Water quality, metal bioaccumulation and parasite communities of *Oreochromis mossambicus* in Loskop Dam, Mpumalanga, South Africa

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Abstract

The principal reason for the construction of Loskop Dam was to provide irrigation water to wheat farmers settling in the Olifants River valley in the 1920s. Agriculture has since developed in the area and today, the Loskop Irrigation Board supplies water to > 700 properties with an area of 25 600 ha farming cotton, wheat, citrus and grapes near the town of Groblersdal. Serious concerns were raised about deteriorating water quality when the crocodile population began to decline and the frequency of large fish kills increased from 2006. Crocodile (*Crocodylus niloticus*) and Mozambique tilapia (*Oreochromis mossambicus*) mortalities were linked to pansteatitis which is characterised by obesity and lipid peroxidation. Known impacts on water quality include eutrophication and acid mine drainage from coal mining with associated increases of soluble metals. The aims of this study were to: i) determine whether pansteatitis could be linked to any specific parameters in the water chemistry and limnology of Loskop Dam; ii) measure concentrations of aluminium, copper, iron, manganese, selenium and zinc in various tissues of *O. mossambicus* to determine whether bio-accumulation was occurring and could be related to pansteatitis; iii) assess the metazoan parasite communities of *O. mossambicus* to determine whether they are effective indicators of ecosystem health in Loskop Dam. Four established sampling sites were used at Loskop Dam and a reference site was located at neighbouring Kranspoort Dam. Surface water quality samples were collected monthly between July and December 2010 from each site and analysed for 27 constituents including nutrients, major ions, total metals, pH and dissolved oxygen using standard methods. Orthophosphate and total inorganic nitrogen results frequently categorised Loskop Dam as eutrophic and the transitional zone of the dam was characterised by very alkaline conditions resulting from algal blooms (median pH 9.67) which increase the solubility of metals like Al. A combination of active and passive biomonitoring techniques were used for fish collection. Fish gills, brain, muscle, liver and bone were analysed for Al, Fe, Mn, Zn, Cu and Se concentrations. The most striking result was an unanticipated significant deficiency in liver Cu concentrations of fish from Loskop Dam (mean 3.4 mg kg^{-1}) compared to fish from Kranspoort Dam (mean 62 mg kg^{-1}). Both endo- and ectoparasites were identified and enumerated on the fish and infection rates were calculated as mean intensity (*I*), mean abundance (*A*) and prevalence (*P*). Fish from Loskop Dam had extremely low infection rates and two fish had no parasites whatsoever. The ratio

between monoxenous and heteroxenous parasites was calculated and was very high in fish from Loskop Dam compared to fish in Kranspoort Dam and Tompi Seleka, indicating a degraded aquatic ecosystem. While no single factor was outstanding as a possible cause of pansteatitis in this study, these findings provide a good foundation from which to formulate further research questions.

Chapter 1

A history of Loskop Dam and important environmental impacts affecting fish and crocodile populations

1.1 Historical overview of Loskop Dam

Loskop Dam was constructed in 1939 to supply water for irrigation to agricultural areas downstream. The irrigation canal system, measuring 480 km, was completed just over 10 years later, in 1948, providing water to the Loskop Irrigation Board. The dam is located about 32 kilometres South of Groblersdal in Mpumalanga province and is surrounded by a nature reserve. Following the construction of impoundments like Witbank Dam located upstream, the dam wall was raised by 9 m in 1979 to its current 49 m height to ensure consistent yield (Van Vuuren, 2008; DWA, 2010).

1.2 The Study Area

The main inflow into Loskop Dam is the Olifants River which drains a large catchment of approximately 11,500 km^2 (Midgley *et al*., 1994) where the two main tributaries are the Wilge and Klein Olifants Rivers (Fig. 1). From Loskop Dam the Olifants River flows North-East through the Drakensberg mountain range, the Kruger National Park and into Massingir Dam in Mozambique before reaching the Indian Ocean. Vegetation in the upper Olifants River catchment consists of sourish mixed bushveld, mixed bushveld and Bankenveld (Acocks, 1975) and the area is a summer rainfall region.

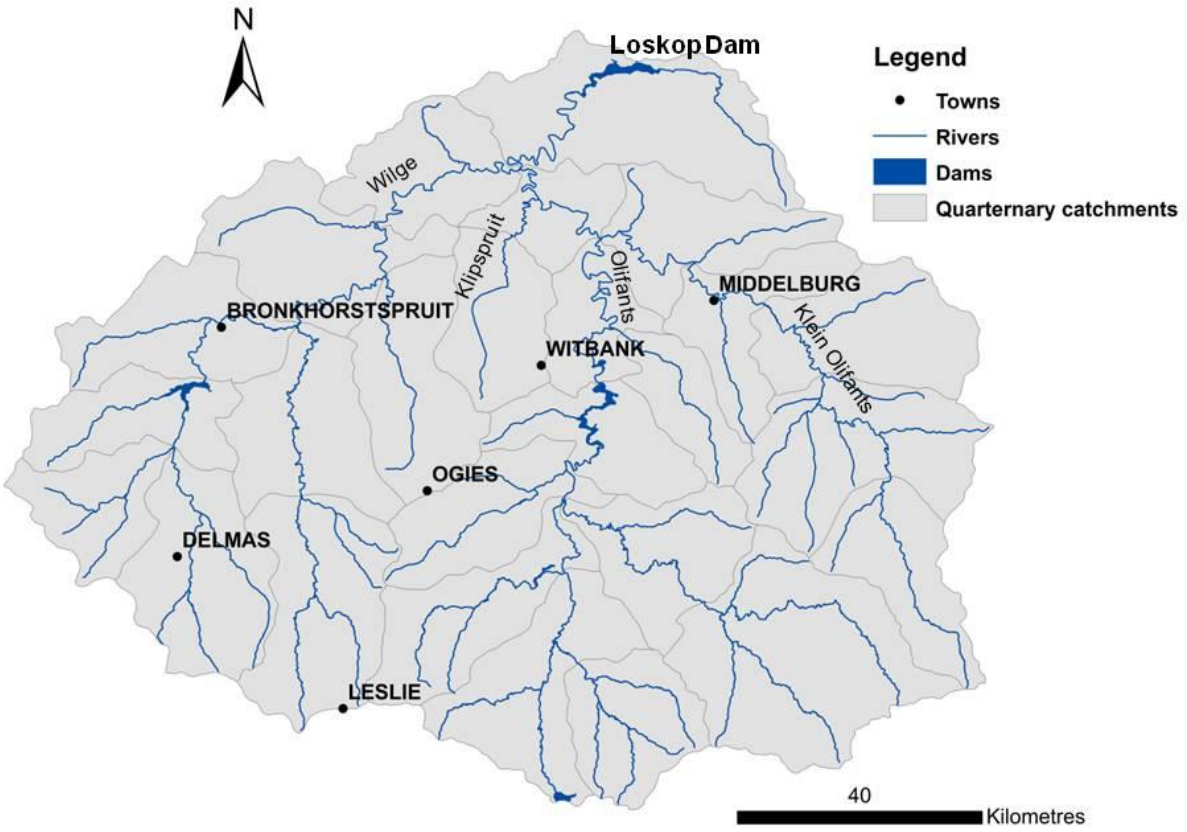

Figure 1. Location map of Loskop Dam (25° 25'1"S 29° 21'30"E) in the upper Olifants River catchment.

1.3 Economic significance of Loskop Dam

A variety of businesses in different sectors rely on Loskop Dam directly or indirectly to sustain their economic activities. The primary purpose of Loskop Dam is the irrigation water supplied via the canal network to the agricultural area in the Olifants River valley in the vicinity of Groblersdal downstream of the dam. The irrigation scheme provides water to > 700 properties covering an area of 25 600 ha. Crops include cotton, wheat, citrus and grapes (Loskop Irrigation Board, 2011).

The dam is used for recreational purposes by visitors to the Forever Resort located on the North-eastern shore of the dam. The resort employs 105 permanent staff and 50 temps and accommodates between 90,000 and 120,000 visitors annually generating over R24 million in gross turnover (pers. comm. C. Wagenaar). Access to the dam for recreational fishing purposes is predominantly through the resort as boat launching within the nature reserve is restricted and the resort regularly hosts major angling competitions at the dam which generate substantial further revenue. The Mpumalanga Tourism and Parks Agency manages the 12,762 ha Loskop Dam Nature Reserve (Eksteen & Bornman, 1990) where approximately 6,000 visitors spend in the region of R800,000 per annum (pers. comm. G. Modau).

1.4 Acute large scale fish kills

First-hand accounts of fish kills at Loskop Dam have been provided by Mr. Jannie Coetzee who was manager of the nature reserve until 2008 (pers. comm. J. Coetzee). He recalls that since 2004 there was an increase in the frequency of fish kill events and the numbers of fish involved. In mid-September 2006 (Fig. 2 A) there was a large multi-species die-off in the transitional zone of the dam followed by another fish kill in February 2007 (Fig. 2 B). On 3 June 2007 (Fig. 2 C) a pollution plume of acid mine drainage was reported in the Wilge River followed by a multi-species die-off in Loskop Dam. In August 2007 (Fig. 2 D) a large die-off dominated by *Labeo rosae* occurred over a period of about 30 days in the lacustrine zone. During this time approximately 14 tons of fish died and there was a strong smell of hydrogen sulphide from the water accompanied by bubbling at the water surface in shallow areas. Dead fish were blown into Lombards Bay and towards the Forever Resort where they collected on the shore. The dam levels during these fish die-offs ranged from around 100% during 2006 to between 26% and 100% during 2007. The drastic differences in these water levels are illustrated in figure (3 a & b) although the dates differ. However the fact that dam levels were consistently low between 2002 and 2005 with no large fish kills suggests that low dilution from low flow is not the only factor involved in fish mortalities.

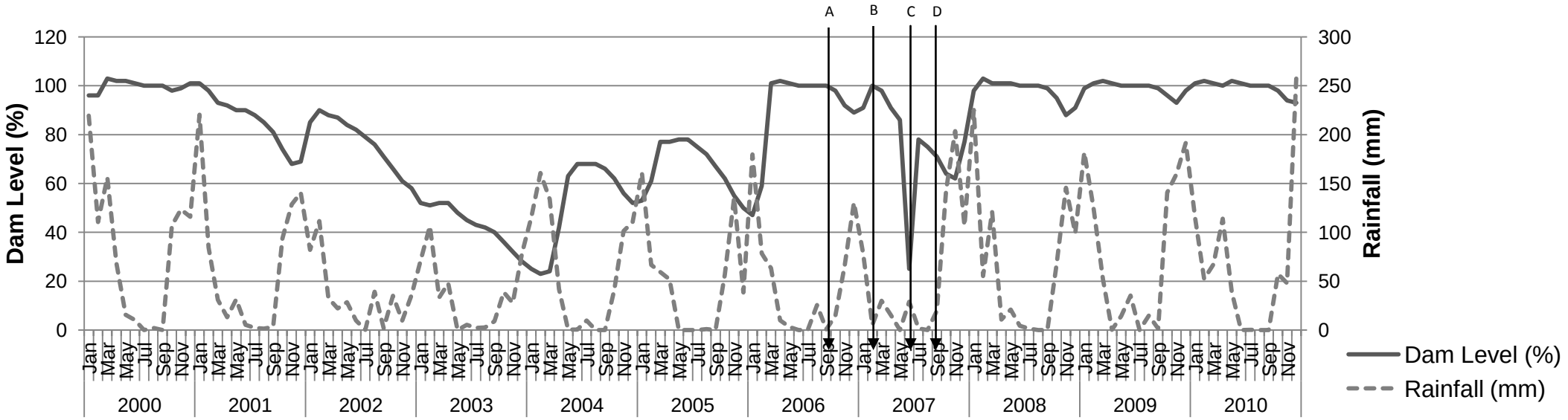

Figure 2. Monthly rainfall levels recorded in Witbank by the South African Weather Service (SAWS) and Loskop Dam levels recorded by the Department of Water Affairs (DWA) between 2000 and 2010. Arrows indicate fish die-off events explained in the text.

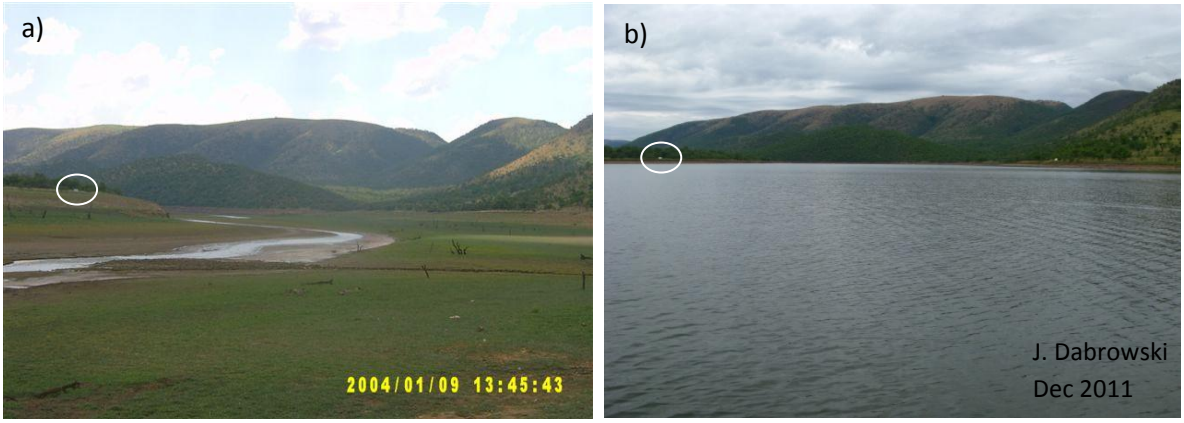

Figure 3. Picture taken at the cable (notice board circled) near sampling site LK3 when Loskop Dam was 26% full in 2004 (a) by Jannie Coetzee and at 100% full in 2011 (b) for comparison of water levels.

1.5 Annual die-offs of <u>Oreochromis mossambicus</u>

Prior to 2004 infrequent mortalities of large *O. mossambicus* around the inflow of the Olifants River were reported (J. Coetzee, pers. comm.; Driescher, 2008) but were not considered important until concerns emerged about the crocodile population at Loskop Dam and possible links to the fish die-offs. Observations made during the current study confirm that particularly during the months of September and October in 2010 there were a number of mortalities of large, predominantly male, *O. mossambicus* in the riverine and transitional zones of the dam. This event was not a large scale fish kill, but numbered around 2-3 new mortalities per day during this period where fish were either found dead and floating at the surface, or were struggling, weak and dying at the surface. After dissection all of these fish were observed to have lesions typical of pansteatitis including abundant abdominal fat with large concentrations of ceroid pigment associated with lipid peroxidation. A similar pattern of events was observed by the author with *Labeo rosae* during this study but to a lesser degree. Specifically 2-3 dead or dying fish were dissected and found to have similar lesions to those observed in *O. mossambicus.* However, these observations have not been further explored and therefore warrant further investigation.

1.6 Overview of crocodile mortalities

Estimates of the crocodile population at Loskop Dam prior to increased mortalities range widely. The highest estimate pre-2000 was reported as approximately 80 individuals with up to 20 crocodiles regularly observed at the inflow of the Olifants River by Driescher (2008) but this was not based on an official survey. Twenty seven years earlier an aerial survey of crocodiles outside of the Kruger National Park recorded six individuals in Loskop Dam and eight upstream of the dam in the Olifants River (Jacobsen, 1984). The author of this study expressed concern because the crocodile population had apparently declined from a previous record of 21 individuals in 1979. Raising of the dam wall and resultant flooding of nesting and basking sites along with water pollution were two possible reasons put forward as explanations for the apparent crocodile population decline. Jacobsen (1984) also reported a crocodile death that occurred simultaneously to a fish die-off in the dam and suggested a possible link between mortalities of these species. During 2010 Botha *et al*. (2011) recorded an estimated number of 6 crocodiles during two spotlight surveys despite the re-introduction of 13 animals to the dam in August 2007. In 2005 a crocodile in poor condition was taken to the Faculty of Veterinary Science at Onderstepoort where it was diagnosed with the condition pansteatitis. The problem is ongoing as another crocodile was found near the inflow of the Kranspoort Spruit in April 2011 (pers. obs.) and after being euthenased and dissected it too was diagnosed with pansteatitis.

1.7 Land use and impacts in the catchment

An overview of the land uses in the upper Olifants River catchment is given in Figure 4 (SANBI, 2009). From a water quality perspective the most important land uses are coal mining, power generation, steel industry, agriculture and wastewater treatment works associated with urban areas.

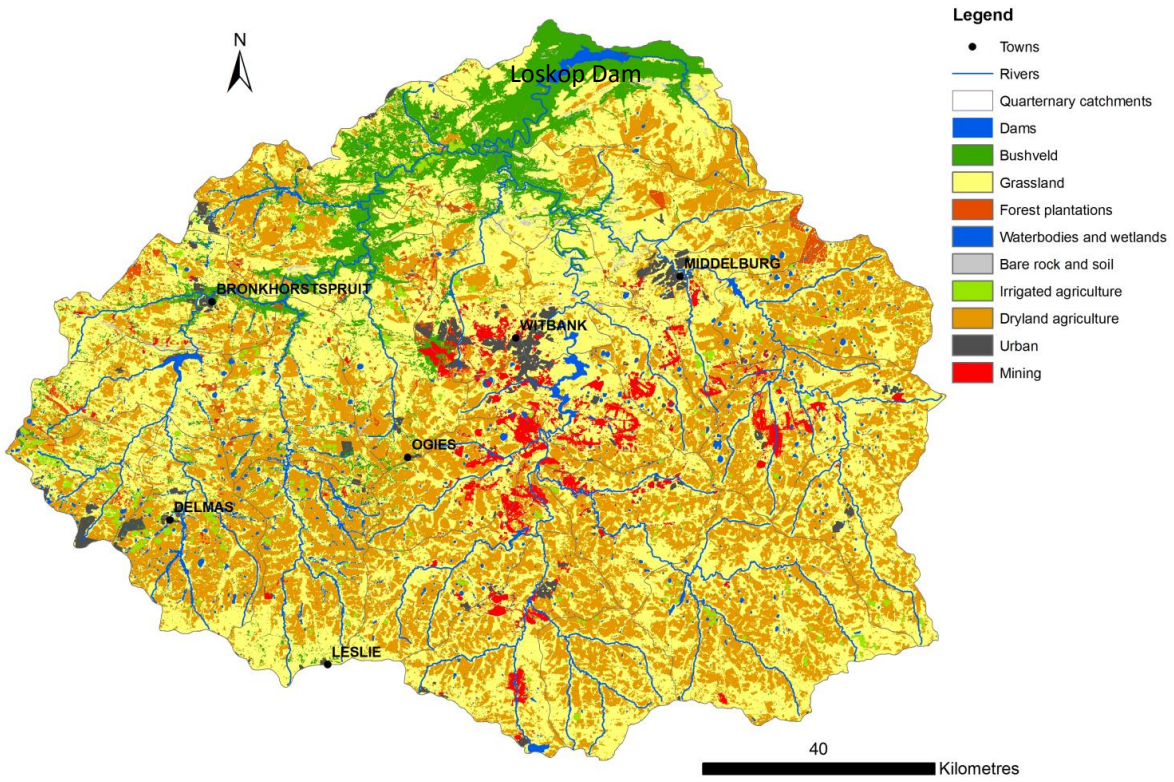

Figure 4. Map of the upper Olifants River catchment showing different land uses.

Coal mining is a dominant activity and the Witbank-Highveld coalfield produces 81% of the coal in South Africa thereby contributing significantly to the South African economy. Coal mining has been ongoing in the catchment since 1870 (Barker, 1999) and typically started using the bord-and-pillar method but has progressed to large opencast mines (Mey & Van Niekerk, 2009). Research has shown that the minerals in the coal and coal-bearing units from the Witbank coalfields do not contain sufficient neutralising constituents to buffer the acid produced through the oxidation of pyrite (FeS_2) associated with the coal seams (Pinetown *et al*., 2007). As a result, acid mine drainage is inevitable as pyrite is oxidised by exposure to air and moisture forming sulphuric acid (H_2SO_4). Decanting water from coal mines has very high sulphate levels with concentrations in some abandoned mine boreholes exceeding 2000 mg l^{-1} (Vermeulen & Usher, 2006). There are no water quality guidelines for dissolved sulphate levels in South Africa, however the Ministry of Environment in British Columnia (Canada) stipulates that dissolved sulphate should not exceed 100 mg l^{-1} in order to protect freshwater aquatic life (Ministry of Environment, 2011). The most important impact of acid mine drainage on aquatic ecosystems is the increased

solubility of metals from surrounding soil and rock which can result in metal toxicity to freshwater biota thus altering ecosystem structure and function (Kelly, 1988). Pollution associated with acid mine drainage has been observed and reported by several authors over many years in the Klipspruit tributary of the Olifants River located upstream of Loskop Dam (Gieskes, 1960; Botha *et al*., 1966; Engelbrecht, 1992; Heath & Claassen 1999) as well as by the current author in 2011 (Fig. 5), and yet little has been done to address sources of this pollution which are predominantly abandoned mines.

Figure 5. An aerial view of the Klipspruit where it joins the Olifants River showing precipitates associated with acid mine drainage.

The release of partially treated and un-treated sewage from various waste water treatment works (WWTW) in the upper catchment is another serious problem resulting in eutrophication of Loskop Dam through increased phosphate loads. This is not a new situation as the discharge of raw sewage from Naauwpoort, Riverview and Ferrobank WWTWs was previously reported by Driescher (2008). The 2011 results of the Department of Water Affairs Green Drop report reflect the poor condition of WWTWs particularly in the Delmas and Emalahleni municipalities (Table 1).

Table 1. Summary of scores from the Green Drop Report (DWA, 2011) for wastewater treatment works in the upper Olifants River catchment.

Wastewater Treatment Works	Municipality	Green Drop Score[1]
Middelburg/Boskrans/Mhluzi	Steve Tshwete	75.8
Kwazamokuhle / Hendrina	Steve Tshwete	82.4
Trichardt	Govan Mbeki	50.7
Kinross	Govan Mbeki	49.1
Kriel (Ga Nala)	Emalahleni	33.9
Rietspruit	Emalahleni	35.9
Ogies (Phola)	Emalahleni	24.9
Naauwpoort	Emalahleni	52.9
Ferrobank	Emalahleni	46
Riverview	Emalahleni	48.7
Klipspruit	Emalahleni	46.7
Botleng	Delmas	29.13
Delmas	Delmas	27.9
Godrich	Kungwini	31
Rethabiseng	Kungwini	37.4
Ekangala	Kungwini	24

[1] Colours indicate categories as follows: Green = Good status, improve where gaps identified to shift to 'excellent'; Black = Average performance, ample room for improvement; Yellow = Very poor performance, need targeted intervention towards gradual sustainable improvement; Red = Critical state, need urgent intervention for all aspects of the wastewater service business.

Phosphate is known to be the limiting nutrient in primary production by phytoplankton in lakes and dams (Wetzel, 1983) and increased phosphate levels in Loskop Dam have lead to intensified blooms of *Microcystis spp.* (Fig. 6) and *Ceratium hirundinella*. While the dysfunctional WWTW's in the upper catchment of the Olifants River are likely to represent a significant source of phosphates, the use of fertilisers in widespread agriculture in the catchment represents an alternative unquantified source.

Figure 6. Aerial view of the cyanobacterium *Microcystis* spp. bloom in the transitional zone of Loskop Dam taken in February 2011.

1.8 Aims of this study

The underlying causes of pansteatitis in *O. mossambicus* have not been determined and with the multitude of impacts affecting water quality in Loskop dam they are likely to be complex in nature involving multiple interactions. This research represents an initial approach towards unravelling the factors that may be affecting fish health and could be involved in development of pansteatitis in fish. Furthermore the effects of deteriorating water quality are possibly impacting the entire aquatic ecosystem at Loskop Dam and fish parasite communities are potentially an effective bio-indicator to confirm this. The study was undertaken in 2010 with the principal aims to i) measure key water quality parameters and describe important limnological aspects of Loskop Dam in relation to pansteatitis-affected fish; ii) measure concentrations of aluminium, copper, iron, manganese, selenium and zinc in various tissues of *O. mossambicus* to determine whether bio-accumulation was occurring and could be related to pansteatitis; iii) assess the metazoan parasite communities of *O. mossambicus* to determine whether they are effective indicators of ecosystem health in Loskop Dam.

Chapter 2

Water Chemistry and Limnology of Loskop Dam: Possible Links to Fish Mortalities

2.1 Introduction

Post-mortems of dead and dying Mozambique tilapia (*Oreochromis mossambicus*) from Loskop Dam have lead to the diagnosis of pansteatitis or yellow fat disease. Seasonal deaths of large individuals (>30 cm) of this species have been occurring in the transitional zone of the dam mostly during spring in the months of September and October. The same disease was implicated in crocodile and fish deaths in the Kruger National Park (Myburgh & Botha, 2009). The cause of the disease in fish from Loskop Dam is as yet unknown, although the disease is well described as being related to a diet high in polyunsaturated fats and insufficient antioxidants in animals bred in captivity (Niza *et al*., 2003). A thorough assessment of current water chemistry and limnology in the dam was an essential starting point in order to determine possible causes of pansteatitis in *O. mossambicus* which was the aim of this study.

Fish mortalities in Loskop Dam are a relatively recent occurrence so it is pertinent to compare current water quality data to published historical data where possible in order to determine significant changes. Water chemistry data has been collected from various permanent sites and over varying time periods in Loskop Dam since 1960 (Gieskes, 1960). In that study the author describes the impoundment as being slightly eutrophic, with high turbidity (secchi disk values < 1 m) that resulted in low light penetration. Reported depth distribution of dissolved oxygen collected bi-monthly over a time period of 12 months showed a lack of complete anaerobiasis during spring and summer months with levels around 4 mg l^{-1} at the time of dam turnover. Contributions of acidic water from the Klipspruit upstream of Loskop Dam were implicated in elevated SO_4^{2-} concentrations of ~ 30 mg l^{-1} in the dam. Subsequently Butty *et al*. (1980) reported physical, chemical and hydrological characteristics of the dam. In this study the SO_4^{2-} levels were consistently recorded above 25 mg l^{-1} and secchi disk readings were frequently below 50 cm. Algal blooms

of *Ceratium hirundinella* and *Microcystis aeruginosa* were observed with corresponding chlorophyll readings above 30 µg l^{-1}. The ratio of inorganic nitrogen: orthophosphate was 14:1 at the inflow indicated that algal growth was limited by phosphate. Depth distribution of dissolved oxygen and temperature showed distinct stratification with anoxic conditions in the hypolimnion.

Deteriorating water quality in Loskop Dam and the upper catchment of the Olifants River has recently prompted research into the major impacts and their relative effects. This was a direct result of the large fish kills and crocodile deaths observed in the dam in 2007 and further downstream in the Kruger National Park from ~ 2008 onwards. A concerning trend is the steady increase in SO_4^{2-} levels since the 1970's that was summarised by de Villiers and Mkwelo (2009). The authors found that routine samples collected by the Department of Water Affairs indicated a > 7-fold increase in SO_4^{2-} at the Loskop Dam wall until 2005 by which stage 18% of samples (N=403) exceeded 100 mg l^{-1}. Metal sulphides like pyrite are abundant in coal-rich lithologies and oxidation there-of results in the release of dissolved sulphate. Therefore it is quite likely that increasing levels of SO_4^{2-} in Loskop Dam are reflective of intensified coal mining in the upper Olifants River catchment (Pinetown *et al*., 2007). Further indications of the impacts of mining were observed in water quality data reported by Oberholster *et al*. (2010) from Jan – Jun 2008. At the inflow of the Olifants River to Loskop Dam the pH was low ranging from 5.9 - 6.8. The increased acidity was attributed to the formation of sulphuric acid through the oxidation of pyrite associated with coal mining. At this time levels of SO_4^{2-} ranged from 290 – 481 mg l^{-1} at the inflow site and from 109 – 181 mg l^{-1} at the dam wall site showing a clear gradient across the dam and a marked increase from previously reported values. In addition the concentrations of aluminium and iron peaked at the inflow measuring 1.56 mg l^{-1} and 1.2 mg l^{-1} respectively in January 2008 which far exceeds the 0.005 mg l^{-1} target water quality range for aluminium (DWAF, 1996). This is further evidence of the impact of mining during this period as the solubility of these metals increases with a decrease in pH resulting in their mobilisation from rock and soil. Increased eutrophication was evident as total phosphate levels at the inflow ranged from 183 – 711 µg l^{-1} which was substantially higher than the range reported at this site by Butty *et al*. (1980) of 6 - 51 µg l^{-1}.

2.2 Methods

2.2.1 Study Sites

Four permanent sites were selected at Loskop Dam (LK1, 2, 3, 4) as historical water chemistry records are available for comparison (Oberholster *et al*., 2010) and any physico-chemical gradients across the dam from the inflow to the dam wall could be detected (Fig.7). A reference site was selected at Kranspoort Dam (KP) located to the East of Loskop Dam on the Kranspoortspruit which also flows into Loskop Dam. The catchment of this dam has comparatively few impacts with no mining, industry or urbanisation and less intense agricultural activity. There have been no recorded fish kills in Kranspoort Dam and preliminary water quality tests indicated the water was relatively unimpacted by pollution.

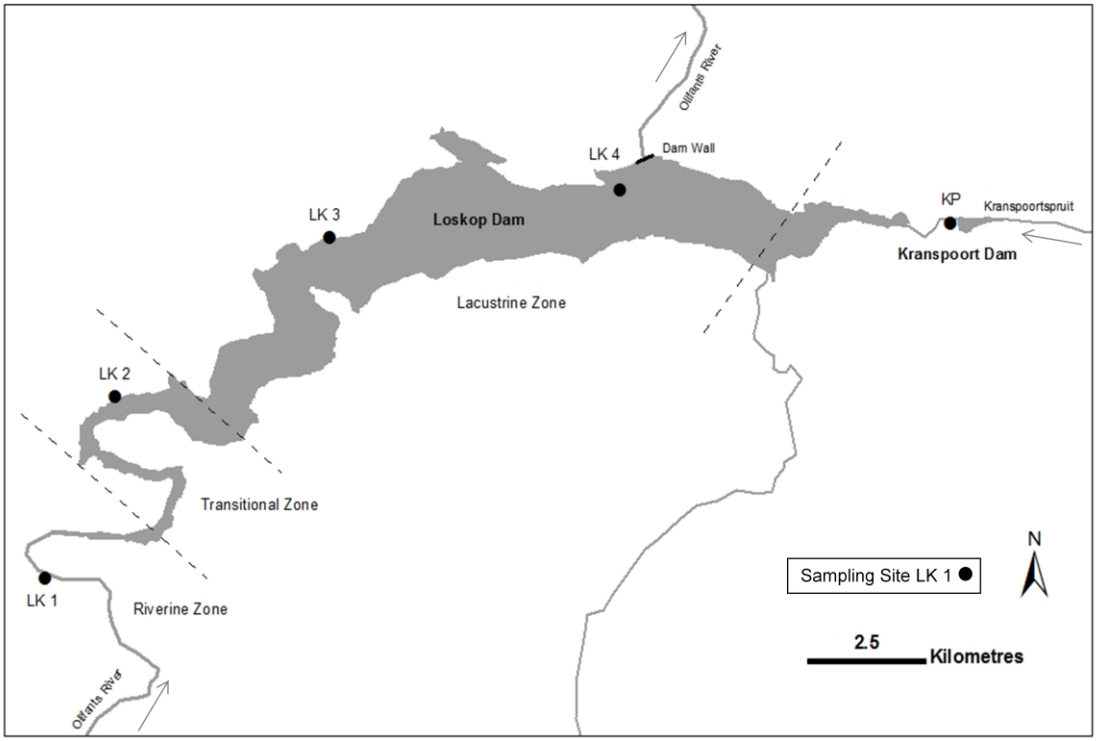

Figure 7. Map showing sampling sites at Loskop and Kranspoort Dams.

2.2.2 Water Chemistry

A one litre integrated surface (2 m depth) water sample was collected on a monthly basis from each site from July to December 2010 in order to cover low flow and high flow periods, as well as the period of periodic mortalities of *O. mossambicus* in October. Samples were kept at 4ºC until they were delivered to a commercial laboratory in Pretoria for analysis. Samples were analysed for 27 constituents including nutrients, major ions and metals using standard methods (APHA, 1992; USEPA, 1983). Unfiltered samples were analysed for total concentrations of Al, Fe, Mn, Zn, Cu, Se and V, as well as major cations using ICP-OES. Alkalinity, ammonia and chloride were determined titrimetrically. Concentrations of total phosphate, nitrite, nitrate and sulphate were determined using spectrophotometric and colorimetric methods while total Kjeldahl nitrogen was analysed titrimetrically. At each site the pH, dissolved oxygen, total dissolved solids, conductivity and temperature of the surface water were measured along with a secchi disk measurement. Measurements were made using a Hach HQ40D multiparameter meter using a pH gel intellical probe, luminescent dissolved oxygen intellical probe and a standard four pole graphite type electrical conductivity intellical probe (US EPA compliant).

2.2.3 Data Analysis

All raw values of the water quality constituents measured during the study period were included in the appendices (Appendix 1). Pertinent sections of the data have been presented in the results. Specific water quality variables were discussed comparing median values as these are insensitive to outliers along with ranges. With no *a priori* assumptions of sample relationships, a principal components analysis (PCA) was used to detect groupings in sites using the water quality data. The PCA was derived from a correlation matrix in order to standardise the variables because the units differ widely. The analysis was completed using Statistica Version 10 (StatSoft, Inc. Tulsa OK). Where possible, results were compared to target water quality ranges (TWQR) set out in the water quality guidelines for ecosystem health in South Africa (DWAF, 1996) for relevant variables. The molar ratios of SO_4^{2-} / Cl^-

were calculated to give an indication of an increase in sulphate relative to chloride which results from pyrite oxidation.

2.2.4 Diel Variation in Dissolved Oxygen and pH

Dissolved oxygen (DO) can fluctuate significantly on a diel basis in highly productive dams as a result of algae switching from photosynthesis during the day to cellular respiration at night (Wetzl, 1983). The result is a reduction in DO which is usually lowest a few hours after dawn. To investigate the magnitude of this reduction, hourly measurements of DO and pH were made at the water surface (1 m depth) over a 24 h period on 13 November 2010 during a *Microcystis* spp. bloom in the transitional zone near site LK2 of Loskop Dam. Measurements began at 13h30 and concluded at 12h30 on 14 November 2010.

2.2.5 Stratification

An important aspect of limnology is thermal stratification which occurs during spring and summer as surface waters absorb heat due to increased solar radiation. Colder water is more dense and therefore remains submerged beneath the warmer, less dense surface water. The lack of mixing during periods of thermal stratification result in de-oxygenation of deep water which is exacerbated by oxygen consuming bacteria as they break down organic matter (Wetzel, 1983). The anoxic conditions created by stratification have important consequences for redox reactions and have a major influence on water chemistry. There is a lack of current knowledge of the limnology of Loskop Dam. The last complete study on the dam was conducted by Butty *et al*. in 1980 in a review of the limnology of a number of South African impoundments. One site near the dam wall was used in that study to measure temperature and dissolved oxygen to a depth of 30 m. While an extensive limnological study was not the present research aim, a few important variables have been measured to aid interpretation of the current situation at the dam.

Measurements of temperature, pH and dissolved oxygen were made at 1 m intervals from the surface to a depth of 30 m at site LK4 in Loskop Dam. This was done on a monthly basis from September to December in order to coincide with previously

observed spring die-offs of *Oreochromis mossambicus.* Measurements were made using the same Hach multiparameter model 40d using 30 m rugged probes with the same specifications as those discussed in the water chemistry methods.

2.3 Results

2.3.1 Water Chemistry

The PCA plot of factor coordinates showed distinct separation of samples collected from Kranspoort Dam and Loskop Dam (Fig. 8). PC1 accounted for 29.46% variation with separation between samples collected from Kranspoort Dam (KP) and sites 2, 3 and 4 at Loskop Dam (L2, 3, 4). PC2 accounted for 17.42% variation with Kranspoort Dam separated from Loskop site 1.

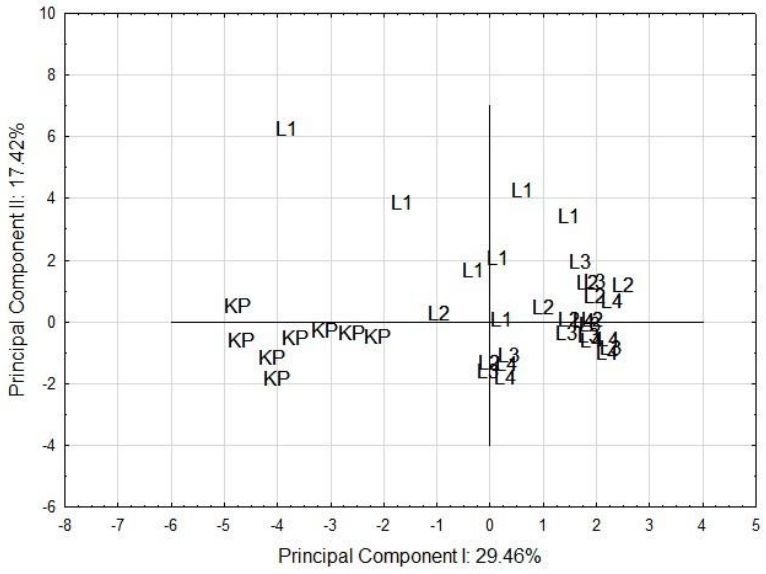

Figure 8. Principal components analysis (PCA) performed using selected water quality variables (Table 2) for 4 sites at Loskop Dam (L1, L2, L3 and L4) and Kranspoort Dam (KP) showing grouping of sites.

The first two component loadings (correlation coefficients) and variances (eigenvalues) have been calculated and presented in Table 2. The proportion of variance accounted for by each component is additive with the first two components accounting for 46.88% of the total variation. Important factor loadings in PCI show that Kranspoort Dam had comparatively high levels of total Al, Fe and Si to Loskop Dam, while sites 2, 3 and 4 at Loskop Dam had high pH, conductivity, secchi depth

and alkalinity as $CaCO_3$ compared to Kranspoort Dam. The 3 most important factor loadings in PCII were for boron, total inorganic nitrogen and manganese which were all high in site 1 at Loskop Dam. Boron is mainly used in the detergent industry as a bleaching agent and as a result is found in relatively high concentrations in domestic wastewater. Concentrations ranged from 0.002 to 0.074 mg l^{-1} at the inflow (LK1) during this study (Appendix 1).

Table 2. Factor loadings of the water quality variables in principal component I and II from the principal components analysis (PCA) in figure 8. Component loadings underlined are > 0.6 and are considered important in interpretation of results (Mahloch, 1974).

Variable	**PC I**	**PC II**
Conductivity (EC)	0.71	0.52
pH	0.76	-0.05
Dissolved O_2 (DO)	0.50	0.0
Temperature (TºC)	0.26	0.48
Secchi Disk (Secchi)	0.73	-0.3
Alkalinity	0.85	-0.12
Total inorganic nitrogen (TIN)	0.01	0.76
Orthophosphate (Ortho-P)	-0.22	0.5
Fluoride (F)	-0.04	-0.41
Silica (Si)	-0.85	-0.17
Aluminium (Al)	-0.78	0.36
Boron (B)	0.24	0.73
Iron (Fe)	-0.86	-0.03
Manganese (Mn)	-0.45	0.62
Zinc (Zn)	-0.07	0.09
Copper (Cu)	-0.11	0.33
Selenium (Se)	0.30	0.5
Vanadium (V)	0.28	0.2
Eigenvalue	**5.3**	**3.13**
Cumulative % Trace	**29.46**	**46.88**

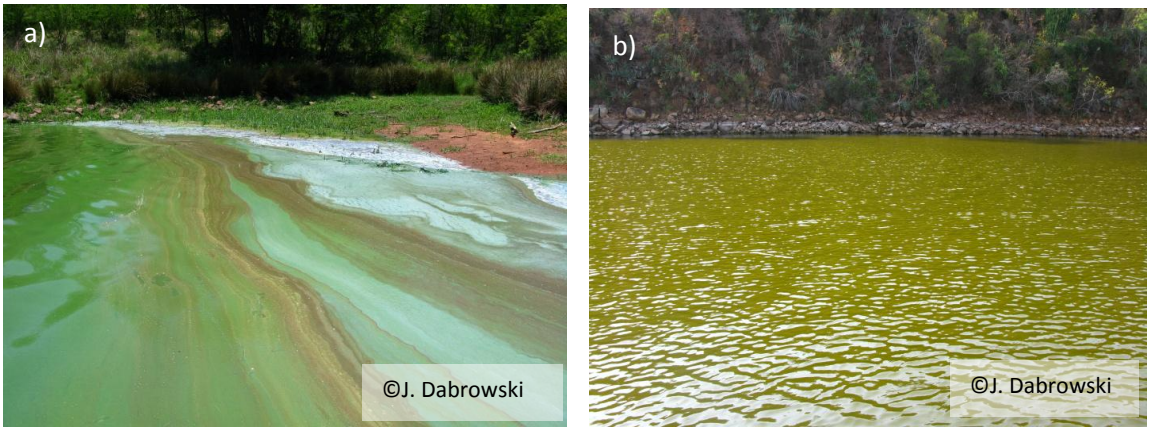

Figure 9. Photographs taken of blooms of *Microcystis* on 16-12-2010 (a) and *Ceratium hirundinella* on 8-10-2010 (b) in the transitional zone (LK2) at Loskop Dam.

During the study period the pH was highly variable across all sites in Loskop Dam. The pH at sites 2, 3 and 4 at Loskop Dam were frequently recorded above 9 (Fig. 10). This was due to the high levels of primary productivity observed at these sites as photosynthesizing algae remove carbonic acid from the water increasing the pH. As this pattern is dependent on algal productivity, it would also be influenced by diel fluctuations (Fig. 16). Site 2 in the transitional zone had the highest recorded pH levels with a median pH of 9.67 (Appendix 1) with frequent blooms of *Ceratium hirundinella* dominating from winter to spring and *Microcystis* spp. more prevalent in summer (Fig. 9). The calculated TWQR values were obtained using the median pH of 7.65 from Kranspoort Dam (*n*=14) and calculating 5% (0.38) of the pH unit to obtain the upper and lower values of 7.27 and 8.03 respectively. This calculation was used as per the TWQR guidelines because it was a more conservative range than the alternative of 0.5 pH units. Guidelines recommend that all values fall within the TWQR. The pH at the inflow (LK1) was in a more neutral range during this study (min. 7.5, max. 8.51) than the more acidic values observed by Oberholster *et al*. (2010) (min. 5.9, max. 6.8) in 2008 at the same site.

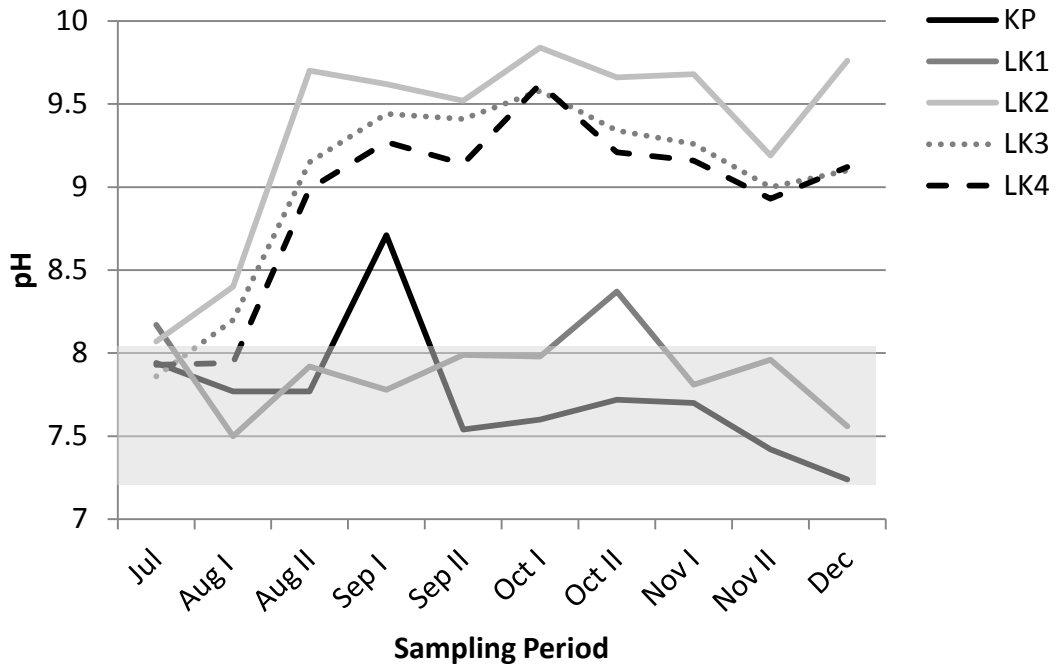

Figure 10. Time series showing pH values from all sites at Loskop and Kranspoort Dams from July to December 2010. Shaded area represents upper and lower limits of the target water quality range (TWQR).

The TWQR for oxygen saturation is between 80 and 120%. This range was applicable to the lowest concentration recorded over a 24-hour period or at just after dawn when dissolved oxygen concentrations in aquatic systems were normally at their lowest levels. The data presented in Figure 11 were collected during daylight hours and are therefore representative of conditions at this time only. Kranspoort Dam and the riverine zone (inflow, LK1) at Loskop Dam were consistently within the TWQR. Sites 2, 3 and 4 at Loskop Dam showed greater fluctuations with very high levels and variation recorded at LK2 (transitional zone) that regularly exceeded the TWQR. A spatial trend of decreasing concentrations from the transitional zone to the dam wall was observed. High pH and dissolved oxygen levels are indicative of active primary production in the surface water (DWAF, 1996). Based on these two parameters site LK2 in the transitional zone was the most productive.

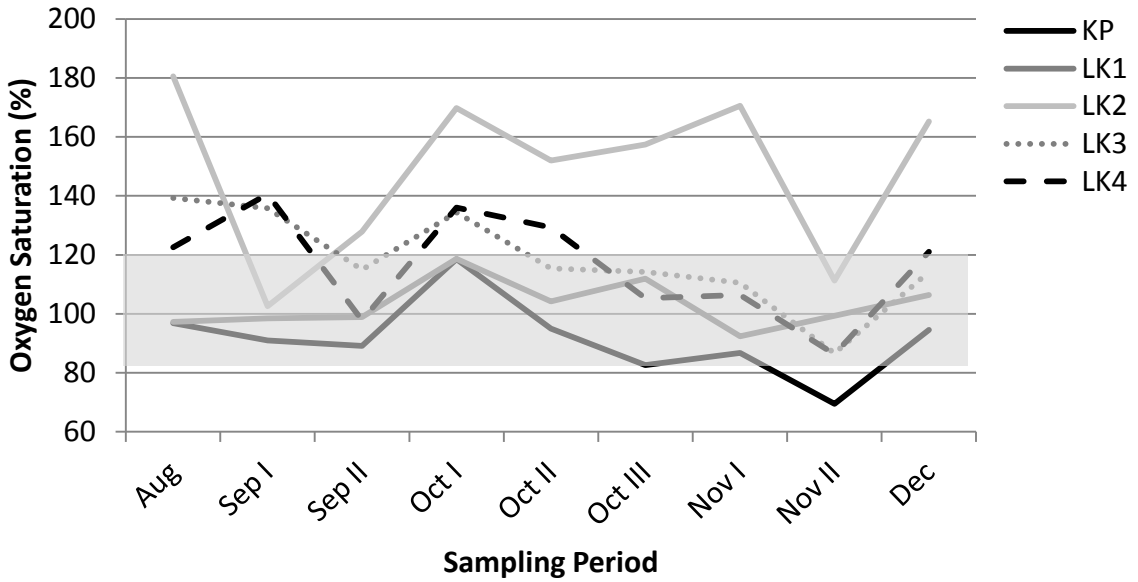

Figure 11. Time series showing oxygen saturation percentages from all sites at Loskop and Kranspoort Dams from August to December 2010. Shaded area represents upper and lower limits of the target water quality range (TWQR) (DWAF, 1996).

Phosphorus may occur in many inorganic and organic forms. As a result orthophosphate values were reported because it is immediately available for uptake by aquatic biota and exerts a strong influence on rates of primary production. The DWAF (1996) water quality guidelines for ecosystem health indicate orthophosphate ranges that are associated with specific symptoms and resulting trophic status of a water body. Oligotrophic conditions occur below 5 µg l^{-1}, mesotrophic conditions between 5 and 25 µg l^{-1} and eutrophic conditions between 25 and 250 µg l^{-1} (Fig. 12) (DWAF, 1996). The guidelines recommend that average summer values be evaluated but this was not possible given the time-frame of this study. The majority of values from both dams fell within the mesotrophic category under which nuisance growth of aquatic plants and cyanobacteria can occur (Fig. 12). The inflow (LK1), transitional (LK2) and lacustrine (LK3) sites at Loskop Dam had periods where the orthophosphate peaked well into the eutrophic category which is characterised by nuisance growth of aquatic plants and algae which may form toxic blooms. The highest peak was observed at sampling sites LK1 and LK3 in November and December 2010 and the concurrent lower concentration at site LK2 may have been

the result of the bloom of *Microcystis* spp. which began to develop at this site and presumably utilise the orthophosphate towards the end of October.

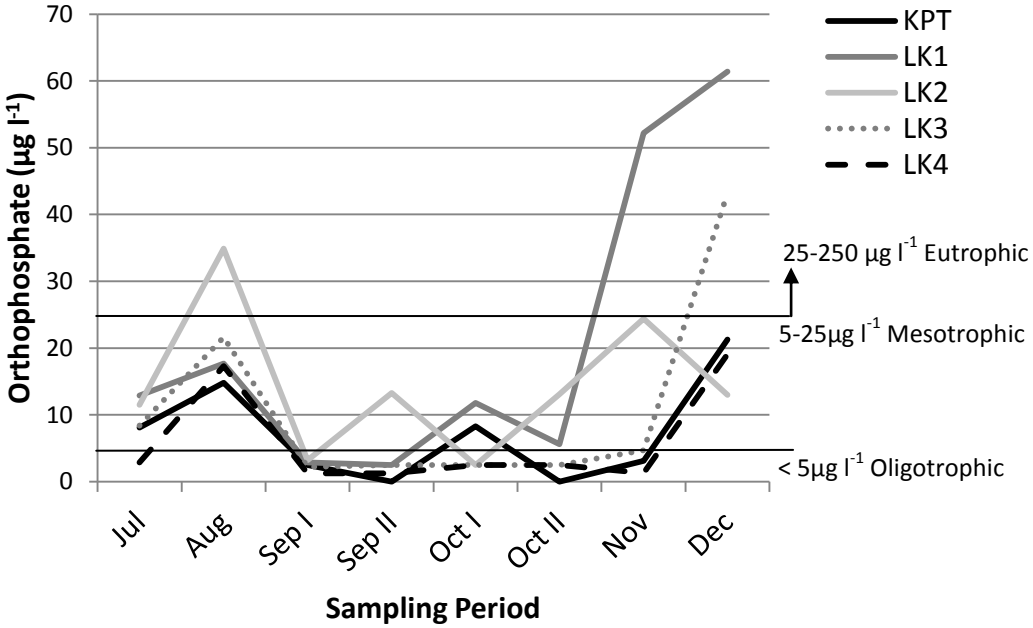

Figure 12. Time series showing orthophosphate from all sites at Loskop and Kranspoort Dams from July to December 2010. Trophic status categories determined by DWAF (1996) are indicated.

Dissolved inorganic nitrogen includes NH_3, NH_4^+, NO_2^- and NO_3^- all of which are available for uptake by aquatic plants and algae. As a result this is an important variable to consider when evaluating the trophic state of a water body. As in the case of orthophosphate, the DWAF guidelines have indicated categories of trophic status assigned to total inorganic nitrogen ranges. These are shown in figure 13. The results showed consistently high levels at the inflow of the Olifants River to Loskop Dam (LK1) indicating eutrophic conditions. These rapidly decrease from site 2 across the dam which was most likely to be reflective of utilisation by the algae during blooms in the transitional zone. The concentrations in Loskop Dam are a lot higher than the average nitrate concentrations of 200µg l^{-1} observed by Gieskes (1960).

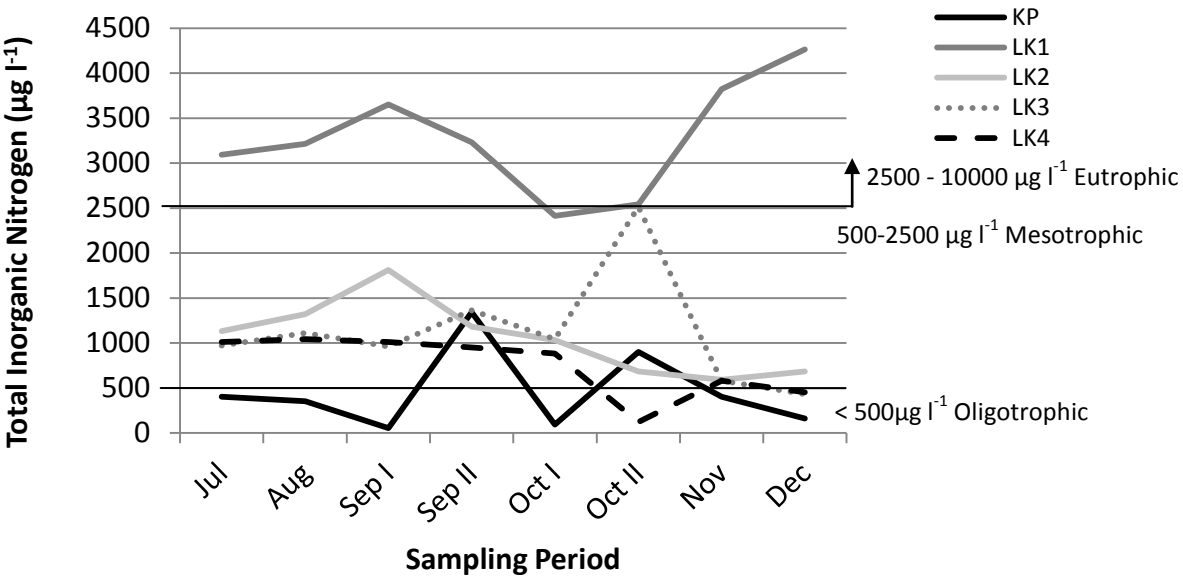

Figure 13. Time series showing total inorganic nitrogen from all sites at Loskop and Kranspoort Dams from July to December 2010. Trophic status categories determined by DWAF (1996) are indicated.

The ratio of total inorganic nitrogen to orthophosphate is often calculated as an indicator of eutrophication because growth of aquatic algae and plants in most unimpacted lakes is phosphate limited. Unimpacted systems have an N:P ratio of 25-40 and impacted systems have an N:P ratio of <10:1 (DWAF, 1996). However because the effluent present in the Olifants River and subsequently the inflow to Loskop Dam (LK1) is rich in both N and P, the ratio does not reflect enrichment with orthophosphate alone as total inorganic nitrogen is not held constant. The results observed in both figure 12 and 13 reflect enrichment with both phosphorus and nitrogen in Loskop Dam.

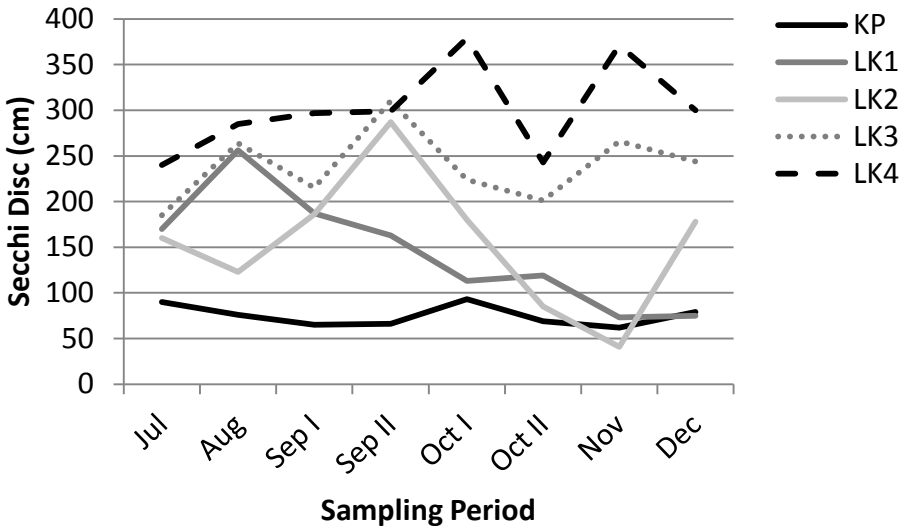

Figure 14. Time series showing secchi disk depth from all sites at Loskop and Kranspoort Dams from July to December 2010.

Secchi disk depths were consistently lowest in Kranspoort Dam which did not show much variation during the study period (Fig. 14). Transparency in the riverine zone (LK1) decreased steadily between September and December which can be attributed to an increase in suspended sediment resulting from the summer rains that commenced in October, the first flush. While this would also influence the decreased visibility observed in the transitional zone (LK2), the variation was a lot higher at this site with periods of low transparency linked to algal blooms of *Ceratium hirundinella* and *Microcystis* spp. The lowest value was observed at site LK2 while at the same time the highest values of orthophosphate and inorganic nitrogen were observed just upstream at the inflow (LK1) indicating a renewed supply of nutrients leading to subsequent algal bloom and reduced visibility. Secchi depth values followed a similar trend in the lacustrine zone and the dam wall with lower visibility at the former as a result of afore-mentioned algal blooms which were less prominent at this site than the transitional zone.

Total dissolved solids showed a decreasing trend from the inflow at Loskop Dam to the dam wall (Fig. 15). All of the major ions showed an increase in concentration relative to Kranspoort Dam. While variation in geology of the catchments of both dams may have an influence on TDS concentrations, the SO_4^{2-} concentrations observed in 1960 (Gieskes, 1960) that ranged from 20-35mg l^{-1} indicate that this ion

has increased substantially to the range observed at 97-138 mg l^{-1} at the same site during this study (Appendix 1).

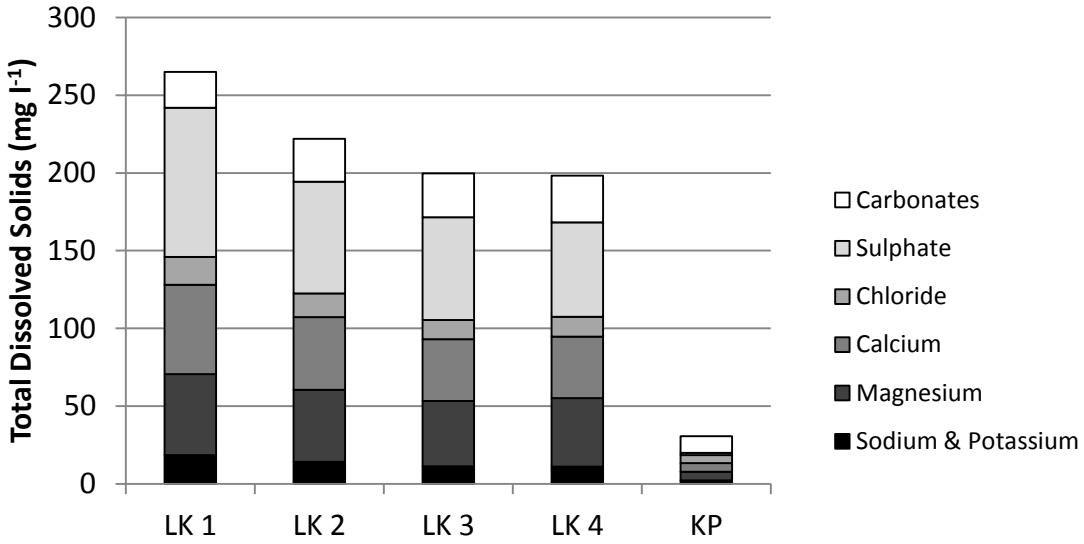

Figure 15. Bar graph showing median total dissolved solids (mg l^{-1}) and the relative composition of cations and anions in milliequivalents per litre at sites in Loskop and Kranspoort Dams.

The ratio of SO_4^{2-} to Cl^- was highly elevated at Loskop Dam ranging from a median of 4.7 at site LK2 to 6.18 at site LK1 which indicated an increase in this ion (Table 3). The ratio at Kranspoort Dam was very low by comparison to Loskop Dam sites at a median of 0.25 which confirmed that there was currently little to no impact of mining in its catchment.

Table 3. Comparison of molar ratios of SO_4^{2-}/Cl^- at sites in Loskop and Kranspoort Dams (*n*=8) calculated from July to December 2010.

	SO_4^{2-}/Cl^- Ratio		
Site	**Median**	**Minimum**	**Maximum**
LK 1 (Riverine zone)	6.18	4.34	6.56
LK 2 (Transitional zone)	4.7	3.73	7.84
LK 3 (Lacustrine zone)	5.27	4.43	5.45
LK 4 (Dam wall)	5.19	4.21	5.99
KP (Kranspoort Dam)	0.25	0.2	0.37

2.3.2 Diel Variation in Dissolved Oxygen and pH

During collection of diel measurements of pH and dissolved oxygen high cell counts of *Ceratium hirundinella* (5001-25000 cells l^{-1}) and an escalating bloom of *Microcystis* spp. (51-250 cells l^{-1}) (determined by Dr. P. Oberholster) were evidenced by the high midday pH of 10.1 and dissolved oxygen measuring 16.09 mg l^{-1} (Fig. 16). The O_2 saturation level at this time was also recorded at the exceptionally high level of 236%. These conditions are ideal for diurnal measurements as they represent a situation where there could be significant fluctuations in dissolved oxygen over a 24 h time period. The results showed a close relationship between the two measured variables. This is to be expected as pH values increase because algae reduce acidity as they absorb carbonic acid from the water in order to photosynthesize during the day (Wetzel, 1983). The lowest level of dissolved oxygen was recorded at 07:30 am measuring 10.28 mg l^{-1}. While this represents a substantial drop from the peak level at midday it is still more than adequate for the survival of fish.

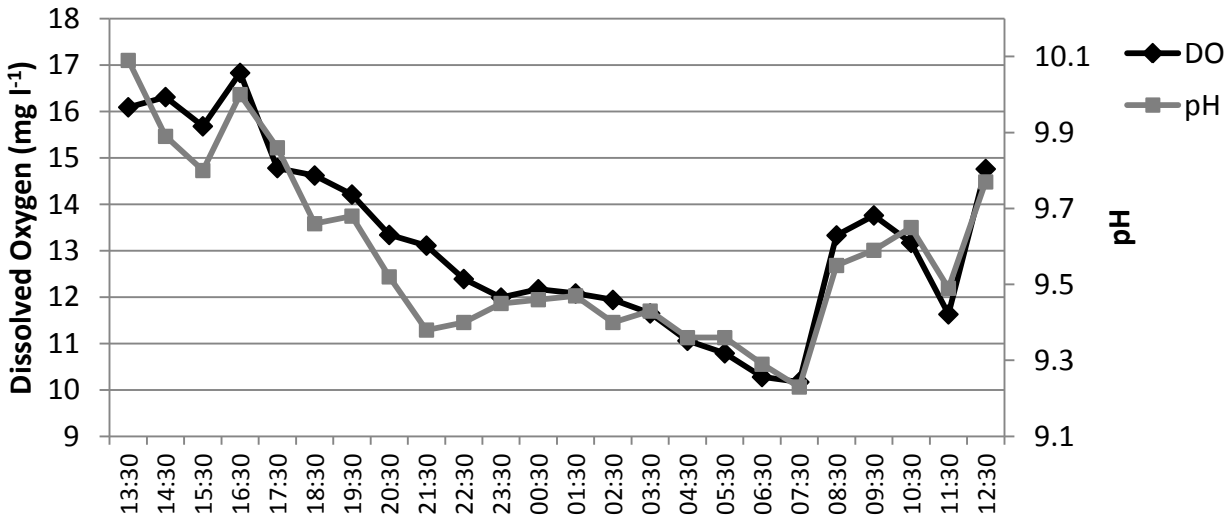

Figure 16. Dissolved oxygen and pH measured hourly over 24 hrs during November 2010 in the transitional zone of Loskop Dam.

2.3.3 Stratification

Depth measurements of DO and temperature were collected between September and December and are representative of the spring turnover period at Loskop Dam. The results followed a predictable pattern for deep lakes that is well described in the literature (Wetzel, 1983) (Fig. 17). Increasing temperatures with the onset of spring resulted in warming of the epilimnion with the development of thermal stratification. Temperatures in the hypolimnion only warmed by < 2ºC during the study period from September to December. The stratum of greatest thermal discontinuity (change >1ºC m^{1}), or thermocline, ranged from 6-9 m and temperature in the epilimnion steadily increased with the onset of summer from October. The depth of thermal gradient increased in November and December as heat conduction and turbulence in the epilimnion was carried into the metalimnion and hypolimnion.

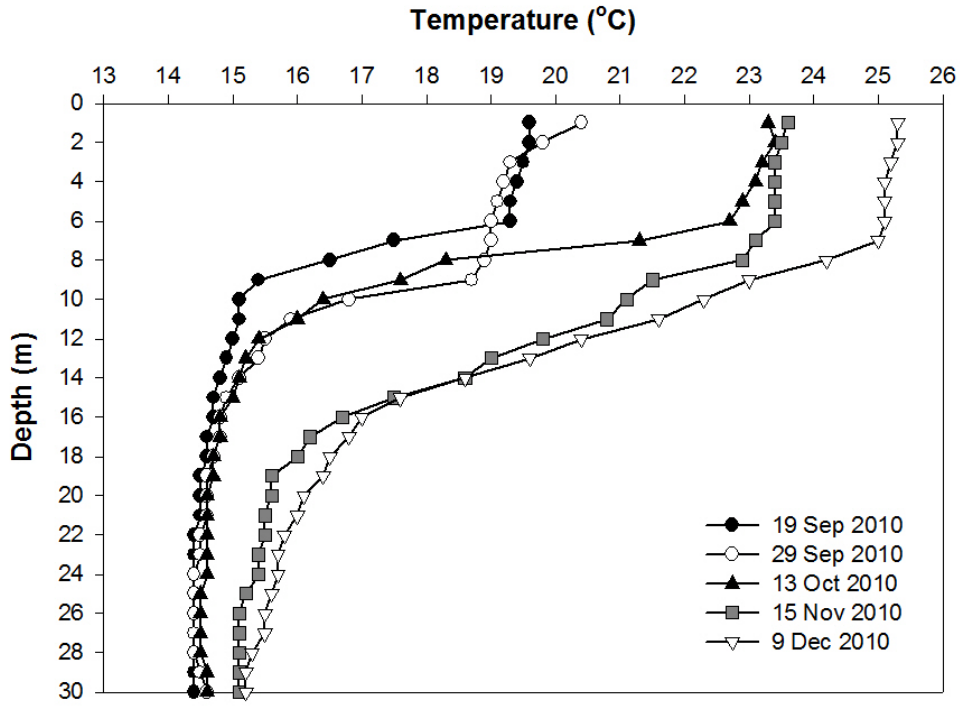

Figure 17. Temperature distribution from the surface to 30 metres depth in Loskop Dam measured at site LK4 near the dam wall between September and December 2010.

In productive lakes the oxygen content of the hypolimnion is depleted rapidly by oxidative processes during summer stratification resulting in a clinograde oxygen

profile (Wetzel, 1983) which was observed at Loskop Dam (Fig. 18). The depth of the oxycline was between 6 and 10 m during this period with progressively anoxic conditions below this depth from September 2010 onwards. The hypolimnion was completely anoxic from October to December. Concentrations of dissolved oxygen in the circulating epilimnion are affected by temperature and primary production with cooler temperatures and increasing primary production result in higher oxygen saturation (DWAF, 1996). The DO in the epilimnion fluctuated widely between September and December unlike the temperature which showed a steady increase over the same period. This suggests that fluctuations in DO were influenced more by algal growth than temperature in the epilimnion.

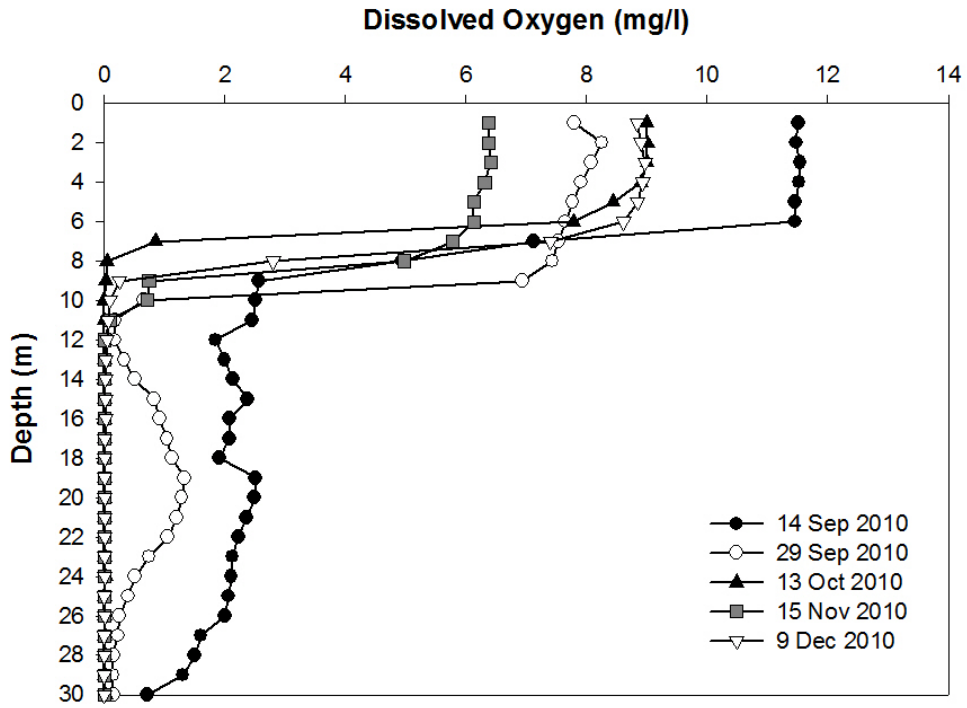

Figure 18. Dissolved oxygen distribution from the surface to 30 metres depth in Loskop Dam measured at site LK4 near the dam wall between September and December 2010.

2.4 Discussion

2.4.1 Water Chemistry

In 1980 Butty *et al.* concluded their report on the limnology of Loskop Dam by saying, "*At present the water quality is compatible with usage, but careful consideration should be given to future development in the catchment since the impoundment is in an extremely sensitive position with regards to pollution by sewage and industrial effluents.*"

The current evaluation of water quality in the dam suggests that eutrophication has increased to unacceptable levels. There are at least fifteen waste-water treatment works in the upper Olifants River catchment and the results of the most recent Green Drop Report found that none scored higher than 60% when audited (DWA, 2011). While the scoring method reflects more than just treatment systems, the results indicate that effluent from these plants is unlikely to have been treated to the phosphate standards for effluent discharge (1 mg l^{-1}). The separation of the inflow site (LK1) at Loskop Dam in the PCA was largely driven by total inorganic nitrogen and boron, both of which are associated with waste-water. Most fish are not very sensitive to boron and the acute LD 50 ranges from 14 to 3400 mg l^{-1} depending on the species (ECETOC, 1996). So while the current boron concentrations may not represent a toxicity threat, they serve as a signature of the presence of waste-water effluent (Vengosh *et al.*, 1999). The high concentrations of orthophosphate observed at this site are also likely to be a result of waste-water pollution. Algal blooms of *Ceratium hirundinella* and *Microcystis* spp. are frequent in the transitional zone thriving on large inputs of nutrients from the Olifants River. As the river enters Loskop Dam the flow reduces from that of a lotic to a lentic environment which is more suited to the growth of phytoplankton making conditions ideal for the development of blooms. If eutrophication continues unchecked at the current rate it is possible that summer blooms of *Microcystis* sp. will cover the entire dam affecting water quality for irrigation, presenting a health hazard for humans and wildlife and impacting on tourism to the dam. From an agricultural perspective this is very important because the Loskop irrigation scheme downstream from Loskop Dam is the second largest irrigation scheme in South Africa. Of concern to fish health in

Loskop Dam is that microcystin-LR toxin produced by *Microcystis aeruginosa* has been identified as a pro-oxidant and hepatotoxin. *Oreochromis niloticus* (Nile tilapia) that had cyanobacterial cells (toxin released) incorporated into their food were found to have elevated levels of lipid peroxidation and antioxidants particularly in their livers (Jos *et al*., 2005). One of the effects of pansteatitis is elevated lipid peroxidation which may be caused by *O. mossambicus* feeding on both live and decomposing cells from *Microcystis* sp. blooms in Loskop Dam. An outbreak of steatitis occurred in > 70 herons and egrets at an agricultural water reservoir in Japan where researchers observed high counts of *Microcystis aeruginosa* in the water, although no definitive links were made and levels of microcystin toxin in the livers of herons were not significantly elevated (Neagari *et al*., 2011).

Furthermore, the frequent algal blooms affect important aspects of water chemistry such as dissolved oxygen and pH resulting in a lack of stability of conditions. Changes in pH to acidic or alkaline extremes have important effects on several aspects of water chemistry relevant to fish health in Loskop Dam. Elevated pH causes an increase in the concentration of the toxic un-ionised form of ammonia (NH_3) relative to the less toxic ammonium ion (NH_4^+). At a pH of 9.5 and a temperature of 25ºC NH_3 will contribute 64% of total ammonia concentrations (DWAF, 1996). Ammonia levels infrequently exceeded the TWQR in the transitional zone of Loskop Dam and at Kranspoort Dam. However given the median pH of 7.65 and temperature of 23.2ºC in Kranspoort Dam, NH_3 would only contribute 1.7% to the total ammonia. While the contribution would increase to 64% in the transitional zone at Loskop Dam where the median pH was 9.67 and temperature was 23.9ºC during this study (Appendix 1). Acidic and alkaline pH values also influence the toxicity and speciation of metals like aluminium.

The sources of high total dissolved solids and SO_4^{2-}/Cl^- ratios in Loskop Dam are likely to be predominantly from coal mining in the upper catchment with smaller contributions possibly from acid precipitation. Acid mine drainage is characterised by water of a low pH, but this was not observed at the inflow to Loskop Dam during the current study period despite previous studies that have reported acidic water at the same site in 2009 (Oberholster *et al*., 2010). Reduced acidity may be due to dilution upstream of Loskop Dam or improved treatment of acid mine drainage prior to

release or decant by mines. It could also reflect seasonal differences as this study was completed from July to December, while the Oberholster *et al*., (2010) study ran from January to June. Release of mine water usually occurs during the high flow period to facilitate dilution so the effects may have been more pronounced later in the high flow period.

Elevated levels of total aluminium at LK1 in the Loskop and Kranspoort Dams may be due to inherent differences between the dams and between the high flow conditions at site LK1 compared to the reduced flow at other sites at Loskop Dam. Kranspoort Dam is shallow (<10 m at the deepest point) compared to Loskop Dam (>30 m at the dam wall) which would permit mixing by wind to continuously resuspend sediments in the water column. This was observed in the consistently low secchi depth observed at Kranspoort Dam. Elevated flow conditions at site LK1 would also carry higher levels of suspended sediment which would settle out of suspension as the dam changed from a lotic to a lentic environment. Aluminium is the third most abundant element on Earth and its chemistry is complex in aquatic environments (DWAF, 1996). Speciation and toxicity of Al is affected by pH. In the range of 6 to 8 Al is relatively insoluble and non-toxic. The solubility and toxicity of Al increases in more alkaline or acidic conditions. It forms inorganic complexes with F^- and SO_4^{2-} which are also dependent on pH (Gensermer & Playle, 1999). Organic complexes can also be formed with humic and fulvic acids that keep Al in solution but reduce its toxicity (Gensemer & Playle, 1999). Given the high pH values observed at sites 2, 3 and 4 in Loskop Dam during this study it is possible that the Al may have been present in a toxic form. This could have important consequences for *O. mossambicus* with pansteatitis as Al has been found to increase activity of xanthine oxidase and reduce activity of the antioxidant enzyme glutathione peroxidase in rats (Moumen *et al*., 2001). The pH values observed at Kranspoort Dam ranged from 6.94 and only exceeded 8 on two occasions which indicates that Al would be largely insoluble and unavailable for uptake by aquatic biota. The source of high levels of Al and Fe that caused separate grouping of the Kranspoort Dam samples in the PCA are unknown, but may be a result of organo-complexing with humic and fulvic acids in the dam (DWAF, 1996). Unfortunately dissolved organic carbon (DOC) was not measured in this study, however abundant aquatic macrophytes and riparian vegetation in Kranspoort Dam may have contributed to

higher levels of dissolved humic and fulvic acids which form strong organic complexes with Al and Fe and could increase their concentrations in the dam water.

2.4.2 Diel Variation in Dissolved Oxygen and pH

The range of dissolved oxygen (DO) in water is an important factor related to the survival of fish. Tilapia are fairly robust with regard to this variable and can withstand DO levels as low as 0.6 mg l^{-1} for brief periods of time as well as supersaturated conditions up to 400% that may result from photosynthesis during algal blooms (Morgan, 1972). *O. mossambicus* has also been found to be resilient to broad fluctuations in pH. They were able to survive with minimal changes to blood sodium levels at a pH of 3.5 in a study investigating the effects of acidification on their health (Yada & Ito, 1997). Tilapia can also tolerate high pH levels up to 11 although a range of 7-9 was found to be optimal for growth by Ross (2000). While the DO and pH levels observed during diel monitoring at Loskop Dam exceeded the TWQR (DWAF, 1996), they did not represent conditions where these factors alone would be sufficient to cause mortalities of *O. mossambicus*. However the results suggest the increased primary production resulting from ongoing eutrophication of the dam may cause diel variation in pH and DO to fluctuate excessively in the future.

2.4.3 Stratification

Loskop Dam is a monomictic water body characterised by summer stratification of DO and temperature and lake turnover in April at which point these variables remain uniform throughout the water column during winter (Butty *et al.,* 1980). The anaerobic conditions in the hypolimnion during summer stratification of Loskop Dam are primarily the result of biological oxidation of organic matter in the dam water and particularly at the sediment-water interface where oxygen consumption is most intense (Wetzel, 1983).

The increasing frequency and abundance of algal blooms at Loskop Dam is an important source of organic matter. This represents a significant change of conditions from those observed by Gieskes (1960) when DO profiles at the same site

collected over 1 year to a depth of 30 m showed a lack of complete anaerobiasis during summer stratification with DO levels at 4 mgl^{-1} at 30 m in December.

One of the observations made by fishermen and officials at Loskop Dam during the largest fish kill (>14 tons) in August 2007 was the distinct odour of rotten eggs and bubbles at the water surface in the main basin (pers. comm. J. Coetzee). The fish were predominantly of the species *Labeo rosae.* This was possibly the result of hydrogen sulphide (H_2S) gas bubbling to the water surface which in low concentrations is highly toxic to fish (Noga, 2000). H_2S is produced in stratified lakes with high primary productivity (Wetzel, 1983). It is produced by sulphur-reducing bacteria (SRB) that use sulphate to oxidise and decompose organic matter in anaerobic environments. Unfortunately the DO and temperature stratification data from this study do not include August, but the September results indicate that the dam was shifting from isothermal and uniform DO and temperature distribution throughout the water column to increasing stratification which was also observed by Butty *et al*. (1980). The production of H_2S can be exacerbated by several factors present in Loskop Dam. The amount of organic matter and availability of sulphate strongly influence the decomposition of organic matter through sulphate reduction (Holmer & Storkholm, 2001). Both factors are present in abundance and are showing an increasing trend with high SO_4^{2-} and algal blooms providing a large source of organic matter once they die off. Near the sediments the H_2S reacts with Fe^{+2} ions to form insoluble FeS which results in a dark brown to black colour of bottom sediments (Holmer & Storkholm, 2001). The toxicity of H_2S is affected by pH. At values below 6 nearly all reduced sulphur is present as toxic H_2S, while above 6 it is mainly HS^- (Gerardi, 2006). The main toxic action of H_2S appears to be respiratory interference causing hypoxia and associated purple-violet gills. Recommended diagnosis of H_2S poisoning in fish is water testing where levels should be < 0.002 mg l^{-1} (Noga, 2001) but unfortunately the water was not tested for H_2S during the fish kill in 2007 and was not tested during this study. Based on the above-mentioned observations, it seems possible that the fish kill was related to H_2S poisoning. Bioturbation by a large shoal of fish feeding in bottom sediments thus releasing H_2S or an influx of acidic water with a pH < 6 may have resulted in increased toxicity of H_2S. This explanation does not suffice for the ongoing annual deaths of large steatitis-affected *O. mossambicus* in the transitional zone. These incidents are isolated.

2.4.4 Conclusions

The ultimate cause of fish mortalities in Loskop Dam appear to involve several factors that range in severity over time and space. During the current study the largest impact on water quality in Loskop Dam was eutrophication and associated algal blooms. The impact of acid mine drainage was far more dominant during the Oberholster *et al.* (2010) study in 2009. The mass mortalities of fish observed ~ 2007 may have been the result of H_2S poisoning or exposure to toxic metal concentrations related to an influx of acidic water exacerbated by low flow conditions. The causes of pansteatitis in fish may be related to high levels of Al or Fe, feeding on *Microcystis* spp. or as yet unexplored avenues like endocrine disruptors that may be present in agricultural or waste-water effluent. It is likely to be a combination of factors as opposed to a single one.

Chapter 3

Trace metal concentrations in the tissues of Mozambique tilapia (*Oreochromis mossambicus*) in Loskop Dam

3.1 Introduction

Intensive coal mining in the upper catchment of the Olifants River has resulted in the release of acid mine drainage from both working and abandoned mines. Associated increases of waterborne metals are of concern as they may be absorbed in excessive quantities by aquatic biota like fish subsequently affecting their survival and reproduction. In a study conducted by Oberholster *et al.* (2010), high levels of aluminium (Al), iron (Fe) and manganese (Mn) were reported in several water samples collected from Loskop Dam, and previous studies have found evidence of Cu and Zn bioaccumulation in tissues of *Oreochromis mossambicus* from Loskop Dam (Kotze *et al*., 1999). The repeated pattern of seasonal die-offs of *O. mossambicus* during September and October involves large (> 40 cm total length) predominantly male fish in the transitional zone of the dam. Mortalities collected during this study and by veterinarians from the Veterinary Faculty at Onderstepoort have found that fish showed macroscopic symptoms of pansteatitis which included large accumulations of abdominal fat with concentrations of ceroid pigment in fat deposits throughout the body which indicate high levels of lipid peroxidation. While it cannot be confirmed that pansteatitis was the final cause of death, the consistent display of symptoms among mortalities makes a strong case for the role of the disease in the deterioration of health and final demise of these fish. Fish primarily store polyunsaturated fat. This is prone to attack by free radicals forming lipid peroxides, which through further oxygen reduction reactions with metals produce malondialdehyde which is both mutagenic and carcinogenic (Valko *et al*., 2005). Oxidative injury may be caused by excessive levels of Cu and Fe under redox-cycling reactions (Valko *et al*., 2005) and the effects of the latter may be further enhanced in synergy with Al (Xie & Yokel, 1996). Thus obesity in pansteatitis-affected *O. mossambicus* in the presence of high dietary or aqueous metal concentrations may help to explain causative mechanisms of the disease.

Like all animals, fish require minerals for normal life processes. They can absorb minerals like calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) from the aquatic environment as well as from dietary sources to satisfy their nutritional requirements (NRC, 1993). The surface of fish gills contain specialised ion-transporting chloride cells and have a net negative charge which results in a high affinity for cationic metals, thus an increase in waterborne metals may interfere with normal gill function. Metal cycling in aquatic systems generally involves phase changes between soluble and insoluble forms which have a major influence on the bio-availability of metals and may result in extreme problems of metal toxicity or deficiency. The speciation of metals such as aluminium (Al) are strongly influenced by pH. The insoluble form of Al, gibbsite, is relatively stable at pH 6 to 8 with solubility and bioavailability increasing under more acidic and alkaline conditions (Gensemer & Playle, 1999). Considering the highly alkaline conditions (median 9.67) in the transitional zone of Loskop Dam discussed in the previous chapter of this thesis, Al would be largely soluble in the form of $Al(OH)_4$ and available for uptake by aquatic biota (Gensemer & Playle, 1999). Adequate mineral concentrations are maintained by homeostatic mechanisms within the fish as they compensate for dietary and environmental fluctuations in intake and availability. Metals absorbed through the gills or across the intestinal wall are distributed via circulation bound to transport proteins where they may be utilised for essential life functions or be detoxified by binding to the protein metallothionein (MT) (Olssen *et al.,* 1998). Toxicity may develop if excessive levels of an element are ingested or absorbed and assimilated so a continuous balance between uptake, storage and excretion must be maintained (Watanabe *et al*., 1997).

Fish make good subjects for active biomonitoring of water quality as their tissues reflect an integrated response to a combination of contaminants. Nile tilapia (*Oreochromis niloticus*) have previously been used for active biomonitoring in cages installed in the Nakivubo Wetland, Uganda where they showed an increase in certain metal concentrations after an exposure period of 6 weeks (Birungi *et al.*, 2007). This practice involves selecting fish from an unpolluted location, translocating them into a polluted one and measuring the response over a period of time ranging usually from 4-6 weeks. Passive biomonitoring by contrast involves the collection of fish from

locations where a natural population exists (Wepener, 2008). Both methods were used in this study.

The overall purpose of the study of fish health in Loskop Dam is to determine the factors that may be responsible for causing pansteatitis in *O. mossambicus*. Information on the underlying causes of the disease in fish are critical if we are to determine their role in the same condition observed in crocodiles in both Loskop Dam and Kruger National Park where populations of these animals are in decline as a result of pansteatitis (Botha *et al*., 2010; Ferreira & Pienaar, 2011). Based on the extensive coal-mining that occurs in the upper catchment of the Olifants River it is possible that metals released with acid mine drainage may be impacting on fish health. Two broad questions about the two major symptoms of pansteatitis in tilapia need to be addressed: (i) How do the fish become obese? (ii) What causes the peroxidation of this excess fat? Exposure to elevated metals through dietary or waterborne sources may result in primary or secondary effects on fish health that help in providing answers to both of these questions. The objectives of this study were to measure concentrations of aluminium, copper, iron, manganese, selenium and zinc in various tissues of *O. mossambicus* to determine whether bio-accumulation was occurring and could be related to pansteatitis.

3.2 Methods

3.2.1 Study Sites

Fish were collected from three sites for the purpose of this study. The first site was Loskop Dam and the reference site was Kranspoort Dam located to the East of Loskop on the Kranspoortspruit (Fig. 7, Chapter 2). The third site was the aquaculture facility at Tompi Seleka agricultural college located adjacent to Flag Boshielo Dam, downstream from Loskop Dam. This site was used to source fish for the biomonitoring cages. The Lolamonthle Dam is the water source for the aquaculture dams and the water quality is good with no recorded fish kills (pers. comm. Andre Hoffman). *Oreochromis mossambicus* stocked in the dams were originally collected from Flag Boshielo Dam and have continued to breed at the

facility. They were fed commercial fish pellets containing 38% protein. Tompi Seleka was considered a reference site for fish in the biomonitoring cages.

3.2.2 Active Biomonitoring

One cage was installed at Kranspoort Dam and three cages were installed in Loskop Dam for the purpose of active biomonitoring using *O. mossambicus*. The cages in Loskop Dam were placed at water quality monitoring sites LK1, LK2 and LK3 (Fig. 7, Chapter 2) where most die-offs of *O. mossambicus* have occurred and where regular blooms of *Microcystis* sp. and *Ceratium hirundinella* have been reported in previous studies (Driescher, 2008; Oberholster *et al.*, 2010). Cage construction was designed with several factors in mind. Firstly, fish required protection from predation and interference from animals like hippos, crocodiles and otters. For this reason a square-shaped outer cage constructed from galvanised welded mesh measuring 2.4 m high by 1.2 m wide with a lid was embedded into the sediment (Fig. 19 a & b). Secondly, the fish needed to be contained in an easily accessible semi-collapsible cage which posed no potential injury to them. We used plastic garden mesh measuring 25 x 25 mm in a circle with a diameter of 1 m and height of 2 m. This cage was placed inside the steel cage with the water level within 0.5 m from the top of it. Thus the fish were in approximately 2 m water depth and had access to both the sediment and water column for feeding purposes. No additional food was provided. The cages were installed in mid-August 2010 and were left for four weeks prior to the introduction of fish so the area could settle.

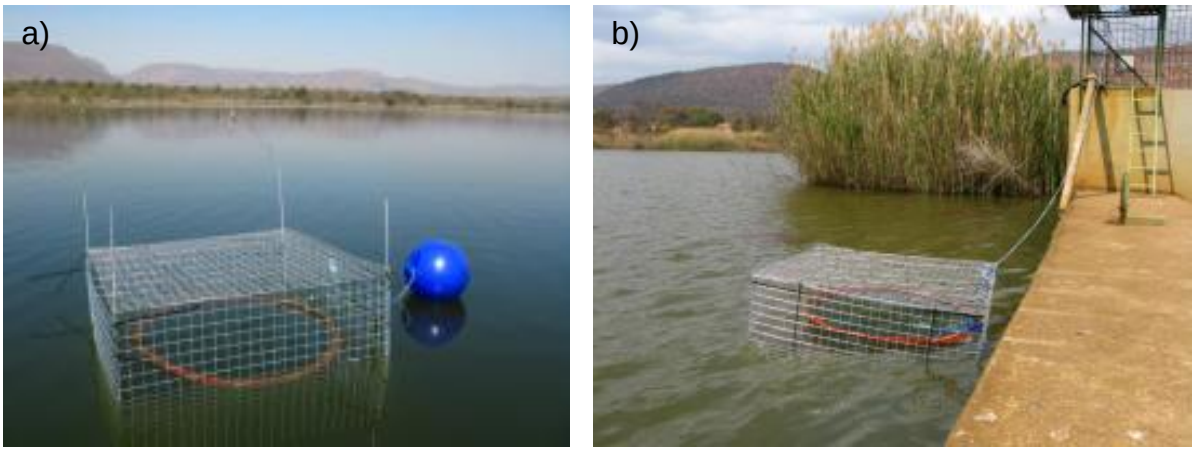

Figure 19. Biomonitoring cages located at Loskop Dam (a) and Kranspoort Dam (b), installed in August 2010.

Fish were collected in mid-September 2010 from Tompi Seleka and 21 of these fish were placed into each cage at Kranspoort Dam and Loskop Dam. Fish were selected in the size class 20-25 cm (total length) and an approximately even sex ratio. Any mortalities within 48 hrs of introduction were attributed to transport stress, and thereafter were deemed to be from other causes. The duration of exposure in the cages was set for a period of six weeks and the fish were removed at the end of October 2010.

3.2.3 Passive Biomonitoring & Mortalities

Oreochromis mossambicus were sampled during September and October 2010 using 20 m long 110 mm stretched mesh gill nets in the transitional zone at Loskop Dam and in Kranspoort Dam. Fish were collected in September 2010 from the aquaculture dams at Tompi Seleka to give an indication of their pre-condition prior to their placement into cages for active biomonitoring. Efforts were made to collect freshly dead or dying *O. mossambicus* during the study period but were restricted by the level of decomposition. Dead fish whose gills were still red were declared freshly dead and were collected along with fish that were dying and visibly struggling at the water surface.

3.2.4 Tissue Metal Concentrations

All fish were weighed and measured to determine their condition factor (Carlander, 1969) using the formula: $K = W \times 100 / L^3$. *K* is the condition factor, W is the weight in grams and L is the total length in cm. Prior to dissection while fish were alive a sample of blood was drawn from the caudal vein in order to determine the packed cell volume (PCV). After centrifugation of whole blood, 0.5 ml of serum was frozen for analysis of sodium concentrations at the Clinical Pathology lab at Onderstepoort. Fish were then killed by severing the spinal cord.

Samples of the gills, liver, brain and muscle were dissected from each fish and immediately frozen. Samples of bone were also collected from all fish except those from Tompi Seleka. Concentrations of Al, Cu, Fe, Mn and Zn were measured in all tissues and Se was measured in liver tissue where adequate samples were

available. Tissue samples were digested following the methods of Jones and Laslett (1994). Once samples were freeze-dried and homogenised, 1 g was microwave digested in nitric acid and hydrogen peroxide (2:1 ratio). Trace metals were detected using inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the CSIR Analytical Laboratory Services in Stellenbosch. Concentrations were reported in mg kg^{-1} dry mass.

3.2.5 Statistical Analyses

Differences in tissue metal concentrations were compared per metal using a multifactor ANOVA with site and tissue as the two factors. A post-hoc Tukey test was used to determine differences between group means and these results are presented in the text. Assumptions were checked and data were log-transformed for analyses to meet the assumption of homogeneity of variance Quinn & Keogh (2002). All analyses were completed using Statistica Version 10 (StatSoft, Inc. Tulsa OK).

3.3 Results

The three replicate cages in Loskop Dam were reduced to a single cage at site LK2 in the transitional zone due to unforeseen events. The cage at site LK1 was vandalised and the fish were eaten, and the cage at site LK3 was blown over in a strong wind and the fish escaped. While replicates had been planned to avoid pseudoreplication from a statistical perspective, these events were unavoidable and the results from the remaining cage still deemed worthy of reporting.

3.3.1 Fish Gender and Size Differences

Results presented in Table 4 include fish sampled from Tompi Seleka and the cages placed in both Loskop and Kranspoort Dam, fish sampled from the natural population in both dams and natural mortalities that were collected during October 2010 in Loskop Dam. Both size and gender can influence patterns of tissue metal concentrations. For this reason attempts were made to ensure an even sex ratio and a similar size range were obtained from each location. However this was not always possible. The fish from Tompi Seleka (and therefore in the cages) were smaller than

those from Loskop Dam (Table 4). Cages were initially stocked with an approximately even sex ratio of 20 fish which after several mortalities were dominated by males at the conclusion of the 6 week active biomonitoring period. Approximately even ratios of males: females were obtained for fish from Tompi Seleka, Kranspoort Dam and Loskop Dam (1.5:1, 1.3:1 and 0.88:1 respectively). Overall the samples collected were dominated by males with a ratio to females of 2.1:1. The skewed sex ratio makes the influence of sex on metal accumulation difficult and in most cases impossible to measure. The fish from Loskop Dam were larger than those from other sites in every aspect (weight, total length, condition factor) (Table 4). Fish from Tompi Seleka, the cages, and Kranspoort Dam all had similar condition factors. As it was not possible to collect a full range of sizes from all locations, size was excluded as a factor in data analysis. A total of four Loskop Dam mortalities were collected during the study and tissue samples were collected where possible but the small sample size restricted extensive statistical analyses.

Table 4. Sample size, sex and mean (± SE) size parameters of *Oreochromis mossambicus* collected from all sites during 2010.

Location (code)	Sample Size	Males	Females	Condition Factor (*K*)	Weight (g)	Total Length (cm)
Tompi Seleka	20	12	8	1.7 (0.03)	243 (17)	24 (0.6)
Kranspoort Cage	11	10	1	1.6 (0.04)	390 (26)	29 (0.85)
Loskop Cage	9	9	0	1.7 (0.02)	427 (35)	29 (0.96)
Kranspoort Dam	7	4	3	1.6 (0.01)	680 (180)	33 (4.32)
Loskop Dam	24	10	14	2.1 (0.04)	1499 (87)	41 (0.85)
Loskop Mortalities	4	3	1	2.2 (0.08)	1909 (103)	45 (0.76)
Total	**75**	**58**	**27**			

3.3.2 Blood parameters

There was no significant difference in the packed cell volume (PCV, %) of fish from any of the sampling locations (Fig. 20). The reference PCV range for tilapia is from 27 – 37 (Hrubec *et al*., 2000) and all of the results in this study were within that range.

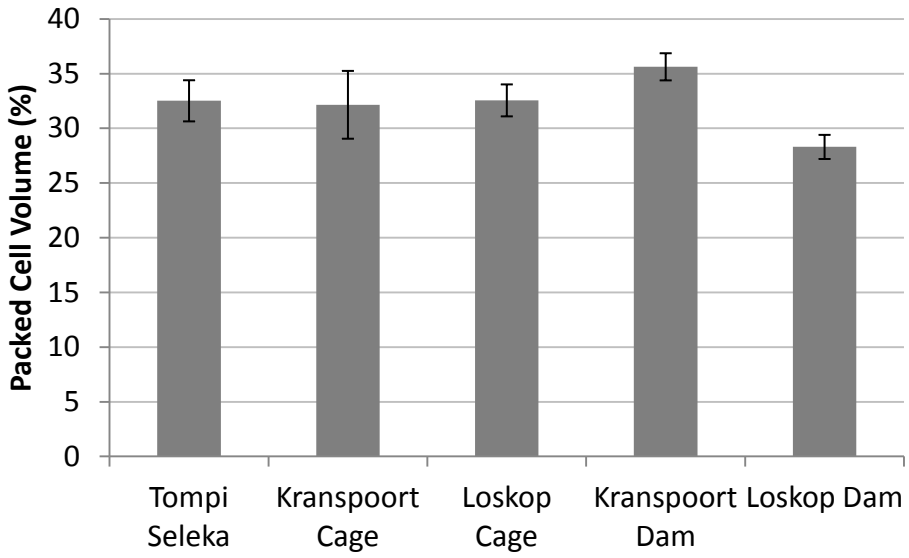

Figure 20. Mean (± SE) packed cell volume (%) values for fish collected during 2010 from each sampling location.

A single factor ANOVA with a Tukey post-hoc test was used to compare blood sodium levels between fish from different sites with mean values presented in figure 21. Blood sodium levels in fish from Tompi Seleka were significantly lower than the fish placed in the Kranspoort cage ($P < 0.001$) and the Loskop cage ($P < 0.05$). The fish in Loskop Dam also had significantly lower levels than fish in the Kranspoort cage ($P < 0.01$) but did not differ significantly to Kranspoort Dam.

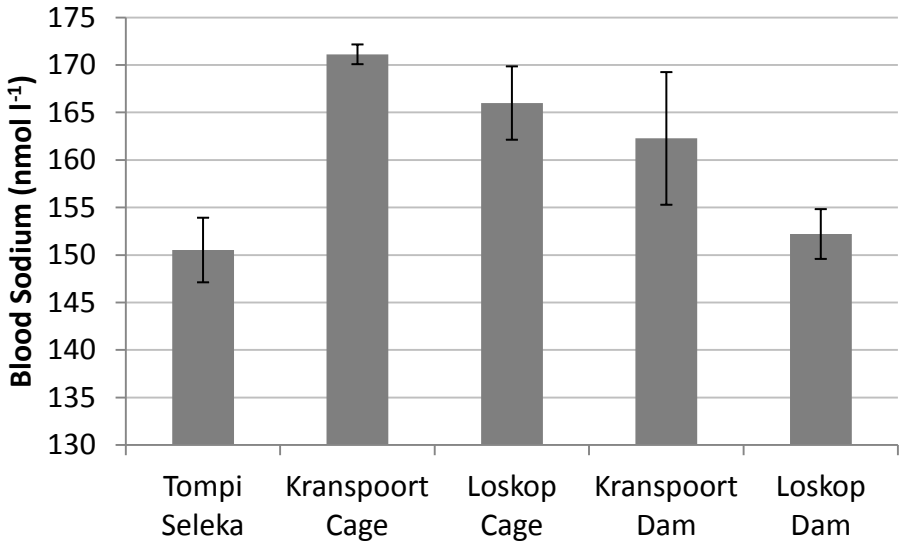

Figure 21. Mean (± SE) plasma sodium levels for *O. mossambicus* collected from each sampling location during 2010.

3.3.3 Tissue Metal Concentrations

A total of 21 *O. mossambicus* were put into each of the biomonitoring cages. Both cages had ≥ 50% mortalities during the exposure period and after 6 weeks there were 9 fish left in the Loskop cage and 11 fish in the Kranspoort Cage of which the majority were males. This confirms that poor water quality was not responsible as deaths occurred in both locations. The cause of death of fish lost from the cages was unknown, but the stress of transport, captivity and adjustment to the new environment would have played a role. Therefore the results obtained from fish that remained alive in this study may have been impacted by factors other than exposure to various compounds through the water, sediment and diet.

Interpretation of the results of the natural mortalities collected from Loskop Dam should be considered with several factors in mind. Firstly the sample size (n=4) was small thus limiting the power of statistical comparisons. Secondly during death there may be extreme physiological responses to stressors which cause biochemical changes that are difficult to interpret. However these changes can also help elucidate what the stressor(s) involved was at the time of death and thus collection of samples was deemed to be useful.

Accumulation of Al for fish from all sites followed a similar decreasing pattern in the order: gills>liver>brain>bone>muscle and values are presented in figure 22. Concentrations of Al in the gills of fish from Tompi Seleka were significantly lower than fish in the Kranspoort cage (P < 0.001), Loskop cage (P < 0.05) and Loskop Dam (P < 0.05). The gills from only two natural mortalities were analysed for Al concentrations so they were omitted from statistical analyses, but the levels were highest in the gills of these fish at 94.4 and 54.9 mg kg^{-1} respectively. Muscle concentrations of Al in fish in the Kranspoort cage were significantly higher than fish in the Loskop cage (P <0.001), Loskop Dam (P < 0.001) and Kranspoort Dam (P < 0.05). Significantly higher concentrations of Al in livers were observed in fish from the Kranspoort cage (P < 0.05) and Loskop Dam (P < 0.001) than those from Tompi Seleka. Levels of Al in the bone of fish in the Kranspoort cage were significantly higher than fish in Loskop Dam (P < 0.001). There were no significant differences between the brain Al concentrations of fish from any of the sites.

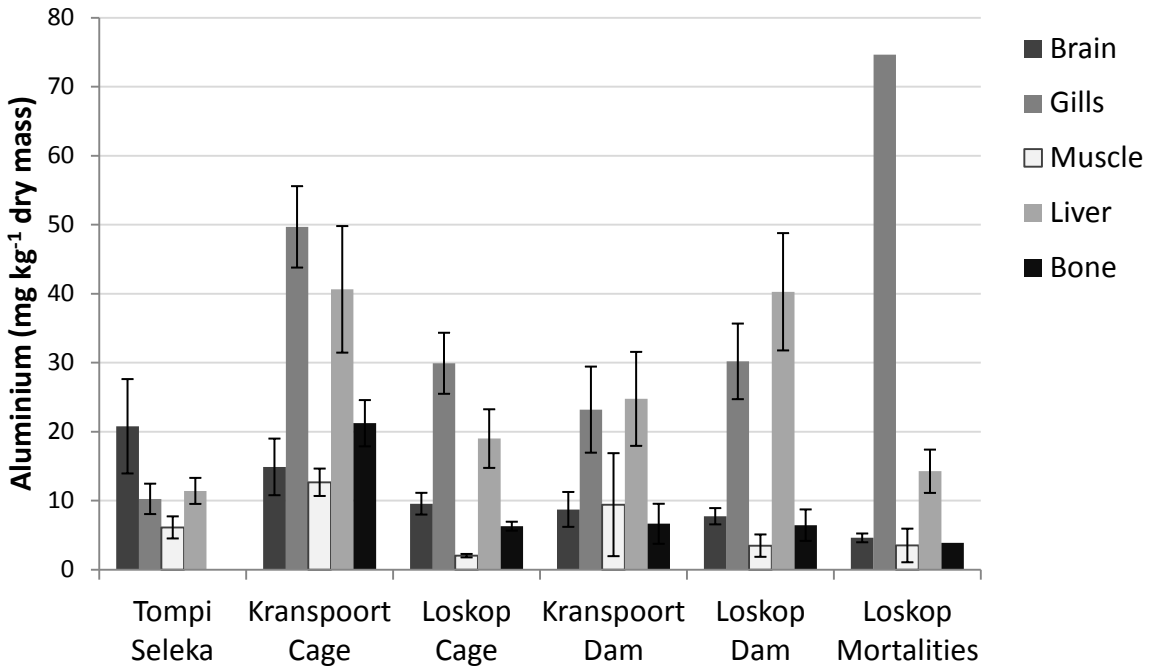

Figure 22. Mean (± SE) aluminium concentrations in the tissues of *O. mossambicus* collected from different sampling sites during 2010.

The general concentration gradient of Mn in tissues for fish from all sites followed the pattern: bone>gills>brain>liver>muscle. Fish in the Kranspoort cage had significantly higher levels of Mn in brain tissue than fish in the Loskop cage ($P < 0.001$), Loskop Dam ($P < 0.001$) and Kranspoort Dam ($P < 0.05$) (Fig. 23). Gill concentrations of Mn were significantly higher in fish from the Kranspoort cage ($P < 0.001$) and the Loskop cage ($P < 0.05$) than fish in the Kranspoort Dam. Significantly higher levels of Mn were found in muscle of fish in the Kranspoort cage ($P < 0.05$) and Tompi Seleka ($P < 0.001$) than fish from Loskop Dam. Unfortunately Mn concentrations in bone could not be compared to a pre-condition from Tompi Seleka fish, but the levels in fish from the Kranspoort cage ($P < 0.001$) and Loskop cage ($P < 0.001$) were significantly higher than in bone from the fish in Kranspoort Dam. There were no significant differences between the liver concentrations between fish from any of the locations.

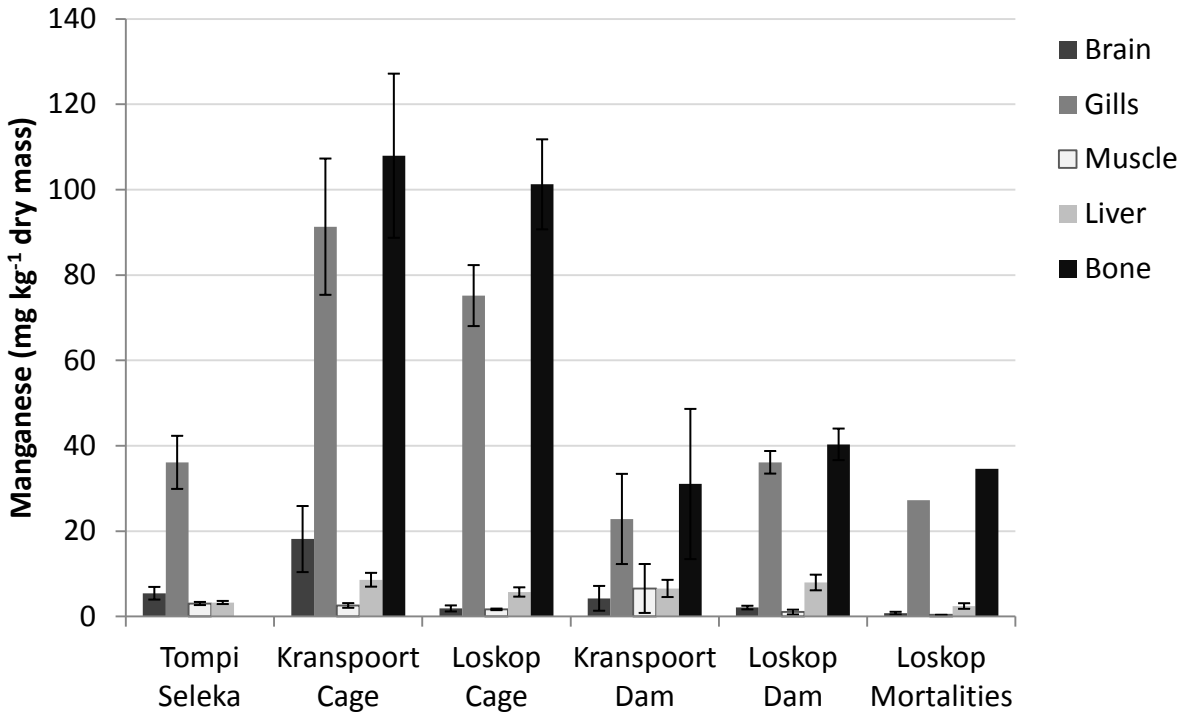

Figure 23. Mean (±SE) manganese concentrations in the tissues of *O. mossambicus* collected from different sampling sites during 2010.

The general pattern of Fe accumulation in fish tissues from all sites followed the decreasing gradient: liver>gills>brain>bone>muscle. The only significant differences in Fe concentrations were lower levels in muscle of fish from Loskop Dam than fish in the Kranspoort cage ($P < 0.001$) and Tompi Seleka ($P < 0.05$). There were no significant differences in gill, liver, brain or bone Fe concentrations between fish from any of the sites (Fig. 24).

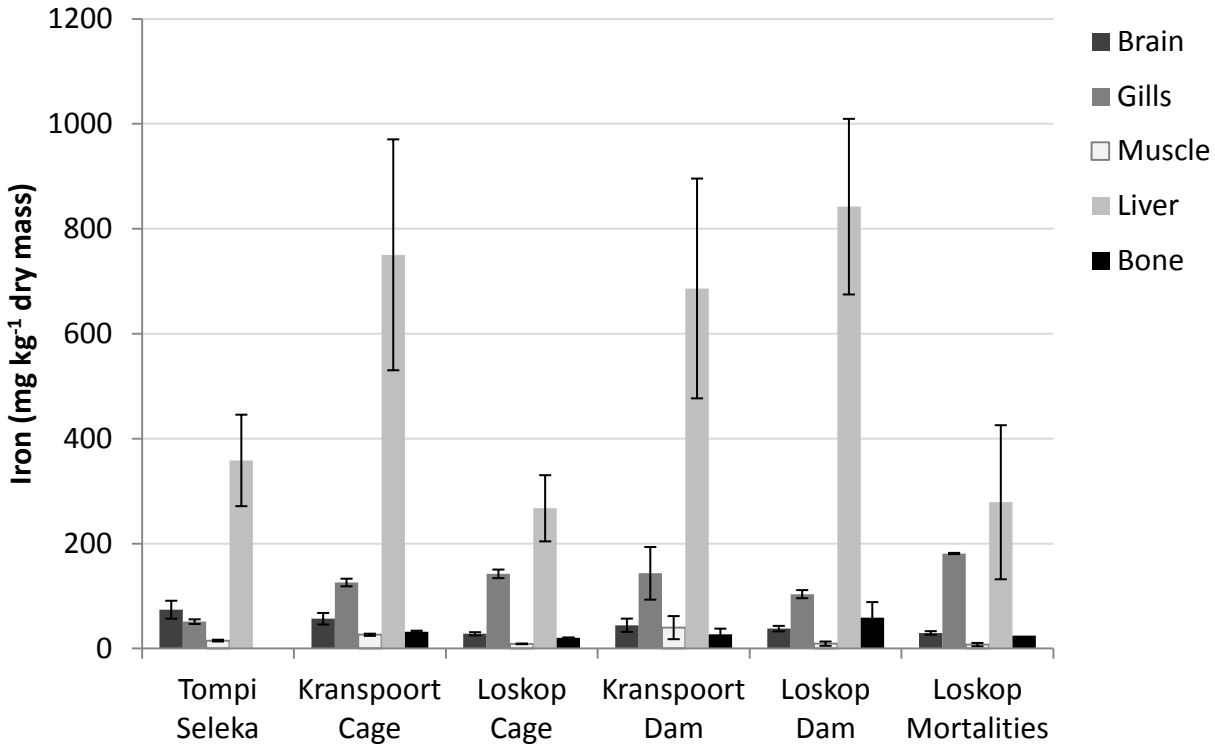

Figure 24. Mean (± SE) iron concentrations in the tissues of *O. mossambicus* collected from different sampling sites during 2010.

The general pattern of tissue Cu accumulation followed the decreasing gradient liver>brain>muscle>gills>bone. There was a striking pattern of Cu deficiency in the livers of fish from Loskop Dam which had significantly lower Cu than fish from all other sites ($P < 0.001$) (Fig. 25). Although the Loskop mortalities were not included in the ANOVA due to the low sample size, these four fish had mean liver Cu values of 2.7 mg kg^{-1} which were similarly low to the livers of fish from Loskop Dam with a mean of 3.4 mg kg^{-1}. These values were also very much lower than the mean concentration of Cu in livers of 120 *O. mossambicus* recorded by Kotze *et al.* (1999) (48 mg kg^{-1} ± 62) in Loskop Dam. Cu concentrations in the brains of fish from Tompi Seleka were significantly higher than fish from Loskop Dam ($P < 0.05$). There were no other significant differences between gill, muscle or bone Cu values in fish from different sites.

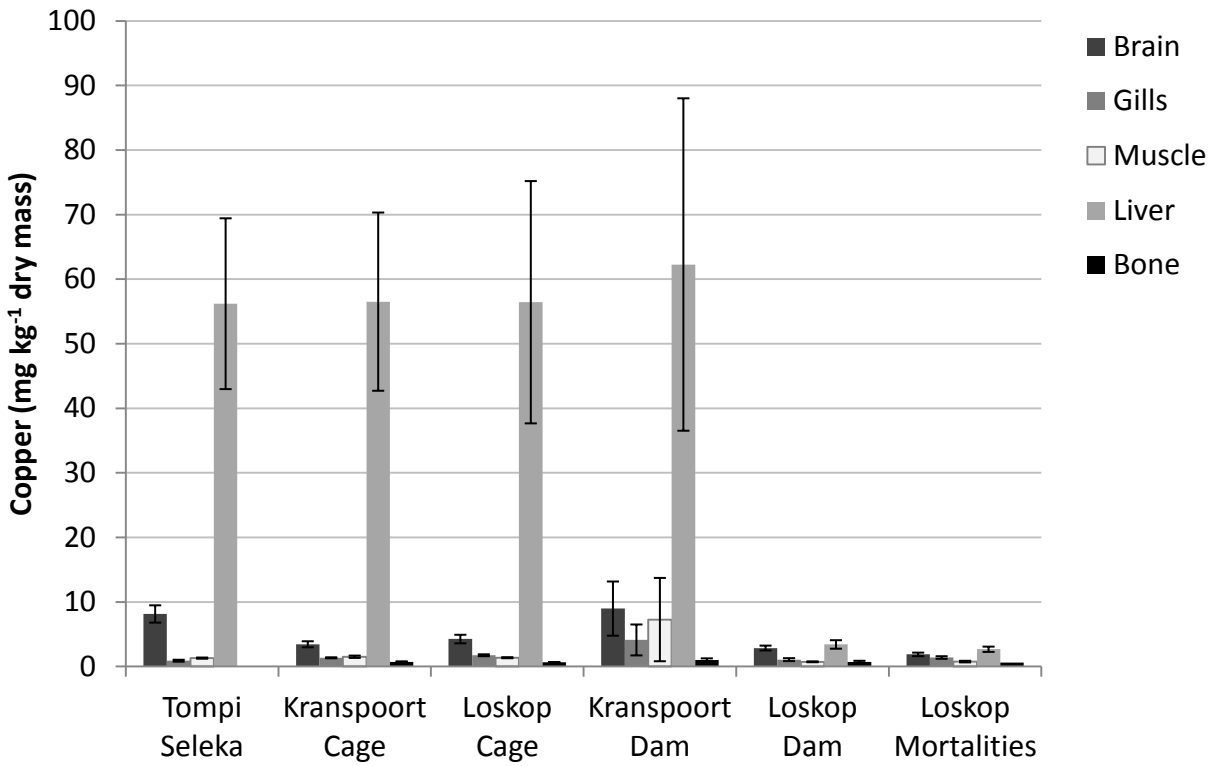

Figure 25. Mean (± SE) copper concentrations in the tissues of *O. mossambicus* collected from different sampling sites during 2010.

There was insufficient brain tissue remaining to measure Zn concentrations however the remaining tissues (gills, muscle, liver and bone) were sufficient for analysis. The general pattern of Zn accumulation in fish tissues followed the decreasing gradient: gills>bone>liver>muscle. The Zn concentrations were significantly higher in gills of fish from the Kranspoort cage ($P < 0.001$), Loskop cage ($P < 0.001$) and Loskop Dam ($P < 0.001$) than fish from Tompi Seleka and Kranspoort Dam (Fig. 26). Fish from Loskop Dam had significantly higher Zn concentrations in their livers than fish from Tompi Seleka ($P < 0.001$). Muscle Zn concentrations were significantly higher in fish from Tompi Seleka than fish from Loskop Dam ($P < 0.05$) and Kranspoort Dam ($P < 0.05$). There were no significant differences in bone Zn concentrations between fish from any of the sites.

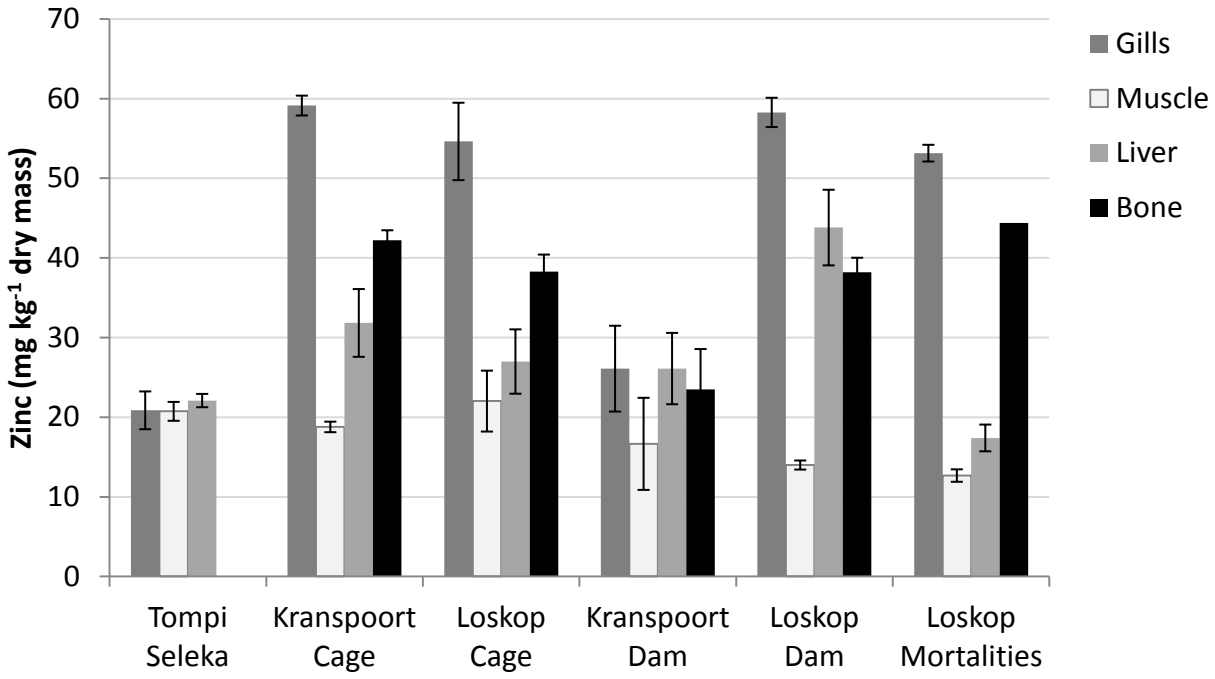

Figure 26. Mean (± SE) zinc concentrations in the tissues of *O. mossambicus* collected from different sampling sites during 2010.

Due to budgetary constraints and limited liver tissue from fish in the bioaccumulation cages not all livers were analysed for Se concentrations. The Se concentrations in the livers of fish from Loskop Dam were significantly lower (*P* 0.02) than those from Tompi Seleka but did not differ significantly from the fish in Kranspoort Dam (Fig. 27).

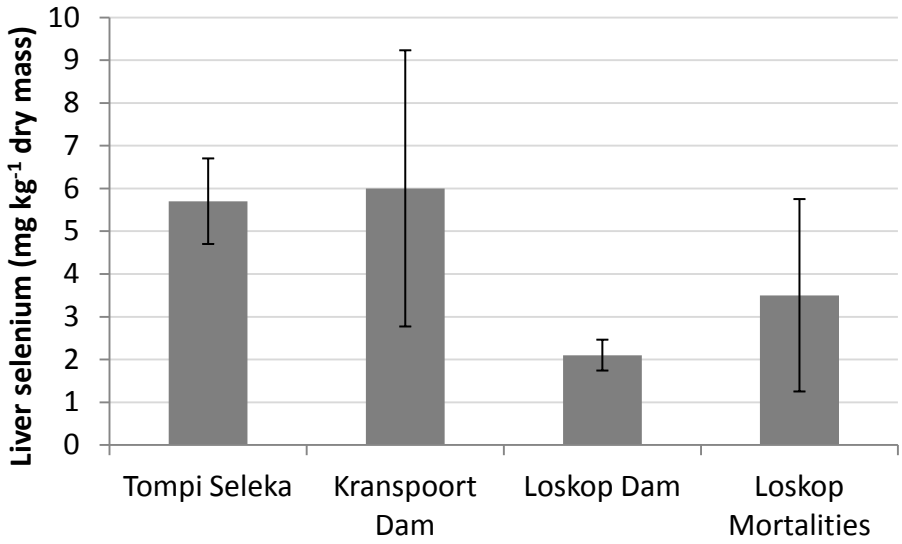

Figure 27. Mean selenium concentrations (±SE) in the livers of *O. mossambicus* collected from different sites during 2010.

3.4 Discussion

The extremely low concentrations of copper observed in both wild fish and natural mortalities from Loskop Dam were unprecedented as accumulation rather than deficiency was initially anticipated in this study. Fish from Kranspoort Dam and Tompi Seleka had the highest Cu concentrations in the liver which is consistent with previous studies of *O. mossambicus* (Robinson & Avenant-Oldewage, 1997; Kotze *et al*., 1999). This was also the case for fish in the cages which suggests that the cause of Cu deficiency in the fish from Loskop Dam occured over a longer time period than six weeks. Along with zinc, copper is an essential co-factor of the metalloenzyme superoxide dismutase (CuZn-SOD) which catalyses the dismutation of superoxide into hydrogen peroxide and molecular oxygen thus protecting cells and functioning as an antioxidant (Halliwell, 2006). Thus a deficiency in copper can have important consequences if lipid peroxidation is occurring as is the case in pansteatitis affected animals. This was demonstrated in rats fed a copper-deficient diet. After iron-induced peroxidation, levels of malondialdehyde (MDA) were significantly higher in both the heart and liver of rats fed the deficient diet (Rayssiguier *et al.* 1993). Similar results were observed by Fields *et al.* (1984) who observed decreased SOD and glutathione peroxidase (GSH-Px) concentrations and increased tissue peroxidation in rats fed a copper-deficient diet. Iron, zinc, cadmium and molybdenum are metabolic antagonists of copper (Watanabe *et al*., 1997) thus a diet enriched with these minerals may interfere with the uptake of copper. It is not possible to confirm whether the low Cu concentrations observed in this study were a primary cause, secondary effect or even related to elevated lipid peroxidation in pansteatitis affected fish in Loskop Dam. However the deficiency warrants further research into the dietary composition and quality as well as antioxidant enzyme status of SOD and GSH-Px in *O. mossambicus* in Loskop Dam.

There was no evidence of bioaccumulation of Al in any of the tissues of fish from Loskop Dam above levels that were present in neighbouring Kranspoort Dam. However it should be noted that the highest Al levels observed in the gills were in the two natural mortalities from Loskop Dam which means that Al bioaccumulation cannot be entirely excluded as a stressor. The gills are a good reflection of concentrations of elements in the water as they are in direct contact with the water whereas liver concentrations represent storage of elements (Arain *et al*., 2008). The

fish in Kranspoort Dam provided the reference condition for Loskop Dam as they did not display any symptoms of pansteatitis. The highest comparative accumulation of Al was by fish in the Kranspoort cage with elevated levels in the gills, muscle, liver and bone. The bioavailability of dietary aluminium is dependent on acidic conditions in the stomach. While the lumen of fish may be strongly acidic, the protective mucous layer that covers the gut maintains circumneutral pH at the epithelial surface causing any soluble aluminium in the lumen to precipitate and bind to the mucous without being absorbed (Handy, 1996). Very little is known about the dietary mechanism of absorption in fish and aluminium is not required for normal biological function. Aluminium has been found to facilitate iron-mediated lipid peroxidation (Xie & Yokel, 1996) and disturb the status of antioxidant enzymes like GSH-Px and xanthine oxidase when it was administered in varying concentrations to rats (Moumen *et al*., 2001). It has also been identified as an endocrine disruptor in female Nile tilapia (*Oreochromis niloticus*) causing changes in lipid mobilization and plasma levels of 17α-hydroxyprogesterone when added to the water which are pH dependent (Correira *et al*., 2010). Aluminium in excess is also known to accumulate in the nervous tissues like the brain (Abubakar *et al*., 2004), and results of this study showed no evidence of elevated levels in the brains of fish from Loskop Dam.

There was no indication that Fe was accumulating above normal levels in any of the tissues in fish from Loskop Dam. Iron is an essential mineral with an important role in cellular respiration and oxygen transport (Ribbins *et al*., 1972). Dietary intake via the intestinal mucosa is thought to be the major site of iron absorption in fish although it can be absorbed through the gills to a certain extent (Watanabe *et al*., 1997). Iron is involved in lipid oxidation as it catalyses the formation of hydroperoxides by providing a free radical initiator in the presence of oxygen and polyunsaturated fatty acids. Ferrous iron is more potent than ferric iron. In a study that investigated the results of feeding catfish (*Clarias gariepinus*) elevated levels of dietary iron (6354 mg kg^{-1}), researchers found these were not directly observed in accumulation in liver or muscle iron concentrations but rather as significantly higher levels of peroxidation measured as liver malondialdehyde (MDA) and lower levels of vitamin E in the liver (Baker *et al*., 1997). This highlights the importance of utilising an effective biomarker or measuring metal concentrations in the appropriate tissue for determining whether

bio-accumulation is occurring or having an effect. While excessive dietary iron altered the antioxidant state of the fish, the other major symptom associated was reduced growth rate which was contrary to observations of the fish in Loskop Dam. Where the actual growth rate is unknown but the abundance of large (> 40 cm) fish would suggest that growth rates are robust. Iron concentrations in the tissues of fish in this study showed no indication of bio-accumulation however, results of the abovementioned study suggest that elevated lipid peroxidation can be indicative of excess dietary exposure to iron. The high levels of lipid peroxidation associated with pansteatitis in fish from Loskop Dam suggest that exposure to excessive dietary iron cannot be ruled out as an important factor and warrants further research.

Manganese deposition in tissues was highest in the bone in this study which is consistent with previous research on tilapia (Lin *et al*., 2008). There was no indication that bioaccumulation of excess manganese was occurring in any of the tissues of the fish from Loskop Dam compared to Kranspoort Dam. Levels of Mn and Zn in the gills and Mn in the bone increased in fish in the biomonitoring cages compared to the reference condition in Tompi Seleka, but it occurred in both cages and may have been related to factors associated with confinement as opposed to differences in exposure.

Zinc plays an important role in normal physiological functioning as it is an integral part of > 70 metalloenzymes (NRC, 1993). There were no statistically significant differences in Zn concentrations in any of tissues when comparing fish from Loskop Dam and Kranspoort Dam which implies that bio-accumulation of Zn is not occurring. However the liver Zn levels were highest in fish from Loskop Dam and given a greater sample size from Kranspoort Dam that difference may have been significant. The levels of zinc observed by Kotze *et al.* (1999) in the gills, liver and muscle tissue of *O. mossambicus* were almost double those observed in each of these tissues of fish from Loskop Dam in this study.

Liver selenium concentrations in fish from Loskop Dam were lower than those from fish collected at Tompi Seleka and Kranspoort Dam. While this difference was statistically significant in the case of Tompi Seleka, the physiological significance is unclear. Selenium is an essential co-factor in many selenoenzymes that regulate

thyroid hormone synthesis and reduce oxidative stress (Arthur & Beckett, 1999). The main role that selenium plays in thyroid hormone regulation is in the metabolism of the prohormone thyroxine (T_4) which is converted to the biologically active triiodothyronine (T_3) by type I iodothyronine de-iodinase (IDI) which requires selenium as a co-factor. Levels of T_3 and T_4 are further regulated by deiodinases type II and type III that also contain selenium (Arthur & Beckett, 1999). In cases of deficiency decreased concentrations of selenium have been detected in liver, kidneys and muscle (Arthur & Beckett, 1999). Another important function of Se is as a co-factor in the enzyme glutathione peroxidase (GSH-Px). The enzyme generally acts intracellularly to detoxify H_2O_2 and lipid hydroperoxides thus protecting cells from oxidative damage. One of the effects of selenium deficiency is severely reduced GSH-Px in the liver. In a study of rats fed a selenium-deficient diet, the activity of GSH-Px was decreased by over 99% in the liver (Bermano *et al*., 1995).

Direct interpretation of metal bioaccumulation is complicated by the influences of metal specificity, environmental influences, exposure route and species-specific characteristics (Luoma & Rainbow, 2005). Seasonal differences in metal concentrations in different tissues further complicate interpretation due to fluctuations in exposure routes (diet and solution) as well as aqueous concentrations and bio-availability. Future studies should attempt to incorporate this variability as this was a limiting factor in the current study. While there was little evidence of significant bioaccumulation of the metals investigated in this study, the results indicate that Al and Zn may be accumulating above normal levels in the gills and livers of *O. mossambicus* respectively in Loskop Dam. However the most significant finding was not one of accumulation but rather deficiency of Cu in the livers of the fish. These findings provide a basis from which to further investigate both the diet and antioxidant status of *O. mossambicus* in Loskop Dam. Cu status may be impacted by dietary antagonists and / or depletion of reserves by increased demand for SOD in the presence of lipid peroxidation associated with pansteatitis.

Chapter 4

Metazoan parasite communities of *Oreochromis mossambicus* as indicators of anthropogenic impacts in Loskop Dam.

4.1 Introduction

Parasites occur at all trophic levels in association with almost all free-living organisms including fish which may serve as definitive, intermediate or transport hosts. They often display complex life cycles involving multiple hosts and thus rely on trophic interactions for transmission. Fish parasite communities are influenced by environmental parameters that can be summarised in three broad categories: 1) the physico-chemical parameters in water and sediment external to the fish; 2) the internal environment of the fish which is defined by its physiological condition; 3) the presence of suitable macroinvertebrates and vertebrates that serve as intermediate hosts for the parasites (Landsberg *et al*., 1998). As a result parasites can provide important information about their hosts as well as ecosystem interactions (Marcogliese, 2005).

Deteriorating water quality in Loskop Dam has affected the aquatic ecosystem on several trophic levels. Ongoing eutrophication has caused primary production by cyanobacteria like *Microcystis aeruginosa* and the dinoflagellate *Ceratium hirundinella* to increase to annual bloom proportions (Oberholster *et al*., 2010). Primary and secondary consumers like Mozambique tilapia (*Oreochromis mossambicus*) and Nile crocodiles (*Crocodylus niloticus*) have been diagnosed with pansteatitis which has resulted in seasonal deaths of both species and a decline in the latter's population at Loskop Dam (Botha *et al*., 2010) and the Olifants River system (Ashton, 2010). In addition to eutrophication caused by untreated sewage released in the upper catchment, acid mine drainage released from working and abandoned coal mines in the upper Olifants River catchment causes fluctuations in pH and associated release of metals like aluminium, iron and manganese (Oberholster *et al*., 2010). While these events represent large-scale perturbations to

the ecosystem, fish parasite communities may reflect more subtle impacts of pollutants.

When using fish parasites as bioindicators it is useful to divide them into two groups known as ‘effect’ and ‘accumulation’ indicators. This dichotomy reflects the multiple levels of exposure of fish parasites to pollution. Endoparasites like intestinal acanthocephalans have been used to demonstrate bioaccumulation of metals such as lead relative to their hosts (Sures *et al*., 1994) and can be described as ‘accumulation’ indicators. Monogenean trematodes on the gills of fish are in direct contact with the water leading to the use of their community structure as an ‘effect’ indicator of ecosystem health (Sures, 2001). Parasites may be further classified into two groups according to their life cycle. Heteroxenous parasites have a complex life cycle with multiple hosts and are thought to be more susceptible to pollution as environmental conditions need to be suitable for all host levels. Monoxenous parasites have a simpler life cycle as they are dependent on a single host, thus environmental conditions need only be favourable for the host and parasite (Dzikowski *et al*., 2003). The free-living stages of parasites face enormous challenges as they must cope with amplified variation in environmental conditions compared to that within their hosts and they also need to locate a suitable host for completion of their life cycle while evading predation. During this stage they are highly susceptible to environmental perturbations caused by pollution (Pietrock & Marcogliese, 2003). Parasites may be affected by environmental changes in a number of ways. If host resistance is compromised or density of intermediate or definitive hosts is increased by the stressor, then parasitism may increase. If the impact results in a decline in intermediate or definitive host densities or direct mortality of the parasites then infection rates will decrease. These apparently opposing community responses can complicate interpretation of results (Lafferty, 1997).

In this study we assessed the parasite communities associated with a single host species, *Oreochromis mossambicus* with a view to using them as ‘effect’ indicators of ecosystem health in Loskop Dam. The only known study reporting parasites on fish in Loskop Dam was from Goldner (1969) who gave a descriptive summary of his observations but not an in depth analysis. Fish parasites have been used in several

studies linking aquatic pollution to changes in host communities both in freshwater and the marine environment (Diamant *et al*., 1999; Galli *et al.,* 2001) and the use of parasites as indicators of ecosystem health has been reviewed and strongly advocated (Marcogliese, 2005). It must be considered however that ecosystems are inherently complex and changes in parasite communities cannot always be unequivocally linked to environmental change or degradation (Kennedy, 1997). The objectives of this study were to: (i) Evaluate parasite communities of *O. mossambicus* in Loskop Dam and compare them to a suitable reference site with good water quality; (ii) Transplant fish from a reference site to Loskop Dam using active biomonitoring in cages for a period of six weeks and describe resulting changes in parasite community structure; (iii) Assess the metazoan parasite communities of *O. mossambicus* to determine whether they are effective indicators of ecosystem health in Loskop Dam.

4.2 Methods

4.2.1 Study Sites and fish collection

The same fish that were collected for metal bioaccumulation analyses in chapter 3 were utilised in this study. The study sites and collection methods therefore remain the same as well as the use of the biomonitoring cages. The only difference was the number of fish that were utilised for analysis of parasite communities and this is described in the results section.

4.2.4 Parasite Collection

After removal from nets or the cages the fish were macroscopically checked for mobile ectoparasites. These were collected in glass containers filled with water from the site and identified in the field laboratory. A slime smear was collected from the skin and fins and examined for parasites with a stereomicroscope. The fish were then checked for ectoparasites inside the opercula and mouth cavity and at the base of the fins and skin. Fish were then killed by severing the spinal cord. During dissection the gills, liver, stomach, intestines, swim bladder, urinary bladder and eyes were placed in separate petri dishes in distilled water and examined for

endoparasites with a Leica EZ4D stereomicroscope. Muscle tissue and the body were examined for encysted parasites. All parasites were counted, their location noted, and identified in the field laboratory.

Degree of parasite infection (prevalence, mean intensity and mean abundance) was calculated according to the method of Bush *et al*. (1997). Prevalence is the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined (expressed as percentage). Mean intensity is the total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample. Mean abundance is the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species in the sample.

4.2.6 Statistical Analyses

The following ecological indices of species richness, diversity and dominance were utilized to compare component parasite communities between sites:

1. Shannon-Weiner index of diversity (H') (Shannon, 1948)
2. Simpson's index of diversity (D) (Simpson, 1949)
3. Shannon-Weiner index of evenness (E)
4. Berger Parker index of dominance (d) (Berger & Parker, 1970)

Site-specific species richness is dependent on sample size. Sample-based rarefaction curves using repeated re-sampling of samples at random and without replacement were calculated for each site using the freeware application EstimateS 8.2.0. (Colwell, 1994 - 2004). Asymptotes of the curves indicate species richness saturation and the point at which sampling may be considered representative of the parasite communities at a site. The ratio between heteroxenous (H) and monoxenous (M) species was calculated according to Diamant *et al*. (1999). In brief the H/M ratio is based on the ratio of heteroxenous to monoxenous parasite individuals per host, while the S_H/S_M ratio is based on the parasite species richness per host. The number of heteroxenous or monoxenous parasite species from fish sampled at each location gave the 'S' (species richness) value. A Principal Components Analysis (PCA) was performed using the CANOCO for Windows package, version 4.5. The decision to use a PCA over a Correspondance Analysis

(CA) was made based on the output of a Detrended Correspondance Analysis (DCA). A DCA calculates the length of gradient in a data set, and is a measure of the degree of unimodality of a latent variable (ordination axis). If a length of gradient of the latent variable is short (less than 4 Standard Deviations), a PCA is recommended (Van Den Brink *et al.,* 2003). Single factor ANOVAs were used to compare fish body measurements and heteroxenous versus monoxenous species data and a Tukey's post hoc test was performed to compare the means of these parameters between sites. Where assumptions of normality were not met, data were log transformed.

4.3 Results

An approximately even sample of 16 fish from Loskop Dam and 15 fish from Tompi Seleka was collected (Table 5). Several attempts were made to collect a similar number of fish from Kranspoort Dam. But despite excellent water quality (eg. Low conductivity, neutral pH and adequate dissolved oxygen levels) and a rigorous sampling effort, the population of *O. mossambicus* appeared to be very low and only 4 fish were collected. Several mortalities of fish occurred in the biomonitoring cages leaving 9 in the Loskop cage and 11 in the Kranspoort cage after they were each initially stocked with 21 fish. Mortalities occurred periodically for the full 6 week exposure period and therefore cannot be attributed solely to transport stress. Attempts were made to obtain an even sex ratio at all sites, but the cages in particular were dominated by males after mortalities. The total length, weight and condition factor of fish in Loskop Dam was significantly higher than fish at the other sites. Fish in the cages from both dams had significantly longer total length and greater weight than the fish from Tompi Seleka, but their condition factors did not change significantly (Table 5). No significant differences in fish size or condition factor were observed between fish from both of the cages.

The diversity indices (Shannon-Wiener and Simpson's) both show the highest diversity at Tompi Seleka and Loskop Dam, followed by the Kranspoort cage and Kranspoort Dam (Table 5). The lowest diversity and evenness was observed in the Loskop cage. Shannon's Evenness and Berger Parker dominance indices show that Tompi Seleka and Loskop Dam had the most even distribution of individuals across

species. The lowest evenness and highest dominance was observed in Kranspoort Dam and both cages.

Table 5. Summary statistics and diversity indices of fish hosts sampled from each site.

	Tompi Seleka (TS)	**Loskop Cage (LC)**	**Kranspoort Cage (KC)**	**Loskop Dam (LD)**	**Kranspoort Dam (KD)**
Sample size (Sex)	15 (10 M, 5 F)	9 (9 M)	11 (10 M, 1 F)	16 (7 M, 9 F)	4 (2 M, 2 F)
No. of parasite species	8	7	5	8	5
Total length (cm)	24 ± 0.6 ^{LC,KC,LD,KD}	29.3 ± 0.9 ^{TS,LD}	29.9 ± 1.1 ^{TS,LD}	41.2 ± 0.9 ^{TS,LC,KC,KD}	30.8 ± 4.1 ^{TS,LD}
Weight (g)	235.1 ± 18.7 ^{LC,KD,LD,KD}	427.6 ± 35.3 ^{TS,LD}	390.4 ± 23.2 ^{TS,LD}	1499.1 ± 86.4 ^{TS,LC,KC,KD}	679.5 ± 180.2 ^{TS,LD}
Condition factor (*K*)	1.66 ± 0.03 ^{LD}	1.65 ± 0.02 ^{LD}	1.58 ±0.04 ^{LD}	2.11 ± 0.05 ^{TS,LC,KC,KD}	1.61 ± 0.01 ^{LD}
Diversity Indices					
Shannon-Weiner (H')	1.61	0.66	1.06	1.54	0.86
Shannon Evenness (E)	0.77	0.53	0.66	0.74	0.53
Simpson's Diversity (D)	0.78	0.38	0.58	0.75	0.51
Berger Parker Dominance	3.01	1.33	1.83	2.71	1.64

Letters in uppercase represent sites where significant differences are $P < 0.05$ using a single factor ANOVA with Tukey post-hoc test.

The calculated intensity, mean abundance and prevalence of parasites on fish from all sites is presented in Table 6. A total of 12 metazoan parasite species were identified with 2145 individuals collected during the survey across all study sites. The intensity and abundance of all parasites on fish from Loskop Dam was generally lower than at all other sites and of the 16 fish inspected for parasites, 2 fish had no parasites on them at all and could not be included in the analysis. The dominant species at each site were *Cichlidogyrus* at Tompi Seleka and Loskop Dam, *Neascus* (black spot) at Kranspoort Dam and *Enterogyrus* in both cages.

The nematode *Contracaecum* was only found at Tompi Seleka and Loskop Dam with a prevalence of 26.7% and 37.5% respectively (Table 6). The cestode gryporynchid (endoparasite) was recorded at all sites, and while infection rates increased in the fish from the Kranspoort Cage, they decreased in the fish from the Loskop cage compared to Tompi Seleka. Gryporynchid cestode infection rates were high in fish from Kranspoort Dam with 100% prevalence compared to 37.5% in Loskop Dam.

A single gyrodactylid was found in a Loskop Dam fish, but not at any other site. Monogenean ectoparasites in the genus *Cichlidogyrus* were found on the gills of fish from all sites. Fish sampled from both cages had the highest infection rates of *Cichlidogyrus* and *Enterogyrus.* The lowest prevalence of *Cichlidogyrus* from all sites was on fish from Loskop Dam (43.75%) however this parasite had the highest prevalence of all genera within the dam. No enterogyrids were found in fish from either Loskop or Kranspoort Dam.

An unidentified digenean metacercaria was found encysted on the skin of fish from Tompi Seleka and in the Loskop cage at a low intensity (1.3 and 2) but was not recorded from the Kranspoort cage. The same species was found at a higher intensity (8.33) and prevalence (75%) at Kranspoort Dam but was absent from Loskop Dam. *Clinostomum* (yellow grub) was recorded at similar intensities from Tompi Seleka and both cages but while the prevalence increased from 46.7% at Tompi Seleka to 63.64% in the Kranspoort cage, it decreased to 22.22 in the Loskop cage. The intensity and abundance of the digenean *Neascus* on the fish from Kranspoort Dam was very high relative to the other sites with 100% prevalence and a mean intensity and abundance of 103.5. By contrast only one fish in Loskop Dam was infected with black spot with an intensity of 2.

The crustacean parasites *Ergasilus* sp., *Argulus japonicus* and *Lernaea cyprinacea* were found at very low intensities at Loskop Dam with the latter also recorded at Tompi Seleka. *Ergasilus* sp. was also recorded on 1 fish from the Loskop cage indicating possible colonisation of this species as it was not recorded on any fish from Tompi Seleka. *Dolops ranarum* was recorded on 2 fish at an intensity of 2 from Kranspoort Dam but not from fish at any other site.

Table 6. Mean intensity (*I*), mean abundance (*A*), and prevalence (*P*) (expressed as %) of metazoan parasites associated with *Oreochromis mossambicus* collected from various sampling sites during 2010.

	Tompi Seleka (*n*=15)			**Kranspoort Cage (*n*=11)**			**Loskop Cage (*n*=9)**			**Kranspoort Dam (*n*=4)**			**Loskop Dam (*n*=16)**		
Taxon	***I***	***A***	***P***	***I***	***A***	***P***	***I***	***A***	***P***	***I***	***A***	***P***	***I***	***A***	***P***
Nematoda[H]															
Contracaecum (m)	1	0.3	26.7	-	-	-	-	-	-	-	-	-	2.33	0.88	37.5
Cestoda[H]															
Gryporynchid (m)	6	1.6	26.7	9.83	5.36	54.55	1	0.11	11.11	57.75	57.75	100	3.33	1.25	37.5
Monogenea[M]															
Gyrodactylus (sk)	-	-	-	-	-	-	-	-	-	-	-	-	1	0.06	6.25
Cichlidogyrus (g)	5.8	4.7	80	20.55	20.55	100	12.67	12.67	100	2	1.5	75	4	1.75	43.75
Enterogyrus (s)	4.8	4.1	86.7	41.64	34.09	81.82	41.44	41.44	100	-	-	-	-	-	-
Digenea[H]															
Digenean *sp.* (sk)	1.3	0.5	40	-	-	-	2	0.22	11.11	8.33	6.25	75	-	-	-
Clinostomum (bc)	1.9	0.9	46.7	2.57	1.64	63.64	1.5	0.33	22.22	-	-	-	-	-	-
Neascus (sk)	10	1.3	13.3	3.67	1	27.27	2	0.22	11.11	103.5	103.5	100	2	0.13	6.25
Crustacea[M]															
Ergasilus (sk & g)	-	-	-	-	-	-	2	0.22	11.11	-	-	-	1.25	0.31	25
Argulus (sk)	-	-	-	-	-	-	-	-	-	-	-	-	1.33	0.25	18.75
Lernaea cyprinacea (sk)	3.3	0.7	20	-	-	-	-	-	-	-	-	-	1	0.06	6.25
Dolops ranarum (sk)	-	-	-	-	-	-	-	-	-	2	1	50	-	-	-

Location: g, gills; s, stomach; m, mesenteries; sk, skin; bc, brain cavity

Development: [H], Heteroxenous; [M], Monoxenous

Species accumulation curves reached an asymptote at Tompi Seleka and in the Kranspoort cage indicating that sample sizes were representative of parasite communities at these locations (Fig. 28). The same appears to be true for Kranspoort Dam despite the small sample collected at that site. Non-asymptote curves from Loskop Dam and the Loskop cage did not achieve sampling saturation which suggests the samples of 16 and 9 fish respectively were inadequate for determination of true species richness at these sites. As all fish in the Loskop cage were assessed, sampling effort could not be increased at that location. However increased sampling at Loskop Dam may yield more species.

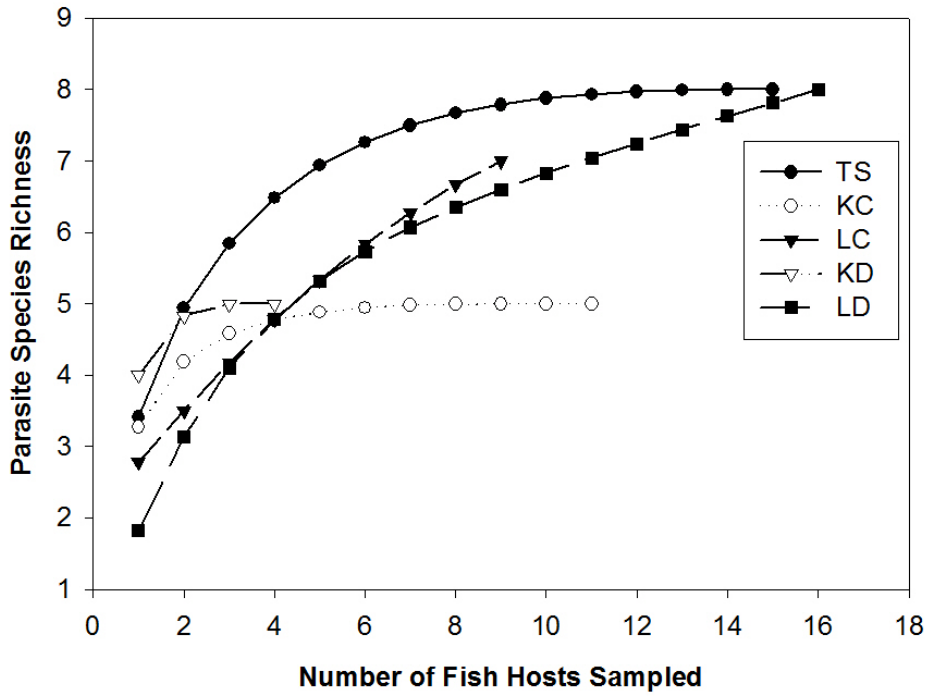

Figure 28. Sample-based rarefaction curves depicting the parasite assemblage as a function of sample size for each site sampled during 2010 (TS, Tompi Seleka; LC, Loskop Cage; KC, Kranspoort Cage; LD, Loskop Dam; KD, Kranspoort Dam).

The Principal Components Analysis showed the parasite species associated with individual hosts at each sampling site (Fig. 29). The amount of inertia (variation) explained by the first axis was 39.6% and the second axis explained 22.5% with a cumulative value of 62.1%. The 2 cages were closely grouped together but showed a slight movement away from Tompi Seleka due to the presence of *Lernaea cyprinacea* at the latter. Kranspoort Dam was clearly separated from the other sites

and was characterised by the presence of *Dolops ranarum,* and the high infection rates of *Neascus,* the unidentified digenean sp. and gryporynchids relative to other sites. The separate grouping of fish from Loskop Dam from those at other sites was based on the presence of *Ergasilus, Argulus* and *Gyrodactylus,* none of which had high infection rates. *Enterogyrus* was not recorded at either of the dams but was found in the fish from Tompi Seleka, with a subsequent increase in abundance in the fish from both cages that further separated them from Tompi Seleka. A similar pattern was observed with *Cichlidogyrus* that occurred at every site but was recorded in highest abundance in the cages in Kranspoort and Loskop Dams (Table 6).

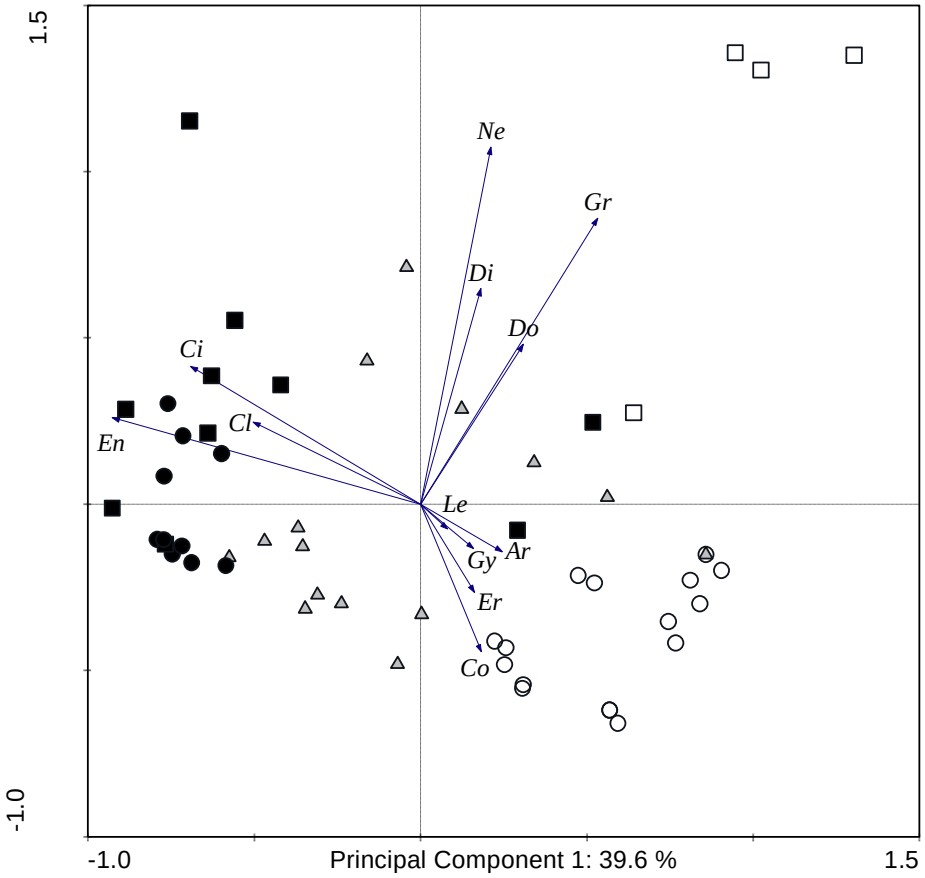

Figure 29. Principal Components Analysis of parasites collected during 2010 from Tompi Seleka (triangles), Kranspoort cage (closed squares), Kranspoort Dam (open squares), Loskop cage (closed circles) and Loskop Dam (open circles). Parasites are Ci, *Cichlidogyrus*; Le, *Lernaea*; Co, *Contracaecum*; Di, *Digenean*; Cl, *Clinostomum*; En, *Enterogyrus*; Gr, *ryporynchid*; Gy, *Gyrodactylus*; Ne, *Neascus*; Er, *Ergasilus*; Ar, *Argulus*; Do, *Dolops*.

Species richness of heteroxenous parasites (S_H) was significantly higher at Kranspoort Dam than at Loskop Dam (P = 0.005) and the Loskop cage (P = 0.01) (Fig. 30). Although S_H was lower in the Loskop cage than at Tompi Seleka and the Kranspoort cage, the difference was not significant. Monoxenous species richness (S_M) was significantly higher from fish at Tompi Seleka (P = 0.017) and the Loskop cage (P = 0.047) than fish from Loskop Dam. The combined S_H and S_M were significantly lower at Loskop Dam than at Tompi Seleka (P = 0.003), Kranspoort Dam (P = 0.012) and the Kranspoort Cage (P = 0.044).

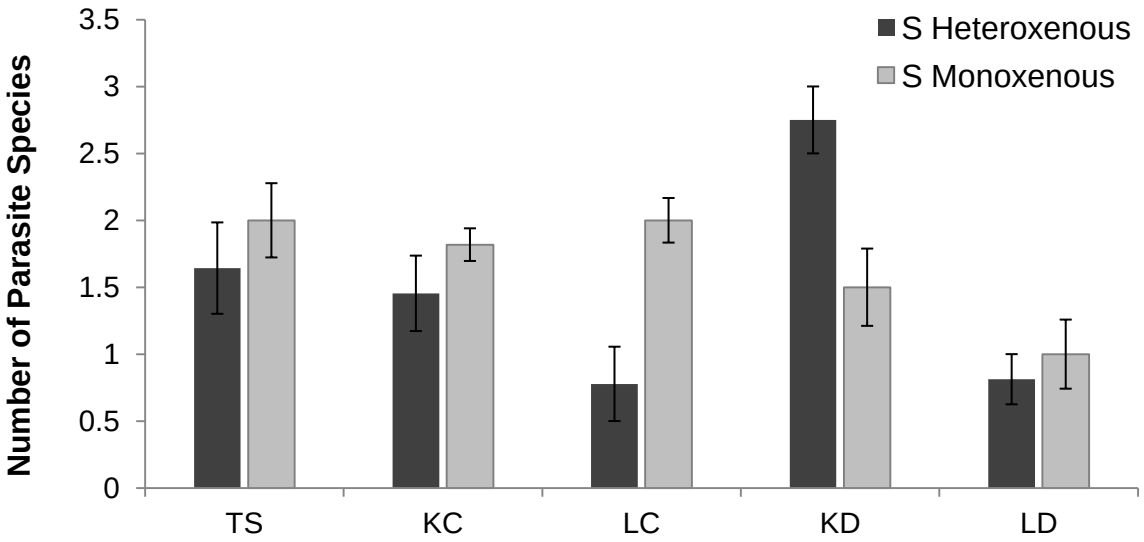

Figure 30. Mean (± SE) monoxenous and heteroxenous parasite species richness (*S*) (± SE) at Tompi Seleka (TS), Kranspoort cage (KC), Loskop cage (LC), Kranspoort Dam (KD) and Loskop Dam (LD).

When calculating ratios of heteroxenous and monoxenous species richness (S_H:S_M) and the quantitative ratio (H:M), 4 fish from Loskop Dam had to be omitted as they had zero monoxenous species making the calculation of a ratio impossible. Influenced by the high abundance of *Neascus*, the H:M ratio at Kranspoort Dam was significantly higher than Loskop Dam (P = 0.003), the Loskop cage (P = 0.007) and the Kranspoort cage (P = 0.037) (Fig. 31). A similar pattern was observed with the S_H:S_M ratio where Kranspoort Dam was significantly higher than all the other sites (P<0.001).

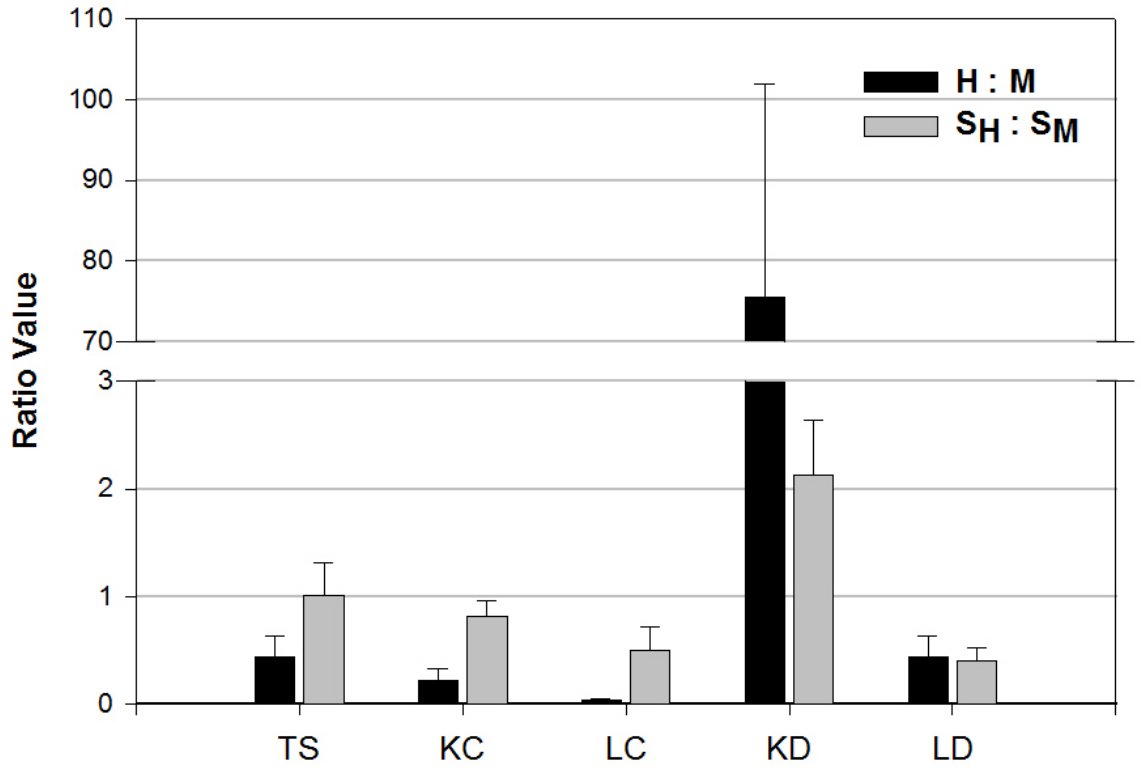

Figure 31. Mean (± SE) heteroxenous and monoxenous parasite ratios of species richness (S_H:S_M) and quantitative ratio (H:M) at Tompi Seleka (TS), Kranspoort cage (KC), Loskop cage (LC), Kranspoort Dam (KD) and Loskop Dam (LD).

4.4 Discussion

Despite the high species diversity and evenness of parasite communities of *O. mossambicus* in Loskop Dam, aspects of the results suggested that community structure at the dam was reflective of an impacted site. Detailed evaluation of the parasite communities showed that the abundance and intensity of all parasites was very low with not a single parasite found on two fish from the sample at Loskop Dam. This is likely to be the reason for the lack of species saturation in the rarefaction curve, and while increased sampling may have yielded more species, it was unlikely to result in increased abundance. In addition, the contribution of monoxenous species to overall richness and abundance was higher than that of heteroxenous species which is considered to be indicative of an environmentally impacted site (Diamant *et al*., 1999). Condition factors are reflective of fat content but in this case

may not have been reflective of good health as pansteatitis lesions were frequently observed in fish with abundant abdominal fat. It is difficult to link the low intensity and abundance of parasites observed on fish in Loskop Dam indisputably to poor water quality, and there may have been causes apart from environmental degradation. Fish at Loskop Dam had a higher condition factor (2.11 ± 0.05) and longer total length (41.2 cm ± 0.9) than fish at other sites and both physical factors have reportedly influenced parasite communities in different ways. A significant correlation between Fulton's condition factor (K) and parasite densities has been reported, with less parasites observed on bluegill sunfish with high conditions factors (Neff & Cargnelli, 2004). In contrast, several studies have reported an increase in the prevalence and intensity of parasites with increased fish size (Khidr, 1990; Timi & Poulin, 2003) due to their larger surface area. However in a meta-analysis of 76 host-parasite species, Poulin (2000) found that although the correlation between intensity of infection and fish length was positive, it was not significant. One of the constraints of the current study was that fish from each location were within distinct size-classes and it was therefore not possible to determine whether total length was an important factor in parasite species parameters within sites.

Another factor that was held constant during this study but which may have influenced fish parasite communities was seasonal fluctuation. *Dolops ranarum* and the *Argulus* sp. recorded in this study are monoxenous species commonly known as fish lice. *D. ranarum* is the only known species of that genus found in Africa (Paperna, 1996) and in this study it was restricted to fish in Kranspoort Dam while *Argulus* was restricted to Loskop Dam. *D. ranarum* have a one year life cycle ending when females leave hosts to deposit eggs on the substratum and die. This was observed by Avenant and Van As (1986) in eutrophic Roodeplaat Dam (Gauteng province, South Africa), where the highest prevalence of *D. ranarum* was in late summer followed by a steady decline until October when none were observed on *O. mossambicus*. In this study fish were collected in mid-October from Loskop and Kranspoort Dams which coincides with the above-mentioned population low point and may explain their absence from Loskop Dam. However their prevalence of 50% in Kranspoort Dam does not support this observation and therefore seasonal influence on the absence of this species from Loskop Dam can be discounted.

Males of the crustacean *Ergasilus* are free-living and feed on nanoplankton while the females are parasitic predominantly on the gills of fish. Their nutritional and environmental requirements appear to limit their establishment in aquaculture ponds, which may explain their absence from Tompi Seleka (Paperna, 1996). Ergasilids were only found on fish (caged and free-swimming) in Loskop Dam in this study and their nutritional requirements during the free-living stage may be the reason for this. High levels of primary productivity associated with eutrophication of the dam provide an ample food source.

There were positive and negative factors involved in the selection of Kranspoort Dam as a reference site for Loskop Dam. Direct comparisons may have been complicated by isolation of the two fish populations, the relatively small size of Kranspoort Dam, and the abundant riparian vegetation and macrophytes compared to Loskop Dam. However, the positive elements were that both dams were in close proximity and linked by the Kranspoortspruit enabling connectivity of intermediate and definitive host populations, and that water quality was considerably better at Kranspoort Dam. Both Kranspoort and Loskop Dam support populations of birds such as darters, cormorants and herons that can theoretically move between the dams to feed and act as definitive hosts for certain parasites. Parasites such as the digenean species and particularly *Neascus* (black spot) were observed at high intensities in Kranspoort Dam, but only two *Neascus* were found on one host at Loskop. Digeneans such as *Clinostomum* (yellow grub) and *Neascus* (black spot) are encysted as metacercariae which precedes the adult stage. The life cycle involves three hosts with the adult stage in the definitive host, usually a piscivorous bird. Free-swimming miracidia emerge from eggs and infect a molluscan host, usually a snail in the genus *Bulinus* (Paperna, 1996). After several larval generations are completed within the mollusc, the cercariae emerge into the water and infect fish which act as a second intermediate host (Shoemaker *et al*., 2006). Although digenean cysts may appear unsightly they usually don't result in any pathology with the only tissue response being to form a fibrous layer around the cyst (Paperna, 1996; Shoemaker *et al*., 2006; Zajac & Conboy, 2006).

The low abundance of digeneans at Loskop Dam may be due to impacts on water quality involving several factors. A review of the environmental conditions that can

negatively impact survival, transmission and encystment of miracidia and cercariae included exposure to metals, sewage sludge, hydrocarbons and various pesticides as well as changes in pH and salinity (Pietrock & Marcogliese, 2003). Fluctuations in pH associated with acid mine drainage and high primary productivity ranging from 5.7 (Oberholster *et al.*, 2010) to a median of 9.67 observed in the transitional zone during this study influence the speciation and bio-availability of metals. Cercariae of the marine trematode *Cryptocotyle lingua* exposed to high concentrations of copper, zinc, iron and manganese had reduced longevity and reduced horizontal swimming rate which are essential for their transmission. The same effects were observed in cercariae released from mollusc hosts collected from metal polluted water (Cross *et al*., 2001). The susceptibility of digenean cercariae to pollutants may be exacerbated by their thin, fragile cuticle as compared to the thick cuticle present on free-swimming stages of the nematodes like *Contracaecum* (Pietrock & Marcogliese, 2003).

An inherent difference between Loskop and Kranspoort Dams was the abundance of macrophytes and riparian vegetation that dominate the latter thus providing a distinct range of habitats that are not present to the same extent in Loskop Dam. Presence of suitable habitat and habitat degradation has been shown to influence populations of intermediate mollusc hosts resulting in declines in heteroxenous parasites like trematode metacercariae (Dzikowski *et al*. 2003(b)). These authors found that reduced lake levels associated with drought and increasing abstraction eliminated habitats that previously supported large populations of *Bulinus* sp. snails. No comprehensive surveys were conducted for snails at Loskop Dam during this study, but there are several records of snails from the dam between 1959 and 1978 in the national collection of freshwater snails at the North-West University (*Bulinus physopsis, B. depressus, Biomphalaria pfeifferi, Lymnaea natalensis, L. columella, Physa acuta*). Therefore the habitat must have been suitable for snails at that stage.

After 6 weeks of exposure during the biomonitoring study, parasite communities changed with increased dominance by *Enterogyrus* and *Cichlidogyrus*, the colonisation of *Ergasilus* and the retention of the digenean sp. in the Loskop cage. Reasons for the high mortalities in the biomonitoring cages are not known as they were ongoing throughout the exposure period, but stress due to confinement may

have been a factor. The monogeneans *Cichlidogyrus* and *Enterogyrus* showed a large increase in infection rates from pre-existing levels at Tompi Seleka during the active biomonitoring period in cages. Monogeneans have a monoxenous life cycle and rely on a fish host to survive (Shoemaker *et al*., 2006). They are often highly host-specific and both *Cichlidogyrus* and *Enterogyrus* are parasitic on fish in the Cichlidae family (Paperna, 1996). The increased abundance of *Cichlidogyrus* on the caged fish may have negatively impacted on their health as they can cause damage at the site of attachment especially if they occur in large numbers (Zajac & Conboy, 2006). Heavy infections with *Cichlidogyrus* have been reported in cichlids kept in confined ponds (Paperna, 1996) and elevated infection levels of *Cichlidogyrus* were observed when *Oreochromis* spp. were injected with hydrocortisone confirming a link between infection levels and stress (Shaharom-Harrison, 1987).

In summary the results of this study show that while the richness and evenness of parasite species at Loskop Dam was relatively high, the very low infection rates coupled with high ratios of monoxenous to heteroxenous species were indicative of environmental degradation.

Chapter 5

Concluding remarks, further research and management recommendations for Loskop Dam

5.1 Conclusions

The three main research questions addressed in individual chapters of this study were:

1) What were the links between water chemistry and limnology of Loskop Dam, and the health of pansteatitis-affected *Oreochromis mossambicus*?
2) Was bioaccumulation of Al, Fe, Mn, Zn, Cu or Se taking place in any of the tissues measured from *O. mossambicus* and could this be related to pansteatitis?
3) Did the metazoan parasite communities associated with *O. mossambicus* serve as effective indicators of ecosystem health in Loskop Dam?

These questions were posed in response to increasing fish and crocodile mortalities at Loskop Dam and their conclusions have helped provide a foundation from which to formulate further research questions. In summary, the water chemistry of Loskop Dam was strongly influenced by algal blooms of *Ceratium hirundinella* and *Microcystis* sp. as a consequence of eutrophication. The alkaline conditions caused by these blooms particularly in the transitional zone (median pH 9.67) of the dam have important implications for fish health. The bio-availability of metals, especially Al, is influenced by pH with increasing solubility above pH 8 which may explain the high Al levels in the gills of natural mortalities of *O. mossambicus* in the transitional zone. While this may not be related to the development of pansteatitis, it may be the final physiological insult to a pansteatitis affected individual causing death. Elevated pH also causes an increase in toxic ammonia (NH_3) which would constitute 64% of the total ammonia given the pH and temperatures observed in this study, creating a further physiological stressor for fish. A further consequence of increasing algal blooms is an altered food web as the bloom represents dominance by singular primary producers. The conspicuous lack of digenean parasites on the fish may also

have been related to fluctuating pH levels and associated water chemistry changes as they are sensitive to metal exposure and pH changes. The extremely low parasite infection rates of *O. mossambicus* at Loskop Dam as well as the high ratio of monoxenous to heteroxenous species were indicative of environmental degradation and were therefore good indicators of ecosystem health. While there was little evidence of substantial metal accumulation in the fish tissues measured, the Cu deficient status of the fish livers is cause for concern due to the important role Cu plays as a co-factor in the antioxidant enzyme superoxide dismutase. Unfortunately no single factor that may be responsible for causing pansteatitis in *O. mossambicus* was outstanding in the water chemistry or bioaccumulation results. It is likely that the condition is caused by a complex combination of factors with primary or secondary effects leading to obesity and lipid peroxidation. Historical acute fish kill events were probably related to H_2S poisoning, the production of which was exacerbated by increasing SO_4^{2-} levels and decomposing organic matter from dying algal blooms particularly in the main basin of the dam where oxygen deficient conditions dominate through winter months. The elevated SO_4^{2-} levels in Loskop Dam confirmed that drainage originating from coal mines was still entering the dam although acidic conditions were not observed in this study. It is possible that the aquatic ecosystem is more vulnerable during periods of drought when dilution is reduced and mine drainage may become a more dominant impact.

5.2 Further research recommendations

Pansteatitis was first reported in Mink and the cause was attributed to a diet high in linolenic acid (polyunsaturated fat) and low in vitamin E (Lalor *et al*., 1951). Since then the condition has been described in several species including horses, domestic cats, pigs, turtles, alligators, herons, tuna, dogs and rainbow trout. In most cases the animals were farmed or kept in captivity and fed formulated diets which were found to be deficient in antioxidants like vitamin E and selenium, and rich in polyunsaturated fats prone to lipid peroxidation. Ultimately the condition represents an imbalance between antioxidant defence systems and free radical damage (Halliwell, 2006).

The question of whether this imbalance occurs as a direct result of the diet of *O. mossambicus* in Loskop Dam needs to be addressed. Further research should determine the main dietary components and their dietary value compared to recommended levels and covering seasonal variation. This should include an investigation into the metal concentrations in the main dietary items as bioaccumulation at a primary production level has not been excluded in Loskop Dam and the large blooms of algae may well be acting as a sink for soluble metals entering the dam thus entering the food chain on a trophic basis. This may explain the Cu deficiency as dietary antagonists may be responsible for reduced Cu uptake.

The antioxidant status of the fish needs to be determined in order to confirm what level of defence is under pressure. This should include an evaluation of vitamin E, superoxide dismutase and glutathione peroxidase levels in the appropriate tissue. Furthermore the oxidative stress levels should be quantified by measuring the product of lipid peroxidation, malondialdehyde, in the liver and fat deposits of the fish along with H_2O_2 levels.

The source of obesity in the fish needs to be determined, and while a complete dietary analysis will go some way towards establishing whether diet alone is a factor, it would be useful to determine whether there is evidence of endocrine disruption occurring. To this end, levels of T_3 and T_4 thyroid hormones should be measured in the plasma as they are involved in regulating metabolic rates and their activity can be affected by selenium status of the fish.

A more detailed study on the limnology of Loskop Dam needs to undertaken including spatio-temporal variation of water chemistry across the dam. This should involve depth sampling at each site, not just the dam wall and include water chemistry samples from the entire water column at each site, not just the surface. Analyses of metals in future water chemistry samples should be conducted after filtration using 45 μm filters to ensure that only the dissolved fraction is reported and can subsequently be compared to DWA guidelines.

An appropriate reference site should be obtained that is more comparable with Loskop Dam. Water quality need not be the determining factor in selection of the site, rather it should be a reservoir of similar size on the Olifants River with the key difference being that pansteatitis is not present in the fish or crocodile populations which are robust and healthy. One recommendation would be the use of Flag Boshielo Dam located downstream of Loskop Dam which has no reports of pansteatitis and a large population of crocodiles. While the dam may not be considered unimpacted, the key difference is that pansteatitis has not been recorded there despite being located on the Olifants River system and this would be the main selection criteria.

5.3 Management recommendations

One of the greatest challenges encountered when attempting to determine causes of historical fish kills was the lack of data collected when the fish kill occurred. During the course of this study several members of the public also reported fish kills in various tributaries of the Olifants River but no conclusions could be made from them as they were reported after the incident occurred. The first recommendation would be to establish a standardised 'fish kill response pack' with detailed instructions on the information and samples that need to be collected at the impacted site. This would include information such as methods of collection, important contacts and information that should be recorded. Additional items could be included such as data sheets, pH strips, buffered formalin and sample containers. These could be distributed to land owners and managers in the upper Olifants River catchment and to managers at Loskop Dam. This would provide a more co-ordinated response to pollution events, empower and involve local stakeholders and ensure valuable data is collected to inform future research.

The ongoing eutrophication of Loskop Dam needs to be urgently addressed. It is clear from the DWA Green Drop report that the status of most wastewater treatment works in the upper Olifants River catchment is very poor and the discharge of partially and untreated sewerage is unacceptably high. The specific reasons for this state of affairs are largely unknown but probably relate to a lack of infrastructure and municipal funding, poor maintenance and lack of skilled labour. These are all

challenges beyond the scope of management recommendations from this report. However, these challenges must urgently be tackled by those responsible if we are to prevent Loskop Dam from becoming permanently eutrophic with year-round blooms of *Microcystis* spp. This would have dire consequences for the aquatic ecosystem as well as the agricultural sector reliant on irrigation water from Loskop Dam.

Drainage associated with operational and particularly historic coal-mines also requires urgent attention. The Klipspruit in particular has been an ongoing and well documented source of acid mine drainage into the Olifants River as early as the 1950's. From the photographs presented in this study it is clear that the problem is ongoing and little is being done to resolve it. Again, the specific management solutions are beyond the scope of this report as they involve the collection and treatment of water from abandoned mines in the catchment at vast expense. However it is irresponsible to allow the situation to continue unchecked for another 50 years at the expense of the integrity of the aquatic ecosystem and all of the associated services upon which our society is dependent.

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Appendix 1

Physical and chemical parameters measured at Kranspoort Dam during 2010.

Parameter	Units	TWQR	10 Mar	15 Mar	23 Apr	4 May	15 Jul	13 Aug	31 Aug	16 Sep	29 Sep	6 Oct	12 Oct	28 Oct	5 Nov	15 Nov	9 Dec
Conductivity	µs/cm		57.1			45.5	54.8	57.5	57.9	61.4	65.6	67.4	67.5	72.9	78.5	81.9	72.8
pH		7.27–8.03	7.2	7.06	**6.94**	**8.9**	7.94	7.77	7.77	**8.71**	7.54		7.6	7.72	7.7	7.42	7.24
Dissolved Oxygen	mg l^{-1}		7.12	6.68	7.69		8.74	8.55	8.14	6.87	7.26	9.03	6.87	6.06	6.14	6.46	7.07
O_2 saturation	%	80 - 120							96.8	91	89.1	118.5	95	82.6	86.7	**69.5**	94.6
Temperature	^{0}C		27.8	25.4	20.1	18.2	12.6	13.8	18	21	20.3	23.4	24.4	23.2	25.8	25.6	25.5
Secchi Depth	cm						90	76		65	66	93	69	58	63	62	79
Potassium (K)	mg l^{-1}			0.9			1.2	1.1		1.1	1		0.9	1.1		1.7	1.5
Sodium (Na)	mg l^{-1}			4			5	5		5	5		5	8		6	5
Calcium (Ca)	mg l^{-1}			4			3	3		4	5		5	5		6	5
Magnesium (Mg)	mg l^{-1}			2			2	2		2	3		3	3		4	3
Ammonia (N)	mg l^{-1}	< 0.007		0.3			ND	ND		1.6	ND		ND	ND		ND	ND
Sulphate (SO4)	mg l^{-1}			2.5			4	4		2.5	2.5		3	2		3	4
Chloride (Cl)	mg l^{-1}			8			8	8		8	7		7	7		9	8
Alkalinity ($CaCO_3$)	mg l^{-1}			20			20	16		20	24		28	32		24	24
Nitrite (N)	µg l^{-1}			ND			ND	ND		ND	ND		ND	ND		ND	ND
Nitrate (N)	µg l^{-1}			2.5			400	350		50	1340		90	900		400	160
Total Kjeldahl Nitrogen	µg l^{-1}			2800			600	800		9000	900		1100	1100		1700	1400
Total Nitrogen	mg l^{-1}			2.8			1	1.1		9	2.2		1.1	2		2.1	1.5
Ortho phosphate	µg l^{-1}			3.6			8.1	14.8		2.5	2,9		8.3	7,9		3.1	21.3
Total Phosphate	µg l^{-1}			2.5			37.5	59.9		39.9	57.8		47.5	30,1		23.2	70
Fluoride (F)	mg l^{-1}	$\leq$ 0.75		0.1			0.5	0.5		0.2	0.3		0.3	0.2		0.1	0.2
Silica (Si)	mg l^{-1}			3.3			3.6	3.6		3.9	3.7		3	2,8		3.6	3.3
Hardness ($CaCO_3$)	mg l^{-1}			18.2			16	15.7		18.2	24.8		24	24.8		31.4	28
Aluminium (Al)	mg l^{-1}	$\leq$ 0.01*		0.184			0.099	0.129		0.198	0.223		0.064	0.03		0.049	0.018
Boron (B)	mg l^{-1}			0.002			0.025	0.002		0.002	0.002		0.002	0.008		0.008	0.008
Iron (Fe)	mg l^{-1}			0.314			0.304	0.409		0.694	0.46		0.564	0.097		0.275	0.149
Manganese (Mn)	mg l^{-1}	0.18*		0.006			0.007	0.022		0.034	0.024		0.045	0.002		0.021	0.01
Zinc (Zn)	mg l^{-1}	$\leq$ 0.002*		0.025			0.025	0.025		0.006	0.025		0.025	0.025		0.025	0.025
Copper (Cu)	mg l^{-1}	$\leq$ 0.001*		ND			ND	ND		ND	0.003		ND	ND		ND	ND
Selenium (Se)	mg l^{-1}	0.002*		ND			ND	ND		ND	ND		ND	ND		ND	ND
Vanadium as V	mg l^{-1}			0.041			ND	ND		ND	ND		ND	ND		ND	ND

* TWQRs for metals are based on dissolved concentrations and therefore a comparison to these results was not possible as samples were not filtered prior to analysis. One sample was collected from Tompi Seleka (TS) aquaculture facility in September 2010. Where possible results are compared to the South African Water Quality Guidelines for Ecosystem Health Target Water Quality Range (TWQR) and figures are presented in bold where these have been exceeded. ND = not detected.

Physical and chemical parameters measured at LK1 (Riverine zone) in Loskop Dam during 2010.

Parameter	Units	TWQR	15 Jul	13 Aug	31 Aug	8 Sep	14 Sep	29 Sep	5 Oct	12 Oct	22 Oct	28 Oct	5 Nov	15 Nov	9 Dec
Conductivity	µs/cm		466	498	460	488	526	557	547	540	586	610	632	625	506
pH		7.27–8.03	**8.17**	7.5	7.92	7.78	7.97	7.99	**8.51**	7.98	7.83	**8.37**	7.81	7.96	7.56
Dissolved Oxygen	mg l^{-1}		9.71	9.25	8.21	7.85	7.69	8.01	8.72	7.32	6.83	8.2	6.73	7.42	7.7
O_2 saturation	%	80 - 120			97.3	97.2	98.5	98.9	118.7	104.2	89.5	111.9	92.4	99.3	106.4
Temperature	^{0}C			12.4	18.3	20.3	23.4	21.3	34.8	27.5		25.4	25.7	25	25.6
Secchi Depth	cm		170	256		187	163	152		113		119	119	73	75
Potassium (K)	mg l^{-1}		4.4	4.6			5.9	5.9		5.3		5.8		5.9	5.9
Sodium (Na)	mg l^{-1}		26	28			33	37		38		47		35	25
Calcium (Ca)	mg l^{-1}		33	35			40	40		41		44		51	37
Magnesium (Mg)	mg l^{-1}		21	22			22	22		21		22		24	20
Ammonia (N)	mg l^{-1}	< 0.007	ND	ND			ND	ND		ND		ND		ND	ND
Sulphate (SO4)	mg l^{-1}		160	159			176	144		141		221		166	160
Chloride (Cl)	mg l^{-1}		18	19			21	23		24		26		23	18
Alkalinity ($CaCO_3$)	mg l^{-1}		44	40			40	36		40		48		36	36
Nitrite (N)	µg l^{-1}		2.5	2.5			2.5	2.5		2.5		2.5		2.5	2.5
Nitrate (N)	µg l^{-1}		3090	3210			3650	3230		2410		2540		3820	4260
Total Kjeldahl Nitrogen	µg l^{-1}		600	1100			600	4700		3400		2800		2800	1400
Total Nitrogen	mg l^{-1}		3.6	4.3			4.2	7.9		5.8		5.3		6.6	5.6
Ortho phosphate	µg l^{-1}		12.9	17.7			2.9	2.5		11.8		5.6		52.2	61.4
Total Phosphate	µg l^{-1}		30.3	24.9			64.6	29.7		42.1		26.8		82.8	113.5
Fluoride (F)	mg l^{-1}	$\leq$ 0.75	0.7	0.7			0.3	0.3		0.3		0.2		0.2	0.3
Silica (Si)	mg l^{-1}		0.5	0.2			1	0.4		0.8		1,6		2.7	3.3
Hardness ($CaCO_3$)	mg l^{-1}		169	180			190.4	190.4		188		200.4		226.2	174.7
Aluminium (Al)	mg l^{-1}	$\leq$ 0.01*	0.087	0.046			0.055	0.061		0.041		0.033		0.096	0.233
Boron (B)	mg l^{-1}		0.006	0.002			0.034	0.046		0.002		0.074		0.019	0.057
Iron (Fe)	mg l^{-1}		0.038	0.034			0.051	0.06		0.086		0.013		0.097	0.294
Manganese (Mn)	mg l^{-1}	0.18*	0.033	0.025			0.055	0.017		0.068		0.002		0.091	0.207
Zinc (Zn)	mg l^{-1}	$\leq$ 0.002*	ND	ND			ND	ND		ND		ND		ND	ND
Copper (Cu)	mg l^{-1}	$\leq$ 0.001*	ND	ND			ND	0.004		ND		ND		ND	ND
Selenium (Se)	mg l^{-1}	0.002*	ND	ND			ND	0.006		ND		0.005		ND	ND
Vanadium (V)	mg l^{-1}		ND	ND			ND	ND		ND		ND		ND	ND

* TWQRs for metals are based on dissolved concentrations and therefore a comparison to these results was not possible as samples were not filtered prior to analysis. One sample was collected from Tompi Seleka (TS) aquaculture facility in September 2010. Where possible results are compared to the South African Water Quality Guidelines for Ecosystem Health Target Water Quality Range (TWQR) and figures are presented in bold where these have been exceeded. ND = not detected.

Physical and chemical parameters measured at LK2 (Transitional zone) in Loskop Dam during 2010.

Parameter	Units	TWQR	15 Jul	13 Aug	31 Aug	8 Sep	14 Sep	29 Sep	5 Oct	12 Oct	18 Oct	22 Oct	28 Oct	5 Nov	15 Nov	9 Dec
Conductivity	µs/cm		390	422	415	421	442	451	461	482	456	452	444	517	492	468
pH		7.27–8.03	**8.07**	**8.4**	**9.7**	**9.62**	**9.13**	**9.52**	**9.96**	**9.84**	**9.84**	**9.75**	**9.66**	**9.68**	**9.19**	**9.76**
Dissolved Oxygen	mg l^{-1}		8.01	9.39	14.5	12.43	7.85	9.92	12.31	10.09	11.47	11.34	11.61	12.11	8.22	12.72
O_2 saturation	%	80 - 120			**180.5**	**157.3**	102.6	**127.9**	**169.8**	**152**	**150.2**	**151.8**	**157.4**	**170.5**	111.3	**165.2**
Temperature	^{0}C		15.8	15.6	20.5	21.1	22.4	22.6	25.2	26.7	23.5	24.3	24.8	27.9	25.7	28.5
Secchi Depth	cm		160	123		186	287	127		180			85	41	167	178
Potassium (K)	mg l^{-1}		4.8	4.7			5.1	5.2		5.1			5		5.4	4.9
Sodium (Na)	mg l^{-1}		20	22			25	26		28			26		30	23
Calcium (Ca)	mg l^{-1}		29	30			33	32		34			31		37	36
Magnesium (Mg)	mg l^{-1}		19	19			20	21		19			19		21	20
Ammonia (N)	mg l^{-1}	< 0.007	ND	0.4			ND	ND		ND			0.5		ND	ND
Sulphate (SO4)	mg l^{-1}		119	170			122	117		120			113		101	142
Chloride (Cl)	mg l^{-1}		16	16			18	20		21			19		20	18
Alkalinity ($CaCO_3$)	mg l^{-1}		56	32			44	48		48			64		48	44
Nitrite (N)	µg l^{-1}		2.5	2.5			2.5	2.5		2.5			2.5		2.5	2.5
Nitrate (N)	µg l^{-1}		1130	1320			1810	1180		1030			680		590	680
Total Kjeldahl Nitrogen	µg l^{-1}		1700	2500			1100	3700		2800			4500		4500	1400
Total Nitrogen	mg l^{-1}		2.8	3.8			2.9	4.8		3.8			5.1		5	2
Ortho phosphate	µg l^{-1}		11.5	34.9			3.2	13.3		<2.5			13.1		24.4	13
Total Phosphate	µg l^{-1}		32.2	63.3			42.6	36.6		46.4			48.5		31.8	47.8
Fluoride (F)	mg l^{-1}	≤ 0.75	0.8	0.7			0.3	0.3		0.3			0.2		0.2	0.3
Silica (Si)	mg l^{-1}		2.3	1.8			0.8	0.1		0.2			0.4		1	1.4
Hardness ($CaCO_3$)	mg l^{-1}		151	153			164.7	166.3		163			155.6		178.8	172.2
Aluminium (Al)	mg l^{-1}	≤ 0.01*	0.014	0.019			0.02	0.027		0.015			0.012		0.031	0.02
Boron (B)	mg l^{-1}		0.002	0.002			0.023	0.029		0.002			0.043		0.008	0.034
Iron (Fe)	mg l^{-1}		0.015	0.073			0.023	0.027		0.046			0.004		0.02	0.026
Manganese (Mn)	mg l^{-1}	0.18*	0.009	0.081			0.009	0.006		0.019			0.001		0.008	0.013
Zinc (Zn)	mg l^{-1}	≤ 0.002*	ND	ND			ND	ND		ND			ND		ND	ND
Copper (Cu)	mg l^{-1}	≤ 0.001*	ND	ND			ND	ND		ND			ND		ND	ND
Selenium (Se)	mg l^{-1}	0.002*	ND	ND			ND	0.004		ND			0.003		ND	ND
Vanadium (V)	mg l^{-1}		ND	ND			ND	ND		ND			ND		ND	ND

* TWQRs for metals are based on dissolved concentrations and therefore a comparison to these results was not possible as samples were not filtered prior to analysis. One sample was collected from Tompi Seleka (TS) aquaculture facility in September 2010. Where possible results are compared to the South African Water Quality Guidelines for Ecosystem Health Target Water Quality Range (TWQR) and figures are presented in bold where these have been exceeded. ND = not detected.

Physical and chemical parameters measured at LK3 (Lacustrine zone) in Loskop Dam during 2010.

Parameter	Units	TWQR	15 Jul	13 Aug	31 Aug	8 Sep	14 Sep	29 Sep	5 Oct	12 Oct	22 Oct	28 Oct	5 Nov	15 Nov	9 Dec
Conductivity	µs/cm		388	393	395	397	410	412	411	415	419	419	427	430	438
pH		7.27–8.03	7.86	8.2	**9.15**	**9.44**	**9.49**	**9.41**	**9.51**	**9.58**	**9.21**	**9.34**	**9.26**	**9**	**9.1**
Dissolved Oxygen	mg l^{-1}		7.45	8.02	11.65	12.23	10.83	9.07	9.97	8.39	8.06	8.53	8.2	6.46	8.79
O_2 saturation	%	80 - 120			**139.3**	**151.6**	**135.8**	115	**134.4**	115.4	108	114.2	110.5	86.6	114.2
Temperature	^{0}C		15.6	16.5	18.7	19.8	20.9	22		24.5	24.2	24.5	25	25.5	25.8
Secchi Depth	cm		185	264		215	310	251		224		201	207	266	244
Potassium (K)	mg l^{-1}		4.8	4.8			5	5		5		5.5		5	4.9
Sodium (Na)	mg l^{-1}		19	20			22	22		23		44		24	21
Calcium (Ca)	mg l^{-1}		28	28			30	30		33		41		33	31
Magnesium (Mg)	mg l^{-1}		19	19			19	20		19		21		20	20
Ammonia (N)	mg l^{-1}	< 0.007	0.2	0.2			0.2	0.2		0.2		0.2		0.2	0.2
Sulphate (SO4)	mg l^{-1}		110	117			118	102		133		178		125	129
Chloride (Cl)	mg l^{-1}		16	16			17	17		18		25		17	18
Alkalinity ($CaCO_3$)	mg l^{-1}		60	56			56	52		52		44		52	56
Nitrite (N)	µg l^{-1}		2.5	2.5			2.5	2.5		2.5		2.5		2.5	2.5
Nitrate (N)	µg l^{-1}		970	1110			960	1360		1040		2510		580	430
Total Kjeldahl Nitrogen	µg l^{-1}		1700	2200			600	2800		1100		3400		3400	1400
Total Nitrogen	mg l^{-1}		2.6	3.3			1.5	4.1		2.1		5.9		3.9	1.8
Ortho phosphate	µg l^{-1}		8.4	21.6			2.2	2.5		2.5		2.5		4.7	43.2
Total Phosphate	µg l^{-1}		23.9	34.6			30.3	24.5		34.5		30		5.9	84.9
Fluoride (F)	mg l^{-1}	$\leq$ 0.75	0.8	0.7			0.3	0.3		0.3		0.2		0.2	0.3
Silica (Si)	mg l^{-1}		2.7	2.3			1.6	0.6		0.2		1.4		0.6	0.1
Hardness ($CaCO_3$)	mg l^{-1}		148	148			153.1	157.2		160		188.8		164.7	159.7
Aluminium (Al)	mg l^{-1}	$\leq$ 0.01*	0.01	0.008			0.009	0.015		0.006		0.012		0.009	0.019
Boron (B)	mg l^{-1}		0.002	0.002			0.017	0.023		0.002		0.051		0.008	0.036
Iron (Fe)	mg l^{-1}		0.011	0.012			0.006	0.009		0.016		0.006		0.009	0.007
Manganese (Mn)	mg l^{-1}	0.18*	0.006	0.02			0.002	0.002		0.003		0.003		0.002	0.007
Zinc (Zn)	mg l^{-1}	$\leq$ 0.002*	ND	ND			ND	ND		ND		ND		ND	ND
Copper (Cu)	mg l^{-1}	$\leq$ 0.001*	ND	ND			ND	ND		ND		ND		ND	ND
Selenium (Se)	mg l^{-1}	0.002*	ND	ND			ND	ND		ND		0.004		ND	0.003
Vanadium (V)	mg l^{-1}		ND	ND			ND	ND		ND		ND		ND	ND

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Physical and chemical parameters measured at LK4 (Lacustrine zone, dam wall) in Loskop Dam during 2010.

Parameter	Units	TWQR	15 Jul	13 Aug	31 Aug	14 Sep	29 Sep	8 Oct	12 Oct	22 Oct	28 Oct	5 Nov	15 Nov	9 Dec
Conductivity	µs/cm		388	388	396	399	403	412	407	416		415	417	442
pH		7.27–8.03	7.93	7.94	**8.99**	**9.27**	**9.14**	**9.62**	**9.62**	**9.21**		**9.16**	**8.93**	**9.12**
Dissolved Oxygen	mg l^{-1}		7.79	7.57	10.08	11.51	7.8	10.2	9.46	8.08		7.88	6.38	8.84
O_2 saturation	%	80 - 120			**122.6**	**140.6**	97.8	**136**	**129.3**	105.3		106.4	86.3	**121**
Temperature	^{0}C		15.8	16.1	20.5	19.6	20.4	24.7	25	23.2		25.2	25.6	25.3
Secchi Depth	cm		240	285		297	299	379	243			279	371	300
Potassium (K)	mg l^{-1}		4.8	4.7		5	5		5.1		4.9		5.1	4.8
Sodium (Na)	mg l^{-1}		19	20		21	21		23		25		23	20
Calcium (Ca)	mg l^{-1}		28	28		29	29		35		30		33	30
Magnesium (Mg)	mg l^{-1}		19	18		20	20		21		19		21	20
Ammonia (N)	mg l^{-1}	< 0.007	0.2	0.2		0.2	0.2		0.2		0.2		0.2	0.2
Sulphate (SO4)	mg l^{-1}		113	105		122	102		97		138		104	122
Chloride (Cl)	mg l^{-1}		16	15		17	17		17		17		17	17
Alkalinity ($CaCO_3$)	mg l^{-1}		60	56		60	52		56		56		56	52
Nitrite (N)	µg l^{-1}		2.5	2.5		2.5	2.5		2.5		2.5		2.5	2.5
Nitrate (N)	µg l^{-1}		1010	1040		1010	950		880		120		580	450
Total Kjeldahl Nitrogen	µg l^{-1}		1100	1100		1700	4700		3400		1700		1100	4200
Total Nitrogen	mg l^{-1}		2.1	2.1		2.7	5.6		5.2		1.8		1.6	4.6
Ortho phosphate	µg l^{-1}		2.9	17.3		1.25	1.25		2.5		2.5		1.3	19.1
Total Phosphate	µg l^{-1}		28.3	33.7		28.4	25.2		26.4		42,3		2.4	43.5
Fluoride (F)	mg l^{-1}	$\leq$ 0.75	0.8	0.7		0.3	0.3		0.3		0.2		0.2	0.3
Silica (Si)	mg l^{-1}		2.8	2.4		1.9	1.1		0.2		0.4		0.5	0.1
Hardness ($CaCO_3$)	mg l^{-1}		148	144		154.7	154.7		173		153.1		168.8	157.2
Aluminium (Al)	mg l^{-1}	$\leq$ 0.01*	0.008	0.004		0.009	0.017		0.006		0.006		0.012	0.012
Boron (B)	mg l^{-1}		0.002	0.002		0.014	0.019		0.002		0.023		0.008	0.035
Iron (Fe)	mg l^{-1}		0.005	0.009		0.005	0.015		0.009		0.004		0.007	0.012
Manganese (Mn)	mg l^{-1}	0.18*	0.005	0.011		0.002	0.004		0.002		0.002		0.003	0.003
Zinc (Zn)	mg l^{-1}	$\leq$ 0.002*	ND	ND		ND	ND		ND		ND		ND	ND
Copper (Cu)	mg l^{-1}	$\leq$ 0.001*	ND	ND		ND	ND		ND		ND		ND	ND
Selenium (Se)	mg l^{-1}	0.002*	ND	ND		ND	0.004		ND		ND		ND	0.003
Vanadium (V)	mg l^{-1}		ND	ND		ND	ND		ND		ND		ND	ND

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Physical and chemical parameters measured at Tompi Seleka during 2010.

Parameter	Units	TWQR	14 Sep	23 Sep
Conductivity	µs/cm		276	210.9
pH		7.27–8.03	**8.6**	**9.27**
Dissolved Oxygen	mg l^{-1}		9.6	10.5
O_2 saturation	%	80 - 120	117.8	
Temperature	^{0}C		20	24
Potassium (K)	mg l^{-1}		2.5	
Sodium (Na)	mg l^{-1}		29	
Calcium (Ca)	mg l^{-1}		10	
Magnesium (Mg)	mg l^{-1}		4	
Ammonia (N)	mg l^{-1}	< 0.007	ND	
Sulphate (SO4)	mg l^{-1}		17	
Chloride (Cl)	mg l^{-1}		18	
Alkalinity ($CaCO_3$)	mg l^{-1}		60	
Nitrite (N)	µg l^{-1}		ND	
Nitrate (N)	µg l^{-1}		270	
Total Kjeldahl Nitrogen	µg l^{-1}		600	
Total Nitrogen	mg l^{-1}		0.8	
Ortho phosphate	µg l^{-1}		9.2	
Total Phosphate	µg l^{-1}		46	
Fluoride (F)	mg l^{-1}	$\leq$ 0.75	0.9	
Silica (Si)	mg l^{-1}		15.1	
Hardness ($CaCO_3$)	mg l^{-1}		41.4	
Aluminium (Al)	mg l^{-1}	$\leq$ 0.01*	0.457	
Boron (B)	mg l^{-1}		0.024	
Iron (Fe)	mg l^{-1}		1.13	
Manganese (Mn)	mg l^{-1}	0.18*	0.084	
Zinc (Zn)	mg l^{-1}	$\leq$ 0.002*	ND	
Copper (Cu)	mg l^{-1}	$\leq$ 0.001*	ND	
Selenium (Se)	mg l^{-1}	0.002*	ND	
Vanadium (V)	mg l^{-1}		ND	

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