0.37	1.25	0	1	49	0	0	0	49
1.95	1.36	8	1.509	162.67	167.74	0.37	1.26	0	1	49	0	0	0	49
1.96	1.37	8	2.585	162.74	167.82	0.37	1.27	0	1	48	0	0	0	50
1.97	1.38	8	3.668	162.82	167.89	0.37	1.28	0	1	48	0	0	0	50
1.98	1.38	8	4.759	162.89	167.97	0.38	1.29	0	1	48	0	0	0	50
1.99	1.39	8	5.857	162.96	168.04	0.38	1.3	0	1	47	0	0	0	51
2	1.40	8	6.963	163.04	168.12	0.38	1.3	0	1	47	0	0	0	51
2.01	1.41	8	8.076	163.11	168.2	0.38	1.3	0	1	47	0	0	0	51
2.02	1.42	8	9.196	163.18	168.27	0.38	1.29	0	1	47	0	0	0	52
2.03	1.43	9	0.324	163.26	168.35	0.39	1.3	0	1	47	0	0	0	52
2.04	1.44	9	1.46	163.33	168.42	0.39	1.31	0	1	47	0	0	0	52
2.05	1.45	9	2.603	163.4	168.5	0.39	1.32	0	1	47	0	0	0	52
2.06	1.46	9	3.753	163.48	168.58	0.39	1.32	0	1	47	0	0	0	53
2.07	1.47	9	4.911	163.55	168.65	0.4	1.33	0	1	46	0	0	0	53
2.08	1.48	9	6.077	163.62	168.73	0.4	1.34	0	0	46	0	0	0	53
2.09	1.49	9	7.25	163.7	168.8	0.4	1.35	0	0	46	0	0	0	53
2.1	1.50	9	8.431	163.77	168.88	0.4	1.35	0	0	46	0	0	0	54
2.11	1.51	9	9.619	163.84	168.95	0.4	1.36	0	0	46	0	0	0	54
2.12	1.52	10	0.815	163.92	169.03	0.41	1.38	0	1	45	0	0	0	54
2.13	1.52	10	2.019	163.99	169.11	0.41	1.39	0	1	45	0	0	0	54
2.14	1.53	10	3.23	164.06	169.18	0.41	1.38	0	1	44	0	0	0	54
2.15	1.54	10	4.449	164.13	169.26	0.41	1.39	0	1	44	0	0	0	55
2.16	1.55	10	5.676	164.21	169.33	0.41	1.39	0	1	44	0	0	0	55
2.17	1.56	10	6.911	164.28	169.41	0.42	1.4	0	1	43	0	0	0	55
2.18	1.57	10	8.153	164.35	169.49	0.42	1.41	0	1	43	0	0	0	55
2.19	1.58	10	9.403	164.43	169.56	0.42	1.41	0	1	43	0	0	0	55
2.2	1.59	11	0.661	164.5	169.64	0.42	1.42	0	1	43	0	0	0	55
2.21	1.60	11	1.927	164.57	169.71	0.43	1.41	0	1	43	0	0	0	56
2.22	1.61	11	3.201	164.65	169.79	0.43	1.42	0	1	43	0	0	0	56
2.23	1.62	11	4.482	164.72	169.87	0.43	1.43	0	1	43	0	0	0	56
2.24	1.63	11	5.772	164.79	169.94	0.43	1.44	0	1	43	0	0	0	57
2.25	1.64	11	7.069	164.87	170.02	0.43	1.44	0	1	43	0	0	0	57
2.26	1.65	11	8.375	164.94	170.09	0.44	1.45	0	1	42	0	0	0	57
2.27	1.65	11	9.688	165.01	170.17	0.44	1.46	0	1	41	0	0	0	57
2.28	1.66	12	1.01	165.09	170.24	0.44	1.46	0	1	41	0	0	0	57
2.29	1.67	12	2.34	165.16	170.32	0.44	1.47	0	1	41	0	0	0	57
2.3	1.68	12	3.677	165.23	170.4	0.44	1.47	0	1	41	0	0	0	57
2.31	1.69	12	5.023	165.3	170.47	0.45	1.47	0	1	41	0	0	0	58
2.32	1.70	12	6.376	165.38	170.55	0.45	1.48	0	1	41	0	0	0	58
2.33	1.71	12	7.738	165.45	170.62	0.45	1.49	0	0	41	0	0	0	59
2.34	1.72	12	9.109	165.52	170.7	0.45	1.49	0	0	41	0	0	0	59
2.35	1.73	13	0.487	165.6	170.77	0.46	1.51	0	1	40	0	0	0	58
2.36	1.74	13	1.873	165.67	170.85	0.46	1.52	0	1	40	0	0	0	59
2.37	1.75	13	3.268	165.74	170.93	0.46	1.53	0	1	40	0	0	0	59
2.38	1.76	13	4.671	165.82	171	0.46	1.53	0	1	40	0	0	0	59
2.39	1.77	13	6.082	165.89	171.08	0.46	1.53	0	0	40	0	0	0	60
2.4	1.78	13	7.502	165.96	171.15	0.47	1.53	0	0	40	0	0	0	60
2.41	1.78	13	8.93	166.03	171.23	0.47	1.53	0	0	40	0	0	0	60
2.42	1.79	14	0.366	166.11	171.31	0.47	1.54	0	0	39	0	0	0	60
2.43	1.80	14	1.811	166.18	171.38	0.47	1.56	0	1	39	0	0	0	60
2.44	1.81	14	3.264	166.25	171.46	0.48	1.56	0	1	39	0	0	0	60
2.45	1.82	14	4.726	166.33	171.53	0.48	1.57	0	1	38	0	0	0	60
2.46	1.83	14	6.196	166.4	171.61	0.48	1.58	0	1	38	0	0	0	60
2.47	1.84	14	7.675	166.47	171.68	0.48	1.58	0	1	38	0	0	0	61
2.48	1.85	14	9.162	166.55	171.76	0.48	1.58	0	0	38	0	0	0	61
2.49	1.86	15	0.658	166.62	171.84	0.49	1.59	0	0	38	0	0	0	61
2.5	1.87	15	2.162	166.69	171.91	0.49	1.59	0	0	38	0	0	0	62

Appendix 4. continued

Max. depth	Ave. depth	Discharge	Width	Wet perimeter	Ave. velocity	Velocity 98%	Distribution (%) of VD. classes						
(m)	(m)	(m3/s)	(m)	(m)	(m/s)	(m/s)	SvS	SS	SD	FVS	FS	FI	FD
2.51	1.88 15	3.675	166.77	171.99	0.49	1.6	0	0	38	0	0	0	62
2.52	1.89 15	5.197	166.84	172.06	0.49	1.63	0	1	37	0	0	0	61
2.53	1.89 15	6.728	166.91	172.14	0.5	1.63	0	1	37	0	0	0	62
2.54	1.90 15	8.267	166.98	172.21	0.5	1.64	0	1	37	0	0	0	62
2.55	1.91 15	9.814	167.06	172.29	0.5	1.65	0	0	37	0	0	0	62
2.56	1.92 16	1.371	167.13	172.37	0.5	1.65	0	0	36	0	0	0	62
2.57	1.93 16	2.937	167.2	172.44	0.5	1.65	0	0	37	0	0	0	63
2.58	1.94 16	4.511	167.28	172.52	0.51	1.65	0	1	36	0	0	0	63
2.59	1.95 16	6.094	167.35	172.59	0.51	1.66	0	1	36	0	0	0	63
2.6	1.96 16	7.521	167.68	172.93	0.51	1.66	0	1	36	0	0	0	63
2.61	1.96 16	8.938	168.04	173.29	0.51	1.67	0	1	36	0	0	0	63
2.62	1.97 17	0.364	168.4	173.65	0.51	1.67	0	1	35	0	0	0	63
2.63	1.97 17	1.799	168.76	174.01	0.52	1.69	0	1	35	1	1	0	63
2.64	1.98 17	3.242	169.12	174.37	0.52	1.69	0	1	35	1	1	0	63
2.65	1.98 17	4.694	169.48	174.73	0.52	1.7	0	1	35	1	1	0	63
2.66	1.99 17	6.154	169.84	175.09	0.52	1.7	0	1	35	1	1	0	63
2.67	2.00 17	7.619	170.21	175.46	0.52	1.69	0	1	35	0	0	0	63
2.68	2.00 17	9.109	170.55	175.81	0.52	1.71	0	1	34	1	1	0	63
2.69	2.01 18	0.609	170.89	176.15	0.53	1.73	0	1	34	1	1	0	63
2.7	2.01 18	2.117	171.23	176.49	0.53	1.72	0	1	34	1	1	0	63
2.71	2.02 18	3.639	171.57	176.83	0.53	1.73	1	1	34	1	1	0	63
2.72	2.03 18	5.17	171.91	177.16	0.53	1.72	0	1	34	1	1	0	63
2.73	2.03 18	6.711	172.24	177.5	0.53	1.73	0	1	34	1	1	0	63
2.74	2.04 18	8.26	172.58	177.84	0.54	1.74	1	1	33	1	1	0	63
2.75	2.05 18	9.961	172.71	177.97	0.54	1.75	1	1	33	1	1	0	63
2.76	2.06 19	1.688	172.82	178.08	0.54	1.74	0	1	33	1	1	0	64
2.77	2.06 19	3.424	172.93	178.19	0.54	1.74	0	1	33	1	1	0	64
2.78	2.07 19	5.17	173.04	178.31	0.54	1.75	0	1	33	1	1	0	64
2.79	2.08 19	6.926	173.15	178.42	0.55	1.76	0	1	33	1	1	1	64
2.8	2.09 19	8.691	173.26	178.53	0.55	1.76	0	1	33	1	1	1	64
2.81	2.10 20	0.466	173.37	178.64	0.55	1.77	0	1	33	1	1	1	64
2.82	2.11 20	2.251	173.48	178.75	0.55	1.77	0	1	33	1	1	1	64
2.83	2.12 20	4.046	173.59	178.86	0.56	1.78	0	1	32	1	1	1	64
2.84	2.12 20	5.85	173.7	178.98	0.56	1.79	0	1	32	0	0	1	65
2.85	2.13 20	7.665	173.81	179.09	0.56	1.81	0	1	32	1	1	1	64
2.86	2.14 20	9.49	173.92	179.2	0.56	1.8	0	1	32	0	0	1	65
2.87	2.15 21	1.324	174.02	179.31	0.56	1.81	0	1	32	0	0	1	65
2.88	2.16 21	3.169	174.13	179.42	0.57	1.83	0	1	31	1	1	1	65
2.89	2.17 21	5.023	174.24	179.53	0.57	1.83	0	1	31	1	1	1	65
2.9	2.18 21	6.888	174.35	179.64	0.57	1.83	0	1	32	0	0	1	66
2.91	2.19 21	8.763	174.46	179.75	0.57	1.84	0	1	31	0	0	1	66
2.92	2.19 22	0.648	174.57	179.87	0.58	1.86	0	1	31	0	0	1	66
2.93	2.20 22	2.543	174.68	179.98	0.58	1.85	0	1	31	0	0	1	66
2.94	2.21 22	4.448	174.79	180.09	0.58	1.86	0	1	31	0	0	1	66
2.95	2.22 22	6.364	174.9	180.2	0.58	1.88	0	1	30	0	0	1	66
2.96	2.23 22	8.29	175.01	180.31	0.59	1.89	0	1	30	0	0	1	66
2.97	2.24 23	0.227	175.12	180.42	0.59	1.88	0	1	30	0	0	1	67
2.98	2.25 23	2.174	175.23	180.53	0.59	1.89	0	1	30	0	0	1	67
2.99	2.25 23	4.131	175.34	180.64	0.59	1.92	0	1	30	0	0	1	67
3	2.26 23	6.099	175.45	180.76	0.59	1.9	0	1	30	0	0	1	67

Appendix 5. Selected metal concentrations (mean ± standard error µg/g dry mass) in sediments from selected sites in the Luvuvhu River, during 4 separate surveys. BCR-A (acid soluble) and B (reducible) and BCR-C (oxidizable) and D (non-bioavailable), derived from a sequential extraction procedure.

	Aluminium (Al) µg/L					Arsenic (As) µg/L					Cadmium (Cd) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
LV-S1-09LF	151	34.39	303.3	23617	24106 ± 12441	0.05	0.04	0.10	0.86	1.04 ± 0.09	0.24	0.01	0.02	0.05	0.32 ± 0.18
LV-S2-09LF	81.36	46.62	220.7	25621	25970 ± 11808	0.04	0.03	0.06	1.14	1.26 ± 0.32	0.05	0.01	0.01	0.10	0.17 ± 0.06
LV-S3-09LF	78	68.80	154.2	4543	4844 ± 1803	0.11	0.07	0.17	2.74	3.08 ± 0.48	0.02	0.01	0.02	0.41	0.45 ± 0.09
LV-S4-09LF	76.57	109.7	132	11477	11795 ± 4856	0.04	0.03	0.20	1.28	1.55 ± 0.31	0.02	0.01	0.02	0.06	0.11 ± 0.01
LV-S1-10HF	1.87	25.49	11.07	2951	2989 ± 1008	0.03	0.03	BD	0.32	0.39 ± 0.13	0.01	BD	0.03	0.03	0.06 ± 0.02
LV-S2-10HF	2.54	35.74	15.55	5207	5261 ± 849.6	0.04	0.05	0.01	0.73	0.83 ± 0.02	0.01	BD	0.01	0.11	0.13 ± 0.04
LV-S3-10HF	7.60	86.36	35.00	16903	17032 ± 2210	0.12	0.07	0.10	1.16	1.44 ± 0.05	0.01	BD	0.01	0.14	0.16 ± 0.02
LV-S4-10HF	7.81	74.60	28.72	9288	9399 ± 1422	0.14	0.06	0.10	1.09	1.38 ± 0.03	0.01	BD	0.01	0.11	0.13 ± 0.01
LV-S1-10LF	4.24	41.72	11.65	6937	6995 ± 1776	0.05	0.09	BD	0.45	0.58 ± 0.07	0.01	BD	0.01	0.09	0.11 ± 0.03
LV-S2-10LF	1.90	39.80	11.54	4287	4341 ± 492.2	0.04	0.10	BD	0.36	0.5 ± 0.15	BD	BD	0.01	0.04	0.05 ± 0.01
LV-S3-10LF	2.89	46.32	11.40	10663	10724 ± 5509	0.04	0.09	0.01	0.79	0.93 ± 0.21	0.01	BD	0.01	0.04	0.06 ± 0
LV-S4-10LF	25.32	230.5	39.77	9453	9748 ± 3139	0.24	0.04	0.17	214.8	215.3 ± 214.5	0.01	BD	0.02	0.20	0.23 ± 0.07
LV-S1-11HF	BD	22.68	8.87	4619	4651 ± 307.3	0.03	0.05	BD	0.61	0.69 ± 0.07	BD	BD	BD	0.04	0.04 ± 0.01
LV-S2-11HF	1.75	47.29	44.57	26125	26219 ± 8872	0.08	0.05	0.10	1.17	1.4 ± 0.1	0.01	BD	BD	0.10	0.11 ± 0.01
LV-S3-11HF	BD	20.20	11.53	4896	4928 ± 262.2	0.04	0.04	0.01	0.74	0.83 ± 0.06	BD	BD	BD	0.04	0.04 ± 0
LV-S4-11HF	2.00	37.49	21.41	9173	9234 ± 1084	0.07	0.05	0.04	0.79	0.95 ±0.1	0.01	BD	BD	0.07	0.08 ± 0.01

Appendix 5. continued

	Chromium (Cr) µg/L					Cobalt (Co) µg/L					Copper (Cu) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
LV-S1-09LF	1.78	0.40	2.85	32.24	37.27 ± 7.96	1.67	0.52	0.46	2.53	5.18 ± 0.16	2.51	0.92	4.74	20.92	29.1 ± 9.16
LV-S2-09LF	0.37	0.33	1.94	71.36	73.99 ± 26.27	1.69	0.44	0.42	4.01	6.55 ± 1.42	1.95	0.90	4.61	36.03	43.48 ± 2.8
LV-S3-09LF	0.28	0.40	7.51	167.1	175.3 ± 13.9	3.16	1.17	1.68	20.67	26.68 ± 2.57	1.55	1.72	7.02	90.99	101.3 ± 14.75
LV-S4-09LF	0.63	0.46	6.40	72.78	80.27 ± 10.6	2.42	0.60	1.54	5.91	10.47 ± 1.7	2.30	1.17	6.10	25.42	34.99 ± 6.37
LV-S1-10HF	BD	0.23	1.06	48.94	50.22 ± 27.24	0.33	0.69	0.25	69.02	70.29 ± 23.5	0.26	BD	0.90	5.72	6.88 ± 2.47
LV-S2-10HF	0.02	0.19	1.54	84.72	86.47 ± 1.26	0.53	1.24	0.29	70.56	72.62 ± 1.89	0.33	BD	1.37	21.77	23.47 ± 4.43
LV-S3-10HF	BD	0.26	2.84	87.78	90.88 ± 3.83	0.73	1.64	0.54	73.07	75.97 ± 2.66	0.19	BD	2.06	31.36	33.6 ± 1.46
LV-S4-10HF	0.22	0.24	2.58	85.44	88.47 ± 4.06	0.89	0.67	0.42	71.14	73.13 ± 2.84	0.18	BD	0.97	24.57	25.72 ± 25.72
LV-S1-10LF	0.02	0.38	1.61	78.75	80.77 ± 0.96	0.52	0.73	0.34	73.24	74.83 ± 8.41	0.40	BD	0.28	13.52	14.2 ± 4.35
LV-S2-10LF	0.05	0.48	1.69	65.70	67.93 ± 17.22	0.49	0.72	0.25	73.35	74.81 ± 4.15	0.32	0.01	0.29	17.12	17.74 ± 3.42
LV-S3-10LF	0.02	0.38	1.52	68.48	70.41 ± 4.79	0.57	0.57	0.28	68.57	69.98 ± 1.84	0.36	0.01	0.29	18.26	18.92 ± 2.59
LV-S4-10LF	0.17	0.21	4.19	74.56	79.12 ± 6.48	1.26	0.33	0.67	75.31	77.58 ± 8.6	0.60	BD	1.61	110.2	112.4 ± 87.71
LV-S1-11HF	BD	0.17	1.33	79.28	80.79 ± 2.12	0.48	1.13	0.34	66.03	67.98 ± 1.78	0.34	BD	0.32	19.54	20.19 ± 4.17
LV-S2-11HF	BD	0.17	5.08	80.53	85.78 ± 2.76	1.93	1.98	1.21	67.07	72.2 ± 1.27	0.84	BD	3.77	35.54	40.16 ± 2.73
LV-S3-11HF	BD	0.10	1.54	87.66	89.3 ± 4.31	0.38	0.80	0.25	72.96	74.39 ± 3.98	0.22	BD	0.38	19.96	20.56 ± 5.25
LV-S4-11HF	BD	0.15	1.92	88.86	90.94 ± 2.67	0.83	0.71	0.40	73.97	75.92 ± 2.33	0.31	BD	0.64	17.17	18.11 ± 1.61

Appendix 5. continued

	Lead (Pb) µg/L					Iron (Fe) µg/L					Manganese (Mn) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
LV-S1-09LF	0.50	0.42	0.66	2.36	3.94 ± 0.35	451.1	116.5	1120	56048	57736 ± 27457	118.2	15.30	5.86	124.7	264.1 ± 69.47
LV-S2-09LF	0.16	0.40	0.51	6.74	7.81 ± 3.02	117.1	246.1	485.8	9912	10761 ± 3102	55.14	4.84	6.08	310.1	376.1 ± 186.6
LV-S3-09LF	0.07	0.39	0.88	9.74	11.09 ± 1.55	104.7	314.3	159.1	5400	5978 ± 764.9	121.8	29.93	28.77	460.7	641.2 ± 81.36
LV-S4-09LF	0.19	0.56	1.02	6.58	8.36 ± 0.81	59.46	466	88.54	12581	13195 ± 4797	121.8	8.49	23.93	370.3	524.5 ± 82.35
LV-S1-10HF	0.03	0.10	0.26	0.29	0.68 ± 0.23	23.20	298.7	88.30	5012	5423 ± 1890	11.43	18.84	1.91	55.34	87.52 ± 30.77
LV-S2-10HF	0.03	0.11	0.24	1.88	2.26 ± 0.06	33.25	499.8	119.7	13121	13774 ± 1627	17.72	41.46	2.85	56.82	118.8 ± 32.93
LV-S3-10HF	0.02	0.13	0.51	1.64	2.3 ± 0.18	13.59	1227	287.8	24033	25561 ± 2353	44.39	48.82	9.26	58.79	161.3 ± 14.69
LV-S4-10HF	0.03	0.10	0.38	1.05	1.55 ± 0.14	72.34	866.8	239.1	14696	15874 ± 1787	39.25	12.48	5.18	57.25	114.2 ± 13.86
LV-S1-10LF	0.05	0.15	0.15	0.83	1.18 ± 0.35	48.92	641.0	108.7	14105	14904 ± 3073	31.63	15.24	3.64	58.06	108.6 ± 5.85
LV-S2-10LF	0.03	0.15	0.13	0.73	1.04 ± 0.09	73.66	528.2	106.4	8981	9689 ± 567	15.35	12.77	1.62	58.30	88.03 ± 2.67
LV-S3-10LF	0.03	0.15	0.14	1.16	1.49 ± 0.43	57.35	767.5	124.1	17761	18710 ± 2415	39.23	8.87	4.30	53.86	106.2 ± 7.28
LV-S4-10LF	0.02	0.13	0.35	1.32	1.83 ± 0.16	227.73	2208	482.6	23565	26484 ± 7455	48.66	5.50	7.92	59.08	121.2 ± 10
LV-S1-11HF	0.02	0.08	0.12	0.59	0.8 ± 0.07	18.75	405.3	43.78	11487	11954 ± 3860	12.09	21.37	1.73	53.13	88.33 ± 18.23
LV-S2-11HF	0.02	0.07	0.61	2.15	2.85 ± 0.25	28.68	2735	509.8	34007	37281 ± 11254	141.3	83.69	15.80	53.95	294.8 ± 33.87
LV-S3-11HF	0.01	0.05	0.14	0.99	1.19 ± 0.26	17.11	523.2	71.02	14292	14903 ± 4841	22.30	24.71	2.78	58.71	108.5 ± 3.73
LV-S4-11HF	0.01	0.06	0.20	0.90	1.18 ± 0.14	17.52	852.3	131.5	11796	12797 ± 3509	39.57	15.62	4.08	59.51	118.8 ± 8.48

Appendix 5. continued

	Nickel (Ni) µg/L					Selenium (Se) µg/L					Silver (Ag) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
LV-S1-09LF	1.67	0.40	1.86	8.30	12.24 ± 0.72	0.01	0.11	0.25	1.51	1.87 ± 0.66	0.76	0.02	0.03	0.39	1.2 ± 0.45
LV-S2-09LF	1.20	0.41	1.34	11.79	14.74 ± 2.23	0.03	0.03	0.16	2.46	2.68 ± 0.15	0.01	0.01	0.03	0.39	0.44 ± 0.04
LV-S3-09LF	4.21	2.35	15.18	142.0	163.7 ± 10.19	0.01	0.02	0.18	1.20	1.4 ± 0.12	0.15	0.02	0.02	1.46	1.65 ± 0.35
LV-S4-09LF	1.91	0.55	11.59	11.47	25.53 ± 4.35	0.01	0.04	0.19	1.28	1.52 ± 0.56	0.13	0.02	0.02	0.51	0.68 ± 0.17
LV-S1-10HF	0.16	0.30	0.65	3.33	4.43 ± 1.53	0.01	0.02	0.15	1.14	1.31 ± 0.5	BD	0.01	0.09	0.03	0.13 ± 0.04
LV-S2-10HF	0.36	0.65	0.86	5.20	7.07 ± 0.31	BD	0.06	0.15	3.63	3.84 ± 0.66	BD	0.01	0.09	0.06	0.15 ± 0.02
LV-S3-10HF	0.98	1.34	3.33	18.61	24.26 ± 1.5	BD	0.05	0.22	2.89	3.16 ± 0.05	BD	0.01	0.09	0.47	0.57 ± 0.17
LV-S4-10HF	1.13	1.04	5.32	20.33	27.82 ± 2.4	BD	0.03	0.20	2.56	2.79 ± 0.18	BD	BD	0.09	0.28	0.37 ± 0.04
LV-S1-10LF	0.23	1.31	0.78	10.65	12.97 ± 6.04	0.02	0.04	0.19	1.62	1.86 ± 0.25	BD	BD	0.09	0.20	0.29 ± 0.13
LV-S2-10LF	0.25	1.62	0.78	3.96	6.6 ± 2.04	0.02	0.05	0.19	3.19	3.45 ± 0.68	BD	BD	0.09	0.07	0.16 ± 0.01
LV-S3-10LF	0.30	1.30	0.97	5.53	8.09 ± 0.89	0.01	0.08	0.20	2.50	2.78 ± 0.28	BD	BD	0.09	0.06	0.15 ± 0.01
LV-S4-10LF	1.62	0.20	8.12	131.4	141.3 ± 105.3	0.03	0.03	0.19	3.00	3.24 ± 0.61	BD	BD	0.09	0.64	0.73 ± 0.15
LV-S1-11HF	0.19	0.50	0.73	4.85	6.26 ± 0.25	BD	0.05	0.16	2.71	2.92 ± 0.45	BD	BD	0.07	0.33	0.4 ± 0.28
LV-S2-11HF	0.91	0.79	1.89	21.95	25.54 ± 3.12	BD	0.08	0.33	2.30	2.71 ± 0.03	BD	BD	0.08	0.21	0.29 ± 0.02
LV-S3-11HF	0.28	0.47	0.99	5.53	7.27 ± 0.56	0.01	0.04	0.16	2.57	2.78 ± 0.69	BD	BD	0.07	0.03	0.11 ± 0.01
LV-S4-11HF	0.60	0.65	2.56	10.85	14.67 ± 2.17	BD	0.04	0.20	1.86	2.1 ± 0.02	BD	BD	0.07	0.15	0.22 ± 0.02

Appendix 5. continued

	Uranium (U) µg/L					Zinc (Zn) µg/L				
Sample	BCR-A	BCR-B	BCR-C	BCR-D	Total	BCR-A	BCR-B	BCR-C	BCR-D	Total
LV-S1-09LF	-	-	-	-	-	14.54	1.98	78.57	22.69	117.8 ± 34.91
LV-S2-09LF	-	-	-	-	-	50.23	2.97	12.61	40.54	106.3 ± 21.81
LV-S3-09LF	-	-	-	-	-	17.01	3.37	26.09	82.57	129 ± 25.08
LV-S4-09LF	-	-	-	-	-	31.69	2.49	33.99	29.11	97.29 ± 17.29
LV-S1-10HF	BD	BD	0.03	0.03	0.06 ± 0.02	0.31	0.49	2.67	9.22	12.69 ± 4.27
LV-S2-10HF	0.01	BD	0.04	0.22	0.26 ± 0.02	0.63	0.54	0.91	37.94	40.02 ± 7.36
LV-S3-10HF	BD	BD	0.07	0.12	0.18 ± 0.03	0.50	0.77	1.64	47.86	50.76 ± 0.98
LV-S4-10HF	BD	BD	0.05	0.08	0.13 ± 0	0.47	0.58	1.01	40.82	42.88 ± 2.95
LV-S1-10LF	0.01	0.01	0.03	0.11	0.16 ± 0.06	2.66	1.73	1.40	17.38	23.18 ± 7.06
LV-S2-10LF	0.01	0.01	0.03	0.14	0.18 ± 0.05	0.62	1.91	1.40	29.97	33.91 ± 5.71
LV-S3-10LF	0.01	0.01	0.03	0.22	0.26 ± 0.07	0.67	1.24	1.07	32.20	35.19 ± 3.48
LV-S4-10LF	0.01	BD	0.06	0.21	0.27 ± 0.03	3.46	1.40	1.03	184.9	190.8 ± 149.4
LV-S1-11HF	0.01	BD	0.03	0.06	0.1 ± 0.01	0.55	0.45	0.62	33.96	35.57 ± 8.94
LV-S2-11HF	0.01	BD	0.08	0.12	0.22 ± 0.01	1.32	0.63	1.42	40.49	43.86 ± 0.19
LV-S3-11HF	0.01	BD	0.03	0.10	0.14 ± 0.04	0.38	0.30	0.61	34.74	36.04 ± 10.2
LV-S4-11HF	0.01	BD	0.04	0.06	0.11 ± 0.01	0.43	0.28	0.75	26.56	28.02 ± 1.68