A COMPARATIVE STUDY OF THE HEALTH OF REDNOSE LABEO BASED ON THE QUANTITATIVE HEALTH ASSESSMENT INDEX, BIOACCUMULATION AND HISTOPATHOLOGY IN THE OLIFANTS RIVER

by

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DISSERTATION

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DECLARATION

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

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J Lebepe Date

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ABSTRACT

The Olifants River System is one of the most polluted river systems in Southern Africa. The Olifants River catchment is characterized by large-scale mining, power generation, heavy industry and inadequate domestic sewage treatment. Fish and crocodile kills have become commonplace over the past decade, especially in the upper catchment and Kruger National Park. The present study was carried out at Loskop and Flag Boshielo dams, two major impoundments in the Olifants River. Water and sediment samples were collected at each dam during winter (July 2011) and summer (November 2011), frozen and sent to an accredited water lab for chemical analysis. Atleast fifteen fish specimens from each dam were collected during each survey using gill nets. To evaluate the health of red-nose labeo, (*Labeo rosae*) in the two dams, fish organs were assessed macroscopically using the fish Health Assessment Index (HAI) protocol and a section of liver tissue and gills were dissected out, frozen and sent to an accredited water lab for metal analysis. The remainder liver samples were fixed in 10% buffered formalin and sent to University of Pretoria Pathology Laboratory for histopathological analysis. The concentration of nutrients and ions were higher at Loskop Dam whereas most metal concentrations were found to be higher at Flag Boshielo Dam. The present study categorised Flag Boshielo Dam as oligotrophic with Loskop Dam being mesotrophic. The concentration of aluminium, copper, antimony, iron, lead, selenium and strontium was higher at Flag Boshielo Dam with manganese, silica and zinc being higher at Loskop Dam. Although the concentrations of these toxic constituents varied, there were no significant differences between localities ($p>0.05$). The constituents that showed high concentrations in sediment were iron and aluminium. The general trend of accumulation in sediment was as follows: Fe > Al > Si > Mn > Zn > Cu > Sb > Sr > Pb > Se at Loskop Dam whereas at Flag Boshielo Dam was as follows: Fe > Al > Mn > Si > Zn > Cu > Sr > Pb > Sb > Se. The liver generally accumulated higher concentrations of metals than the other tissue. General trend of liver > gills > muscle was reported for Al, Cu, Fe, Pb, Sb, Se, Si and Zn with gills > liver > muscle trend being reported for Mn and Sr at both localities. Macroscopic abnormalities were observed for some gills and liver at both localities. Parasite (*Lernaea cyprinacea*) induced lesions on the skin and mild erosion on the tail fin of some fish were recorded at Flag Boshielo Dam. Most of the histopathological alterations were common at both localities but hydropic glycogen and hyaline droplets were observed only at Loskop Dam, with haemosiderin being observed at Flag Boshielo Dam. Both quantitative HAI results and histopathology have shown that the fish population from Flag Boshielo Dam are in better condition/health than the population from Loskop Dam. There might be a correlation between the nutrient levels and fish health. The overall ecological state is better at Flag Boshielo Dam than Loskop Dam.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND INFORMATION

Freshwater ecosystems are the most threatened ecosystems in South Africa suffering from over-extraction of water, flow regulation and the most common problem, pollution. Water pollution can be defined as the altering of features of water to the detriment of its users or inhabitants (Davies & Day 1998). The adverse effects of all these are the decline of water quality and availability, loss of native species and change in the distribution and structure of the aquatic biota (Dallas & Day 2004). The Olifants River System is currently one of the most threatened river systems in South Africa. This river has been used and abused for the past five decades, and pollution is getting progressively worse. This can be seen in the character of the water quality, which has worsened markedly over the years (Van Vuuren 2009).

In South Africa, water quality management mainly focused on measuring chemical and physical water parameters. Dallas (2000) suggested that aquatic biota and physico-chemical monitoring should actually be viewed as complementary. Physico-chemical analysis can give very accurate measure of the amount of individual substances in the water of a river, but they only consider the water passing at the moment of collection; they are thus instant ‘snapshots’ of the environment (Davies & Day 1998). A biomonitoring programme, the River Health Programme (RHP) were thus developed in 1997 in order to incorporate several components of the biota, including macro-invertebrates, fish and riparian vegetation with the physico-chemical components (Dallas 2000). Although biomonitoring is less detailed, it provides a bigger picture of both the past and the present conditions of a river. This is because the organisms that live in the river must have been able to survive whatever chemical and physical conditions the river has been subjected to in the recent past (Davies & Day 1998; Palmer *et al.* 2004).

In essence, biomonitoring is a scientific procedure which uses the aquatic biota to monitor the ecological state of an aquatic ecosystem (Dallas 2000; Van Dyk 2003b). Furthermore, biomonitoring is used to provide resource information by repetitive measurement, continued observation and evaluation of environmental data (Van Dyk 2003b). Two distinct approaches of biomonitoring are stressor and response monitoring. Stressor monitoring focuses on the stressors that are assumed to be associated with the cause of pollution and ecological change whereas response monitoring refers to the monitoring of bioindicators (including biomarkers) in order to characterize the response of the environment to disturbances (Davies & Day 1998; Palmer *et al.* 2004).

Bioindicators are organisms or communities that can integrate and reflect the effects of chemical and physical parameters over an extended period of time (Palmer *et al.* 2004). Bioindicators can be subdivided into two groups; effect indicator and accumulation indicator (Sures 2001). Several indices were developed to monitor the health of aquatic ecosystems in South Africa e.g., Riparian Vegetation Index (RVI), South African Scoring System (SASS 5) and Biological Diatom Index (BDI) (Chutter 1998; Kemper 1999; De la Rey 2008). All these indices were based on the fact that different organisms have different tolerance levels. Palmer *et al.* (2004) further emphasized that, in any ecological sample collected from a specific ecosystem, the presence or absence of sensitive organisms, or simply a change in community composition, can indicate a change in water chemistry that may not be detected by the chemical data record.

Another bioindicator that has proved to be a successful monitoring tool is the health of fish population. Fish health as bioindicator were introduced by Adams *et al.* (1993) based on the assumption that the ecological health of an ecosystem can be reflected by the health of organisms that reside in that system. Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem (Van Dyk 2003a). Goede and Barton (1990) developed a field necropsy method that provides a health profile of fish based on the percentages of anomalies observed in the tissues and organs of individuals sampled from a population. The major limitation of this necropsy or health profile method was that it could not provide quantitative results that were amenable to statistical comparison of data among sites, species, or years. Adams *et al.* (1993) modified and refined the necropsy method to provide a quantitative index so that statistical comparisons can be made between data sets and the index termed quantitative Health Assessment Index (HAI) was developed.

This quantitative HAI entails the macroscopic examination of various fish organs. Scores are assigned to fish organs depending on the degree of abnormalities observed. The sum total of values awarded being the index value for that fish and the mean calculated for all fish in the sample being the index value for that locality. An increase in index value correlates with decreased water quality, and hence increased stress (Crafford & Avenant-Oldewage 2009). Other variable of HAI is the parasite load whereby scores are assigned based on the number of parasites recovered. In the original HAI parasites were recorded as present or absent. Based on the premise that contaminants have different influences on endo- and ectoparasites, they were incorporated as separate variables in the HAI tested in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001). The quantitative HAI has been successfully applied in the Olifants River system (Avenant-Oldewage *et al.* 1995) in South Africa. Crafford and Avenant-Oldewage (2009) further applied the macroscopic HAI in the Vaal River, and it has proved to be effective in distinguishing two localities known to differ with regard to water quality.

Furthermore, fish are located near, or at the top of the food chain and are known to bioaccumulate toxicants. Bioaccumulation is an increase in the concentration of a chemical in specific organs or tissues at a level higher than would normally be expected (Barbour *et al.* 1999; Van Dyk 2003a). The process of bioaccumulation repeat at each level of the food chain, with the toxins becoming more and more concentrated until the top organisms accumulate a lethal dose; the phenomenon called biomagnification (Davies & Day 1998). The metal concentration in fish organs may tell the concentration that the fish has been exposed to in the environment. Nevertheless, the organs with the ability to accumulate toxicants could be overwhelmed by an elevated concentration and could subsequently result in structural damages of cells and tissues of a target organ. Furthermore, the macroscopic abnormalities of a fish are the net results of microscopic abnormalities (histopathological alterations) occurring at the lower biological levels, i.e., cellular and tissue levels (Van Dyk 2003b). Therefore, the early toxic effects of pollution in fish may, however, be evident on cellular or tissue level before significant changes can be identified at the higher levels, i.e., fish organ or behaviour.

1.2 MOTIVATION

Heavy metal pollution in South Africa's aquatic ecosystems, especially river systems, is a major environmental concern. It is therefore necessary to investigate not only the possible organism and population response to heavy metal pollution, but also the underlying causes of the specific response, for example histological alteration or damage caused by the specific exposure (Van Dyk 2003b). The Olifants River is one of the country's hardest working rivers and very little information is available on the histology and histopathology of endemic fish species from this river (Van Vuuren 2010; Van Dyk 2003a). It is becoming unfortunate that the area which was supposed to offer sanctuary to organisms is now becoming dangerous to them (Van Vuuren 2010).

The Loskop and Flag Boshielo dams are both situated in the Olifants River System. The river has been subjected to prolonged and cumulative ecosystem stress as a result of anthropogenic activities, which is thought to have resulted in the death of aquatic biota (Van Vuuren 2009). Recent investigations indicated that many of the fish caught in the Olifants River show multi-organ pathology, presumably caused by poor water quality (Van Vuuren 2009). The scope of the current study is to investigate the ecological state of the two dams, the Loskop and Flag Boshielo dams based on the quantitative macroscopic HAI, microscopic HAI (histopathology) and bioaccumulation of selected metals in fish tissues.

The rednose labeo, (*Labeo rosae*) are commonly found in both dams. In 2006 Loskop Dam experienced its largest fish kills to date, with thousands upon thousands of indigenous fish being found dead along the shoreline of the dam (Fig. 1.1), including a big number of *Oreochromis mossambicus* for which Loskop Dam was famous in the past, as well as rednose labeo (Legrange 2007; Van Vuuren 2009).

Figure 1.1 Fish died at the shoreline of Loskop Dam (from Legrange 2007).

The upper catchment of the Olifants River is characterised by extensive rainfed cropping and stock farming, coal mining and coal-fired power generation (De Lange *et al.* 2003). Loskop Dam serves as a repository for pollutants from the upper catchment of the Olifants River System (Grobler *et al.* 1994). The middle Olifants River catchment comprised of an intensively irrigated area growing a variety of crops, with a trend towards permanent high value crops, including large citrus plantations and export table grape production under hail netting (De Lange *et al.* 2003). Therefore Flag Boshielo Dam is subjected to pollutants from agricultural activities. The lower Olifants River is characterised by game farms and industrial activities which are abundant at the Phalaborwa town. The tributaries such as the Ga-Selati River carry the industrial effluents from the Phalaborwa industrial complex into the Olifants River. The impact of these effluents on the quality of water entering the Kruger National Park (KNP) is of particular concern to conservationists (De Lange *et al*. 2003; Van Vuuren 2009).

Despite signs that water quality in the Olifants River has been deteriorating as a result of industrial, mining and agriculture activities, the trigger for episodic fish and crocodile deaths in the river system remains elusive (De Villiers & Mkwelo, 2009). According to Van Dyk (2003a), fish will either adapt to environmental change or may slowly vanish. In order to manage healthy fish populations, it is necessary to identify early detectable warning signs of damage on cellular and tissue level (Van Dyk 2003a). Taking the above mentioned into consideration the study is currently relevant and crucial to determine the effects toxicants may have on the health status of fish at the two dams in the Olifants River.

Since fish are located near or on top of the food chain and are known to accumulate toxins, histopathological investigations may prove not only to be an indicator of prior exposure to toxins but also a cost-effective tool to determine and monitor the health of a fish, hence reflecting the health of the entire freshwater ecosystem (Barbour *et al.* 1999; Van Dyk 2003b). Furthermore, histopathological investigation could provide valuable information to help access the potential for human exposure to the environmental pollutants and for predicting human health risks (Van Dyk 2003a).

1.3 HYPOTHESES

- The sediment and water quality at Flag Boshielo Dam is more acceptable than at Loskop Dam.
- The *Labeo rosae* population at Flag Boshielo Dam is healthier than at Loskop Dam.
- Fish can be used as an accumulation indicator of heavy metals in an aquatic ecosystem.
- Elevated concentration of metals can cause gross alterations and histological alterations in organs and tissues of fish.

1.4 AIM

To evaluate the impact of water quality on the health of *Labeo rosae* based on the quantitative HAI, bioaccumulation levels and histopathology at the Loskop and Flag Boshielo dams of the Olifants River System, South Africa.

1.5 OBJECTIVES

- To determine selected water quality constituents at Loskop and Flag Boshielo dams during the two seasons.
- To determine the concentration levels of selected metals in the sediment, muscle, gills and liver of *Labeo rosae* from Loskop and Flag Boshielo dams.
- To compare the health of *L. rosae* populations from Loskop and Flag Boshielo dams by applying the HAI.
- To compare the condition factor (CF) and hepatosomatic index (HSI) of *L. rosae* populations between the two localities.
- To identify parasites found in/on *L. rosae* from Loskop and Flag Boshielo dams (forms part of HAI).
- To compare statistical infestation of parasites and Parasite Index (PI) between the two localities.
- To describe and quantify the histopathological alterations in the gills and livers of *L. rosae*.

1.6 RESEARCH QUESTIONS

- Does the sediment and water quality differ between Loskop and Flag Boshielo dams?
- Is the health of fish populations from Loskop and Flag Boshielo dams affected by different water quality?
- Is the fish parasite diversity higher at Flag Boshielo Dam than Loskop Dam?
- Is there any difference on histopathological alterations of gills and liver between the two localities?
- Are the bioaccumulation levels in fish from Loskop Dam higher than fish from Flag Boshielo Dam?

1.7 DISSERTATION OUTLINE

Chapter 1 comprises the introduction to the ecological state of freshwater ecosystems in South Africa. It outlines the background of water quality monitoring, how biomonitoring has emerged and various indices that are used in biomonitoring. It also describes the ecological status of the Olifants River with respect to fish and crocodile deaths reported recently. Furthermore, this chapter includes the hypotheses, aim and objectives of the study. Chapter 2 describes the materials and methods used to conduct this study. It includes the description of study areas, selected fish species and all equipment used to achieve the objectives, water sampling and the procedure of tissue processing for bioaccumulation analysis and histopathology. This chapter further described the various indices are that used in the current study. Chapter 3 contains water quality results, discussion and conclusion for Loskop and Flag Boshielo dams. Chapter 4 provides bioaccumulation results including bioaccumulation factors for both dams. Chapter 5 contains the results for HAI, PI, CF and HSI as well as discussion and conclusion. Chapter 6 gives histopathology results of fish from both Loskop and Flag Boshielo dams. Chapter 7 gives a summary for the overall results, conclusions as well as recommendations.

CHAPTER 2

MATERIALS AND METHODS

2.1 SAMPLING LOCALITIES

The Olifants River catchment is about 54 750 km^2 with mean annual rainfall of 2 400 x 10^6 m^3 (De Villiers & Mkwelo 2009). The river originates from the east of Johannesburg but the larger part lies in the Limpopo and Mpumalanga provinces. The Olifants River catchment has been divided into the upper, middle and lower reaches. Loskop Dam is located in the upper reach while Flag Boshielo Dam is found in the middle reach (Fig. 2.1) (De Lange *et al.* 2003).

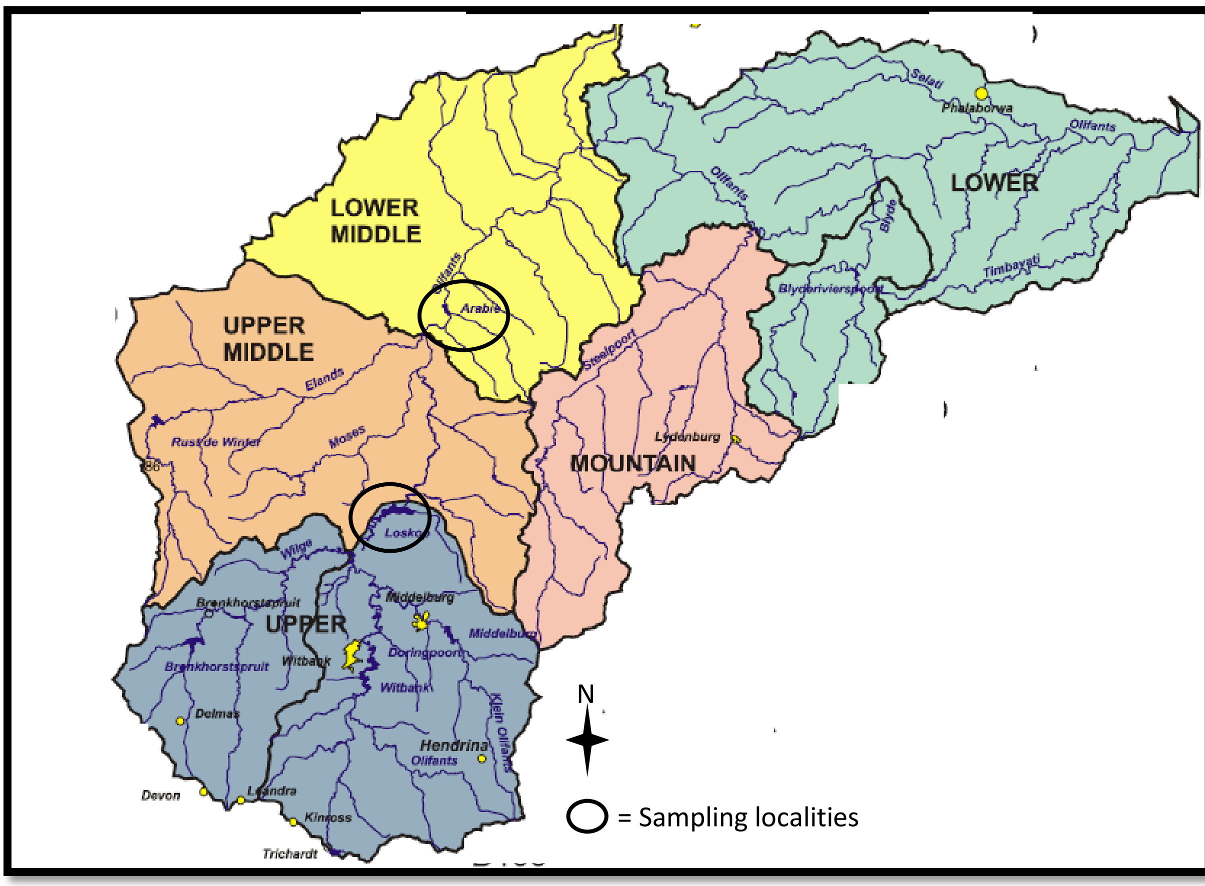

Figure 2.1 Map showing all reaches of the Olifants River System (modified from De Lange *et al.* 2003).

2.1.1 Loskop Dam

Loskop Dam (LD) is located in the upper reach of the Olifants River within the Loskop Nature Reserve (Figs 2.1 and 2.2). The dam is found approximately 32 km south (upstream) of the town of Groblersdal in Mpumalanga province, South Africa. The dam was constructed in 1938 by the Department of Water Affairs and in 1979 the wall was raised to its current height of 54 m above the foundation (Botha *et al.* 2011). The impoundment has a surface area of 24.27 km^2 and a volume of 374 X 10^6 m^3 at full supply capacity, and was designed primarily to supply water for agricultural irrigation downstream of the dam wall (DWAF 2004). But the dam serves as a repository for pollutants from the upper catchment of the Olifants River System (Grobler *et al.* 1994).

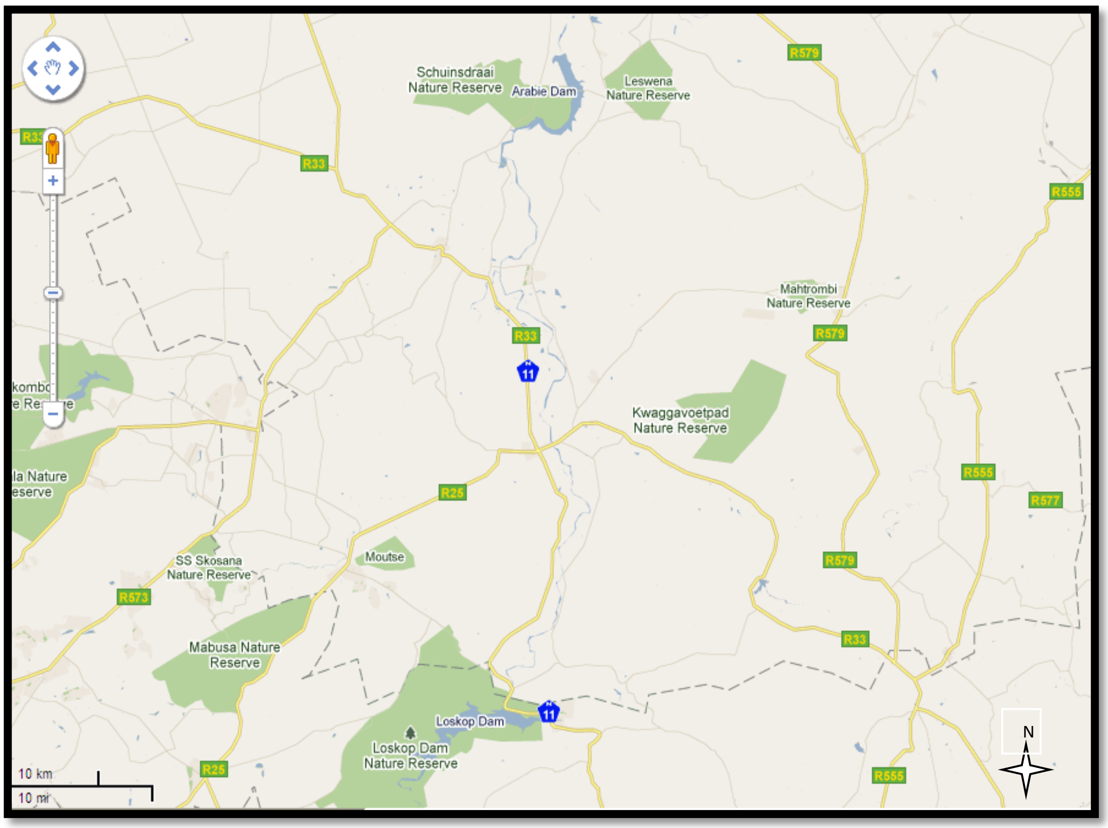

Figure 2.2 Map showing the two sampling localities (Google Earth 2011).

The upper Olifants River catchment is characterized by large-scale coal mining, coal-fired power generation plants, extensive agriculture, diverse array of heavy and light industries as well as several towns and smaller urban centres (Driescher 2008; Oberholster 2009). Several kills of aquatic biota such as fish and crocodile have been reported over the past 15 years and has become more frequently since 2003 at Loskop Dam (Driescher 2008; Botha *et al.* 2011).

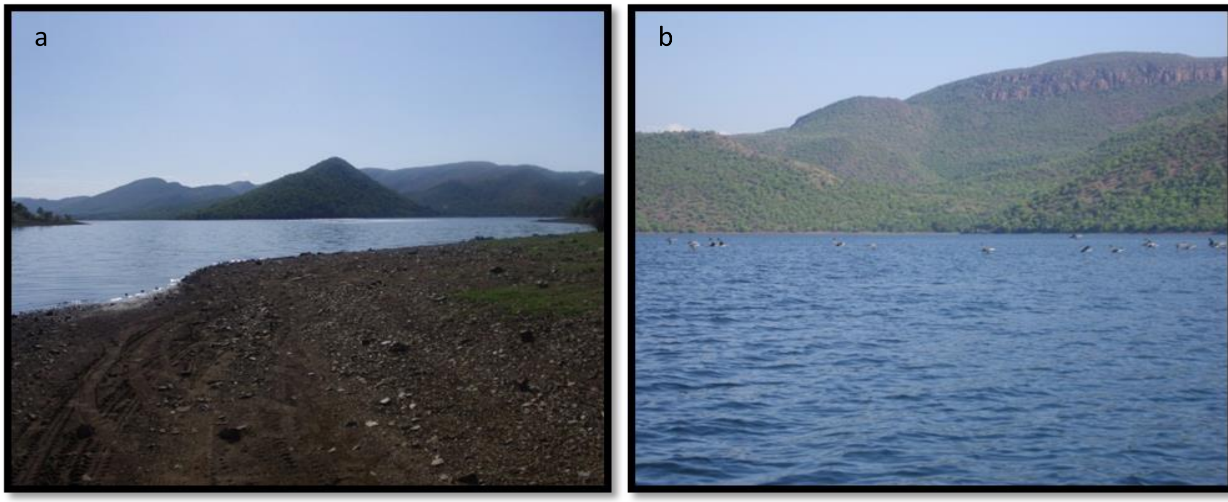

Figure 2.3 a and b: Loskop Dam.

2.1.2 Flag Boshielo Dam

Flag Boshielo Dam (FBD) is situated in the lower middle reach of the Olifants River System (Fig. 2.1). The dam is located about 85 km downstream (north) of Loskop Dam and approximately 25 km north-east of Marble Hall (Botha 2005). The dam was built to provide water for irrigation, for domestic and industrial supply and also for recreational purposes (McCartney *et al.* 2004). It was constructed in 1987 and raised by 5 meters in 2005 (Ashton 2010). The dam was originally known as Mokgomo Matlala Dam and later changed to Arabie Dam (Botha 2005). The dam was further renamed in 2001, to Flag Boshielo Dam.

Figure 2.4 a and b: a – Flag Boshielo Dam; b – An island in the middle of the dam with crocodile and birds.

Flag Boshielo Dam is home to vertebrates such as crocodiles and birds as well as various fish species. There are commercial and subsistence agriculture as well as numerous point and diffuse sources of industrial pollution along the Olifants River towards the dam (Heath & Claassen 1999).

2.2 SELECTED FISH SPECIES

Labeo rosae is a small headed, compressed fish. The base colour is golden green with silver pink scales, reddish eye and snout with red tubercles (Fig. 2.5). Juveniles are silvery with a black caudal peduncle spot (Skelton 2001). They are most common in the lowveld reaches of the Limpopo, Incomati and Phongolo river systems in South Africa, Botswana, Mozambique, Zimbabwe and Swaziland (Skelton 2001; Bills *et al.* 2007).

Figure 2.5 The rednose labeo, *Labeo rosae.*

Labeo rosae prefers sandy stretches of larger perennial and intermittent rivers. The species is prevalent in Flag Boshielo and Loskop dams. It feeds on detritus, algae and small invertebrates. It is an active fish, leaping at barriers when migrating upstream in swollen rivers to breed in summer (Reid 1985; Skelton 2001). It attains sexual maturity at about 150 mm total length (Skelton 2001). *Labeo rosae* is susceptible to the impacts of weir and net fishing as well as sedimentation (Bills *et al.* 2007). Numerous incidents of fish kills have been recently reported at Loskop Dam (Van Vuuren 2010).

2.3 WATER SAMPLING

The water samples were collected seasonally (summer and winter) at Loskop and Flag Boshielo dams. Loskop and Flag Boshielo dams are very huge; the sample from one site would not represent the entire dam. Therefore, the samples were collected from the dam wall, middle and the inflow. The collected water samples were stored in acid treated sampling bottles and frozen. Frozen water samples were sent to an accredited water laboratory (WATERLAB (PTY) LTD) in Pretoria for chemical analysis. The following parameters were analysed: alkalinity, aluminium, ammonia, antimony, arsenic, calcium, chloride, copper, iron, lead, magnesium, manganese, nitrates, nitrites, phosphorus, potassium, sodium, sulphate, total dissolved solids (TDS), total hardness and zinc. These parameters were selected because of their importance in fish health and their toxicity. Dissolved oxygen (DO), electrical conductivity (EC), salinity, pH and water temperature were measured *in situ* by using a handheld multi parameter instrument (YSI 556 Multi Probe System). The evaluation of water quality parameters was done by comparing the results with the TWQR, AEV and CEV for aquatic ecosystem and aquaculture suggested by DWAF (1996a) where applicable and available.

2.4 BIOACCUMULATION

Sample of gills, liver and muscle were dissected out from each fish, wrapped with plastic or put in small bottles and kept in the freezer. The samples were sent to the accredited laboratory in Pretoria for metal analysis. The following parameters were analysed: aluminium, antimony, copper, iron, lead, manganese, selenium, silicon, strontium and zinc. These parameters were selected because of their importance in fish health and toxicity of these heavy metals. Furthermore, in fish tissues these metals are of potential concern to consumer (human) health (Heath *et al.* 2004). The bioaccumulation factor (BAF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in the medium (Mohamed 2008). The BAF was calculated as used by Authman & Abbas (2007) and adapted by Mohamed (2008).

$$BAF = \frac{\text{Pollutant concentration in fish organ (mg/kg)}}{\text{Pollutant concentration in water (mg/l)}}$$

2.5 FISH SAMPLING

Fish were sampled using gill nets with different stretched mesh sizes (30 - 110 mm). Relevant permit were obtained from .Twenty hosts were collected at each sampling site during each survey. If many other fish species were caught, they were returned to the dams. The specimens were put in a holder tank filled with dam water, transported to a place where a temporary field laboratory was set up. The holder tanks were aerated to increase the oxygen concentration and minimize stress.

Figure 2.6 a and b: a – *Labeo rosae* caught by gill net; b – holder tanks aerated by air pump.

2.6 HEALTH ASSESSMENT INDEX

As mentioned previously fish were removed from the gill nets and kept in a holder tank filled with aerated dam water. Blood were drawn from the fish using a syringe to fill capillary tubes. The capillary tube was plugged at one end using commercial Critoseal™ clay. The tubes were centrifuged in a micro-haematocrit centrifuge for approximately ten minutes to separate the blood plasma from white blood cells (WBC) and red blood cells (RBC) and the haematocrit values were obtained.

The fish was sacrificed by severing the spinal cord. Mass (g) and the length (cm) (standard length, folk length and total length) of the fish were measured. The fish were examined externally by using the revised HAI method (Table 2.1) and recorded on a HAI data sheet (Heath *et al.* 2004; Jooste *et al.* 2005). Depending on the degree of stressor-induced abnormalities, a numerical value were awarded to examined fish tissues and organs as suggested by Adams *et al.* (1993) and Jooste *et al.* (2005) (Table 2.1). The fish was then dissected and gills, eyes and the alimentary canal were removed and placed in separate Petri-dishes filled with dam water. All internal organs were assessed with the help of a colour chart developed by Watson (2001) and values were assigned as indicated in the revised HAI table (Table 2.1).

2.6.1 Calculation of the Health Assessment Index

Original field designations of all variables from the necropsy-based system were substituted with comparable numerical values into the HAI (Table 2.1). Each organ was assigned a score for its condition, according to the categories suggested by Adams *et al.* (1993) and Jooste *et al.* (2005). The variables of the HAI were presented with a value ranging from 0-30, depending on the condition of the organs tested, with normal conditions indicated by 0 and abnormal conditions indicated by 30. To calculate HAI for each fish within a sample, numerical values for all variables were summed (Adams *et al.* 1993). The HAI for a sample population was calculated by adding all individual fish HAI values and dividing it by the total number of fish examined for that sample.

2.6.2 Condition factor

The length and weight of the fish were measured. The condition factor determines the condition of fish in a habitat by correlating the length and weight of the fish. The condition factor was determined for each fish to ascertain the possible impacts of pollution on the fish. The correlation between the condition factor and the HAI for each fish were assessed to find out if there was a correlation among the two variables. Condition factor was calculated as suggested by Heath *et al.* (2004).

$$CF = \frac{(W \times 10^5)}{L^3}$$

W = Weight in g

L = Total length in mm

Table 2.1. Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy-based system (Adams *et al.* 1993; Jooste *et al.* 2005).

Variables	Variable condition	Original field designation	Substituted value for the HAI
External variables			
Length	Total length in millimetres	mm	-
Weight	Weight in grams	g	-
Eyes	Normal	N	0
	Exopthalmia	E1/E2	30
	Haemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins[a]	No active erosion or previous erosion healed over	0	0
	Mild active erosion with no bleeding. (>10 parasite cysts)[a]	1	10
	Severe active erosion with haemorrhage/secondary infection. (>50 parasite cysts)[a]	2	20
Skin[a]	Normal, no aberrations	0	0
	Mild skin aberrations. (>10 parasite cysts)[a]	1	10
	Moderate skin aberrations. (>50 parasite cysts)[a]	2	20
	Severe skin aberrations	3	30
Opercules	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	OT	30
Pseudobranch	Normal	N	0
	Swollen	S	30
	Lithic	L	30
	Swollen and lithic	P	30
	Inflamed	I	30
	Other	OT	30

Table 2.1 Continued.

Variables	Variable condition	Original field designation	Substituted value for the HAI
Thymus[b]	No haemorrhage	0	0
	Mild haemorrhage	1	10
	Moderate haemorrhage	2	20
	Severe haemorrhage	3	30
Internal variables (necropsy)			
Mesenteric fat[b]	(Internal body fat expressed with regard to the amount present)		
	None	0	-
	Little where less than 50% of each cecum is covered	1	-
	50% of each cecum is covered	2	-
	More than 50% of each cecum is covered	3	-
	Cecae are completely covered by large amount of fat	4	-
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Other	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal	N	0
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30
Liver	Red	A	0
	Light red	B	30
	“Fatty” liver, “coffee with cream” colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other	OT	30

Table 2.1 Continued.

Variables	Variable condition	Original field designation	Substituted value for the HAI
Bile[b]	Yellow or straw colour, bladder empty or partially full	0	-
	Yellow or straw colour, bladder full, distended	1	-
	Light green to "grass" green	2	-
	Dark green to dark blue-green	3	-
Blood (Haematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30
Blood (plasma protein)	Normal range	30-69mg/dL	0
	Above normal range	>70mg/dL	10
	Below normal range	<30mg/dL	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10
Endoparasites[a]	No observed endoparasites	0	0
	Observed endoparasites <100	0	10
	101 – 1000	1	20
	>1000	3	30
Ectoparasites[a]	No observed ectoparasites	0	0
	Observed ectoparasites 1 – 10	1	10
	11 – 20	2	20
	>20	3	30

a – refinement of the HAI.
b – no values were assigned to these values in the original HAI.

2.6.3 Hepatosomatic Index

The liver and fish were separately weighed and the masses used to calculate the Hepatosomatic Index (HSI). Hepatosomatic Index correlates the mass of the liver and the whole body mass. An organ or tissue can adapt to any changes occurring in the surrounding environment by undergoing hypertrophy, hyperplasia or atrophy (Van Dyk, 2003a). The index can tell whether the liver has undergone hyperplasia, hypertrophy or atrophy or the body mass has increased. In a normal fish the index range from 1 – 2% (Munshi and Dutta, 1996). The formula is as follows:

HSI = (liver mass/body mass) x 100

2.6.4 Histopathology

Immediately after euthanizing the fish, a gill arch and approximately a third of the liver were dissected out and fixed in 10% neutral buffered formalin. The samples were submitted to the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria for routine histological processing and haematoxylin and eosin (H&E) staining (Bancroft, 2003).

a. Tissue processing

The samples were dehydrated through a series of ethanol (70%, 80%, 96%, 100%). Xylene was used to clear the samples and making them transparent. The samples were infiltrated through increasing concentrations of Tissue-Tek® III wax in a 60˚C oven. According to the methods by Humason (1962), once thoroughly infiltrated, samples were embedded in Tissue-Tek® III wax blocks with careful orientation.

Each block was sectioned at 4-5 µm using a rotary/sliding microtome (Reichert-Jung 2040). The samples were floated using gelatine and distilled water solution, and then mounted on glass microscope slides and air dried in 60˚C oven (Humason 1962). Dried samples were prepared for light microscopy analysis using standard technique for Haematoxylin and Eosin staining. The stained section was mounted with cover slips using Entellan.

b. Quantitative histological assessment

Prepared histological sections were examined using a standard light microscope. Histopathological assessment of gill and liver tissue was performed using the protocol proposed by Bernet et al. (1999). Histological alterations were classified into five reaction patterns. Each reaction pattern involves several alterations which concern either functional units of the organ or an entire organ (Table 2.2).

i. Five reaction patterns

Reaction pattern 1 (rp_1): circulatory disturbances

Circulatory disturbances can be caused by pathological conditions of blood and tissue fluid flow. The pathology includes:

- Haemorrhage: blood leaking from blood vessels.
- Hyperaemia: congestion of blood in an organ caused by venous as well as arterial processes.
- Aneurysm: well out-lined dilations of arterial blood vessels.

- Intercellular oedema: the stagnant tissue fluid which has leaked from capillaries in to tissues (Bernet *et al.* 1999).

Reaction pattern 2 (rp_2): regressive changes

Alterations in this reaction pattern include:

- Architectural and structural alteration: changes in tissue structure as well as the shape and arrangement of cells.
- Plasma alterations: changes in cellular plasma caused by hyaline droplets, colloidal droplets, degenerative fatty vacuolization or hydropic glycogen droplets, calcareous degeneration, and thickening of the fine fibres of connective tissue.
- Deposits: intercellular accumulations of substances primarily caused by degenerative processes.
- Nuclear alteration: changes in the nuclear shape and structure of chromatin.
- Atrophy: reduction in number and volume of cells and/or a degreasing amount of intercellular substances.
- Necrosis: morphological state of a cell or a tissue which appears after irrevocable loss of cell function.

Reaction pattern 3 (rp_3): progressive changes

Typical lesions in this reaction pattern are:

- Hypertrophy: enlargement of cell volume or tissue without increase in cell number.
- Hyperplasia: enlargement of tissue or organ by a greater number of cells without change in volume of the cells.

Reaction pattern 4 (rp_4): inflammation

Although it is often difficult to attribute inflammatory changes to one single reaction pattern, Bernet *et al.* (1999) used the term inflammation in a very strict sense which involves only three alterations.

- Exudate: fluid containing a high protein concentration, and a large amount of cellular debris exuded from blood and lymph vessels.
- Activation of reticuloendothelial system (RES): hypertrophy of the RES, which consists of endothelial cells and macrophages that line small blood vessels.

- Infiltration: leucocytes penetrating the walls of blood vessels and infiltrating the surrounding tissue.

Reaction pattern 5 (rp_5): tumour (neoplasm)

Tumour is an uncontrolled cell and tissue proliferation. Two classes of tumour are:

- Benign tumour: differentiated cells which replace or displace the original tissue; these tumour cells resemble the cells of the normal tissue.
- Malignant tumour: poorly differentiated, rapidly multiplying cells which invade and destroy resident tissues, metastasis may be observed.

ii. Two variables used when calculating lesion indices

Importance factor (*w*)

According to Bernet *et al.* (1999), importance factor ranges from 1 to 3 depending on how the alteration affect the organ functioning and the ability of the fish to survive.

- 1 = for minimal pathological importance, when the lesion is easily reversible as exposure to irritants ends.
- 2 = moderate pathological importance, when the lesion is reversible in most cases if the stressor is neutralized.
- 3 = for marked pathological importance, when the lesion is generally irreversible, leading to partial or total loss of the organ function.

Score value (*a*)

The score ranged from 0 to 6, depending on the degree and extent of alteration: (0) unchanged; (2) mild occurrence; (4) moderate occurrence; and (6) severe occurrence. However, intermediate values are also considered (Bernet *et al*. 1999).

iii. Calculation of lesion indices

Once the importance factor (*w*) and score value (*a*) has been assigned to each alteration identified in an organ, four different indices can be calculated:

Organ index (I_{org})

Organ index aims to determine the degree of injury to a single organ. It is calculated by the sum (∑) of the multiplied importance factor (w) and score value (*a*) of all alterations (alt) identified in all the reaction patterns (rp) of a specific organ (org) (Table 2.3). A high index value indicates a high degree of injury.

$$\mathbf{I_{org}} = \Sigma_{\mathbf{rp}} \Sigma_{\mathbf{alt}} (\mathbf{a}_{\mathbf{1}\ \mathrm{org}\ \mathbf{all}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}} \times \mathbf{w}_{\mathbf{1}\ \mathrm{org}\ \mathbf{all}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}})$$

Reaction index ($I_{org\ rp}$)

Determine the degree of injury within a single reaction pattern to a single organ. It is calculated by the sum (∑) of the multiplied importance factor (w) and score value (*a*) of all alterations (alt) identified in a single reaction patterns (rp) of a specific organ (org) (Table 2.3). A high index value indicates a high degree of injury.

$$\mathbf{I_{org\ rp}} = \Sigma_{\mathbf{alt}} (\mathbf{a}_{\mathbf{1}\ \mathrm{org}\ \mathbf{1}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}} \times \mathbf{w}_{\mathbf{1}\ \mathrm{org}\ \mathbf{1}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}})$$

Table 2.2 Histopathological assessment tool, an importance factor with scores assigned for every alteration (Bernet *et al.* 1999).

Organ	Reaction pattern	Functional unit of the tissue	Alteration	Importance factor	Score value	Index
Gills	Circulatory disturbance		Haemorrhage/hyperaemia/aneurysm	$w_{GC1} = 1$	a_{GC1}	I_{GC}
			Intercellular oedema	$w_{GC2} = 1$	a_{GC2}	
	Regressive changes	Epithelium	Architectural and structural alterations	$w_{GR1} = 1$	a_{GR1}	I_{GR}
			Plasma alterations	$w_{GR2} = 1$	a_{GR2}	
			Deposits	$w_{GR3} = 1$	a_{GR3}	
			Nuclear alterations	$w_{GR4} = 2$	a_{GR4}	
			Atrophy	$w_{GR5} = 2$	a_{GR5}	
			Necrosis	$w_{GR6} = 3$	a_{GR6}	
			Rupture of the pillar cells			
		Supporting tissue	Architectural and structural alterations	$w_{GR7} = 1$	a_{GR7}	
			Plasma alterations	$w_{GR8} = 1$	a_{GR8}	
			Deposits	$w_{GR9} = 1$	a_{GR9}	
			Nuclear alterations	$w_{GR10} = 2$	a_{GR10}	
			Atrophy	$w_{GR11} = 2$	a_{GR11}	
			Necrosis	$w_{GR12} = 3$	a_{GR12}	
	Progressive changes	Epithelium	Hypertrophy	$w_{GP1} = 1$	a_{GP1}	I_{GP}
			Hyperplasia	$w_{GP2} = 2$	a_{GP2}	
		Supporting tissue	Hypertrophy	$w_{GP3} = 1$	a_{GP3}	
			Hyperplasia	$w_{GP4} = 2$	a_{GP4}	
	Inflammation		Exudate	$w_{GI1} = 1$	a_{GI1}	I_{GI}
			Activation of RES	$w_{GI2} = 1$	a_{GI2}	
			Infiltration	$w_{GI3} = 2$	a_{GI3}	
	Tumour		Benign tumour	$w_{GT1} = 2$	a_{GT1}	I_{GT}
			Malignant tumour	$w_{GT1} = 3$	a_{GT2}	
Liver	Circulatory disturbance		Haemorrhage/hyperaemia/aneurysm	$w_{LC1} = 1$	a_{LC1}	I_{LC}
			Intercellular oedema	$w_{LC2} = 1$	a_{LC2}	

Table 2.2 Continued.

	Regressive changes	Liver tissue	Architectural and structural alterations	$w_{LR1} = 1$	a_{LR1}	I_{LR}
			Plasma alterations	$w_{LR2} = 1$	a_{LR2}	
			Deposits	$w_{LR3} = 1$	a_{LR3}	
			Nuclear alterations	$w_{LR4} = 2$	a_{LR4}	
			Atrophy	$w_{LR5} = 2$	a_{LR5}	
			Necrosis	$w_{LR6} = 3$	a_{LR6}	
			Rupture of the pillar cells			
		Interstitial tissue	Architectural and structural alterations	$w_{LR7} = 1$	a_{LR7}	
			Plasma alterations	$w_{LR8} = 1$	a_{LR8}	
			Deposits	$w_{LR9} = 1$	a_{LR9}	
			Nuclear alterations	$w_{LR10} = 2$	a_{LR10}	
			Atrophy	$w_{LR11} = 2$	a_{LR11}	
Liver	Regressive changes	Interstitial tissue	Necrosis	$w_{LR12} = 3$	a_{LR12}	I_{LR}
		Bile duct	Architectural and structural alterations	$w_{LR13} = 1$	a_{LR13}	
			Plasma alterations	$w_{LR14} = 1$	a_{LR14}	
			Deposits	$w_{LR15} = 1$	a_{LR15}	
			Nuclear alterations	$w_{LR16} = 2$	a_{LR16}	
			Atrophy	$w_{LR17} = 2$	a_{LR17}	
			Necrosis	$w_{LR18} = 3$	a_{LR18}	
	Progressive changes	Epithelium	Hypertrophy	$w_{LP1} = 1$	a_{LP1}	I_{LP}
			Hyperplasia	$w_{LP2} = 2$	a_{LP2}	
		Interstitial tissue	Hypertrophy	$w_{LP3} = 1$	a_{LP3}	
			Hyperplasia	$w_{LP4} = 2$	a_{LP4}	
		Bile duct	Hypertrophy	$w_{LP5} = 1$	a_{LP5}	
			Hyperplasia	$w_{LP6} = 2$	a_{LP6}	
			Wall proliferation of bile ducts or ductules			
	Inflammation		Exudate	$w_{LI1} = 1$	a_{LI1}	I_{LI}
			Activation of RES	$w_{LI2} = 1$	a_{LI2}	
			Infiltration	$w_{LI3} = 2$	a_{LI3}	
	Tumour		Benign tumour	$w_{LT1} = 2$	a_{LT1}	I_{LT}
			Malignant tumour	$w_{LT2} = 3$	a_{LT2}	

(G) gills; (L) liver; (C) circulatory disturbances; (R) regressive changes; (P) progressive changes; (I) inflammation; (T) tumour. The importance factors ($w_{org\ rp\ alt}$) ranging from 1 to 3 is assigned to every alteration: it is composed of the respective organ (org), the reaction pattern (rp) and the alteration (alt)

Total organ index ($TotI_{org}$)

Determine the cumulative degree of damage to all target organs examined. It is calculated by the sum (∑) of the multiplied importance factor (w) and score value (*a*) of all alterations (alt) identified in all reaction patterns (rp) of all organs (org) (Table 2.3). This index represents a measure of the overall health status based on histological alterations.

$$\mathbf{TotI_{org}} = \Sigma_{\mathbf{org}} \Sigma_{\mathbf{rp}} \Sigma_{\mathbf{alt}} (\mathbf{a}_{\mathbf{all}\ \mathrm{org}\ \mathbf{all}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}} \times \mathbf{w}_{\mathbf{all}\ \mathrm{org}\ \mathbf{all}\ \mathrm{rp}\ \mathbf{all}\ \mathrm{alt}})$$

Total reaction index ($TotI_{rp}$)

Determine the degree of injury within a single reaction pattern to all organs. It is calculated by the sum (∑) of the multiplied importance factor (w) and score value (*a*) of all alterations (alt) identified in a single reaction patterns (rp) of all organs (org) (Table 2.3). This index represents the quality of the histological alterations in all examined organs of an individual fish.

$$\mathbf{TotI_{rp}} = \Sigma_{\mathbf{rp}} \Sigma_{\mathbf{alt}} (\mathbf{a}_{\mathbf{all}\ org\ \mathbf{1}\ rp\ \mathbf{all}\ alt} \times \mathbf{w}_{\mathbf{all}\ org\ \mathbf{1}\ rp\ \mathbf{all}\ alt})$$

Table 2.3 Lesion indices (from Bernet *et al.*1999).

Organ	Reaction pattern					
	rp_1	rp_2	rp_3	rp_4	rp_5	I_{org}
Organ 1 Organ 2	$I_{org1\ rp1}$ $I_{org2\ rp1}$	$I_{org1\ rp2}$ $I_{org2\ rp2}$	$I_{org1\ rp3}$ $I_{org2\ rp3}$	$I_{org1\ rp4}$ $I_{org2\ rp4}$	$I_{org1\ rp5}$ $I_{org2\ rp5}$	I_{org1} I_{org2}
$TotI_{.rp}$	$I_{.rp1}$	$I_{.rp2}$	$I_{.rp3}$	$I_{.rp4}$	$I_{.rp5}$	Tot-I

$I_{org\ rp}$ = Reaction index of an organ; I_{org} = Organ index; $TotI_{.rp}$ = the total reaction index; Tot-I = the total index

2.6.5 Parasites

Immediately after removing fish from the gill nets, examinations for visible moving ectoparasites were done on the boat. If ectoparasites were collected, the collected hosts were marked in order to keep record of all parasites recorded from that specific host. The body surface, mouth cavity and fins of the fish were examined for ectoparasites.

Skin smears were made by holding the fish firmly and scraping the skin on both sides with glass slides. The slides were examined carefully for parasites with the aid of a stereomicroscope. The fish were sacrificed by severing the spinal cord behind the head and opened ventrally. The body cavity and mesenteries were examined for parasites. Organs such as gills, eyes, gastrointestinal tract, liver, spleen, brain and kidneys were placed in petri-dish filled with dam water for parasite examinations.

Monogeneans were placed in a small Petri dish filled with dam water and fixed by adding hot (±70°C) alcohol-formalin-acetic acid (AFA) fixative and stored in 70% ethanol. They were mounted by using glycerine jelly. Digeneans were fixed flat in AFA for approximately 10 minutes and preserved in 70% ethanol to which 5% glycerine were added. Cestodes were fixed and stored in warm buffered formalin. Nematodes were fixed in glacial acetic acid for approximately 2 minutes and preserved in 70% ethanol with 2% glycerine added. Copepods were kept alive in dam water, fixed by adding 70% ethanol to the water in small quantities over a period of approximately one hour, where after they were stored in 70% ethanol.

a. Ecological terms used in infestation statistics

Three ecological terms (prevalence, mean intensity and mean abundance) were calculated as suggested by Bush *et al.* (1997). **Prevalence** refers to the number of infested individuals of a host species divided by the number of hosts examined, expressed in percentage. **Mean intensity** is the total number of particular parasite species divided by the number of infested hosts. **Mean abundance** refers to the total number of particular parasite species divided by the total number of hosts sampled.

b. Parasite Index (PI)

In the original HAI (Adams *et al.* 1993), parasites were recorded as being present or absent. The inserted PI distinguished between the presence of ecto- and endoparasites. The PI was refined to distinguish further between the number of ecto- and endoparasites present (Table 2.4) (Crafford & Avenant-Oldewage 2009). In the refined PI the higher numbers of either endo- or ectoparasites were given higher score values. For ectoparasites, the refined PI was contradicting with the HAI because higher number of ectoparasites denote healthy ecosystem, so in order to incorporate inverted PI with the HAI, the PI was inverted. When inverting the PI, the larger numbers of ectoparasites were given a lower score for this correlation to be reflected in the HAI value (Table 2.4). The inverted PI is based on the premise that ectoparasites are more directly exposed than endoparasites to the effects of water quality. It follows that more ectoparasites are found when water quality is good. In turn, good water quality correlates with a low HAI value (Crafford & Avenant-Oldewage 2009). The PI were calculated as suggested by Jooste *et al.* (2005) and IPI as suggested by Crafford & Avenant-Oldewage (2009).

Table 2.4 The revised Parasite Index (PI) (Jooste *et al.* 2005) and Inverted Parasite Index (IPI) (Crafford & Avenant-Oldewage 2009).

Ectoparasites	PI	IPI	Endoparasites	PI
Zero parasites observed	0	30	Zero parasites observed	0
1-10	10	20	<100	10
11-20	20	10	101-1000	20
>20	30	0	>1000	30

2.6.6 Statistical analysis

The mean abundance, mean intensity and prevalence for parasites were calculated as suggested by Bush *et al.* (1997). The indices i.e. CF, BAF and HSI were calculated and the results were presented as mean value ± standard deviation. Analysis of variance (ANOVA) was used to determine differences between the localities and seasons. The significance level was $p<0.05$. For quantitative histological assessment the organ index, total organ index, reaction index and total reaction index were calculated as proposed by Bernet *et al.* (1999) and the results were presented as mean value ± standard deviation.

CHAPTER 3

WATER QUALITY

3.1 INTRODUCTION

Water occupies about 70% of the earth's surface, but out of all that water, only 2.5% of it is fresh with the remainder brackish and salty seawater. On the little freshwater available, about 70% of it is locked away in the polar ice caps. The huge amount of unfrozen freshwater are hidden away as groundwater and only a small amount (0.01%) is found in streams, rivers and lakes (Van Vuuren 2011). Surface water is very limited, and the conservation of its quality and quantity is becoming of particular concern in South Africa but also globally. Water quality describes the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems (DWAF 1996a). The quality of the water differs from continent to continent, and even from region to region, as a result of differences in climate, geomorphology, geology and soils, and biotic composition (Dallas & Day 2004). The properties of water are controlled or influenced by the constituents that are either dissolved or suspended in water (DWAF 1996a).

Water quality constituents can be divided into physical attributes such as temperature, turbidity, total suspended solids (TSS); and chemical constituents such as the total dissolved solids (TDS) and the concentration of solutes such as gases and ions (Dallas & Day 2004). However, DWAF (1996a) divided water quality constituents into four categories based on the effects that the constituents may have on aquatic biota. **System variables** described as the constituents that regulate the essential ecosystem processes such as spawning and migration e.g. pH and dissolved oxygen (DO). **Nutrients** are generally not toxic but stimulate eutrophication if present in excess e.g. NO_3, NO_2, NH_4, PO_4 and SO_4. **Non-toxic constituents** are described as the constituents that cause toxic effects at extreme concentrations e.g. TDS, TSS, electrical conductivity (EC), salinity and total water hardness. **Toxic constituents** seldom occur in high concentrations in unimpacted systems e.g. Cu, Fe, Pb, Al, Zn and Mn.

South Africa is diverse in climate, geomorphology, geology and soils, and aquatic biotas, and so the different regions exhibit quite considerable differences in water quality, even when unaffected by human activity (Dallas & Day 2004). Aquatic ecosystems are very fragile; they have limited ability to absorb continual pollutant loads before a dramatic change takes place in the structure and functioning of the ecosystem components. If the system consistently moves beyond a critical threshold, it is likely to change dramatically and start to behave in a different way, often with unforeseen or undesirable consequences for people who rely on the system for water supplies (Oberholster 2009). The altering of the features or constituents of water quality to the detriment of its inhabitants or users is called water pollution (Davies & Day 1998). Water pollution occurs when conditions exceed the aquatic ecosystem's ability to compensate for the changes (Chapman 1996). There are two kinds of water pollution, i.e. point source and non-point source pollution. Point source pollution occurs when the effluents are discharged from pipes or stormwater drains into the river, lakes and wetland or directly into the sea. The quantity of pollutants can be measured and it is fairly easy to control. Non-point source occurs when the pollutants enter the water body through runoff from urban and industrial areas, diffusion from the atmosphere, seepage from mines and leaching from domestic and solid waste disposal sites. This type of pollution is difficult to quantify and control due to irregular discharges (Davies & Day 1998; Heath & Claassen 1999; Dallas & Day 2004). According to Svobodóva *et al.* (1993), water pollution may reduce the ability of aquatic biota e.g. fish to maintain an effective immunological response system, so the pollution may increase the susceptibility of organisms to different diseases.

Fish live in water for their entire life; therefore they are continuously exposed to the external water environment. Good quality water is the key to flourishing fish health. Poor water quality can kill fish directly; moreover, it is a major contributing factor to disease outbreaks since the fish's immune system is lowered in response to the poor conditions (Novotny 2003). Furthermore, Van Dyk (2003a) reported that the health and reproductive potential of a fish may reflect the quality of the water they live in. Numerous incidents of fish and crocodile kills have been reported in the Olifants River System (Oberholster 2009).

The Olifants River System originates from the east of Johannesburg and initially flows northwards before curving eastwards towards the Kruger National Park (KNP) where it is joined by the Letaba River before flowing into Mozambique (Heath *et al.* 2010). The river is one of the hardest working river systems in South Africa, particularly at the upper catchment which is characterised by elevated levels of copper and zinc (Grobler 1994; Van Vuuren 2010). It has been used and abused for the past five decades, and this can be seen in the character of the water quality, which has worsened markedly over the years (Van Vuuren 2009). The water quality in the Olifants River has been deteriorating as a result of mining, industrial and agricultural activities. Mining activities consist mainly of coal mining in the upper reaches of the catchment, platinum and chrome mining in the middle as well as copper and phosphorus mining in the lower catchment

(Claassen *et al.* 2002). Other activities that play a role on the deterioration of water quality in the Olifants River include power generations, agricultural runoff, informal rural settlements and urban runoff (Nussey *et al.* 2000). The recent investigations by an independent fish pathologist, Dr David Huchzermeyer, indicated that most of the fish caught in the Olifants River are not healthy and their internal organs and gills are badly affected by bad quality water (Van Vuuren 2009).

Loskop Dam is found in the upper catchment of the Olifants River. The dam has been described as a repository for pollutants from the upper catchment (Grobler *et al*. 1994). Numerous incidents of fish kills has been reported during the past five years which were thought to be caused by the acid mine drainage flowing into the dam (Oberholster 2009). Flag Boshielo Dam is located about 85 km downstream of Loskop Dam and approximately 25 km north-east of Marble Hall (Botha 2005). There are commercial and subsistence agriculture as well as numerous point and diffuse sources of industrial pollution along the Olifants River towards the dam (Heath & Claassen 1999). Nevertheless, no recent fish and crocodile kills have been reported from Flag Boshielo Dam.

3.2 RESULTS AND DISCUSSION

3.2.1 System variables

These are the variables that regulate essential ecosystem processes such as spawning and migration. System variables depend on the climate, geomorphology, geology and the aquatic biota of the area (Davies & Day 1998). The aquatic biota is usually adapted to the natural seasonal cycles of changing water quality which characterizes their systems. Anthropogenic changes in the amplitude, frequency and duration of these cycles may cause severe disruption to the ecology and physiological functions of aquatic organisms, and hence the ecology of the system (DWAF 1996a). These variables include temperature, DO and pH.

Temperature

All water bodies are subjected to natural daily and seasonal variations in temperature (Dallas & Day 2004). The temperatures of inland waters in South Africa generally range from 5 - 30°C (DWAF 1996a). All organisms have a specific temperature or range of temperatures at which optimal growth, reproduction and general fitness occur (Palmer *et al.* 2004). The metabolic rate of aquatic organisms is related to temperature, and in warm waters, respiration rates increase leading to increased oxygen consumption and increased decomposition of organic matter (Bartram & Ballance 1996). Dallas and Day (2004) reported that the increase in water temperature decreases oxygen solubility and also increase the toxicity of certain chemicals, both which result in increased stress in the associated organisms.

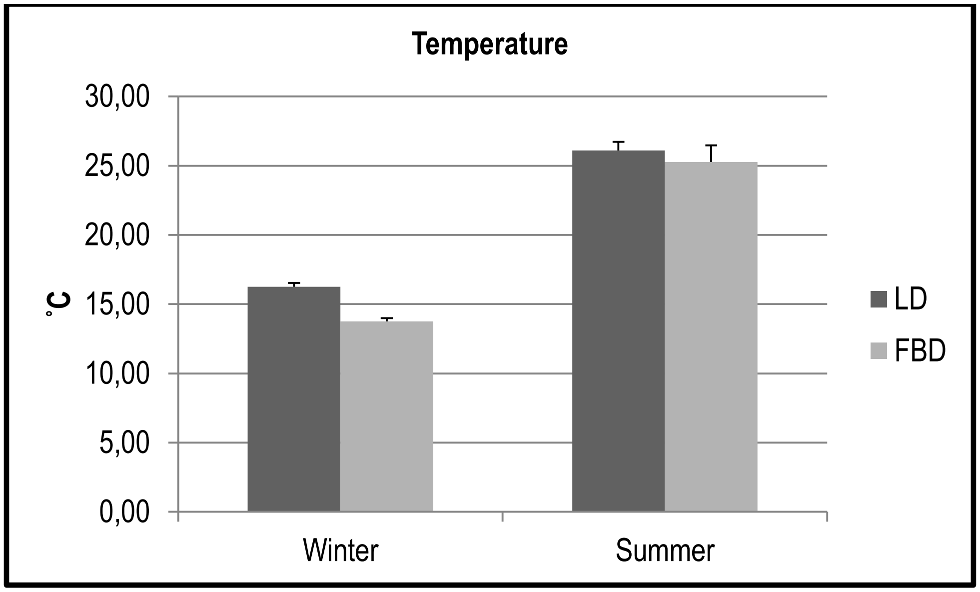

Figure 3.1 Temperature recorded at Loskop and Flag Boshielo dams.

Since water temperature depends mostly on climatic conditions, it tends to change from time to time with water depth throughout the day and it depends on the vegetation cover as well. A temperature of 16.25˚C and 26.09˚C was recorded at Loskop Dam during winter and summer respectively with 13.76˚C and 25.27˚C being recorded at Flag Boshielo Dam (Fig 3.1). There were no significant differences between the water temperature of the two localities ($p>0.05$). According to Svobodóva *et al.* (1993), fish can easily tolerate natural fluctuation of water temperature within the water body. However, the human induced water temperature fluctuation may have negative effects if the temperature becomes 12˚C colder or warmer than the original water temperature. Due to the natural diel fluctuation of water temperature, there is no specific target water quality range (TWQR), however, it has been suggested for aquatic ecosystems that the temperature should not be allowed to vary from the background average daily water temperature considered to be normal for that specific site and time of day, by > 2˚C, or by > 10% (DWAF 1996a).

Dissolved oxygen

Dissolved oxygen is one of the most important abiotic factors relating to the survival of most aquatic organisms (Dallas & Day 2004). It is defined as the amount of oxygen dissolved in the water at a given atmospheric pressure, temperature and salinity. The DO is expressed in mg/ℓ or as a percentage of the saturation concentration at the time of sampling (DWAF 1996a). The major sources of oxygen are photosynthesis by plants and phytoplankton as well as atmospheric aeration during turbulent mixing (Mmualefe & Torto 2011). Aquatic organisms use DO during respiration and if DO levels decline, some sensitive species may become weak, migrate or die (DWAF 1996a; Dallas & Day 2004). A concentration of 80% - 120% saturation is suitable for protection of all life stages of most southern African aquatic biota endemic to or adapted to aerobic warm water habitats (DWAF 1996a).

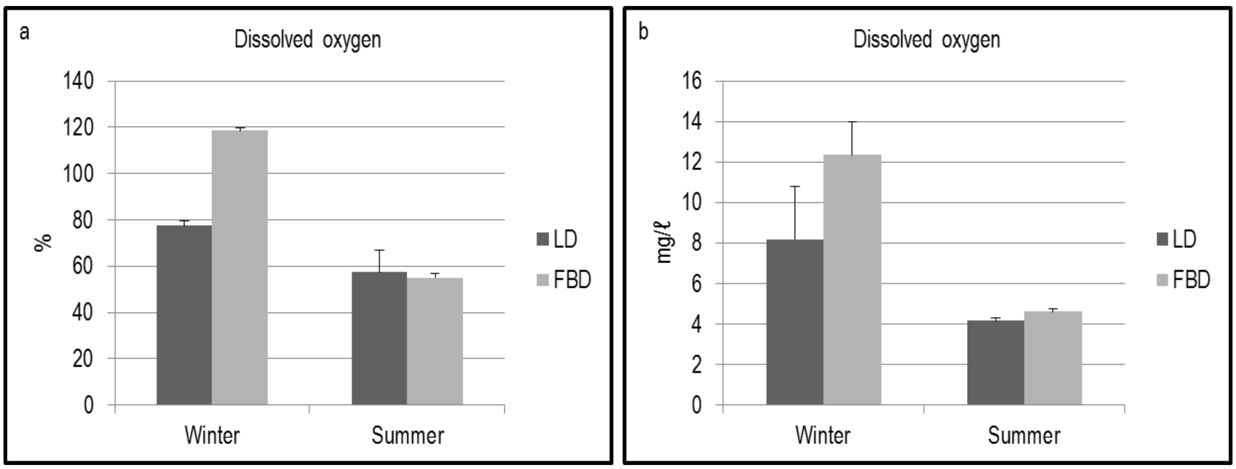

Figure 3.2 Dissolved oxygen in % (a) and mg/ℓ (b) recorded at Loskop and Flag Boshielo dams.

As mentioned previously, DO depend on water temperature and since there were no significant differences for the water temperature between the two localities, the same applied for DO ($p>0.05$). The concentrations of 8.17 mg/ℓ and 4.17 mg/ℓ of DO were recorded at Loskop Dam during winter and summer respectively with 12.35 mg/ℓ and 4.62 mg/ℓ being recorded at Flag Boshielo Dam (Fig. 3.2a&b). In percentage of saturation, a concentration of 77.47% and 57.47% were recorded at Loskop Dam during winter and summer with 118.43% and 55.07% being recorded at Flag Boshielo Dam (Fig. 3.2a&b). The mean DO recorded was found to be 6.17 mg/ℓ at Loskop Dam and 8.49 mg/ℓ at Flag Boshielo Dam. Different fish species have different requirements for concentration of DO and cyprinids can survive in lower concentration. Cyprinid fishes can thrive in water containing 6-8 mg/ℓ and show signs of suffocation only when the DO falls to 1.5-2 mg/ℓ (Svobodóva *et al.* 1993). The DO at both localities was between 4 mg/ℓ and 12.5 mg/ℓ, therefore, it was good for red nose labeo.

During summer, the concentrations were good to protect all life stages of most southern African aquatic biota endemic to, or adapted to aerobic warm water habitats as suggested by DWAF (1996a). The DO was also sufficient for growth of warm water fish for aquaculture (DWAF 1996b). Despite the direct impact of DO variation to aquatic biota, many toxic constituents such as ammonia, cadmium, zinc etc. may become increasingly toxic as DO concentrations get reduced, and when the temperature increases (Palmer *et al.* 2004).

pH

The pH is defined as a measure of the hydrogen ion activity in a water sample. It is effectively the negative logarithm of the hydronium ion (H_3O^+) activity (DWAF 1996a&b). The mean of pH cannot be worked out since it is a logarithmic value. The pH of natural waters is determined largely by the geological and atmospheric influences. For surface water, pH values typically range between 4 and 11. According to DWAF (1996a), most freshwaters in South Africa are relatively well buffered and more or less neutral, with pH ranges between 6 and 8. The pH values above 10.8 and below 5.0 may be rapidly fatal to cyprinids (Svobodóva *et al.* 1993). Furthermore, the direct effect of pH on an organism includes alterations in the rate and type of ion exchange across the body surface (DWAF 1996a).

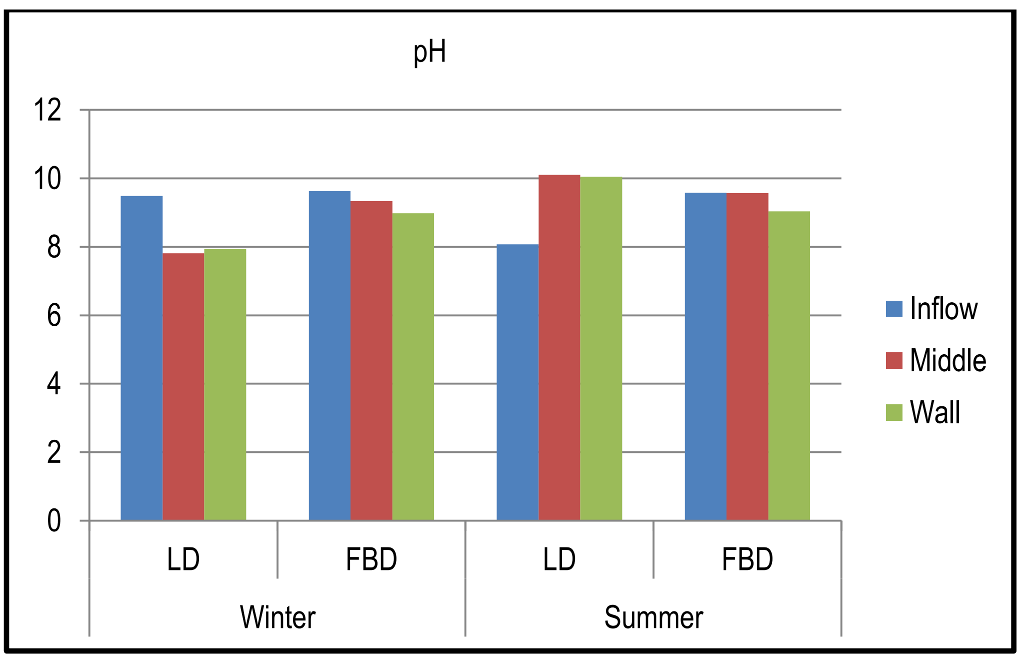

Figure 3.3 pH recorded during winter and summer at LD (Loskop Dam) and FBD (Flag Boshielo Dam)

Alkaline pH values were recorded throughout the study with the highest pH value of 10.10 being recorded at Loskop Dam during summer. The slight difference of pH values were recorded between the inflow, middle and dam wall at both localities. The pH values ranged from 8.98 to 9.63 at Flag Boshielo Dam and 7.81 to 10.10 at Loskop Dam (Fig. 3.3). However, there were no significant differences between the two localities ($p>0.05$). There is no pH TWQR set for aquatic ecosystem, however, most species will tolerate and reproduce successfully in the pH range of 6.5-9 in aquaculture (DWAF 1996b). An increase in pH may be affected by an increase in biological activities and photosynthetic activities of algae which are the results of industrial effluents and anthropogenic eutrophication (Palmer *et al.* 2004). According to Svobodóva *et al.* (1993), only alkaline pH values above 10.8 may have deadly effects to cyprinids. Therefore, in the present study, the pH was not deadly for *L. rosae.*

3.2.2 Non-toxic inorganic constituents

Total Dissolved Solids

Total dissolved solids are natural constituents in a water body; mostly depend on geochemical, physical and hydrological processes. The TDS concentration is defined as a measure of the total amount of soluble materials in a sample of water while the total dissolved salts concentration is a measure of the quantity of all dissolved compounds carrying an electric charge (DWAF 1996a; Dallas & Day 2004). In natural aquatic ecosystems, TDS is determined by the degree of weathering and the chemical composition of rocks and the relative influences of evaporation and rainfall in the catchment (Davies & Day 1998). Dallas and Day (2004) reported that anthropogenic activities such as industrial effluents, irrigation and water re-use lead to increases in TDS. The constituents that form the bulk of TDS include the cations sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and the anions chlorine (Cl^-), sulphate ($SO_4{}^{2-}$), bicarbonate (HCO_3), and carbonate ($CO_3{}^{2-}$). The TDS and electrical conductivity (EC) usually correlate closely for a particular type of water (Davies & Day 1998).

Total dissolved solids concentrations of 255.66 mg/ℓ and 345.80 mg/ℓ were recorded at Loskop Dam during winter and summer respectively with the concentrations of 274.95 mg/ℓ during winter and 310.70 mg/ℓ during summer being recorded at Flag Boshielo Dam (Fig. 3.4). There were no significant differences on the TDS levels between the two localities ($p>0.05$). The seasonal mean TDS concentration at Loskop Dam was found to be 300.73 mg/ℓ and 292.83 mg/ℓ at Flag Boshielo Dam. Throughout the study higher TDS concentrations were recorded during summer and it might be attributed to the evaporation since the dams were sampled prior to rain. Little is known regarding the effects of increased TDS levels on freshwater organisms, but juvenile stages are often more sensitive than adults. Thus, increased TDS levels may have some negative impacts in areas where the organisms are adapted to pure waters (Davies & Day 1998). Dallas and Day (2004) reported that TDS concentration that is too high or too low may limit growth and may lead to the death of many aquatic organisms. There are no TDS TWQR set for aquatic ecosystem but the concentrations should not be changed by > 15% from the normal cycles of the water body under unimpacted conditions at any time of the year (DWAF 1996a).

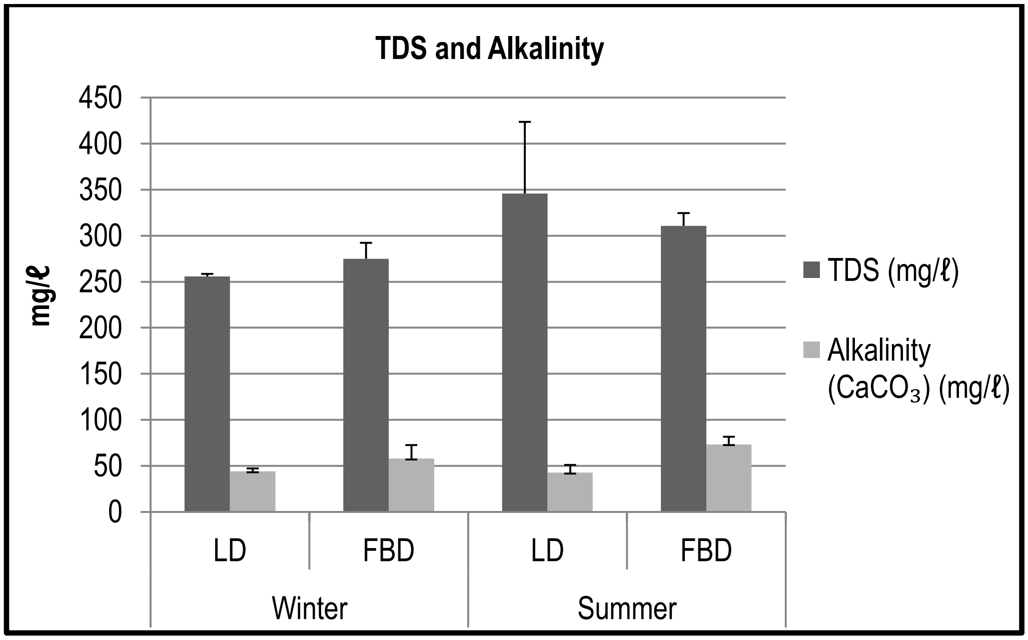

Figure 3.4 Total dissolved solids and alkalinity levels recorded at Loskop and Flag Boshielo dams.

Alkalinity

Alkalinity is defined as a measure of the number of H^+ ions that have reacted over a given pH range during an acid titration, that is, a measure of the water's ability to neutralise acid (DWAF 1996a). It is determined as 'acid neutralising capacity' which in freshwaters is usually due to HCO_3^- and CO_3^{2-} ions (Bartram & Ballance 1996). The chemical composition of rocks and soils strongly influences the natural alkalinity of water, which can range from very low values to several hundred milligrams per litre (DWAF 1996b). Alkalinity pollution may result from certain industrial effluents and anthropogenic eutrophication and is less common in South Africa (Palmer *et al.* 2004).

Alkalinity levels at Flag Boshielo Dam were insignificantly higher than at Loskop Dam ($p>0.05$). The concentrations of 58.0 mg/ℓ and 73.3 mg/ℓ were recorded at Flag Boshielo Dam during winter and summer respectively with 44.0 mg/ℓ and 42.67 mg/ℓ being recorded at Loskop Dam (Fig. 3.4). Oberholster *et al.* (2012) recorded the mean alkalinity of 53.0 mg/l at Loskop Dam from June to September 2009. The lower alkalinity at Loskop Dam might be attributed to the acid inputs from mining or industrial activities as well as atmospheric pollution. The higher alkalinity concentration at Flag Boshielo Dam might have been caused by the agricultural practices occurring in the middle catchment. However, there were no significant difference on the alkalinity concentrations between the localities and they were within the TWQR suggested by DWAF (1996b) for aquaculture. DWAF (1996b) emphasized that water in close proximity to intensive agriculture may have a measurable phosphate-based alkalinity. There is no TWQR set for aquatic ecosystem and domestic use.

Electrical conductivity

Electrical conductivity is defined as a measure of the water sample to conduct electric current and is expressed in millisiemens per meter (mS/m) (Dallas & Day 2004). This ability is a result of the presence of ions such as sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), chlorine (Cl^{-}), sulphate (SO_4^{2-}), nitrate (NO_3^{-}), bicarbonate (HCO_3^{-}), and carbonate (CO_3^{2-}) all of which carry electric charge (DWAF 1996a). The EC of most freshwaters ranges from 0.1-10 mS/m but may exceed 10 mS/m, especially in polluted waters, or those receiving large quantities of land run-off. Electrical conductivity is sensitive to variations in dissolved solids, mostly mineral salts (Chapman 1996). Furthermore, DWAF (1996a) reported that the EC increases as the concentration of dissolved salts increases in an aquatic ecosystem.

In the present study it has been found that lower EC was recorded during winter at both localities. An EC of 39.33 mS/m and 53.20 mS/m was recorded at Loskop Dam during winter and summer respectively with 40.80 mS/m and 47.80 mS/m being recorded at Flag Boshielo Dam (Fig. 3.5). There were no significant difference in the EC between the two localities ($p>0.05$). The EC mean were found to be 46.27 mS/m at Loskop Dam and 44.30 mS/m at Flag Boshielo Dam. Although Chapman (1996) reported a range of conductivity in natural waters as 0.1-10 mS/m, there are no health effects associated with the conductivity of < 45 mS/m (DWAF 1996c). The EC TWQR suggested for domestic use range from 0-70 mS/m, and the EC at both localities were within that range. According to Dezuane (1997), EC is a useful test in raw and finished water for quick determination of minerals.

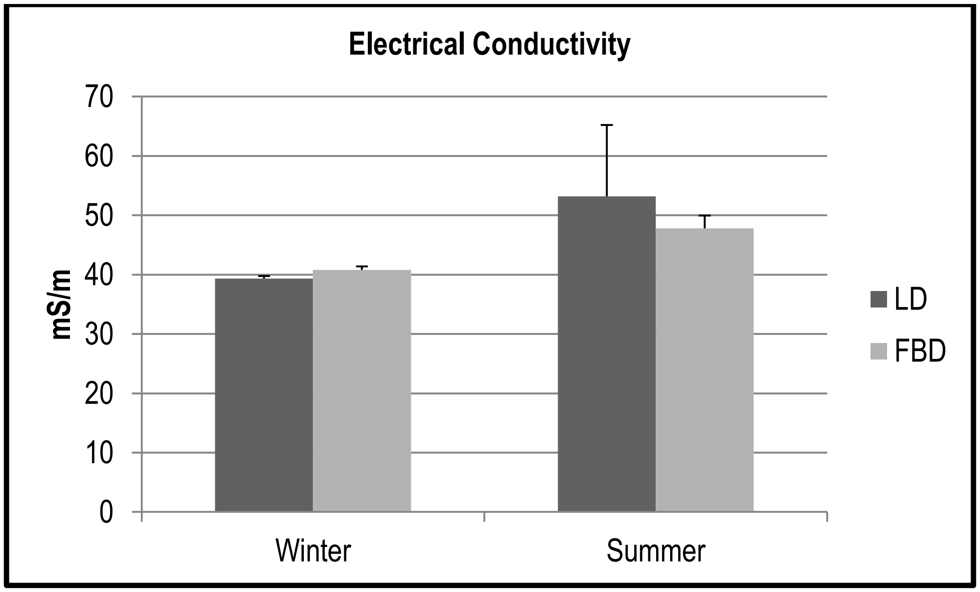

Figure 3.5 Electrical conductivity recorded at Loskop and Flag Boshielo dams.

Salinity

The term salinity often refers to the saltiness of water. Salinity is defined as the mass (in grams) of the dissolved inorganic solids in 1 kg of sea water and the unit is often given as ‰ (parts per thousand). Sea water has a salinity of 35, or 35‰, or 35 g/ℓ. Since the quantity of dissolved organic matter in sea water is very small relative to the amount of inorganic matter, salinity and TDS are virtually identical in sea water. In freshwater, however, the proportion of dissolved organic matter may form a significant fraction of contaminants. In this case, values for salinity and TDS may vary quite significantly for a single sample (Dallas & Day 2004). Salinity and TDS are both measures of the mass of solutes in water; however they differ in the components they measure. Salinity measures only the dissolved inorganic content where as TDS is the mass of the dissolved inorganic and organic compounds in water (DWAF 1996b). Both parameters are of vital importance to fish health as they impact directly on the metabolic and physiological processes of fish (Jooste *et al.* 2005). Salinity is a natural component of an aquatic ecosystem. In a natural system salinity may be influenced by the geological characteristics; it may come from the dissolution of salts from land surface, soil and aquifer material by the rising groundwater (Chapman 1996).

The variation pattern of salinity values recorded during this study was similar to the variation pattern of electrical conductivity with seasons and localities. Thus, salinity and conductivity depend on total dissolved salts in a water body. A salinity concentration of 0.19‰ and 0.26‰ was recorded at Loskop Dam with 0.20‰ and 0.23‰ being recorded at Flag Boshielo Dam (Fig. 3.6). The salinity difference was not significant between the localities ($p>0.05$) and it was within the TWQR set by DWAF (1996b) for aquaculture. There is no TWQR set for aquatic ecosystem since very little information is available on the salinity tolerances of freshwater organisms. In general, it seems that many species are able to survive and even flourish at relatively high salinities. Furthermore, it has been reported that a certain amount of salt in water can either protect aquatic organisms from, or sensitize them to, various pollutants such as heavy metals and biocides (Dallas & Day 2004).

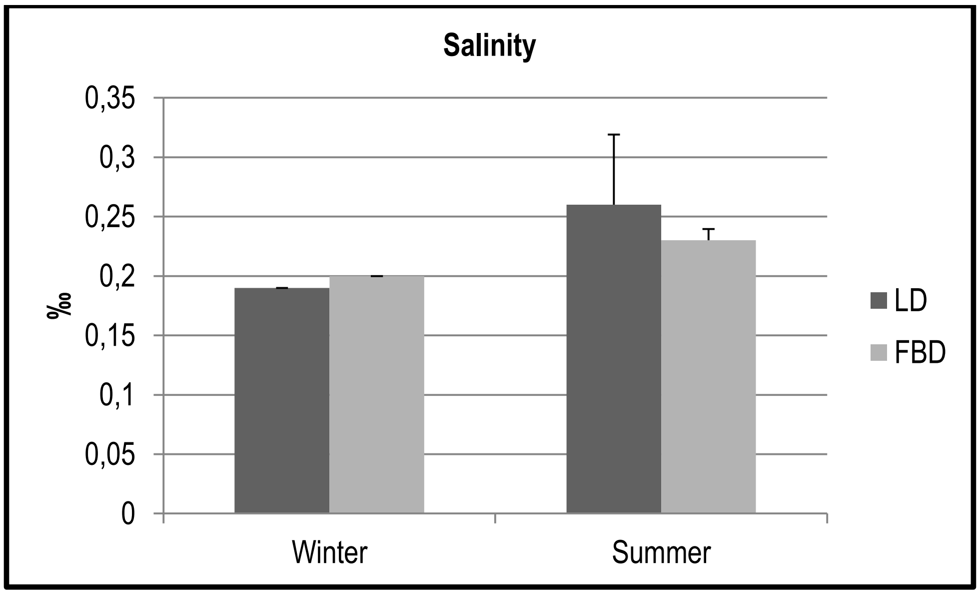

Figure 3.6 Salinity levels recorded at Loskop and Flag Boshielo dams.

Turbidity

Turbidity in water is caused by the presence of suspended matter which usually consists of a mixture of inorganic matter, such as clay and soil particles, as well as organic matter. It is defined as a measure of the light-scattering ability of water and is indicative of the concentration of suspended matter in water. Furthermore, turbidity of water is related to clarity, a measure of the transparency of water (DWAF 1996c). Continuous increase in turbidity may have serious consequences on the aquatic biota. Since light penetration is getting reduced, primary production also decreases and the food production decreases. In natural waters, turbidity can range from <1 NTU in very clear water to more than 1000 NTU in turbid, muddy water (DWAF 1996b).

In the present study, Flag Boshielo Dam showed to be more turbid than Loskop Dam. However, the differences were not significant ($p>0.05$). Turbidity values of 4.93 NTU and 7.93 NTU were recorded at Loskop Dam during winter and summer respectively with 6.5 NTU and 14.67 NTU being recorded at Flag Boshielo Dam (Fig. 3.7). The lower turbidity at Loskop Dam might be attributed to the bottom substrate which is dominated by rocks. Both localities however, were above the TWQR suggested for domestic use but only Flag Boshielo Dam showed to be >10 NTU during summer. According to DWAF (1996c), turbidity of >10 NTU may have severe aesthetic effects in terms of taste and odour. Furthermore, water carries an associated risk of disease due to infectious disease agents and chemicals adsorbed onto particulate matter.

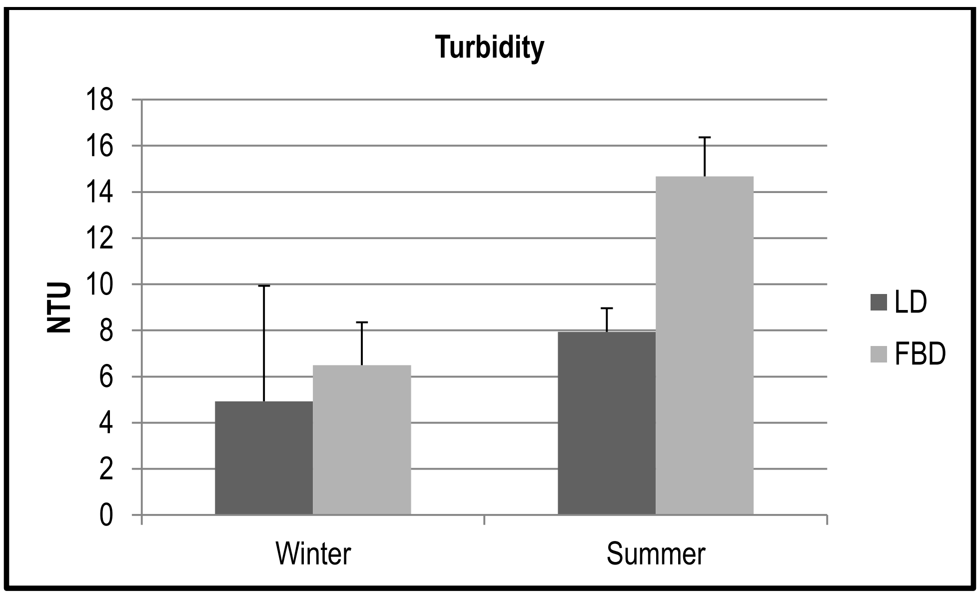

Figure 3.7 Turbidity recorded at Loskop and Flag Boshielo dams.

3.2.3 Nutrients

Ammonia

Ammonia is produced naturally by the biodegradation of nitrogenous matter. It may be present in the free, un-ionized form (NH_3) or in the ionized form as the ammonium ion (NH_4^+). Both are reduced forms of inorganic nitrogen derived mostly from aerobic and anaerobic decomposition of organic material (DWAF 1996a). The ratio between these two ammonium forms depends on the pH, temperature and the DO of the water. Their toxicity increases as temperature and pH increases therefore, the lower the DO, the greater the toxicity of ammonia (Svobodóva *et al.* 1993; Palmer *et al.* 2004). Furthermore, Dallas and Day (2004) reported that natural waters contain ammonia and ammonium concentrations below 0.1 mg/ℓ.

In this study, ammonium concentrations of 1.0 mg/ℓ and 1.1 mg/ℓ were recorded at Loskop Dam during winter and summer respectively with 0.27 mg/ℓ and 0.20 mg/ℓ being recorded at Flag Boshielo Dam (Fig. 3.8). There were significant differences for ammonia concentration between the two localities ($p<0.05$). The seasonal mean concentration for both localities was found to be 1.05 mg/ℓ at Loskop Dam and 0.23 mg/ℓ at Flag Boshielo Dam (Fig. 3.9). Ammonium availability in a water body may be of organic origin by means of domestic sewage, agricultural wastes or the reduction of nitrates and nitrites by bacteria in anoxic water or of inorganic origin by means of industrial effluents from gas works, cooking plants and power stations (Svobodóva *et al.* 1993). The ammonia concentration at both localities was above the TWQR suggested for aquatic ecosystem (DWAF 1996a). However, the concentration at Flag Boshielo Dam was within the TWQR suggested for aquaculture. The possible sub-lethal effects in warm-water fish occur in the range of 0.3-0.8 mg/ℓ (DWAF 1996b). The concentration of ammonia at Loskop Dam was above this range as well as the AEV for aquatic ecosystem. The mesotrophic condition at Loskop Dam was due to an elevated concentration of ammonia. However, the increased concentration of ammonia did not cause any serious threat to the health of fish at Loskop Dam.

Nitrite

Nitrite (NO_2) is the inorganic intermediate, and nitrate (NO_3) the end product, of the oxidation of organic nitrogen and ammonia (DWAF 1996a). Nitrite co-occur with nitrate and ammonia but they are usually found at low concentration because they are readily oxidized to nitrate or reduced to ammonia, both chemically and biochemically by bacteria. The conversion of nitrogen from one form to another in the water and soil involve two processes, nitrification and denitrification. During these processes, two groups of highly aerobic, autotrophic bacteria, mainly *Nitrosomonas* spp. and *Nitrobacter* spp. oxidise ammonia to nitrite, and nitrite to nitrate (DWAF 1996b).

A nitrite concentration recorded at Loskop Dam was only at the inflow during summer whereby the concentration of 0.1 mg/ℓ was recorded with the middle and dam wall localities showing concentrations of below detection levels (< 0.1 mg/ℓ) (Fig. 3.8). The nitrite concentration at Flag Boshielo Dam was found to be below detection value throughout the study. The low concentration of nitrite might be due to the fact that it is readily oxidized to nitrate or is reduced to ammonia. According to Davies and Day (1998), nitrite is toxic to aquatic organisms even at low concentrations. In aquaculture practices, a concentration ranging from 0.06-0.25 mg/ℓ is considered to be safe for a number of warm water fish (DWAF 1996b). Therefore, the NO_2 concentration of 0.1 mg/ℓ recorded at the inflow of Loskop Dam during summer might still be safe for fish.

Nitrate

Nitrates are the final product of the aerobic decomposition of organic nitrogen compounds. Davies and Day (1998) stated that nitrate is seldom abundant in natural surface waters because it is incorporated into cells or is chemically reduced by microbes and converted into atmospheric nitrogen. Nitrate is the least toxic of the inorganic nitrogen compounds to fish but concentrations in excess of 10 mg/ℓ may indicate pollution of water by industrial and agricultural wastes which may contain toxic substances, even though the NO_3 at this concentration is not toxic (DWAF 1996b).

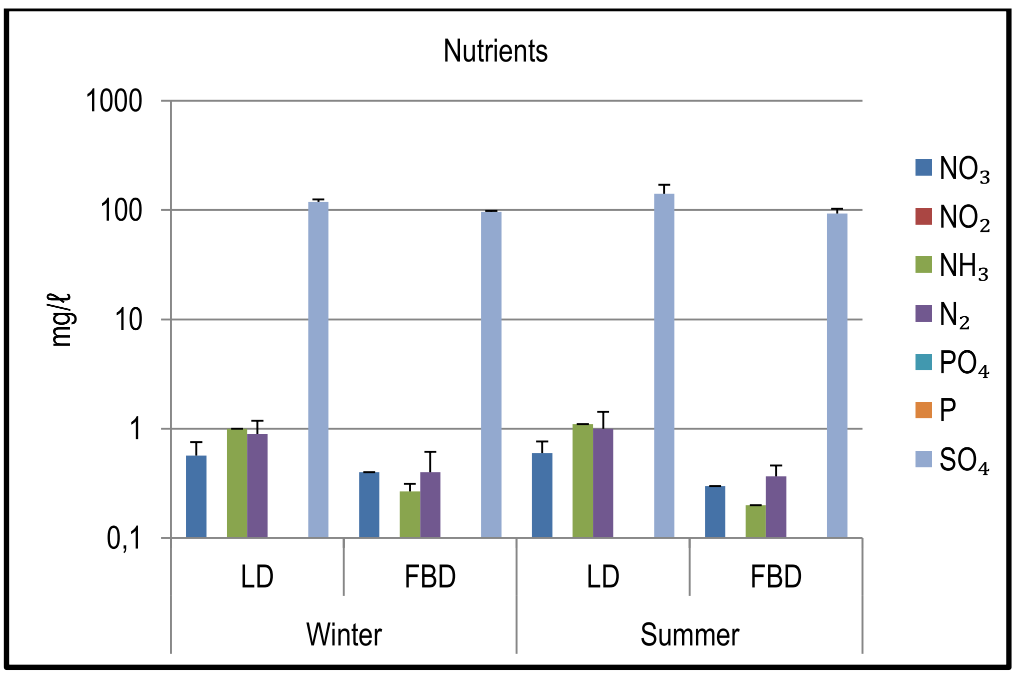

Figure 3.8 Nutrients levels recorded at Loskop and Flag Boshielo dams.

The NO_3 concentrations of 0.56 mg/ℓ and 0.60 mg/ℓ were recorded at Loskop Dam during winter and summer respectively (Fig. 3.8). At Flag Boshielo Dam a concentration of 0.40 mg/ℓ has been recorded during winter and 0.30 mg/ℓ during summer (Fig. 3.8). The seasonal mean concentration of NO_3 at Loskop Dam was found to be 0.58 mg/ℓ and Flag Boshielo Dam being 0.35 mg/ℓ (Fig. 3.9) and it was within the TWQR suggested for aquaculture. An elevated concentration of NO_3 at Loskop Dam might be attributed to the sewage pollution and extensive agricultural activities that occur in the upper Olifants River catchment (Oberholster *et al.* 2009). In aquaculture there are no known adverse effects for NO_3 concentrations below 300 mg/ℓ. Furthermore, Svobodóva *et al*. (1993) reported that the toxicity of nitrates to fish is very low, and mortalities have only been recorded when concentrations have exceeded 1000 mg/ℓ.

Total Nitrogen

Nitrogen (N_2) occurs abundantly in nature and is an essential constituent of proteins, which include the enzymes that catalyse all biochemical processes, and is therefore a major component of all living organisms (Dallas & Day 2004). The total nitrogen includes all the major inorganic nitrogen components (NH_3, NH_4^-, NO_2 and NO_3) present in water. Inorganic nitrogen may enter aquatic ecosystems in effluents from industry but mostly from sewage and agricultural activities which include intensive animal culture like dairy farming (Davies & Day 1998). Low concentrations of nitrogen is essential for the growth of aquatic plants but high concentrations can cause rapid growth of algae and plants that may clog waterways and result in blooms of toxic blue-green algae (Mmualefe & Torto 2011). In South Africa, inorganic nitrogen concentrations in unimpacted aerobic surface waters are usually below 0.50 mg/ℓ but may increase to above 5-10 mg/ℓ in highly enriched waters. The presence of inorganic nitrogen and its components is regulated by the temperature, DO and pH of water (DWAF 1996a).

The total nitrogen of 0.90 mg/ℓ and 1.00 mg/ℓ was recorded at Loskop Dam during winter and summer respectively (Fig. 3.8). A concentration of 0.40 mg/ℓ was recorded at Flag Boshielo Dam during winter and 0.37 mg/ℓ during summer (Fig. 3.8). There were significant differences for the N_2 levels between the two localities ($p<0.05$). The seasonal mean concentrations of N_2 were found to be 0.95 mg/ℓ at Loskop Dam and 0.38 mg/ℓ at Flag Boshielo Dam (Fig. 3.9). Although the N_2 concentration at Loskop Dam was found to be higher, it was below 2.5 mg/ℓ which categorise the water body as mesotrophic with Flag Boshielo Dam being oligotrophic (<0.5 mg/ℓ). Oberholster *et al.* (2012) reported eutrophic condition at Loskop Dam between June and September 2009 whereby the mean concentration was 3.67 mg/ℓ. In the present study, the N_2 concentration was still acceptable for both aquatic ecosystems and domestic use at both localities.

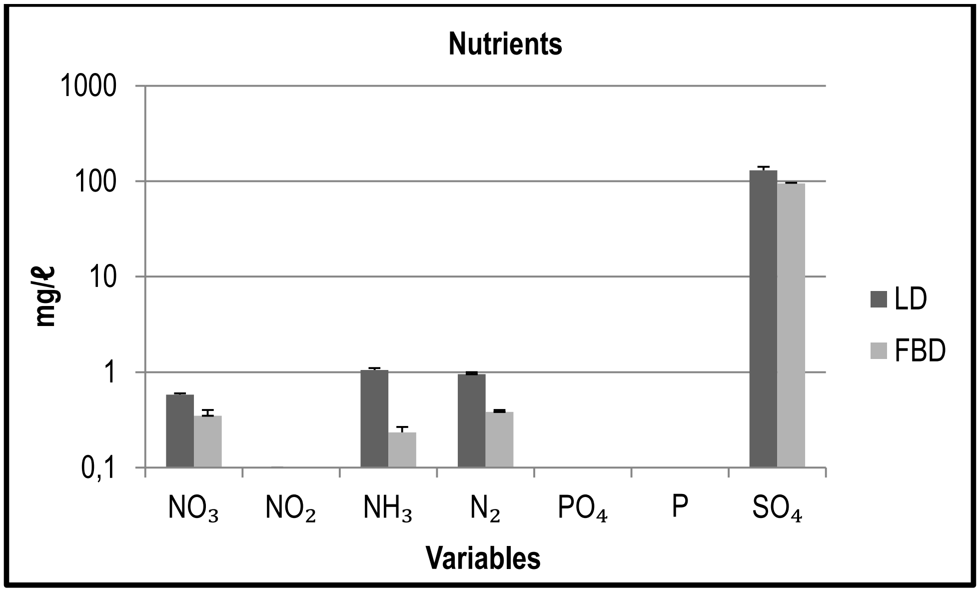

Figure 3.9 The mean nutrient values recorded at Loskop and Flag Boshielo dams.

Phosphorus

Phosphorus is required in numerous life processes and is an integral part of DNA (Davies & Day 1998). It is an essential macronutrient, and is accumulated by a variety of living organisms. Phosphorus stimulates growth of both algae and aquatic macrophytes. The element phosphorus exists as orthophosphates, polyphosphates, metaphosphates, pyrophosphates and organically bound phosphates in natural waters. Naturally, phosphate enters a water body by the weathering of rocks and the subsequent leaching of phosphate salts into surface waters, as well as the decomposition of organic matter (DWAF 1996a&b). Anthropogenic activities that release phosphates into the water include domestic and industrial effluents, atmospheric precipitation, urban runoff as well as drainage from agricultural practices (Dallas & Day 2004).

In the study conducted by Oberholster (2008) at Loskop Dam phosphorus concentration was found to be 0.7 mg/ℓ (Oberholster 2009). However, the present study showed that the concentrations of phosphorus as well as phosphate have dropped to below detectable levels (<0.2 mg/ℓ) at both localities. Both localities can be categorised as oligotrophic. The low concentration of phosphorus may be attributed to the fact that surface waters receive most of their phosphorus (P) in surface flows rather than in groundwater since phosphates bind to most soils and sediment (Correll 1998). The phosphate ion is a highly surface-active species that is readily adsorbed to clay particles in the sediment and water column. Thus during low-flow periods, sediments act as a sink for P entering the stream at high concentrations from point sources (Webster *et al.* 2001).

Sulphate

Sulphate (SO_4^{2-}) is an abundant ion in the earth's crust and its concentration in water can range from a few milligrams to several thousand milligrams per litre (Bartram & Ballance 1996). It is an oxy-anion of sulphur in the +VI oxidation state and forms salts with various cations such as potassium, sodium, calcium, magnesium, barium, lead and ammonium. Furthermore, sulphate occurs naturally in freshwaters; it arises from the dissolution of mineral sulphates in soil and rock, particularly calcium sulphate (gypsum) and other partially soluble sulphate minerals (DWAF 1996c). Anthropogenic sources of sulphate include acid mine wastes and many other industrial processes such as tanneries, textile mills and processes using sulphuric acid and sulphate (Bartram & Ballance 1996; DWAF 1996c).

Naturally, the sulphate concentration in surface waters tends to be 5 mg/ℓ or less, although concentrations of several 100 mg/ℓ may occur where dissolution of sulphate minerals or discharge of sulphate rich effluents from acid mine drainage takes place. In the present study, SO_4^{2-} concentrations of 119.0 mg/ℓ and 141.77 mg/ℓ were recorded at Loskop Dam during winter and summer respectively (Fig. 3.8). At Flag Boshielo Dam, a SO_4^{2-} concentration of 96.0 mg/ℓ was recorded during winter and 93.3 mg/ℓ during summer (Fig. 3.8). There were significant differences in the SO_4^{2-} concentrations between the two localities ($p<0.05$). Although the water bodies showed significant difference in (SO_4^{2-}) concentrations, they were all within the TWQR suggested by DWAF (1996c) for domestic use. There is no sulphate TWQR available for aquaculture and aquatic ecosystems in South Africa. Bartram and Ballance (1996) reported that the World Health Organisation (WHO) does not recommend any guideline value for sulphate since it is known as one of the least toxic anions.

3.2.4 Ions

Chloride

Chloride (Cl^-) is the major anion in sea water and in many inland waters, particularly in South Africa (Dallas & Day 2004). It is found in various forms such as elemental chlorine, hypochlorites, chloramines, and hypochlorite-based compounds such as calcium hypochlorite and dichloroisocyanurates (DWAF 1996b). Naturally, it enters surface water with the atmospheric deposition of oceanic aerosol and with the weathering of some sedimentary rocks (mostly rock salts deposits). Anthropogenic sources include industrial and sewage effluents, agricultural and road run-off (Chapman 1996; DWAF 1996a). Chlorine is used extensively as a biocide for disinfecting and sterilizing drinking water (DWAF 1996b). The toxicity of chloride to fish increases as the DO concentration in water decreases (Svobodova *et al.* 1993). Chapman (1996) revealed that pristine freshwaters has a chloride concentration of <10 mg/ℓ and sometimes <2 mg/ℓ. However, it is only detectable by taste at concentrations exceeding approximately 200 mg/ℓ (DWAF 1996c).

In this study, Cl^- concentrations of 24.00 mg/ℓ and 27.67 mg/ℓ were recorded at Flag Boshielo Dam during winter and summer respectively (Fig. 3.10). There were significant differences on Cl^- concentration between the two localities ($p<0.05$). A concentration of 14.33 mg/ℓ was recorded at Loskop Dam during winter and 16.67 mg/ℓ during summer (Fig. 3.10). The higher concentrations of chloride at Flag Boshielo Dam might be attributed to the agricultural activities in the middle catchment. The mean concentrations of Cl^- were found to be 15.5 mg/ℓ at Loskop Dam and 25.84 at Flag Boshielo Dam (Fig. 3.11). The Cl^- concentration was within the TWQR suggested for domestic use but above the TWQR, CEV and AEV suggested for aquatic ecosystem. However, in aquaculture, there are no known adverse effects of chloride concentration of <1000 mg/ℓ (DWAF 1996b). Chapman (1996) reported that high concentrations of chloride can make waters unpalatable and therefore, unfit for drinking or livestock watering.

Calcium

Calcium (Ca^{2+}) is an alkaline earth metal and exists as the doubly positively-charged ion, Ca (II) (DWAF 1996c). It is one of the most essential elements in living organisms. The element Ca^{2+} is found as a structural material in the bones, teeth, mollusc shells and crustacean (e.g. crab) exoskeletons. It is vital for muscle contraction, nervous activity, energy metabolism and a great variety of other biochemical interactions (Dallas & Day 2004). It is one of the components that contribute to the total hardness of the water together with magnesium (Chapman 1996). Its solubility is usually governed by the carbonate/bicarbonate equilibrium and is thus strongly influenced by pH and temperature (DWAF 1996c). The primary source of calcium may be the geological characteristics of the area where mineral deposits of calcium are common, usually as calcium carbonate, phosphate or sulphate (DWAF 1996c; Jooste *et al.* 2005).

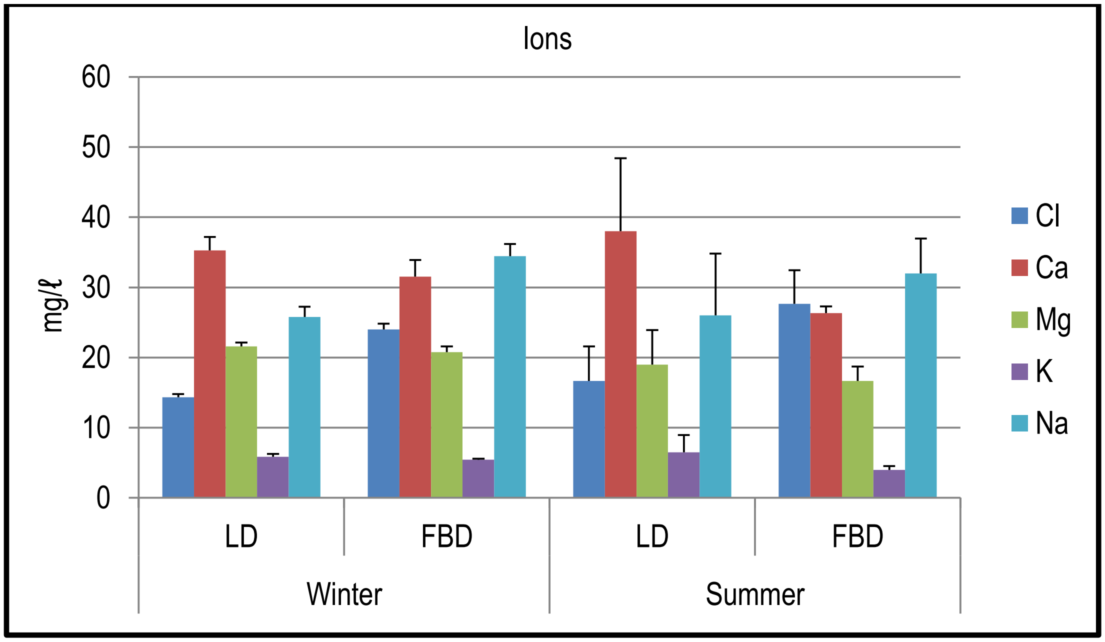

Figure 3.10 Concentration of Ions recorded at Loskop and Flag Boshielo dams.

Bartram and Balance (1996) reported that Ca^{2+} dissolves from almost all rocks and is consequently detected in many waters. Waters associated with granite or siliceous sand will usually contain a Ca^{2+} concentration of <10 mg/ℓ. Many waters from limestone areas may contain 30-100 mg/ℓ and those associated with gypsiferous shale may contain several hundred milligrams per litre. In the present study, a concentration of 35.30 mg/ℓ and 38.00 mg/ℓ was recorded at Loskop Dam during winter and summer respectively with 31.53 mg/ℓ and 26.33 mg/ℓ being recorded at Flag Boshielo Dam (Fig 3.10). There where no significant difference on Ca^{2+} concentration between the two localities ($p>0.05$). The mean concentrations were found to be 36.65 mg/ℓ at Loskop Dam and 28.93 mg/ℓ at Flag Boshielo Dam (Fig 3.11). The TWQR suggested for domestic use ranges from 0-32 mg/ℓ, but the concentration of >80 mg/ℓ may still have no severe effects (DWAF 1996c). Furthermore, Jooste *et al.* (2005) reported that Ca levels up to 250 mg/ℓ may still be acceptable for all users.

Magnesium

Magnesium (Mg^{2+}) is a relatively abundant element in the earth's crust and hence a common constituent of natural waters (Bartram & Balance 1996). Together with calcium it forms the main constituents of total water hardness which is defined as the sum of calcium and magnesium concentration, expressed as calcium carbonate (DWAF 1996a). The main source of Mg^{2+} is the weathering of rocks containing ferromagnesium minerals and some carbonate rocks. Natural concentrations of magnesium in freshwaters may range from 1 to >100 mg/ℓ, depending on the rock types within the catchment. Although magnesium is used in many industrial processes, these contribute relatively little to the total magnesium in surface waters (Chapman 1996).

Magnesium concentrations of 21.61 mg/ℓ and 19.00 mg/ℓ were recorded at Loskop Dam during winter and summer respectively with 20.79 mg/ℓ and 16.67 mg/ℓ being recorded at Flag Boshielo Dam (Fig. 3.10). The Mg^{2+} mean concentration was found to be 20.31 mg/ℓ at Loskop Dam and 18.73 mg/ℓ at Flag Boshielo Dam (Fig. 3.11). There is no Mg^{2+} TWQR suggested for both aquaculture and aquatic ecosystems, however, the concentration was within the TWQR for domestic use (0-30 mg/ℓ). The difference between the two localities was not significant ($p>0.05$).

Potassium

Potassium (K) is an alkali metal which reacts violently with water to form positively-charged potassium ions (DWAF 1996c). According to Chapman (1996), potassium is a relatively abundant element but its concentration in natural freshwater is usually less than 10 mg/ℓ. In addition, Bartram and Balance (1996) reported that potassium concentration in natural freshwater is usually less than 20 mg/ℓ. The low concentration of potassium in natural freshwaters is due to the fact that the rocks which contain potassium are relatively resistant to weathering. However, potassium salts are widely used in industries and in fertilizers for agriculture; therefore, it may enter freshwaters with industrial discharges and run-off from agricultural land (Chapman 1996).

The potassium concentration between the two localities did not differ significantly ($p>0.05$) but a higher concentration was recorded at Loskop Dam. A potassium concentration of 5.87 mg/ℓ and 6.50 mg/ℓ were recorded at Loskop Dam during winter and summer respectively, with 5.45 mg/ℓ and 4.00 mg/ℓ being recorded at Flag Boshielo Dam (Fig. 3.10). The mean concentrations were found to be 6.19 mg/ℓ at Loskop Dam and 4.73 mg/ℓ at Flag Boshielo Dam (Fig. 3.11). Potassium concentrations were within the TWQR set for domestic use at both localities. There is no TWQR for potassium available for aquatic ecosystems and aquaculture. Potassium together with sodium is the natural salts dissolved in freshwaters and they might have been contributed significantly to the TDS at both localities.

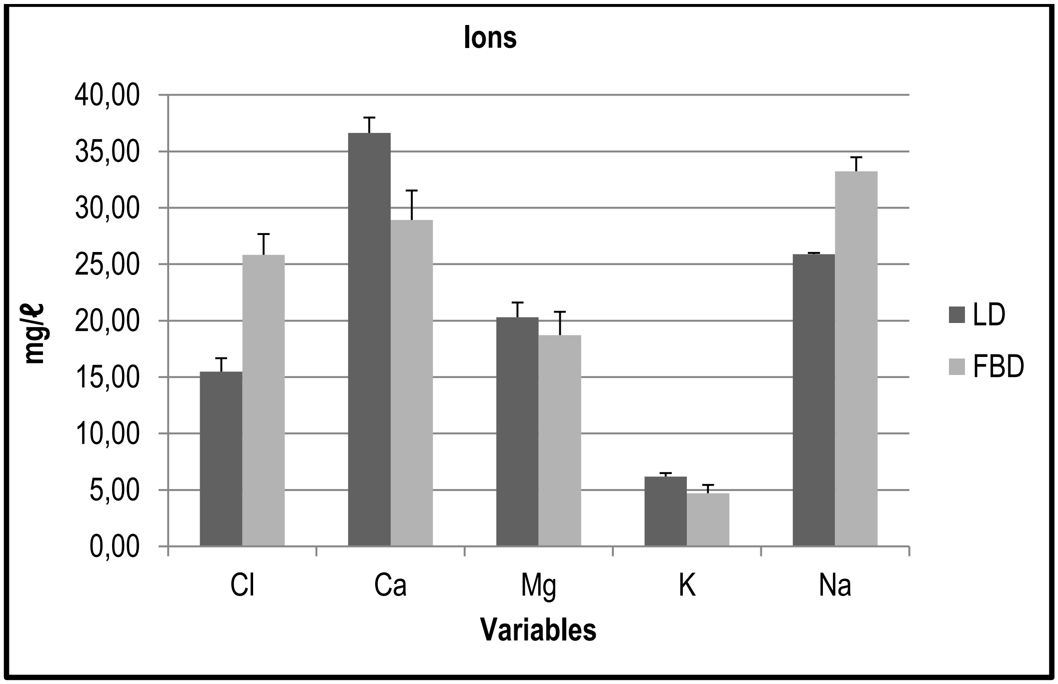

Figure 3.11 Mean values of ions recorded at Loskop and Flag Boshielo dams.

Sodium

Sodium (Na) is one of the most abundant elements on earth and all natural waters contain some sodium since sodium salts are highly water soluble. The Na concentration in natural surface waters depends on the geology of the catchment. The anthropogenic sources include sewage and industrial effluents (Chapman 1996). Sodium and potassium are known to be the most important extracellular and intracellular cations respectively, and vital to all living organisms (DWAF 1996c). According to Chapman (1996), Na is commonly measured where the water is to be used for drinking or agricultural purposes, particularly irrigation. Elevated Na in certain soil types can degrade soil structure thereby restricting water movement and affecting plant growth. Sodium is probably the least toxic metal cation and its effects on aquatic systems are almost entirely as a major contributor to TDS (Dallas & Day 2004). There is no Na TWQR available for aquatic ecosystems and aquaculture.

Sodium concentrations of 25.81 mg/ℓ and 26.00 mg/ℓ were recorded at Loskop Dam during winter and summer respectively with 34.47 mg/ℓ and 32.00 mg/ℓ being recorded at Flag Boshielo Dam (Fig. 3.10). There were significant difference on Na concentration between two localities ($p<0.05$). Although Flag Boshielo Dam had higher concentrations of Na than Loskop Dam, they were all within the TWQR for domestic use. Therefore, mining and industrial activities at the upper catchment as well as the agricultural activities at the middle catchment have not severely elevated the concentration of Na at both localities.

3.2.5 Toxic constituents

Toxic constituents seldom occur in high concentration in healthy aquatic ecosystems, however, their toxicity depend on physico-chemical properties of the water such as pH, temperature, TDS etc. (Svobodóva *et al.* 1993; DWAF 1996a). Various toxic constituents find their way into the aquatic ecosystem as a result of anthropogenic activities such as mining. The toxic constituents cause a wide range of damage to vertebrates as well as invertebrates (Davies & Day 1998). Although the Olifants River is regarded as the third most polluted river in South Africa, most of the tested metals were detected during winter with only few being detected during summer at both Loskop and Flag Boshielo dams (Table 3.1).

Aluminium

Aluminium (Al) is the third most abundant element in the earth's crust but it is present in only trace concentrations in natural waters (DWAF 1996a). It is practically present in all surface water because its primary source is the geological characteristics of an area (Bartram & Balance 1996). The solubility of Al in water is strongly pH dependent; it becomes more soluble and toxic in an acidic pH. Under neutral pH conditions, Al becomes partially soluble. However, as the pH increases, Al undergoes hydrolysis resulting in a series of hydroxide complexes and decreases in solubility and toxicity (DWAF 1996a; Chapman 1996). Therefore, Al becomes more deleterious in acidic conditions. Svobodóva *et al.* (1993) revealed that freshly flocculated Al (as a colloid) may be toxic but fully flocculated hydroxide has a low toxicity similar to that of suspended solids in general.

An Al concentration of 0.04 mg/ℓ was recorded during winter but no Al were detected during summer at Loskop Dam (Fig. 3.12 & Table 3.1). However, concentrations of 0.05 mg/ℓ and 0.11 mg/ℓ were recorded at Flag Boshielo Dam during winter and summer respectively (Fig. 3.12 & Table 3.1). There were no significant differences for Al concentration between the two localities ($p>0.05$). At Loskop Dam, the pH ranged from 7.81-9.49 during winter and 8.07-10.1 during summer whereas at Flag Boshielo Dam it ranged from 9.34-9.63 throughout the study all of which were thus an alkaline pH (Fig. 3.3).

Table 3.1 Concentration of toxic constituents at Loskop and Flag Boshielo dams.

Constituents (mg/l)	Winter		Summer	
	Loskop Dam	Flag Boshielo Dam	Loskop Dam	Flag Boshielo Dam
Al	0.0387	0.0543	-	0.1110
Ag	0.0010	0.0020	-	-
As	0.0037	0.0040	-	-
B	0.1065	0.0767	0.0420	0.1110
Ba	0.0533	0.0443	0.0580	0.0513
Be	0.0043	0.0043	-	-
Cd	0.0010	0.0010	-	-
Cr	0.0010	0.0010	-	-
Cu	0.0020	0.0023	-	-
Fe	0.0767	0.1187	-	0.1130
Li	0.0153	0.0090	-	-
Mn	0.0867	0.0343	0.2190	0.0250
Pb	0.0100	0.0110	-	-
Sb	0.0047	0.0050	-	-
Se	0.0100	-	-	-
Si	2.0677	0.8333	6.2267	5.2000
Sr	0.1860	0.1807	0.1730	0.1490
V	0.0020	0.0023	-	-
Zn	0.0040	0.0040	-	-

(-): not detected

The pH of 8.07 at Loskop Dam was recorded at the inflow with 10.1 and 10.05 being recorded at the middle and dam wall respectively. Therefore, the insolubility of aluminium at Loskop Dam during summer might be attributed to the higher pH in the middle and at the dam wall. All the detected concentrations were above the TWQR suggested for aquatic ecosystems and aquaculture but within the TWQR for domestic use. Svobodóva *et al.* (1993) revealed that high concentrations of aluminium may significantly reduce the growth of a fish in an aquatic ecosystem. However, DWAF (1996a) reported that in alkaline pH values, Al is present as soluble but biologically unavailable hydroxide complexes or as colloids and flocculants. Therefore, an Al concentration was not toxic to aquatic life since the pH was alkaline at both localities throughout the study.

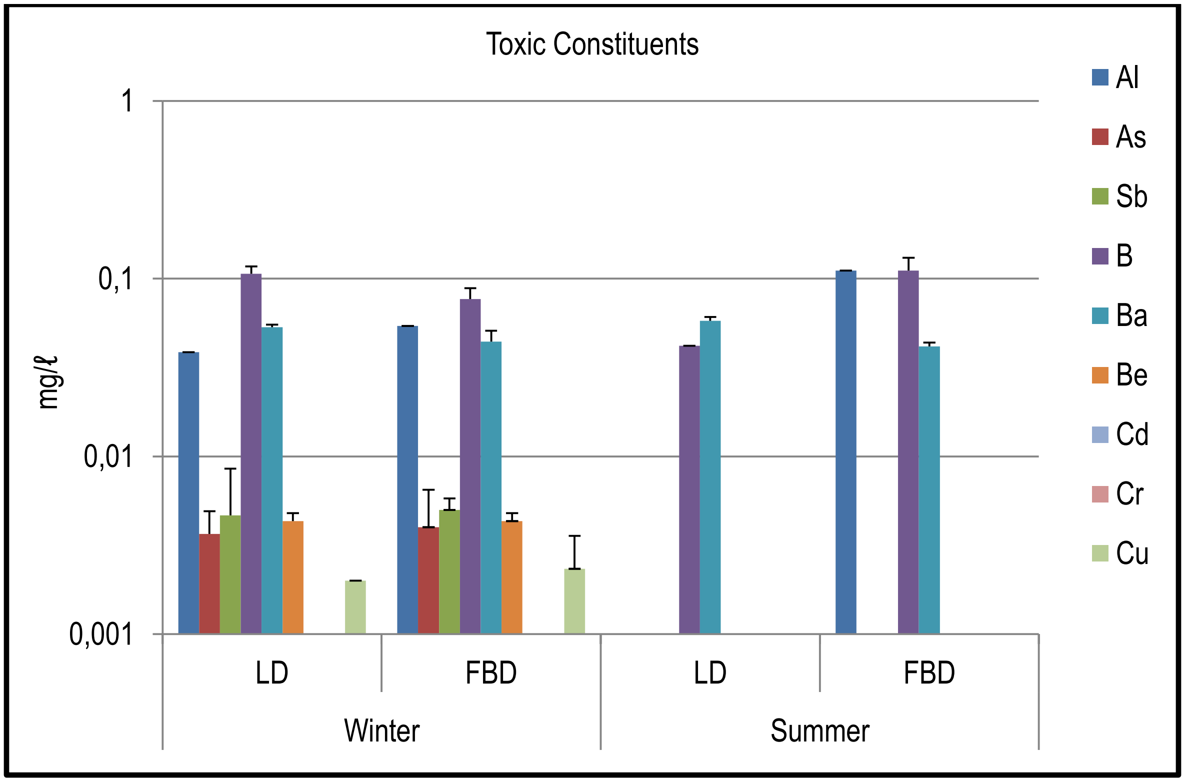

Figure 3.12 Concentration of toxic constituents at LD (Loskop Dam) and FBD (Flag Boshielo Dam).

Copper

Copper (Cu) is a common metal in the rocks and minerals of the earth's crust and it enters the aquatic environment naturally as a result of weathering processes or from dissolution of Cu minerals and native Cu (DWAF 1996a). Copper occurs in three oxidation states, as metallic copper(0), cuprous copper(I) and cupric copper(II). Its toxicity depends on the solubility and chemical species of the Cu present in water. Free cupric copper ions (Cu^{2+}) are considered most toxic, whereas complex forms are the least toxic to aquatic organisms (DWAF 1996b). The mobility and solubility of Cu is high in acidic pH water and it precipitates in alkaline water and is thus not toxic. Furthermore, its toxicity may be reduced as water hardness increases and in the presence of Zn, Mo and SO^{2+} (Dallas & Day 2004).

According to DWAF (1996a), Cu is correlated with water hardness as follows:

Water Hardness (mg $CaCO_3$/ℓ)	< 60 (soft)	60-119 (medium)	120-180 (hard)	>180 (very hard)
TWQR	0.0003	0.0008	0.0012	0.0014

The water hardness ranged from 120-180 mg/ℓ throughout the study, therefore water was categorised as hard at both localities. Copper concentration of 0.0020 mg/ℓ was recorded at Loskop Dam and 0.0023 mg/ℓ at Flag Boshielo Dam during winter (Fig. 3.12 & Table 3.1). Copper could not be detected during summer at both localities. Copper is easily adsorbed and precipitated in sediments at alkaline pH (DWAF 1996a). However, water hardness seems to influence the solubility and toxicity of Cu. According to DWAF (1996a), the toxicity of Cu decreases with the increase in water hardness. The copper concentration was within the TWQR suggested for aquaculture but above for aquatic ecosystem at both localities. Furthermore, Cu was detected during winter at both localities and it was not toxic since the pH was alkaline.

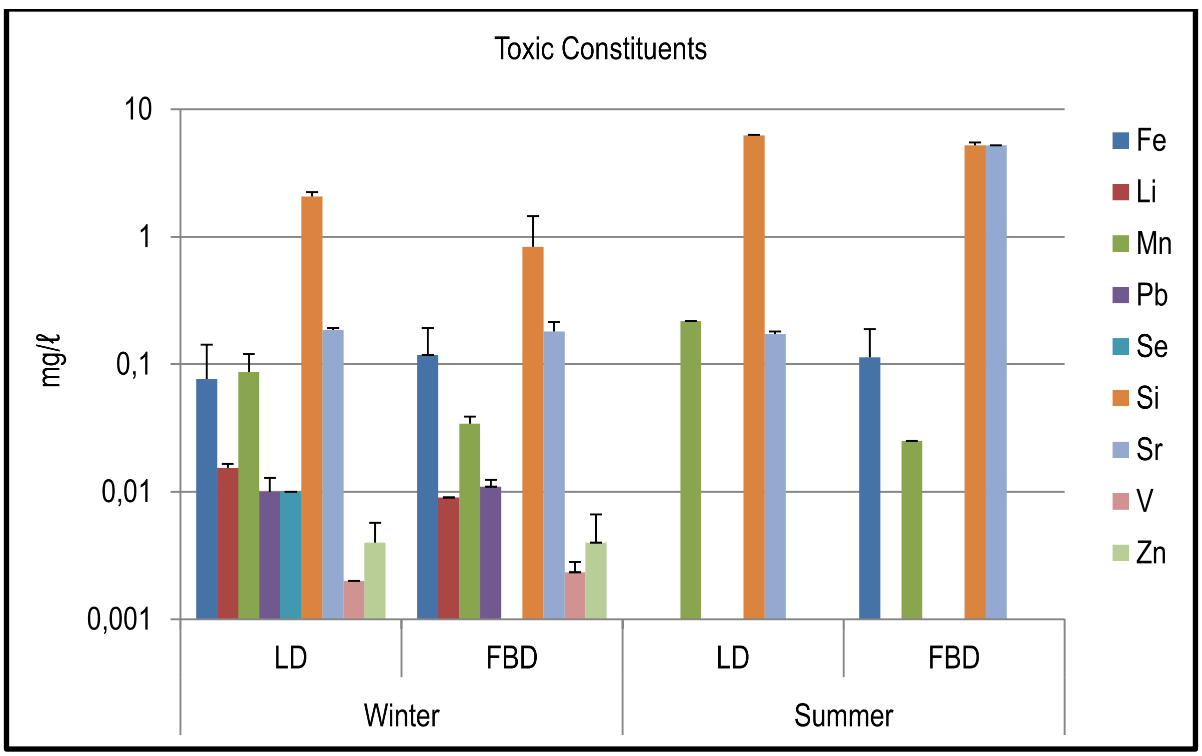

Figure 3.13 Toxic constituents concentrations recorded at Loskop and Flag Boshielo dams.

Iron

Iron (Fe) occurs in a natural system as a result of weathering of sulphide ores and igneous, sedimentary and metamorphic rocks. Leaching from sandstones releases iron oxides and iron hydroxides to the environment. However, anthropogenic activities such as burning of coke and coal, acid mine drainage, mineral processing, sewage, landfill leachates and the corrosion of Fe and steel can release Fe to the environment (DWAF 1996a). It occurs in two common states, as reduced ferrous (Fe^{2+}) and the oxidized ferric (Fe^{3+}) state. The form and solubility of Fe in natural waters are strongly dependent upon the pH and the oxidation-reduction potential of the water. Ferric iron is found in solution only at a pH of < 3 (Bartram & Ballance 1996). According to DWAF (1996c), the Fe concentration in unpolluted surface waters ranges from 0.001-0.5 mg/ℓ.

Iron concentration of 0.08 mg/ℓ was recorded at Loskop Dam during winter while the concentration was below detection level during summer (Fig. 3.13 & Table 3.1). Concentrations of 0.12 mg/ℓ and 0.11 mg/ℓ were recorded at Flag Boshielo Dam during winter and summer respectively (Fig. 3.13 & Table 3.1). The concentration of Fe was within the TWQR as suggested for domestic use and above for aquaculture at both localities. In aquatic ecosystems, Fe concentrations should not be allowed to vary by more than 10% of the background dissolved Fe concentration for a particular site or case, at a specific time. However, it is generally accepted that the concentration of soluble ionized forms of Fe should not exceeds 0.2 mg/ℓ for cyprinids fish (Svobodóva *et al.* 1993).

Lead

Lead (Pb) is a common and toxic trace metal which has the capability of being accumulated in living tissues, and in vertebrates, to become immobilized in bone, where it does not exhibit toxic effects (Dallas & Day 2004). Lead toxicity to fish and other aquatic organisms is significantly influenced by the water quality and depends on the solubility of Pb compounds and on the concentration of Ca^{+} and Mg^{2+} in water (Svobodóva *et al.* 1993). Sources of Pb include industrial and municipal wastewaters discharge, mining, combustion of fossil fuel etc. Lead enters the aquatic ecosystem and largely accumulates at the bottom sediment at concentration about four times greater than in the water. Decreasing pH values increases the bioavailability of divalent Pb, which is accumulated by aquatic biota (DWAF 1996a). The water solubility of Pb compounds is reduced with increasing alkalinity at a constant pH. Furthermore, the toxicity of Pb is known to be reduced with increasing Ca^{+} and Mg^{2+} concentrations in water (Svobodóva *et al.* 1993; DWAF

1996a). Lead is correlated with the water hardness (DWAF 1996a) and the aquatic ecosystem TWQR was set with respect to water hardness and the table below shows how these two constituents are correlated:

Water Hardness (mg $CaCO_3$/ℓ)	**< 60** (soft)	**60-119** (medium)	**120-180** (hard)	**>180** (very hard)
TWQR	0.0002	0.0005	0.001	0.0012

The Pb concentration could only be detected during winter at both localities. The concentrations recorded were 0.010 mg/ℓ at Loskop Dam and 0.011 mg/ℓ at Flag Boshielo Dam (Fig. 3.13 & Table 3.1). The Pb concentrations were above the TWQR for aquatic ecosystem but within the range for aquaculture and domestic use. Since the solubility of Pb strongly depend on physico-chemical properties of the water, the variation of system variables, non-toxic constituents, ions and nutrients might have had an impact on the insolubility of Pb at both localities.

Manganese

In aquatic ecosystems, manganese (Mn) does not occur naturally as a metal but is found in various salts and minerals, frequently in association with Fe compounds. Manganese exists in two forms, as soluble manganous (Mn^{2+}) form, but is readily oxidised to the insoluble manganic (Mn^{4+}) form (DWAF 1996a). Natural sources of Mn include soils, sediments and metamorphic and sedimentary rocks and anthropogenic sources are sewage and industrial effluents, and acid mine drainage (Bartram & Ballance 1996). Similar to Fe, the concentration of dissolved Mn is influenced by changes in redox potential, dissolved oxygen, pH and organic matter. In a natural system, the concentration of Mn ranges from 0.00002-0.13 mg/ℓ (DWAF 1996b). According to Dallas and Day (2004), high concentrations of Mn are toxic to vertebrates leading to disturbances in various metabolic pathways.

Manganese concentrations of 0.09 mg/ℓ and 0.22 mg/ℓ were recorded at Loskop Dam during winter and summer respectively with a concentration of 0.03 mg/ℓ being recorded at Flag Boshielo Dam during both seasons (Fig. 3.13 & Table 3.1). The Mn concentrations were within the TWQR which is 0.18 mg/ℓ for aquatic ecosystem except for Loskop during summer. The seasonal mean concentrations at both localities were found to be 0.16 mg/ℓ at Loskop Dam and 0.03 mg/ℓ at Flag Boshielo Dam. The solubility of Mn decreases as the water hardness ($CaCO_3$) increases (DWAF 1996a). There were no significant differences in the Mn concentration between the two localities ($p>0.05$).

Selenium

Selenium (Se) is a non-metallic element which occurs in five oxidation states, namely, -II, 0, II, IV and VI, of which the tetravalent state is the most common. It occurs naturally as ferric selenite, calcium selenate, as elemental Se and in organic compounds derived from decayed plant tissue (Bartram & Ballance 1996; DWAF 1996a; Dallas & Day 2004). It may occur at increased concentrations in water bodies subject to industrial pollution, or in the vicinity of industrial activities utilising or discharging Se or Se compounds (DWAF 1996a). Selenium interacts with sulphur (S), Fe and As and with metals such as Cu, Cd and mercury (Hg). Selenium speciation depends on pH and redox potential of an aquatic ecosystem. A decrease in water pH decreases the solubility of Se and hence, decreases in pH have very little impact on the toxicity of Se (DWAF 1996a). In aquatic environments with low pH values or high reducing capacity, elemental Se is reduced to selenides (Se^{2-}). At high pH and/or under oxidizing conditions, elemental Se is oxidized to selenite (SeO_3^{2-}) and then selenate (SeO_4^{2-}), which represent Se(IV) and Se(VI), respectively (DWAF 1996b).

Selenium was only detected at Loskop Dam during winter throughout the study. The concentrations were above the TWQR suggested for aquatic ecosystem. At Flag Boshielo Dam it could not be detected during both seasons. According to DWAF (1996b), dietary Se interacts with As and Cd and it decreases their toxicity. Arsenic and Cd were only detected during winter at both localities. Arsenic concentrations of 0.0037 mg/ℓ and 0.0040 mg/ℓ were recorded at Loskop and Flag Boshielo dams respectively. Cadmium concentration of 0.001 mg/ℓ was recorded at both localities (Fig. 3.13 & Table 3.1). The solubility of Cd is high under acidic conditions and low under neutral and alkaline conditions, therefore, pH might have had an influence on Cd concentration in this study. The toxicity of As and Cd depends on physico-chemical properties such as water temperature, pH, chemical speciation etc. (DWAF 1996a).

Silicon

Silicon (SI) occurs predominantly as silica or as various silicates (DWAF 1996d). It is an essential element for certain aquatic plants (principally diatoms). It is taken up during cell growth and released during decomposition and decay giving rise to seasonal fluctuations in concentrations, particularly in lakes (Chapman 1996). Silica is a major constituent of igneous and metamorphic rocks, of clay minerals such as kaolin, and of feldspars and quartz. It is likely that most of the dissolved silica in water originates from the chemical breakdown of silicates in the processes of metamorphism or weathering (Bartram & Ballance 1996). Furthermore, Si may be discharged into water bodies with wastewaters from industries using siliceous compounds in their processes such as potteries, glass works and abrasive manufactures (Chapman 1996). The concentration of Si in most natural waters is in the range of 1-30 mg/ℓ (Bartram & Ballance 1996; Chapman 1996).

A higher concentration of silicon was recorded at Flag Boshielo Dam than Loskop Dam with concentrations of 6.23 mg/ℓ and 5.20 mg/ℓ being recorded during winter and summer respectively (Fig. 3.13 & Table 3.1). A concentration of 2.07 mg/ℓ was recorded at Loskop Dam during winter and 0.83 mg/ℓ during summer (Fig. 3.13 & Table 3.1). There were significant difference in the Si concentration between the two localities ($p<0.05$). As mentioned previously, Bartram and Ballance (1996) and Chapman (1996) reported that the Si concentration in most natural waters ranges from 1-30 mg/ℓ. Struyf *et al.* (2011) reported that Si is a key nutrient in determining the species composition of aquatic and coastal phytoplankton communities but there is no TWQR available for aquatic ecosystems. However, the concentration at Flag Boshielo Dam was above the TWQR suggested for industrial use and values at Loskop Dam were within the range. Riparian vegetation are often characterized by large standing stocks of biomass and can contain a large amount of Si so it can serve as a source of Si in an aquatic ecosystems particularly lakes or dams (Struyf *et al.* 2011).

Zinc

Zinc (Zn) is an essential nutritional trace element for plants and animals. Humans have a high tolerance level to elevated Zn concentrations, while fish are highly susceptible to Zn poisoning (DWAF 1996c). Zinc occurs in rocks and ores and is readily refined into a pure stable metal. It can enter aquatic ecosystems through both natural processes such as weathering and erosion, and through industrial activity. It occurs in two oxidation states in aquatic ecosystems, namely as the metal, and as Zn(II). Zinc(II) ion is toxic to aquatic ecosystems particularly to fish even at a relatively low concentration (DWAF 1996a). The toxicity of Zn to fish is influenced by the chemical characteristics of water; in particular, increasing Ca concentrations reduce the toxicity of Zn (Svobodóva *et al.* 1993). The concentration of Zn in inland waters is usually low, typically about 0.015 mg/ℓ. Elevated Zn concentrations arise at neutral and alkaline pH (DWAF 1996b). However, the lethal Zn concentrations are around 0.5 to 1.0 mg/ℓ for cyprinids fish (Svobodóva *et al.* 1993).

Zinc concentrations of 0,004 mg/ℓ were recorded at both localities during winter (Fig. 3.13 and Table 3.1). The concentration at both localities was below detection levels during summer. According to DWAF (1996a), the greatest dissolved zinc concentrations will occur in water with low pH, low alkalinity and high ionic strength, hence, chemical speciation of zinc is affected primarily by pH and alkalinity. The zinc concentrations recorded during winter was above the TWQR for aquatic ecosystem (0.002 mg/ℓ) but within the range for aquaculture (1 mg/ℓ) and domestic use (3 mg/ℓ).

3.3 CONCLUSION

The Olifants River System is known as one of the most polluted river systems in South Africa with Loskop Dam serving as a repository for pollutants from mining and industrial activities in the upper catchment. In the present study, lower concentration of metals were recorded at Loskop Dam than Flag Boshielo Dam but the difference were not significant ($p>0.05$). However, Ca, K and Mg ion concentrations were higher at Loskop Dam with Na and Cl^- being higher at Flag Boshielo Dam. All nutrients were higher at Loskop Dam than Flag Boshielo Dam and Oberholster *et al.* (2010) noted eutrophic to hypertrophic condition at Loskop Dam. However, the present study noted mesotrophic state at Loskop Dam with Flag Boshielo Dam being oligotrophic. Sources of pollution in the upper Olifants River include acid mine drainage emanating from a number of abandoned coal mines and the discharge of treated, partially treated and untreated domestic and industrial sewage from municipal sewage treatment works (Oberholster 2009). But, these anthropogenic activities did not severely elevate the nutrients levels at Loskop Dam during the present survey.

Some metals are insoluble in alkaline pH, so they tend to precipitate in some cases and sink to the bottom sediment. Water quality does not reflect the overall chemical condition within the catchment; therefore, metal accumulation analysis was also carried in the sediment and fish tissues. Water quality study is a very complex study, because the solubility of constituents depends on one another. A high concentration of some metals can reduce the solubility and toxicity of another. For example, the toxicity of copper is reduced in the presence of Zn, Mo and SO^{2+} (Dallas & Day 2004). The present study showed that the water quality is in acceptable conditions at both localities although some constituents were above TWQR for aquatic ecosystems.

CHAPTER4

ACCUMULATION OF SELECTED METALS

4.1 INTRODUCTION

All metals are natural constituents of the environment and are found in varying levels in all ground and surface waters. However, anthropogenic activities such as industries, mining, agriculture etc. may release contaminants into the environment which increase the concentration levels of metals. Metals tend to accumulate in sediment through adsorption and precipitation processes; therefore, sediments are considered to be the ultimate sink for contaminants (Coetzee *et al.* 2002; Wepener & Vermeulen 2005). Sediment may accumulate excessive quantities of contaminants that directly disrupt aquatic ecosystems (Burton 2002). Furthermore, metals can be reintroduced into the water column in a bioavailable form and organisms such as fish absorb these metals from the water by means of gills or epithelial tissues and accumulate them in their body tissues (Coetzee *et al.* 2002).

Some of these metals are required for the normal metabolism of aquatic organisms, while others are non-essential and play no significant biological role. However, organisms can accumulate metals to levels above which are required for normal physiological functioning (Coetzee *et al.* 2002). According to Moiseenko and Kudryavtseva (2001), a significant deviation from the natural ratio of these substances in an organism causes negative, frequently deadly consequences. Davies and Day (1998) reported that the concentration of a particular chemical substance in the body of an organism is seldom directly proportional to the concentration of it in the surrounding water.

Metals may enter a fish's body in five possible ways; through the body surface, gills, ingestion of food, non-food particles and oral consumption of water (Muhammad 2005). Once the metals are absorbed, they are transported by the blood to either a storage point or to the liver for transformation and storage (Ayandiran *et al.* 2009). The toxicity of the metals may not be easily defined due to the number of factors that may influence or modify them. Some of the factors that can influence metal toxicity include: the metal species in the water, the presence of other metals, abiotic factors such as water temperature, dissolved oxygen (DO), pH, water hardness and salinity, biotic factors such as age, size and sex, stage in life history and adaptive capabilities as well as behavioural responses (Van Vuren *et al.* 1994a). Ayandiran *et al.* (2009) further revealed that the toxicity of metals may vary along the food chain.

Aquatic biota accumulate metals along the food chain with the organisms higher in the food chain accumulating more until the organisms at the top of the food chain accumulate lethal dose, the phenomenon called biomagnification. The organisms at the top of the food chain may accumulate a million times more than the organisms at the bottom (Davies & Day 1998). Fish is one of the organisms situated near or at the top of the food chain and they are known to accumulate metals within their organs and tissues (Barbour *et al.* 1999). Therefore, in an ecological study, fish can be used as an accumulation indicator. According to Abel (1996), the levels of pollutants within the tissues of living organisms can be used to indicate the degree of contamination of the waters in which they live.

This chapter present the results on accumulation of metals (aluminium, antimony, copper, iron, lead, manganese, selenium, silicon, strontium and zinc) in the muscle, gills and liver of *L. rosae* as well as in sediment.

4.2 RESULTS AND DISCUSSION

4.2.1 Metal accumulation in sediment

Sediment tends to accumulate metals from surface water through adsorption and precipitation processes whereby under some circumstances, e.g. change in pH, the metals can be reintroduced into the water column in a bioavailable form (Coetzee *et al.* 2002). In despite, benthic organisms in close proximity to the sediment can assimilate metals bound to sediment. Some of these organisms may then be subjected to predation, thus transferring metals throughout the food web and/or food chain, a process known as biomagnification (Barker 2006). Metal accumulation in sediment occurs over a long period of time; therefore, sediment can provide information on the contamination history of the system. Sediments are considered to be the ultimate sink for pollutants (Greenfield *et al.* 2007). The results of metal accumulation in sediment at Loskop and Flag Boshielo dams are presented in Figure 4.1a&b.

The metals that showed high concentrations in sediment were iron and aluminium. The general trend of accumulation at Loskop Dam was as follows: Fe > Al > Si > Mn > Zn > Cu > Sb > Sr > Pb > Se whereas at Flag Boshielo Dam was as follows: Fe > Al > Mn > Si > Zn > Cu > Sr > Pb > Sb > Se. Sediment showed to have accumulated higher concentrations of iron and aluminium with lesser concentration of selenium. Osman and Kloas (2010) reported that the toxicity, mobility and bioavailability of metals depend on physico-chemical properties of water such as pH, temperature, water hardness, etc.

The levels of aluminium, copper, iron, manganese, lead, silicon and strontium were higher at Flag Boshielo Dam with antimony, selenium and zinc being higher at Loskop Dam (Figs 4.1a&b). Despite selenium concentration being below detection level in the water at Flag Boshielo Dam, the concentration of 0.0034 mg/g could be detected in sediment. Svobodóva *et al.* (1993) described selenium as a metal with high bioaccumulation capacity. Therefore, the concentration of this metal in the water may not provide a true indication of the total concentration in the system; it is better to integrate sediment as well as tissues of predatory organisms found at the top of the food chain as indicators. All other metals could be detected in the water, sediment and fish tissues.

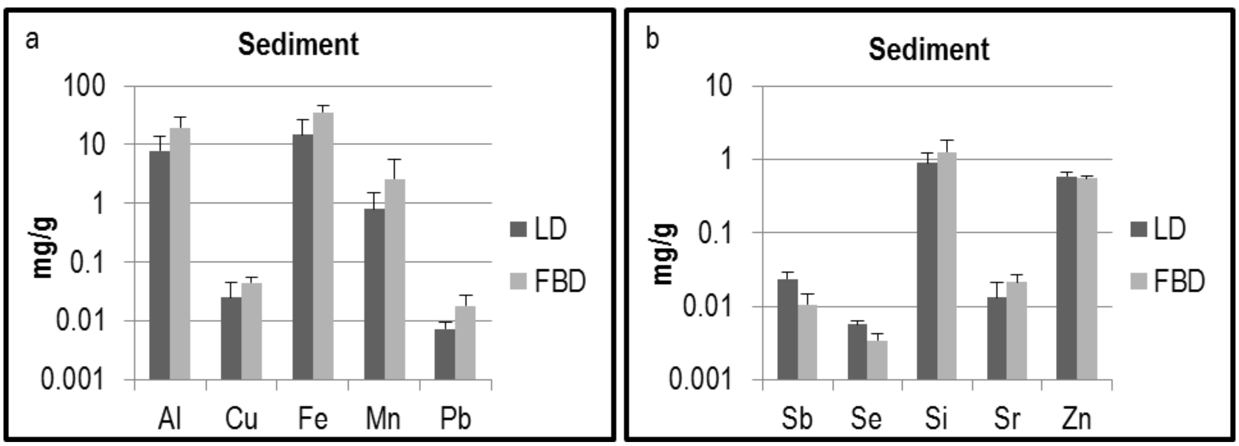

Figure 4.1 Mean concentration of metals in sediment from Loskop and Flag Boshielo Dam.

4.2.2 Bioaccumulation in fish tissues

Aluminium

Higher concentration of aluminium (Al) was recorded in the liver followed by gills and muscle respectively at both localities (Fig. 4.2a & Figs 4.3a&b). The liver and gills of fish from Flag Boshielo Dam exhibited higher Al concentration than the liver and gills from Loskop Dam with muscle having higher Al concentration at Loskop Dam than Flag Boshielo Dam. Aluminium concentration was higher in the water and sediment at Flag Boshielo Dam than at Loskop Dam and these might have resulted in the Al concentration in the liver and gills being higher at Flag Boshielo Dam than at Loskop Dam. However, there were no significant differences between the Al concentrations in the liver and gills between the two localities ($p>0.05$). In contrast, Al concentration in the muscle was significantly higher at Loskop Dam than at Flag Boshielo Dam ($p<0.05$).

Coetzee *et al.* (2002) noted gills of *L. umbratus* accumulating high levels of Al followed by liver and muscle respectively. Consistent with Coetzee *et al.* (2002), Crafford and Avenant-Oldewage (2010) recorded higher Al concentration in the gills, followed by liver and muscle respectively from *Clarias gariepinus*. Aluminium is highly pH dependent, and its bioavailability may as well be influenced by water hardness and temperature (DWAF 1996a). The pH of the water was alkaline (> 7.5) at both localities; therefore, the variation of Al levels within different tissues and localities might have been influenced by its solubility, bioavailability and mobility.

Table 4.1 Mean concentration of metals in the muscle, gills and liver of *Labeo rosae* collected from Loskop and Flag Boshielo dams.

Metals (mg/g)	**Muscle**		**Gills**		**Liver**	
	Loskop	Flag Boshielo	Loskop	Flag Boshielo	Loskop	Flag Boshielo
Al	0.1051 ± 0.02	0.0779 ± 0.01	0.2334 ± 0.28	0.4335 ± 0.58	1.0604 ± 1.58	2.3902 ± 3.97
Cu	0.0018 ± 0.002	0.0119 ± 0.01	0.0001 ± 0.0004	0.0494 ± 0.07	2.0442 ± 2.55	0.4635 ± 0.42
Fe	0.0901 ± 0.07	0.0818 ± 0.05	0.3243 ± 0.34	0.4583 ± 0.66	7.0999 ± 12.22	10.8468 ± 7.37
Mn	0.0038 ± 0.003	0.0041 ± 0.002	0.0761 ± 0.09	0.1164 ± 0.15	0.0731 ± 0.12	0.0388 ± 0.04
Pb	0.0039 ± 0.01	0.0019 ± 0.002	0.0069 ± 0.005	0.0059 ± 0.01	0.0308 ± 0.07	0.1425 ± 0.22
Sb	0.1194 ± 0.02	0.0069 ± 0.008	0.2470 ± 0.31	0.0212 ± 0.03	1.2692 ± 1.89	1.5564 ± 2.23
Se	0.0186 ± 0.01	0.0075 ± 0.005	0.0336 ± 0.05	0.0204 ± 0.30	0.2749 ± 0.36	0.1528 ± 0.24
Si	0.1247 ± 0.10	0.0785 ± 0.04	0.5759 ± 0.86	1.0068 ± 1.23	1.1019 ± 1.52	3.6450 ± 4.71
Sr	0.0088 ± 0.004	0.0097 ± 0.004	0.3605 ± 0.49	0.5231 ± 0.67	0.0362 ± 0.008	0.0433 ± 0.06
Zn	0.4873 ± 0.13	0.3052 ± 0.03	1.0356 ± 1.19	1.3606 ± 1.65	5.0261 ± 7.30	6.4553 ± 8.85

Copper

Higher concentrations of copper (Cu) were recorded from the liver than other organs with higher levels being recorded at Loskop Dam than Flag Boshielo Dam (Fig. 4.2b & Table 4.1). The muscle of fish at Loskop Dam has accumulated a higher concentration of Cu than the gills while gills at Flag Boshielo Dam accumulated higher concentration than the muscle (Fig. 4.2b & Table 4.1). No definite pattern was observed for Cu accumulation among the three tissues. However, there were significant differences between the Cu concentration in the liver, gills and muscle between the two localities ($p<0.05$). Wepener *et al.* (2001) reported the gills of *Tilapia sparrmanii* accumulating Cu from the water at a faster rate than the liver.

Luus-Powell (1997) revealed that the liver of *L. rosae* accumulating high concentrations of Cu, followed by gills with muscle accumulating a lower level. Consistent with Luus-Powell (1997), Coetzee *et al.* (2002) noted higher Cu accumulation in the liver followed by gills and muscle in *L. umbratus* from Klein Olifants River in Mpumalanga. Although Robinson and Avenant-Oldewage (1997) have studied *Oreochromis mossambicus*, similar pattern (liver > gills > muscle) was reported for Cu accumulation. In very alkaline water, Cu forms hydroxides of low solubility, and in waters with a high bicarbonate/carbonate concentration, Cu precipitates as poorly soluble or insoluble cupric carbonate (Svobodóva 1993). In the present study the water was alkaline at both localities, with the pH ranging from 7.8 to 10.1. This pH levels might have had an influence on the bioavailability of Cu.

Table 4.2 The ranking of metal concentration in the tissues and sediment in a descending order.

Metals	Loskop Dam	Flag Boshielo Dam
Al	sediment > liver > gills > muscle	sediment > liver > gills > muscle
Cu	liver > sediment >muscle > gill	liver > gills > sediment > muscle
Fe	sediment > liver > gills > muscle	sediment > liver > gills > muscle
Mn	sediment > gills > liver > muscle	sediment > gills > liver > muscle
Pb	liver > sediment > gills > muscle	liver > sediment > gills > muscle
Sb	liver > gills > muscle > sediment	liver > gills > sediment > muscle
Se	liver > gills > muscle > sediment	liver > gills > sediment > muscle
Si	liver > sediment > gills > muscle	liver > gills > sediment > muscle
Sr	gills > liver > sediment > muscle	gills > liver > sediment > muscle
Zn	liver > gills > sediment > muscle	liver > gills > sediment > muscle

Iron

Both localities showed a similar pattern for the accumulation of iron (Fe). Liver has accumulated a higher concentration of iron followed by gills and muscle respectively (Fig. 4.2c & Table 4.1). The liver and gills from Fag Boshielo Dam showed to have accumulated higher amount of Fe than the liver and gills from Loskop Dam (Fig. 4.2c). However, muscle showed to have accumulated more iron at Loskop Dam than at Flag Boshielo Dam (Fig. 4.2c). The difference of Fe concentrations in the tissues was not significant between the two localities ($p>0.05$). The liver has accumulated higher concentrations of Fe than gills and muscle whereby mean concentrations of 7.1 mg/g and 10.8 mg/g were recorded at Loskop and Flag Boshielo dams respectively. The concentration in gills and muscle were below 1 mg/g.

Luus-Powell (1997) also recorded higher Fe concentration in the liver followed by gills and muscle in *L. rosae* from the lower Olifants River. Furthermore, Coetzee *et al.* (2002) noted the same trend (liver > gills > muscle) for Fe accumulation in *L. umbratus* and attributed the high Fe concentrations in the liver tissue to the iron-containing enzymes and the extensive vascular system of the liver, as the haemoglobin in the blood binds approximately three quarters of the Fe in the body. Robinson and Avenant-Oldewage (1997) revealed a higher concentration of Fe in the liver of *Oreochromis mossambicus* followed by gills and muscle. The higher levels of Fe in the liver might as well be due to liver detoxification function which makes it a target organ for various xenobiotic substances (Van Dyk 2003a).

Manganese

The manganese (Mn) concentration was found to be higher in the gills, followed by liver and muscle respectively (Fig. 4.2d & Table 4.1). The concentrations in the gills were found to be 0.12 mg/g at Flag Boshielo Dam and 0.08 mg/g at Loskop Dam (Table 4.1). There were no significant differences in the Mn concentration in the liver, gills and muscle between the two localities ($p>0.05$). The concentration variation between gills and liver were minimal with a far lesser concentration being recorded in the muscle. The muscle and gills from Flag Boshielo Dam showed to have accumulated higher concentrations of Mn than the muscle and gills from Loskop Dam (Fig. 4.2c). In contrast, the Mn concentration in the liver was higher at Loskop Dam that at Flag Boshielo Dam (Fig. 4.2c).

Coinciding with this study, Luus-Powell (1997) recorded higher Mn concentration in the gills with lower concentration being recorded in the muscle. Furthermore, Nussey *et al.* (2000) and Coetzee *et al.* (2002) supported the fact that Mn accumulation in *Labeo* sp. may be higher in the gills, followed by liver and muscle respectively. Robinson and Avenant-Oldewage (1997) observed a similar trend (gills > liver > muscle) for Mn in *O. mossambicus* from the lower Olifants River inside the Kruger National Park (KNP). The Mn concentration in the water at Loskop Dam was higher than at Flag Boshielo Dam and so was Ca^{2+} concentration. According to Robinson and Avenant-Oldewage (1997), Ca^{2+} ions compete with Mn^{+} ions for absorption through the gills; therefore the lower Mn levels in the gills at Loskop Dam might be attributed to the higher Ca^{+} concentration recorded in the gills.

Lead

The muscle and gills showed to have accumulated higher concentration of lead (Pb) at Loskop Dam than at Flag Boshielo Dam with liver having accumulated higher levels at Flag Boshielo Dam than at Loskop Dam

(Fig. 4.2e). The general trend showed that liver has accumulated more Pb than gills and muscle (Figs 4.2e & 4.4a&b). The mean concentrations of 0.0308 mg/g and 0.1425 mg/g were recorded for Pb at Loskop and Flag Boshielo dams respectively in the liver (Figs 4.2e & Table 4.1). There were no significant difference in the Pb concentration in the tissues between the two localities ($p>0.05$).

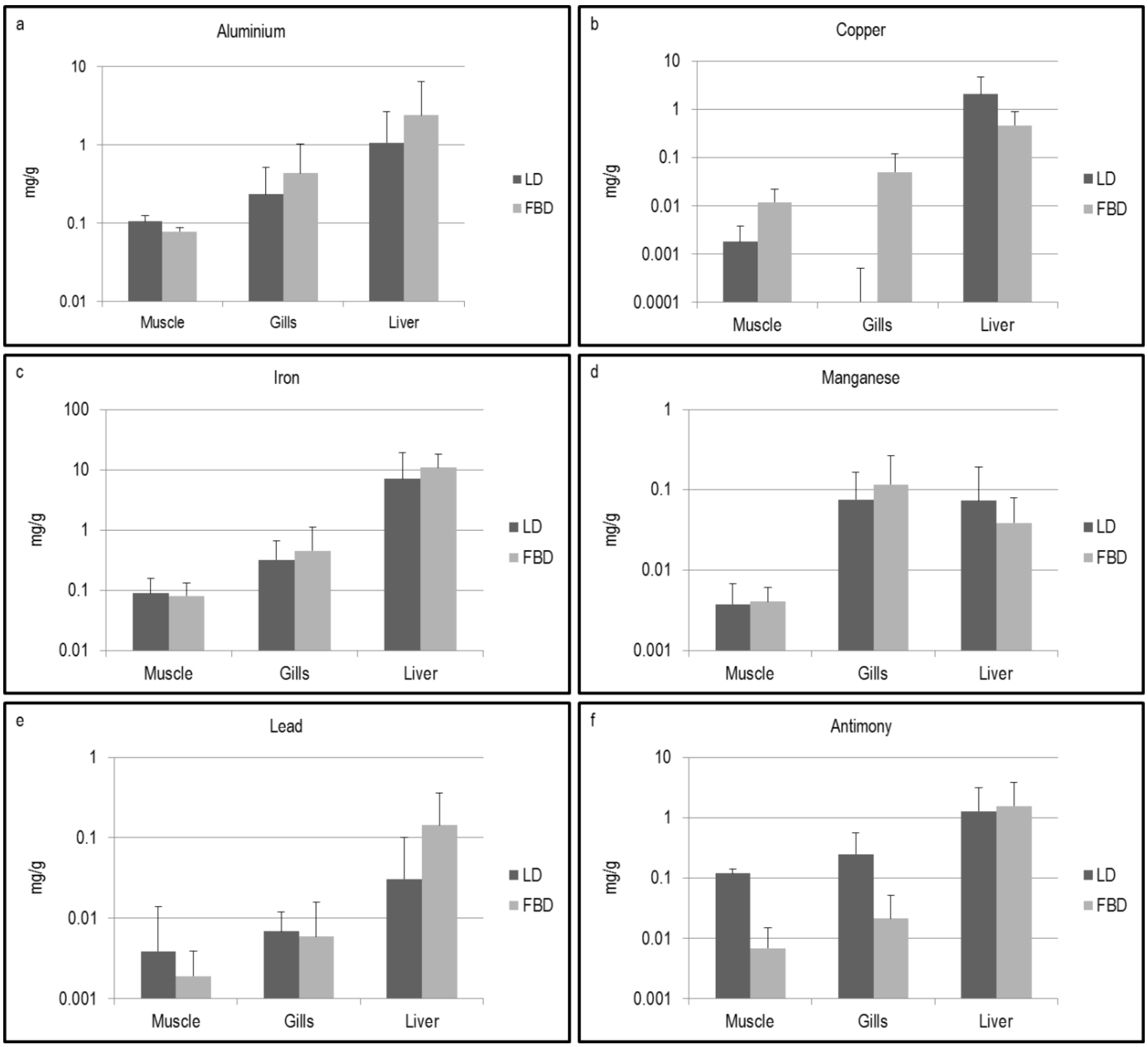

Figure 4.2 Concentration of metals in the muscle, gills and the liver of *Labeo rosae* from Loskop and Flag Boshielo dams.

Luus-Powell (1997) noted variation of Pb concentration levels between tissues of *L. rosae.* Coetzee *et al.* (2002) reported higher Pb concentration in the gills followed by liver and muscle respectively for *Labeo* *umbratus*. In despite, Nussey *et al.* (2000) reported a higher amount of Pb in the gills followed by muscle and liver. Therefore, Pb shows no specific patterns of bioaccumulation in different tissues of fish. The toxicity and bioavailability of Pb is inversely related to the calcium content of water, with Pb less toxic at high calcium concentrations. Elevated concentration of Pb can pose serious threat to aquatic ecosystem as well as to human who consume fish from Pb contaminated water body. Heath *et al.* (2004) reported that Pb can damage the nervous system, kidneys and reproductive system but the most sensitive is the central nervous system, particularly in children. In the present study, Loskop Dam had higher concentration of Ca^{2+} as compared to Flag Boshielo Dam; therefore, the bioavailability of Pb was lower at Loskop Dam than at Flag Boshielo Dam.

Antimony

Higher concentrations of antimony (Sb) were recorded in the liver, compared to gills and muscle of fish (Figs 4.2f & 4.4a&b). The liver concentrations were found to be 1.2692 mg/g at Loskop Dam and 1.5564 mg/g at Flag Boshielo Dam (Table 4.1). Although Fu *et al.* (2010) reported that Sb is generally not readily mobilized in the environment despite its high concentrations in the soil at smelter and mining sites; the present study has recorded Sb concentration in the water, sediment and fish tissues at both localities. There were significant difference on the Sb concentrations in gills and muscle between the two localities with higher concentration recorded at Flag Boshielo Dam ($p<0.05$). In contrast, no significant difference were observed in the Sb concentrations in the liver between the two localities ($p>0.05$).

Antimony pollution is a global issue because of its toxicity to humans and its role in causing diseases of liver, skin, and respiratory and cardiovascular systems. The International Association for Cancer Research (IARC) has reported that inhalation of Sb oxides can be carcinogenic in vertebrates (IARC, 1989). Other studies have shown that Sb is a human carcinogen and it has been listed as a priority pollutant of interest in both the United States and European Union (Wu *et al.* 2011). However, little has been done in South Africa on the bioaccumulation of Sb in aquatic biota.

Selenium

Selenium (Se) could not be detected in the water at Flag Boshielo Dam whereas a mean concentration of 0.01 mg/ℓ was recorded at Loskop Dam. However, concentrations of 0.0075 mg/g, 0.0204 mg/g and 0.1528 mg/g were recorded in the muscle, gills and liver of fish at Flag Boshielo Dam (Fig. 4.3a & Table 4.1). Muscatello *et al.* (2008) also recorded high concentration of Se in the fish and sediment even though the concentration in the water was below detection level. The liver, gills and muscle from Loskop Dam had higher Se concentration than the liver, gills and muscle from Flag Boshielo Dam (Fig 4.3a). Significant difference of Se concentration was only observed in the muscle between the two localities ($p<0.05$) and no significant difference was noticed in the liver and gills ($p>0.05$). The general pattern of Se accumulation has shown liver accumulating a higher concentration of Se followed by gills and muscle respectively (Fig. 4.3a).

According to Lemly (1999), once Se is released into the aquatic environment, it can be removed from the water column and deposited into sediments by adsorption, complexation, and coprecipitation processes, as well as, absorption by aquatic organisms. Furthermore, Muscatello *et al.* (2008) stated that even though the uptake of Se by aquatic organisms can occur through water or diet, dietary uptake is usually the dominant pathway of Se accumulation in upper trophic levels such as in fish. Therefore, Se might have been accumulated through dietary uptake at Flag Boshielo Dam.

Silica

The general pattern of silica (Si) accumulation showed liver accumulating more silica followed by the gills and muscle respectively at both localities. The Si concentration in the liver was found to be 1.1019 mg/g at Loskop Dam and 3.6450 mg/g at Flag Boshielo Dam. The concentrations in the muscle and gills exhibited minimal variation although gills had a higher Si concentration than the muscle. In the gills and liver, the concentrations of Si were found to be higher at Flag Boshielo Dam with Loskop Dam exhibiting high Si concentration only in the muscle (Fig. 4.3b). There were no significant difference in the Si concentration in the liver and gills between the two localities ($p>0.05$). Only Si concentration in the muscle has showed to differ significantly ($p<0.05$) between the two localities.

Strontium

The present study showed strontium (Sr) concentrations being higher in the gills, followed by liver and muscle respectively at both localities (Fig. 4.3c). The difference in Sr concentration in the liver, gills and muscle was not significant between the two localities ($p>0.05$). Although Loskop Dam had higher concentration of strontium in the water, Flag Boshielo Dam exhibited higher concentration in the liver, gills and muscle of fish. According to Seymore *et al.* (1995), when calcium concentration in the water is high, calcium will compete with Sr in the uptake process, resulting in lower Sr accumulation by fish. Therefore, the higher concentration level of Sr in tissues at Flag Boshielo Dam might be attributed to the fact that Ca level was lower in the water at Flag Boshielo Dam than at Loskop Dam.

Furthermore, Chowdhury and Blust (2002) reported that the uptake of Sr is suggested to occur through Ca transport systems located in the chloride cells of gills and enterocytes of the intestine in fish. No study has been done in South Africa on Sr bioaccumulation in *L. rosae*; the only study done was on *Labeobarbus marequensis* (formerly known as *Barbus marequensis*) in the lower Olifants River. Seymore *et al.* (1995) recorded higher level of Sr in the gills, followed by muscle and liver respectively in *L. marequensis*. However, Crafford and Avenant-Oldewage (2010) recorded higher Sr concentration in the gills, followed by liver and muscle in *Clarias gariepinus* from Vaal River System and gills > muscle > liver trend were observed at Vaal River Barrage.

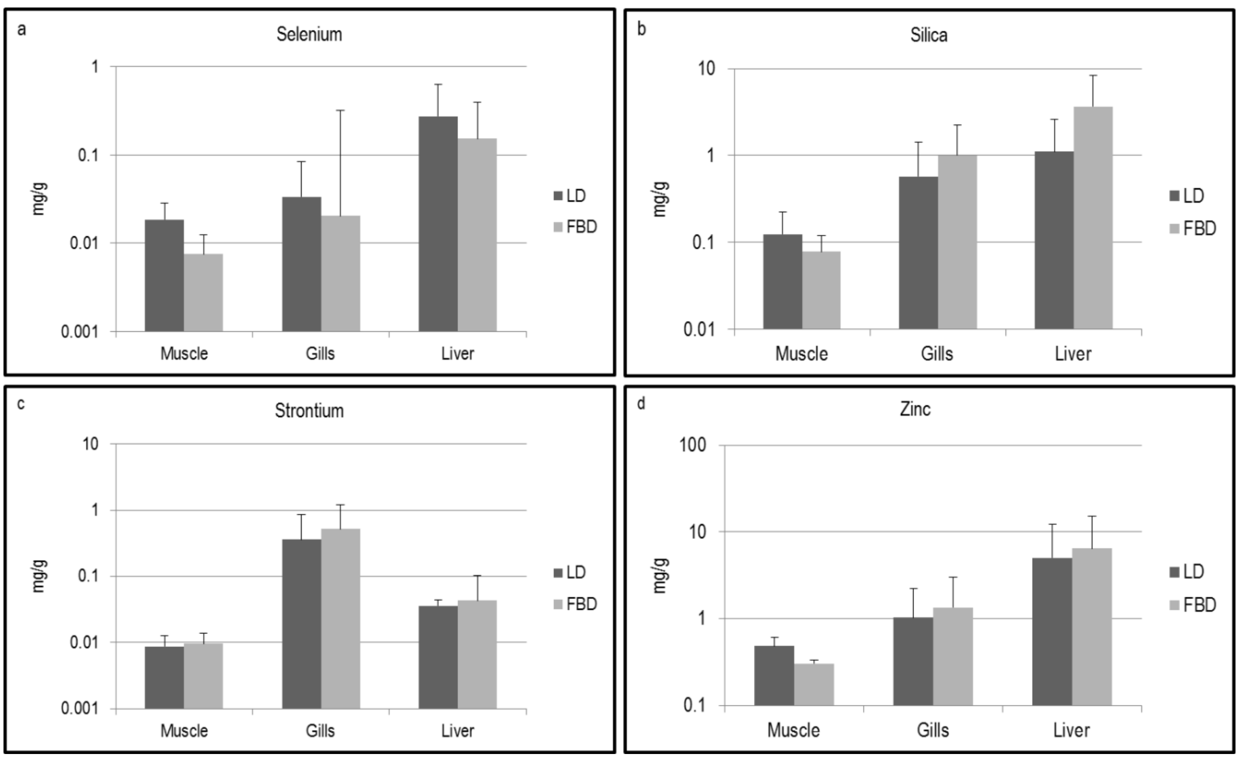

Figure 4.3 Concentration of metals in the muscle, gills and the liver of *Labeo rosae* from Loskop and Flag Boshielo dams.

Zinc

The liver showed to have a far higher concentration of zinc (Zn), followed by gills and muscle respectively at both localities (Fig. 4.3). The concentrations recorded in the liver were 5.0261 mg/g at Loskop Dam and 6.4553 mg/g at Flag Boshielo Dam. The liver and gills accumulated more Zn at Flag Boshielo Dam than at Loskop Dam while the muscle exhibited higher Zn concentration at Loskop Dam than at Flag Boshielo

Dam. There was a significant difference in the concentration of Zn accumulated in the muscle at both localities ($p<0.05$). However, the Zn concentration in the liver and gills showed to differ insignificantly ($p>0.05$). Luus-Powell (1997) recorded higher concentration of Zn in the gills, followed by liver and muscle in *L. rosae*. Coinciding with Luus-Powell (1997), Coetzee *et al.* (2002) also recorded a higher concentration of Zn in the gills, followed by liver and muscle respectively in *L. umbratus*.

According to Osman and Kloas (2010), fish assimilate Zn directly from the water by mucus and gills, therefore, gills are the primary target organ for Zn. However, the relative proportion of Zn uptake from each route varies with the bioavailability of the metal in water and diet (Sappal *et al.* 2009). Osman & Kloas (2010) recorded higher concentration of Zn in the liver of *Clarias gariepinus* and suggested that the higher Zn concentration in the liver of various fish species may be due to the role of Zn as an activator of numerous enzymes present in the liver.

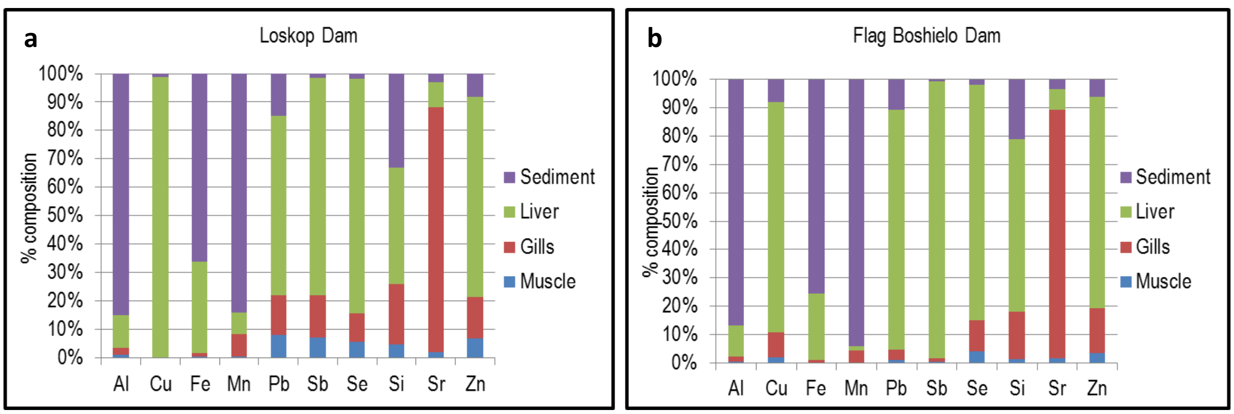

Figure 4.4 Percentage composition of each metal in the water, sediment, liver, gills and muscle of *Labeo rosae* from Loskop and Flag Boshielo dams.

4.2.3 Bioaccumulation factor

The bioaccumulation factor (BAF) can be seen as a constant of proportionality between the concentration of a certain metal in fish tissue and the concentration in the water (BAF_W) and/or sediment (BAF_S) (Coetzee *et al.* 2002). In addition, Arnot and Gobas (2006) defined the bioaccumulation factor as the ratio of chemical concentration in an organism to the chemical concentration in the media. The bioaccumulation factor provides some indication of the bioavailability of metals to the fish from the water and/or sediment (Coetzee *et al.* 2002). Bioaccumulation factors for Al, Cu, Fe, Pb, Sb, Se, Si and Zn were high for liver, followed by gills and muscle at both localities (Tables 4.3 & 4.4). In contrast, Mn and Sr deviated from the liver > gills >

muscle pattern whereby higher bioaccumulation factors were recorded for gills followed by liver and muscle respectively (Tables 4.3 & 4.4).

Table 4.3 Bioaccumulation factors between water and tissues (BAF_w) of *Labeo rosae* at Loskop and Flag Boshielo dams.

Constituents	Flag Boshielo Dam			Loskop dam		
	muscle	gills	liver	muscle	gills	liver
Al	928.20	5685.85	23465.77	2718.28	6037.89	27423.66
Cu	5122.51	18882.86	197658.94	2746.43	199.16	3066341.65
Fe	734.03	4166.60	94797.16	1136.21	4088.59	89494.84
Mn	142.35	3998.33	1635.56	24.73	497.93	478.29
Pb	131.35	478.95	10111.31	398.12	692.94	3081.94
Sb	1686.07	3180.45	262662.23	25604.65	52936.59	271976.38
Se	-	-	-	2790.27	5041.70	41238.66
Si	14.02	138.76	540.89	85.96	397.02	759.68
Sr	63.60	2789.94	228.07	48.04	1966.38	197.50
Zn	126979.66	488059.35	2317640.15	182743.41	388352.75	1884786.28

(-): concentration in water was below detection level.

The concentrations of metals in the tissues were far higher than the concentration in the water resulting in very high BAF_w. The lowest BAF_w recorded was 14.02 for Si in muscle whereas the highest was 2317640.15 for Zn in the liver (Table 4.3). The BAF_w recorded by Seymore *et al.* (1995) for Mn, Pb and Sr ranged from 0.7 to 23533 in various tissues of *L. marequensis*. According to Mohamed (2008), bioaccumulation factor gives an indication about the accumulation efficiency for any particular pollutant in any fish organ.

The lowest BAF_s recorded was 1.63 for Mn in muscle with 123897.28 for Sb recorded in the liver (Table 4.4). There was no vast difference between the concentration of metals recorded for sediment and fish tissues. As mentioned earlier, metals tend to accumulate in sediment through adsorption and precipitation processes and fish has the ability to accumulate metals within their tissues (Coetzee *et al.* 2002; Wepener & Vermeulen 2005). The BAF between water and tissues (BAF_w) were higher than the BAF between sediment and tissues (BAF_s). Similar results were obtained by Seymore *et al.* (1995) and Coetzee *et al.* (2002) on *Labeobarbus marequensis*, and *C. gariepinus* and *L. umbratus*. The BAF_s recorded for Mn, Pb and Sr by Seymore *et al.* (1995) ranged from 0.001 to 7.

Table 4.4 Bioaccumulation factors between sediment and tissues (BAF_s) of *Labeo rosae* at Loskop and Flag Boshielo dams.

Constituents	Flag Boshielo Dam			Loskop dam		
	muscle	gills	liver	muscle	gills	liver
Al	4.01	24.56	101.36	13.41	29.79	135.29
Cu	266.80	983.48	10294.74	73.83	5.35	82428.54
Fe	2.29	13.00	295.79	6.13	22.04	482.54
Mn	1.63	45.89	18.77	4.68	94.17	90.46
Pb	80.27	292.69	6179.13	545.36	949.23	4221.84
Sb	795.32	1500.21	123897.28	5063.07	10467.69	53780.64
Se	2052.85	5616.35	38776.64	3263.47	5896.73	48232.36
Si	63.79	631.23	2460.62	140.81	650.34	1244.39
Sr	480.75	21088.29	1723.87	672.31	27519.35	2764.00
Zn	541.66	2081.91	9886.34	836.31	1777.26	8625.53

4.3 CONCLUSION

Various metals precipitate to the bottom sediment in alkaline conditions (Svobodóva *et al.* 1993). In the present study, Al, Fe and Mn were higher in sediment than in water, liver, gills and muscle at both localities (Figs 4.4a&b). The higher concentration of Al, Fe and Mn in the sediment might be attributed to the alkaline pH at both localities. The present study proved that sediment can serve as a source of pollution as suggested by Van Vuren *et al.* (1994b). According to Van Vuren (1994b), metals are not permanently fixed by sediments; therefore, sediments act as carriers and thus possible source of pollution since metals can be released back into the water column by changes in environmental conditions such as pH, redox potential or the presence of organic chelators. Due to the acid mine drainage that drains into the upper Olifants River, these higher concentrations of Al, Fe and Mn in sediments might become a threat to the ecological state of Loskop and Flag Boshielo dams.

Moreover, the present study deduces that metals at elevated levels do accumulate in the tissues and organs of the organisms in an aquatic ecosystem. *Labeo rosae* has proved to be a reliable bioindicator for bioaccumulation study. Liver is a good indicator of metal pollution due to its capability of bioaccumulation and detoxification function. However, the direct contact with the external water environment of the gills and their capability of bioaccumulation make them a good indicator of prior exposure to both metals and suspended irritant materials. The Cu, Pb, Sb, Se, Si and Zn concentrations were higher in the liver than in the gills, muscle and sediment at both localities. Gills showed a high concentration of Sr at both localities. The liver and gills have proved their effectiveness in reflecting the chemical condition of an aquatic ecosystem. Muscle showed to have accumulated lesser metal concentrations than gills and liver, however, the concentrations of Al, Cu, Sb, Se and Zn were higher in the muscle than in the water at both localities. Furthermore, muscle is the tissues that are consumed by human. Therefore, muscle cannot be excluded in bioaccumulation studies.

Water quality gives a snapshot at the time of sampling while bioaccumulation can provide information on the contamination history. The inclusion of bioaccumulation in aquatic ecosystem monitoring provides a bigger picture on the chemical condition that the system has been through. Furthermore, bioaccumulation factor is a reliable tool in estimating the extent of bioaccumulation relative to the medium. Since little has been done regarding bioaccumulation of *L. rosae* in the Olifants River, this study will serve as baseline information for the future studies.

CHAPTER 5

HEALTH ASSESSMENT INDEX AND PARASITE INDEX

5.1 INTRODUCTION

The ecological health of freshwater ecosystems cannot be measured directly but rather by the health of the biota found in the system (Robinson 1996). This statement emphasizes that, if the organisms in a particular ecosystem appear normal, there is a high probability that the ecological state of that particular system is good. Although fish in their natural environment are subjected to numerous stressors such as fluctuating water temperature, low dissolved oxygen, limited food availability and high sediment load, they are relatively sensitive to anthropogenic changes such as pollution (Adams *et al.*1993; Van Dyk 2003b). Fish health may thus reflect and give a good indication of the health status of a specific aquatic ecosystem (Van Dyk 2003a).

A variety of approaches have been used over the past years to evaluate the effects of contaminants on the health of fish populations and most of them could not be rapidly and inexpensively applied to field studies (Adams *et al.* 1993). As an alternative to the more sophisticated approaches, Goede & Barton (1990) developed a field necropsy method that provides a health condition of fish based on the percentages of anomalies observed in the tissues and organs of individuals sampled from a population. Adams *et al.* (1993) improved the method of Goede & Barton (1990) by developing a quantitative Health Assessment Index (HAI), intending to minimize the limitations of the necropsy-based system.

The quantitative HAI is an index that allows statistical comparisons of fish health among data sets (Adams *et al.* 1993). The fish HAI comprises the evaluation of the external condition of fish (any aberrations of the skin, fins, opercules and eyes) as well as all internal organs and assigning values based on the degree of severity, i.e. 0 represent normal conditions while abnormal condition can assume values of 10, 20 or 30, depending on the degree of severity. The sum total of values awarded being the index value for that fish and the mean calculated for all fish in the sample being the index value for that locality (Crafford & Avenant-Oldewage 2009). An increase in index value correlates with decreased water quality, and hence increased stress (Adams *et al.* 1993; Crafford & Avenant-Oldewage 2009).

When using an autopsy-based system such as the HAI, the following assumptions are required:

I. When all organs and tissues appear normal according to the autopsy criteria, there is a good probability that the fish is normal.

II. When fish are exposed to elevated levels of contaminants (fish under stress), tissue and organ function will change in order to maintain homeostasis.

III. If a change in function persists in response to continuing stress, there will be a gross change in the structure of organs and tissues and

IV. If the appearance of an organ or tissue system departs from the normal or from a control condition, the fish is responding to changes brought about by the environmental stressor (Goede & Barton 1990).

The HAI has been successfully tested in United States of America in the pulp-polluted Tennessee River basin contaminated with polychlorinated biphenyls and in the Pigeon River that receives effluents from a bleached kraft mill (Adams *et al*. 1993). Avenant-Oldewage and Swanepoel (1993) were the first to suggest the use of fish health studies in South Africa. Subsequently, the fish HAI has been applied and adapted for local conditions, through studies on the Olifants River system (Avenant-Oldewage *et al*. 1995, Jooste *et al*. 2005). The indicator species used are *Clarias gariepinus* (Marx 1996; Watson 2001; Jooste *et al.* 2005; *Labeo* spp. (Luus-Powell 1997) and *Oreochromis mossambicus* (Robinson 1996; Jooste *et al.* 2005). After successful application in the Olifants River, the HAI was also tested in the Vaal River system on *C. gariepinus* (Crafford & Avenant-Oldewage 2009). Furthermore, Madanire-Moyo *et al.* (2012) successfully applied HAI with *C. gariepinus* as an indicator species in the Limpopo River system.

Other variables of the HAI that receive assigned values based on the severity of condition are parasite loads. Contaminants have different influences on endo- and ectoparasites and therefore these were incorporated as separate variables in the HAI tested in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001). In the original HAI by Adams *et al*. (1993), parasites were recorded as present or absent. The inserted Parasite Index distinguished between the presence of ecto- and endoparasites. The refined Parasite Index (PI) was then introduced to distinguish further between the number of ecto- and endoparasites present (Table 2.4). Due to the premise that ectoparasites are more directly exposed to the effects of water quality than endoparasites, the Inverted Parasite Index (IPI) was introduced. Larger numbers of ectoparasites are indicative of better water quality and should be given a lower score for this correlation to be reflected in the HAI value (Table 2.4) (Crafford & Avenant-Oldewage 2009).

5.2 RESULTS AND DISCUSSION

5.2.1 Health Assessment Index

Fish live in water for their entire life span. In aquatic ecosystems, fish are continuously exposed to natural variation of environmental factors as well as anthropogenic pollutants. Consequently, the health of the fish can reflect the quality of the water they live in. If the pollution level reaches a critical concentration, changes may be seen in the fish organs while some fish species may die, migrate or fail to reproduce (Hinton & Laurén 1990). As mentioned earlier, some of the assumptions required when applying the HAI is that, when fish are exposed to elevated levels of contaminants, tissue and organ function will change in order to maintain homeostasis and if the appearance of an organ or tissue system departs from the normal condition, the fish is responding to changes brought about by the environmental stressor (Goede & Barton 1990).

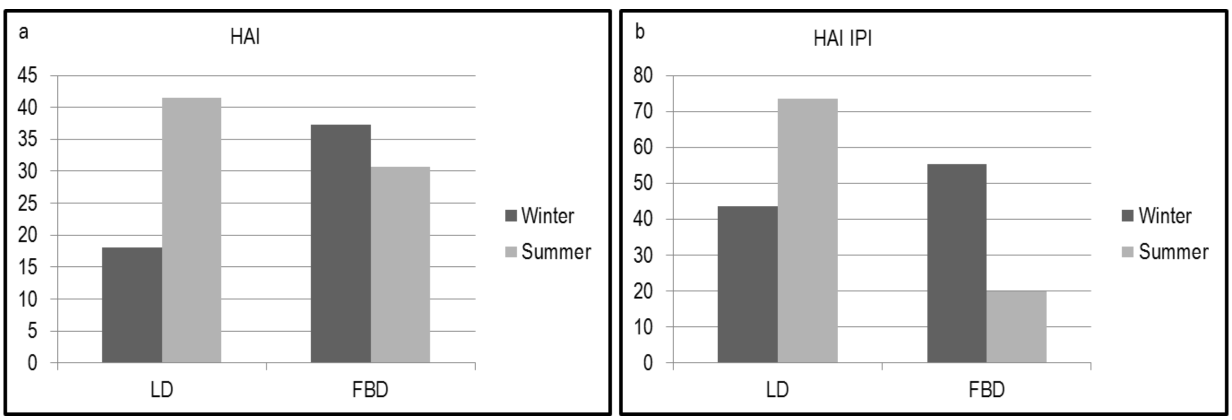

Figure 5.1 Health Assessment Index values for *Labeo rosae* at Loskop and Flag Boshielo dams.

The highest HAI value, which signifies poorer quality water, was recorded at Loskop Dam during summer (Fig. 5.1a). The HAI values of 18 and 41.5 were recorded during winter and summer respectively at Loskop Dam with 37.3 and 30.7 being recorded at Flag Boshielo Dam (Fig. 5.1a). The mean index value at Loskop Dam was 29.7 while at Flag Boshielo Dam was 34. The HAI showed that differences between the two localities were not significant ($p > 0.05$). Due to the discrepancy between the refined PI and the HAI, refined PI was inverted so that the higher number of ectoparasites will be given lower score for compatibility with the HAI. For the HAI incorporated with the IPI, a lower index value was recorded at Flag Boshielo Dam with 37.65 as the index mean value. The highest index value of 73.5 was recorded at Loskop Dam during summer (Fig 5.1b) and the mean index value was found to be 58.5. The HAI incorporated with the IPI showed that the difference between the two localities were significant ($p<0.05$).

A high prevalent of abnormalities were observed at Flag Boshielo Dam with liver, fins and skin abnormalities being recorded the most. The fish at Flag Boshielo Dam were medium sized with the maximum mass of 546.6 g being recorded. A maximum fish mass of 2080.9 g was recorded at Loskop Dam with little abnormalities being observed for the gills and livers. When fish are exposed to environmental stressors, their vulnerability may differ according to the competence of their immune systems and other inherent biochemical and physiological factors. Furthermore, factors such as fish size and sex may influence the inherent variability of stress responses in fish (Adams *et al.* 1993). These factors might have had an influence on the health condition of fish from Flag Boshielo and Loskop dams.

a. Variables of HAI

i. External variables

Skin

The skin is extremely important for the ability of fish to maintain proper osmoregulatory as well as for protection. The skin is directly exposed to contaminants and acts as the initial barrier to infections (Roberts 2012). Although covered by scales, the skin has been described as the first organ to be attacked by numerous environmental factors and parasites (Bowser 1999). According to Adams *et al.* (1993), skin abnormalities are rated as mild aberrations, moderate aberrations and severe aberrations.

In the present study no skin infection has been observed at Loskop Dam during summer and winter whereas several skin infections by *L. cyprinacea* were observed at Flag Boshielo Dam during winter. The skin abnormalities recorded at Flag Boshielo Dam were parasite induced lesions (Fig. 5.2a). No pollutant induced skin abnormalities were observed at both localities during both seasons.

Fins

Fins are in direct contact with the water environment and they are subjected to all dissolved and suspended materials in the water bodies. According to Jooste *et al.* (2005), forked fins and abnormally long fins may be a result of genetic variation or they may originate from environmental influences. Pelis & McCormick (2003) further reported that factors that are associated with fin abnormalities include overcrowding, water quality, temperature, feed type, malnutrition, bacterial infection, handling, and exposure to excessive sunlight and environmental contaminants.

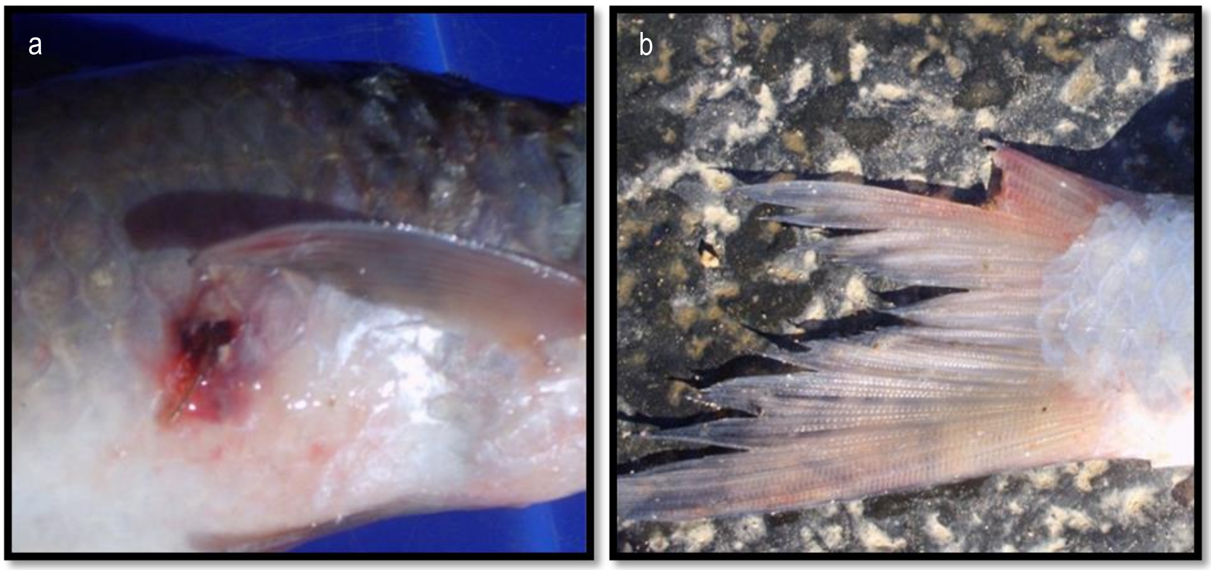

Figure 5.2 a. *Lernaea cyprinacea* infections on the skin of *Labeo rosae*; b. mild erosion on the tail fin of *Labeo rosae* at Flag Boshielo Dam.

Mild erosion of fins has been observed on one fish from Flag Boshielo Dam during this study (Fig 5.2b). Sindermann (1979) reported that fin rot and red sores are generalized disease signs and may be characteristic of fishes resident in degraded aquatic systems where environmental stresses of toxic chemicals exist. No parasites infections or cysts were recorded on the fins at both localities during winter and summer. Loskop Dam showed to be mesotrophic with Flag Boshielo Dam being oligotrophic; however, various metals were found to be above TWQR suggested by DWAF (1996a) for aquatic ecosystem at both localities. Therefore, the lower diversity and abundance of ectoparasites might be attributed to the elevated metals concentrations at both localities.

Eyes

The eyes are organs that indicate the well-being of fish in several ways (Goede & Barton 1990). There are numerous lesions occurring in the eyes of fish and the most frequent lesions involve swelling of the orbit or discolouration of the cornea (Roberts 2012). Reichenbach-Klinke (1973) revealed that severe internal illness, starvation, chlorine poisoning, unfavourable conditions during winter, etc. may cause sunken eyes in fish. Adams *et al.* (1993) and Crafford and Avenant-Oldewage (2009) rated the eye abnormalities as follows; opaque eye, swollen protruding, haemorrhagic, blind or missing (Chapter 2: Table 2.1).

No eye abnormality was recorded throughout the study at both localities, but several *Nematobothrium* sp. parasites were recorded in the eye orbits of fish at both localities. According to Reichenbach-Klinke (1973), bacterial or parasitic infection of the eye usually results in lesions of the peri-orbital tissue. Roberts (2012) further reported that the lens can be affected by a number of pathological processes, all leading to progressive degenerative cataract formation. No cater act or haemorrhagic eyes were recorded for *L. rosae* during this study.

Opercula

The opercula are the hard bony flap covering and protecting the gills. In most fish, the rear edge of the operculum roughly marks the division between the head and the body (Zapata *et al.* 1996). According to Reichenbach-Klinke (1973) shortened and perforated opercula might be observed in fish as a result of calcium deficiency, environmental damage or predation, or it might be of genetic origin. No shortening or perforations were recorded during this study at both localities. Although most of the gills were infected by monogeneans, the gill abnormalities did not have an effect on the morphology of the opercula.

Gills

Gills are respiratory organs found in many aquatic organisms including fish. According to Ackermann (2008), the gill is a system for bringing the blood haemoglobin into close contact with the water, so that oxygen can be absorbed and carbon dioxide released. Apart from respiratory function, the gills are responsible for regulating the exchange of salts and water, and play a major role in the excretion of nitrogenous wastes products. They are among the most delicate organs of the fish. Their vulnerability is thus considerable because their external location and necessarily intimate contact with the external water environment means that they are liable to damage by any irritant materials, whether dissolved or suspended in water (Roberts 2012). Hinton and Lauren (1990) reported that gills are sensitive indicators of environmental stress, including exposure to harmful compounds present in aquatic ecosystems as a result of human activities.

Variety of gills abnormalities were observed at Flag Boshielo Dam during summer and winter whereas at Loskop Dam this condition was only observed during summer (Figs 5.3a&b). Many monogeneans were recorded at Loskop Dam during summer with only few being recorded during winter. Most gill lesions are more frequently associated with lethal, rather than sublethal, exposure to irritants and are sensitive primary target organs for a variety of stressors including metals (Mallatt 1985; Van Dyk *et al.* 2009). Quantitative HAI does not tell whether the anomalies were pollutants or parasites induced, in this regards the histopathological analysis were done and the results are discussed in Chapter 6.

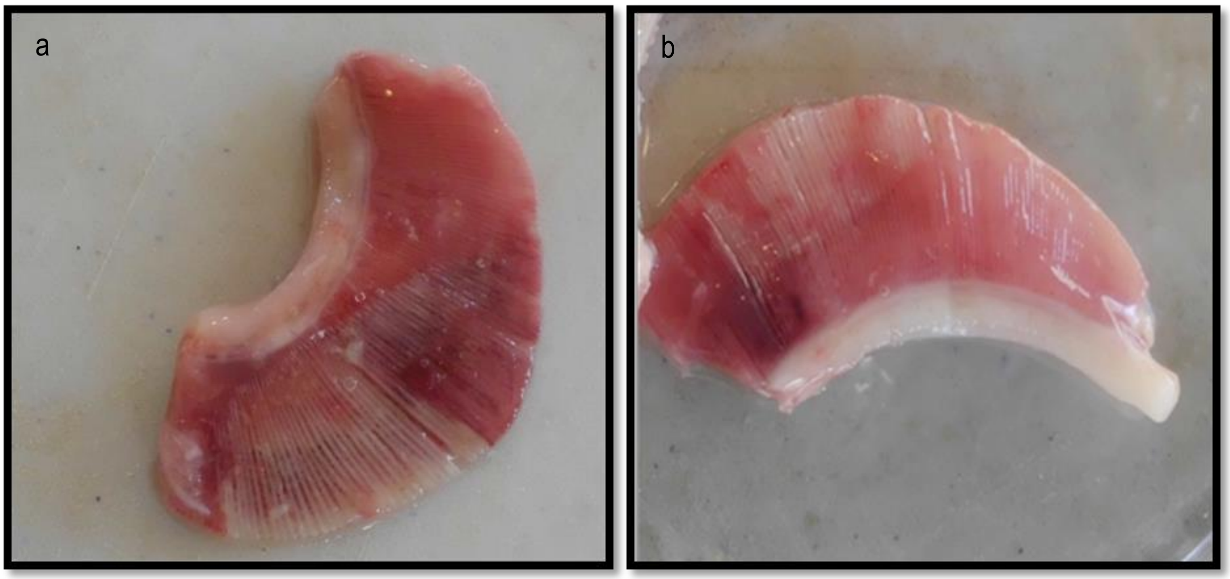

Figure 5.3 a & b. Frayed gills of *Labeo rosae* at Loskop dam.

ii. Internal variables

Liver

The liver is unique among organs, because it is composed of a large mass of glandular tissues. It is a dense organ located ventrally in the cranial region of the body cavity (Van Dyk 2003a). The liver is important in many aspects of nutrition including lipids and carbohydrates storage. It is further known as the detoxification organ which is essential for the metabolism and excretion of toxic substances (Hinton & Lauren 1990). Although the liver has the ability to degrade toxic compounds, its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds and subsequently results in structural damage (Van Dyk 2003a). The normal colour of a fish liver is considered to be red or light red and the abnormal manifestations of the liver include fatty liver, light tan in colour, liver nodules, focal discolouration and general discolouration (Goede 1992).

Various abnormalities of the liver have been recorded at both localities with more severe abnormalities being recorded at Flag Boshielo Dam. Some of the livers were found to be yellowish to orange in colour with some few nodules (Fig. 5.4a). The liver of fish from Loskop Dam exhibited some abnormalities, although the conditions were better than at Flag Boshielo Dam (Fig. 5.4a). No parasites or parasite induced anomalies were observed on the liver at both localities throughout the study. In the present study the liver has proved to be a good indicator and reliable on distinguishing localities with different water quality.

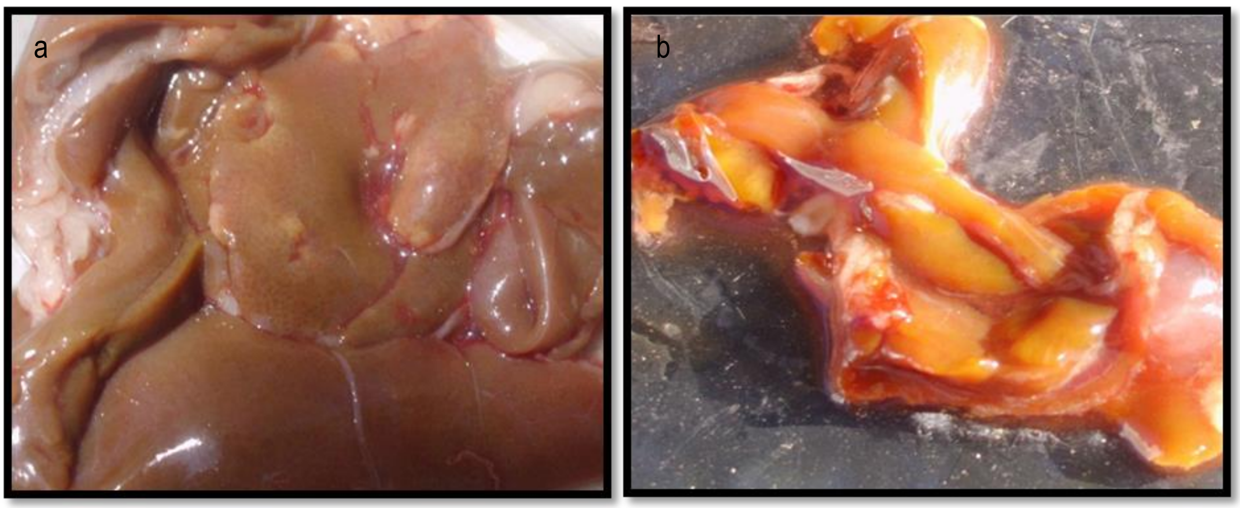

Figure 5.4 a. Liver of *Labeo rosae* at Loskop Dam; b. discoloured liver of *Labeo rosae* at Flag Boshielo Dam.

Kidney

The fish kidney is a mixed organ comprising haemopoietic, reticuloendothelial, endocrine and excretory elements (Roberts 2012). The primary function of the kidney in freshwater fish is to excrete any excessive water and is also known as the primary haemopoietic organ (Ellis *et al*. 1978). Thophon *et al.* (2003) reported that the teleostean kidney is one of the first organs to be affected by contaminants in the water. The most common alterations found in the kidney of fish exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space (Takashima & Hibiya 1995). Normal kidneys have a light to dark brown colour and might appear dark red to black in some species (Ellis *et al.* 1978). In the present study, no kidney anomalies were recorded at both localities.

Spleen

The spleen is the only lymph-node like organ to be found in the teleost fish. The normal colour ranges from dark red to black and under normal conditions, spleen has sharply defined edges. Although usually single, it may in some species be divided into two or more smaller spleens (Roberts 2012). The histological changes, including increased vacuolation of hemoblasts and swelling, can be expected in the splenic blood cells of some fish during stress conditions (Peters & Schwarzer 1985). No spleen anomalies were recorded in this study from either locality.

Bile and mesenteric fats

Bile is produced by the liver and stored in the gall bladder. It is difficult to tell if the bile or mesenteric fats were normal or abnormal because bile can take on different colours depending on feeding regimes of the fish (time since last meal, meal quantity and quality, etc.) and mesenteric fat deposits in the fish, or the lipid index, can vary widely depending not only on food availability and feeding regimes, but on other interacting factors such as fish size, sex, time of year, and stress level (Adams *et al.* 1993). Fat abnormality that has been a deadly incident over the last few decades and has even led to an increasing attention in the Olifants River is pansteatitis which is the hardening yellow discolouration of the fat.

The mesenteric fats were recorded based on the caecum coverage. The fish at Loskop Dam were larger with greater than 50% of the caecum being covered by fats. Less than 50% of the caeca of fish were covered at Flag Boshielo Dam (Fig 5.5b). No pansteatitis signs were observed for *L. rosae* during this study at both localities. The colour of the bile varied from yellow to dark blue green depending on the feeding regime.

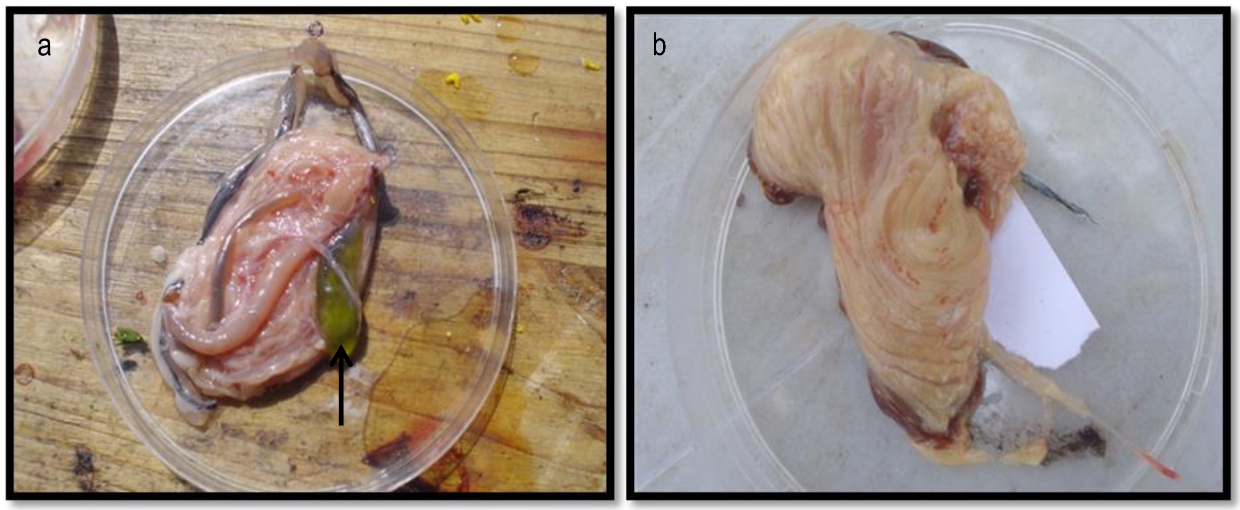

Figure 5.5 a. Bile of *Labeo rosae* from Flag Boshielo Dam (arrow); b. Mesenteric fats (more than 50% of cecum covered) of *Labeo rosae* from Flag Boshielo Dam.

Hindgut

The hindgut is the last part of the intestine; it is the site where the final digestion and absorption of mineral salts occur before defecation of waste products. In some fish species, during cyclical periods of starvation, spawning and migration, the cells of the intestine become shrunken; the intestinal folds flatten and become darkly stained and extensive epithelial necrosis become present (Ellis *et al.* 1978). However, most of the hindgut anomalies can be as a result of parasite infections (Goede & Barton 1990). No parasites or pollutants induced anomalies were recorded in the hindgut of fish at both Loskop and Flag Boshielo dams.

Blood (haematocrit)

Fishes are poikilothermic and many external factors operating in the aquatic environment can therefore modify their haematological constants. These have made haematological parameters a great concern on ecological impact studies. The use of haematological parameters in fish has became increasingly more prevalent in the assessment of environmentally stressful conditions because the properties of blood are very sensitive to physiological as well as pathological changes in fish (Alexander *et al*. 1980).

As mentioned in chapter 2, the capillary tubes were filled with blood, plugged on one side using CritocealTM commercial clay. The tubes were centrifuged using microhaematocrit centrifuge for about 7 minutes and the readings were obtained. When a blood sample taken with a capillary tube from the fish is centrifuged to separate the cells from the serum, the ratio of the cellular fraction to the total blood volume is called a haematocrit. According to Schuett *et al.* (1997), haematocrit values reflect the percentage of red blood cells to total blood volume. The haematocrit value will vary depending on the health and physiological condition of the individual fish (Jawad *et al.* 2004). According to Cyriac *et al.* (1989), haematocrit values increase when fish are exposed to metals for longer than 24 hours. A high haematocrit value can result from acute stress while a low haematocrit value may indicate a diseased state (Barton *et al.* 1985). Furthermore, Ots *et al*. (1998) reported that starvation and high parasite infestation may lower haematocrit levels.

Although the normal range for haematocrit levels is from 30% – 45%, Adams *et al.* (1993) reported that normal ranges should be established for each species or groups of similar species for major geographical areas of the country (e.g., southeast, northwest). In the present study, haematocrit values ranged from was 21% - 31% during winter and 10% - 29% during summer at Flag Boshielo Dam. At Loskop Dam the haematocrit values ranged from 18% - 44% during winter and 18% - 37% during summer. No significantly higher haematocrit values were recorded during this study. According to Goede and Barton (1990), low haematocrit levels indicate the presence of disease in a population and can also be due to parasitic infestations. The higher infestation of ecto- and endoparasites were observed at Flag Boshielo Dam than Loskop Dam, therefore, the parasite infestations might have had an impact on the lower haematocrit values at Flag Boshielo Dam.

5.2.2 Parasites

Almost all free-living organisms are host to parasites and parasitism. In its broadest sense, parasitism is considered to be the most common life style on earth. Parasites are ubiquitous, they occur in almost all food webs at all trophic levels (Marcogliese 2005). Fish parasites are the indigenous components of healthy ecosystems and their presence or absence can serve as an indicative tool of ecosystem health (Sures 2001).

A total number of 64 *Dactylogyrus pienaari* (Fig. 5.7a) was recorded at Loskop Dam throughout the study with a total of 195 being recorded at Flag Boshielo Dam (Table 5.1). The other ectoparasites recovered from *L. rosae* at Flag Boshielo Dam include *Lambroglena* sp., *Ergasilus* sp., *Paradiplozoon* sp. and *Lernaea cyprinacaea* (Fig. 5.6a-f). In contrast, *Dactylogyrus pienaari* was the only ectoparasites recorded at Loskop Dam (Table 5.1). The endoparasite species recorded for *L. rosae* included *Nematobothrium* sp. and *Paracamallanus cyathopharynx* (Figs 5.7b-d). Only *Nematobothrium* sp. was recorded at Loskop Dam with *Nematobothrium* sp. and *Paracamallanus cyathopharynx* being recorded at Flag Boshielo Dam. A total number of 78 *Nematobothrium* sp. were recorded at Loskop Dam with 13 *Nematobothrium* sp. and 5 *Paracamallanus cyathopharynx* being recorded at Flag Boshielo Dam (Table 5.1).

Table 5.1 Number of parasites recovered from the different organs of *Labeo rosae* at Loskop and Flag Boshielo dams.

	Name of Parasite	Site/organ	Number of parasites in seasons		Total
Loskop Dam					
			Summer	Winter	
Ectoparasites	*Dactylogyrus pienaari*	Gills	47	17	64
Endoparasites	*Nematobothrium* sp.	Eye orbit	49	29	78
Flag Boshielo Dam					
Ectoparasites	*Dactylogyrus pienaari*	Gills	170	25	195
	Lernaea cyprinacaea	Skin	0	8	8
	Lambroglena sp.	Gills	2	0	2
	Paradiplozoon sp.	Gills	0	1	1
	Ergasilus sp.	Skin	0	2	2
Endoparasites	*Nematobothrium* sp.	Eye orbit	4	9	13
	Paracamallanus cyathopharynx	Intestine	3	2	5

Higher numbers of ectoparasites as well as a higher diversity were recorded at Flag Boshielo Dam than at Loskop Dam. However, a higher number of endoparasites was recorded at Loskop Dam. The infestation statistics showed that a prevalence of 30% for ectoparasite was recorded at Loskop Dam and 81% at Flag Boshielo Dam during summer (Fig. 5.8a). For endoparasites, a prevalence of 25% was recorded at Loskop with 47% being recorded at Flag Boshielo Dam during summer (Fig. 5.8b). The prevalence of ectoparasites were always higher at Flag Boshielo Dam than at Loskop Dam with prevalence of endoparasites being higher at Loskop Dam than at Flag Boshielo Dam (Figs 5.8a&b). The mean abundance of ectoparasites was higher at Flag Boshielo Dam than Loskop Dam throughout the study (Fig. 5.8c). In contrast, the mean abundance of endoparasites was higher at Loskop Dam than Flag Boshielo Dam during both seasons (5.8d). The mean intensity for ectoparasite was higher at Flag Boshielo Dam than Loskop Dam with endoparasites showing a higher mean intensity at Loskop Dam (Fig. 5.8e&f).

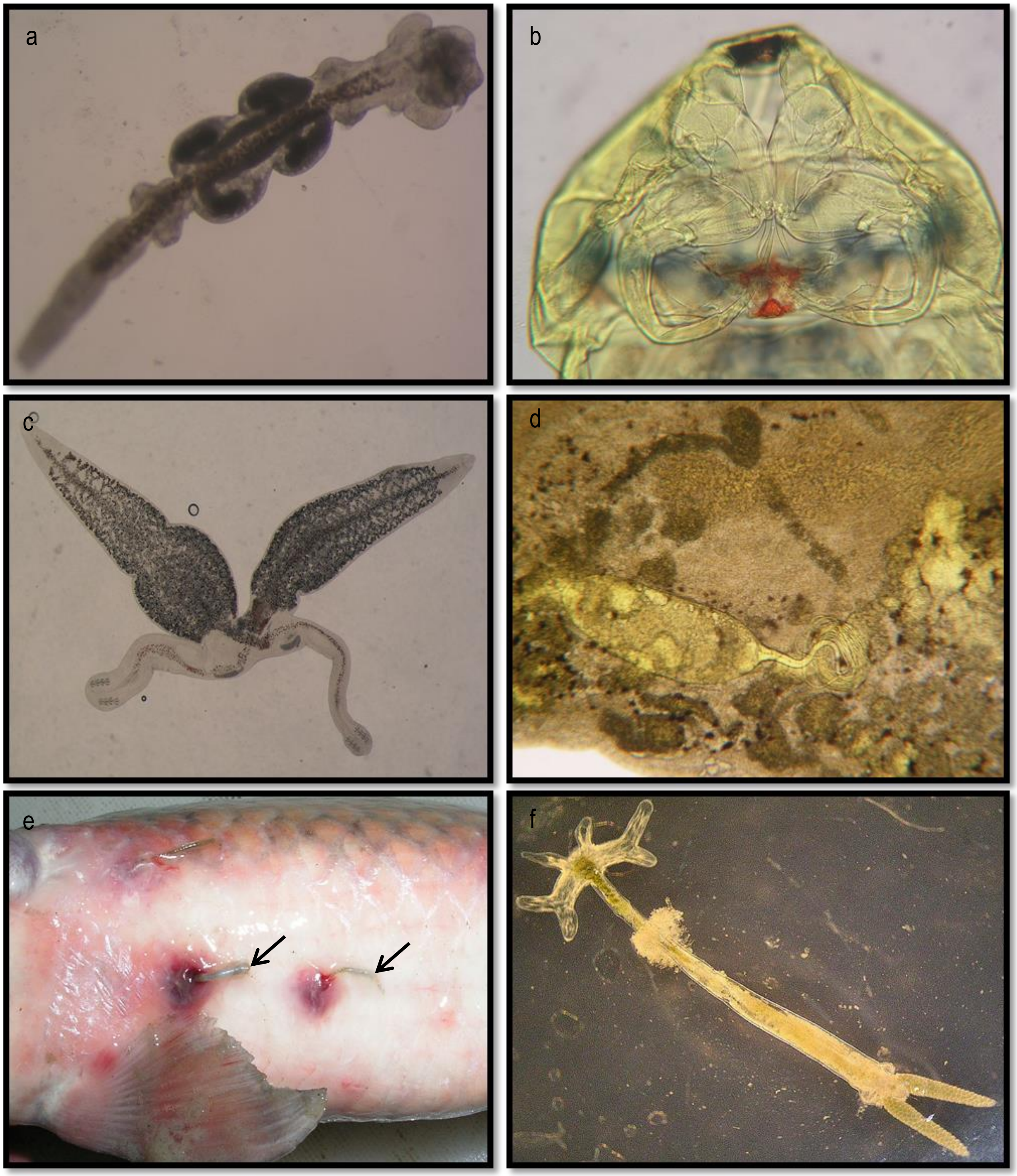

Figure 5.6 Ectoparasites recorded from *Labeo rosae*: a. *Lambroglena* sp.; b. *Ergasilus* sp.; c & d *Paradiplozoon* sp.; e. *Labeo rosae* infected by *Lernaea cyprinacea* (arrowed); f. *Lernaea cyprinacea* (showing modified antennae on the anterior end).

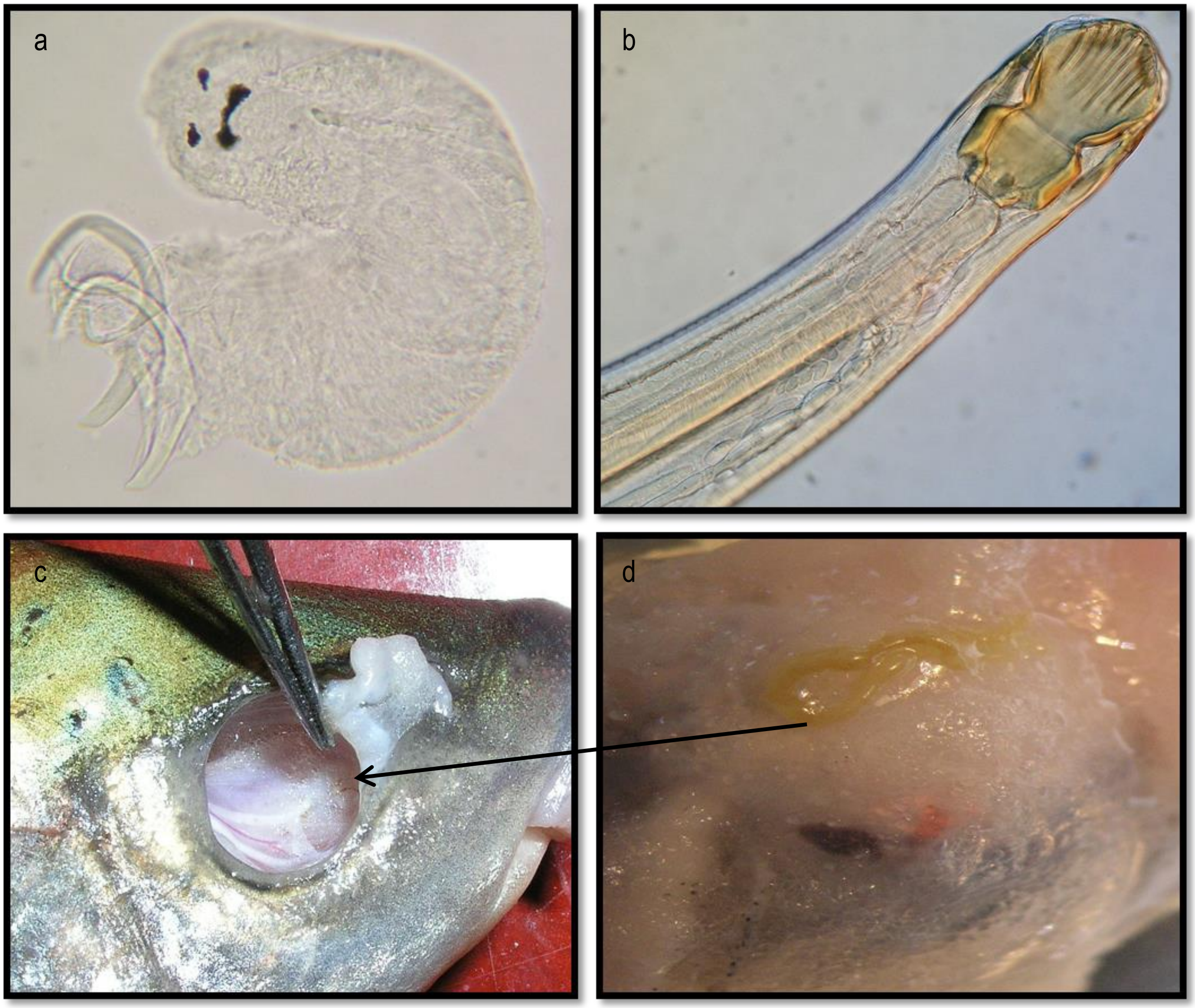

Figure 5.7 Ecto- and endoparasites recorded from *Labeo rosae*: a. *Dactylogyrus pienaari*; b. *Paracamallanus cyathopharynx*; c. Eye orbit of *Labeo rosae* infected by *Nematobothrium* sp.; d. *Nematobothrium* sp.

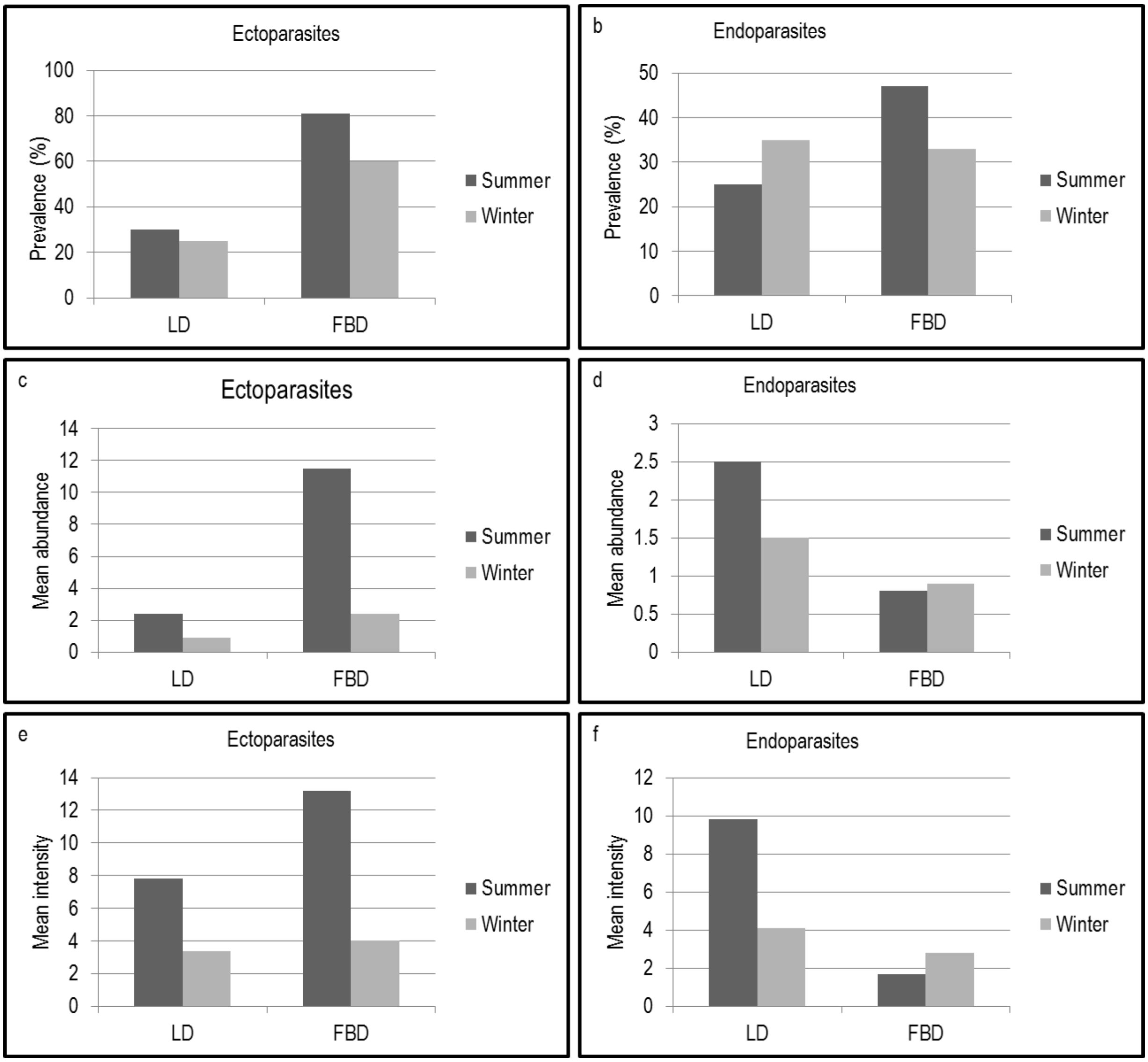

Figure 5.8 Statistical infestation of *Labeo rosae* from Loskop and Flag Boshielo dams: a. Ectoparasite prevalence; b. Endoparasite prevalence; c. Ectoparasite mean abundance; d. Endoparasite mean abundance; e. Ectoparasite mean intensity; f. Endoparasite mean intensity.

Parasite Index

Avenant-Oldewage (1998) described the Parasite Index (PI) as a useful biomonitoring tool which provides a reliable indication of water quality. In the original HAI (Adams *et al.* 1993), parasites were recorded as being present or absent. Due to their great diversity in terms of number of species and their number of life stages, there is an increasing interest in using parasites as ecological indicators of their fish host life conditions (Marcogliese 2005). Furthermore, Blanar *et al.* (2009) reviewed the specific use of aquatic parasites data in environmental studies. Given the relevance of parasite data in environmental monitoring, the original HAI notation was thus expanded and developed into a PI tested in conjunction with the HAI (Marx 1996).

According to Jooste *et al.* (2005), ectoparasites are in a direct contact with the external water environment and some ectoparasitic groups tend to reduce when water is heavily polluted. A higher number of ectoparasites should be expected in good quality water while the number of some endoparasites, on the other hands, tends to increase in polluted water. However, some ectoparasites e.g. *Trichodina* (protozoans) tend to increase in number in organic pollution (Ogut & Palm 2005). Due to the variety of response that the parasites might exhibit when exposed to contaminants, endo- and ectoparasites were incorporated as separate variables in the HAI tested in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001).

Ectoparasites are expected to range from 1 to greater than 20 (Table 2.8.1). The absence of parasites is indicated by zero. Due to the fact that zero denotes ectoparasites absence and a high number of ectoparasites denote good quality water, the ecto PI was inverted so that it can be incorporated in the HAI. On the IPI, the presence of more than 20 ectoparasites were denoted by 0 (because 0 indicate good water quality) and the absence denoted by 30 (Crafford & Avenant-Oldewage 2009). Endoparasites are usually much higher in number as compared to ectoparasites, such that, more than 1000 endoparasites can be observed in a single host (Jooste *et al.* 2005).

A higher ecto-PI value (which denotes good quality water) was recorded at Flag Boshielo Dam during summer. Ecto-PI values of 4 and 2.5 were recorded at Loskop Dam during summer and winter respectively with 15 and 6 being recorded at Flag Boshielo Dam (Fig. 5.11a). For the ecto IPI, the highest index value was recorded at Loskop Dam whereby values of 26 and 27.5 were recorded during summer and winter respectively. At Flag Boshielo Dam, an ecto-IPI value of 15 was recorded during summer with a value of 24 being recorded during winter (Fig. 5.11b). The mean values for the ecto-IPI were found to be 27.75 at Loskop Dam and 19.5 at Flag Boshielo Dam. The differences were found to be highly significant between the two localities ($p<0.05$).

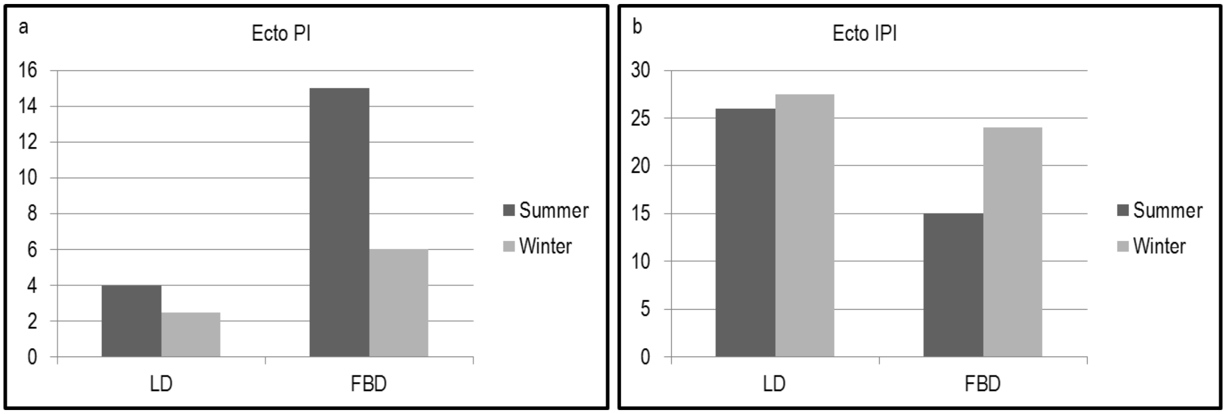

Figure 5.11 Ecto-PI and IPI of parasites of *Labeo rosae* at Loskop and Flag Boshielo dams.

Endo-PI values of 4 and 3.3 were recorded at Flag Boshielo Dam during summer and winter respectively with values of 2.5 and 3.5 being recorded at Loskop Dam (Fig. 5.12). The mean value at Flag Boshielo Dam was 7.3 and 6 at Loskop Dam. The endo-PI mean values did not differ much, therefore, statistical differences between the two localities were found to be insignificant ($p>0.05$). Endoparasites are protected by the body of the host; their abundance should increase when the immune system of the host is impaired due to polluted conditions (Luus-Powell 1997).

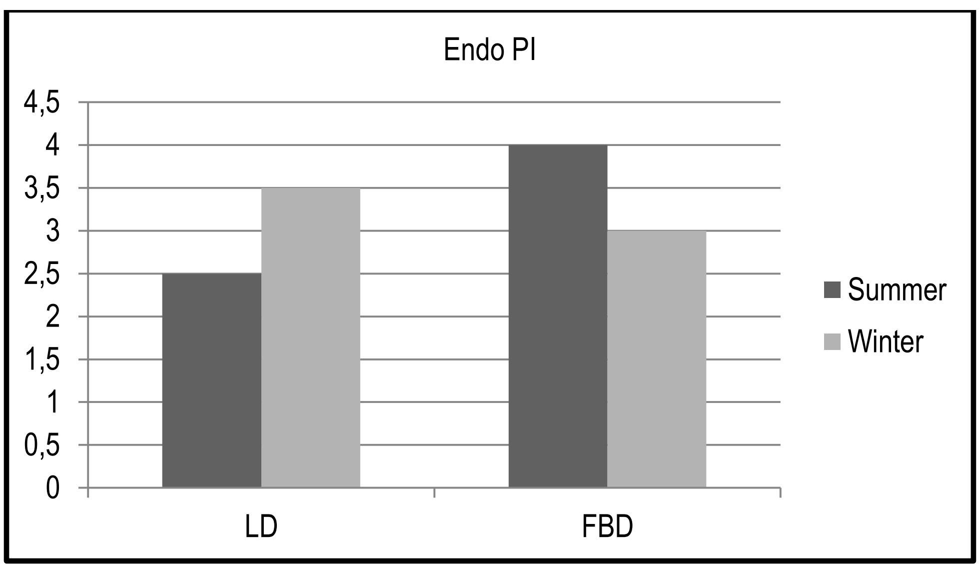

Figure 5.12 Endo-PI of *Labeo rosae* at Loskop and Flag Boshielo dams.

5.2.3 Condition Factor

The length-weight relationship has been widely used in fish biology with several purposes i.e. to estimate the mean fish weight based on the known length, morphometric interspecific and intrapopulational comparison and to assess the index of well being of the fish population (Da Costa & Araújo 2003). Jenkins (2004) reported that heavier fish are in better condition than lighter fish of the same length. The condition factor is strongly influenced by the biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene 2005). Any stresses in the natural environment can have an effect on fish overall health and condition. Nevertheless, there are natural fluctuations in condition factor of fish due to locality, species, sex and season (e.g. temperature, spawning, photoperiod, prey quantity/quality) (Jenkins 2004). Yousuf and Khurshid (2008) further stated that the condition factor may vary for the same fish from different localities or for the same fish at different seasons. According to Gomiero and Braga (2005), drops in condition factor values may indicate the reproductive period and/or changes in the foraging habits of certain species.

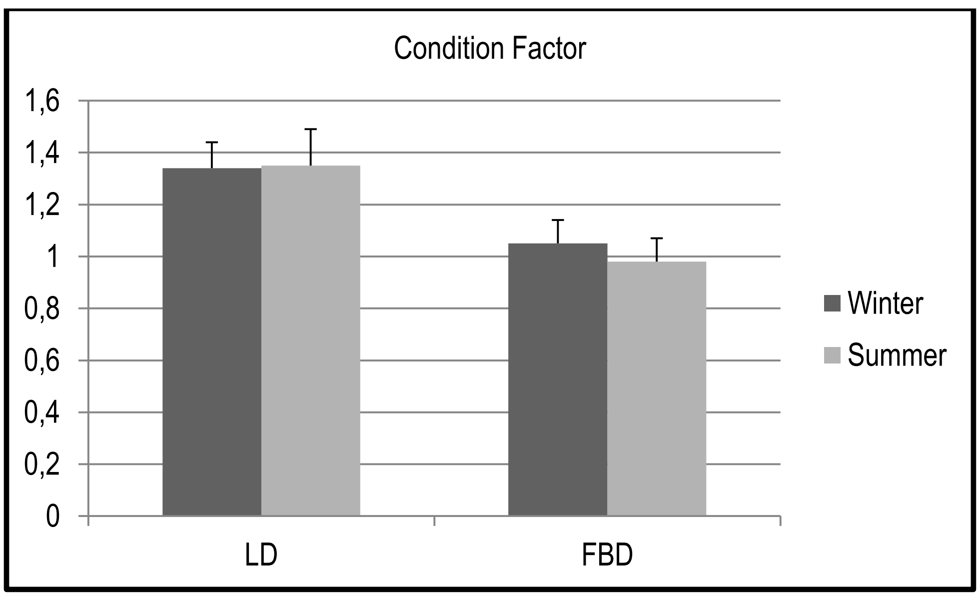

Figure 5.13 Condition Factor for *Labeo rosae* at the Loskop and Flag Boshielo dams.

Condition factors are believed to be good indicators of the general well-being or fitness of fish populations (Bolger and Connolly 1989) and are often used as indicators of pollutant exposure and the effect thereof (Kloepper-Sams *et al*. 1994). The condition factor allows quantitative comparison of the condition of fish populations from different localities, but it is important to sample the individuals or populations at the same time of the year so that the individuals or populations were at the same stage of the reproductive cycle (Barnham & Baxter 1998).

According to Barnham and Baxter (1998), the condition factor of 1.60 indicate an excellent condition (trophy class fish) while 0.80 indicate extremely poor fish resembling a barracouta, big head and narrow, thin body. However, Jooste *et al.* 2005 reported that condition factor of fish is classified as ideal when a value of one is recorded. In the present study, condition factors of 1.34 and 1.35 were recorded at Loskop Dam during winter and summer respectively while 1.05 and 0.98 were recorded at Flag Boshielo Dam during winter and summer (Fig. 5.13). There were significant differences between the two localities ($p<0.05$). The fish at Loskop Dam were bigger than the fish at Flag Boshielo Dam. According to Barnham and Baxter (1998) and Jenkins (2004), the condition factor is greatly influenced by the age of fish, sex, season, stage of maturity, fullness of gut, type of food consumed, amount of fat reserve, degree of muscular development and stage of development of the reproductive organ. In the present study, condition factor indicates that the fish from both localities were in a fairly good condition. A bit higher condition factor at Loskop Dam might be attributed to the fact that the fish from Loskop had heavier body weights than at Flag Boshielo Dam. The heavier body weight of fish from Loskop Dam could be due to nutrients enrichment, hence more food availability at Loskop Dam.

The overall health of *L. rosae* seems to be fairly better at Flag Boshielo Dam than Loskop Dam (based on HAI). However, the condition factors were not very indicative in distinguishing the two localities. The limitations of condition factor as that it can change with physiological development and sexual maturation (degree of maturity influencing weight) (Strange & Pelton 1987). Furthermore, Jooste *et al.* (2005) emphasised that it is not justifiable to compare the condition factor on length criteria only due to the existence of a big overlap in length between different age groups in a fish species.

5.2.4 Hepatosomatic Index

Liver has the ability to degrade toxic compounds, but it may be overwhelmed by elevated levels of these compounds, therefore hyperplasia or hypertrophy may be an adaptive response to increase the detoxification capacity (Salamat & Zarie 2012). The hepatosomatic index of each fish specimen was calculated to determine any deviation in terms of ration of liver weight to body weight. Marchand (2006) reported that the normal HSI values for Osteichthyes range from 1 – 2%. The HSI values may increase or decrease due to hypertrophy (increase in cell size), atrophy (decrease in cell size), hyperplasia (increase in cell number) and cellular necrosis (Salamat & Zarie 2012).

The HSI values recorded at Loskop Dam ranged from 0.57% – 1.19% with the mean values found to be 0.75% and 0.91% during winter and summer respectively (Fig. 5.14). At Flag Boshielo Dam the HSI values ranged from 0.62 – 1.8% with the mean values found to be 0.9% during winter and 1.2% during summer (Fig. 5.14). Although the HSI values between the two localities varied, they did not differ significantly ($p>0.05$). Marchand (2006) reported that a decrease in HSI may be a direct response to starvation since it drops drastically during fasting period. Due to the fact that the fish are actively feeding during summer than winter, the higher HSI values were recorded during summer at both localities than in winter. The index values at Flag Boshielo Dam were less than the normal range, but they were close to one which tells that there was no drastic change on the ration of body to liver weight.

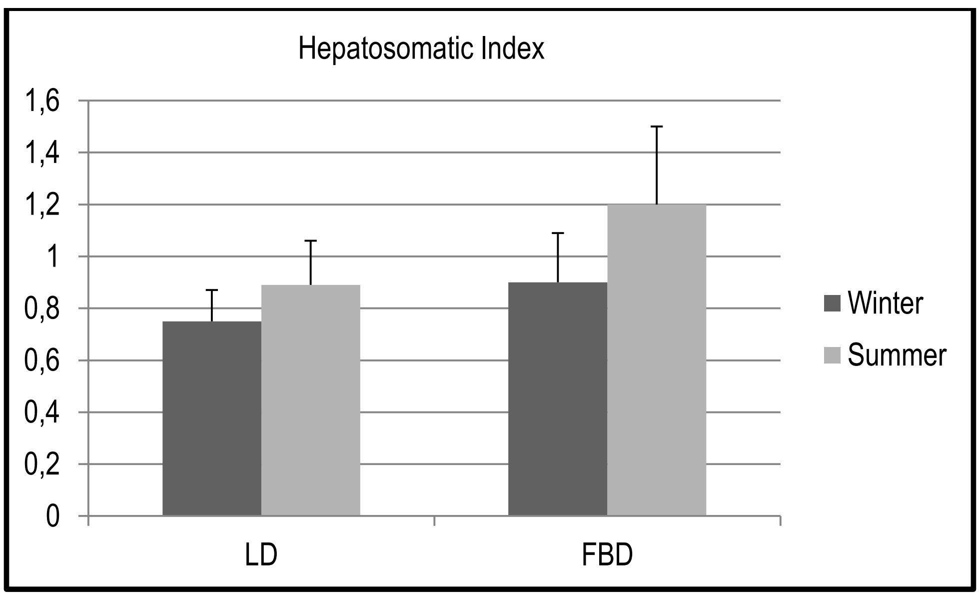

Figure 5.14 Hepatosomatic Index values for *Labeo rosae* at the Loskop and Flag Boshielo dams.

5.3 Conclusion

In the present study, HAI proved to remain a rapid and inexpensive method to indicate the occurrence of change in an aquatic ecosystem. Heath *et al.* (2004) mentioned the primary objective of this method as not to understand the reasons for a change in population health or condition, but to document in a relatively rapid and inexpensive manner the occurrence of a change in a fish population. Furthermore, the HAI cannot be used for the identification of type of chemicals or compounds to which the fish respond in the same way as physical-chemical techniques. Jooste *et al.* (2005) reported that the HAI is not designed to be a substitute for the other methods, to be diagnostic or to solve specific problems related to fish health or environmental conditions. In addition, Heath *et al.* (2004) reported that applying HAI with other biological indices such as SASS 4 simultaneously should increase the value of all.

The HAI on its own is unlikely to provide sufficient information for environmental decision-making purposes unless consistent and dramatic differences are observed (Jooste *et al.* 2005). In the present study, there is no definite trend on the health condition of the fish and the concentration of water quality constituents between the two localities. Avenant-Oldewage (2001) emphasized that it should not be attempted to compare different environment with one another and that the index is valuable when it is used repetitively over a period of time in the same locality with the survey carried out at the same time of the year and the same species being used as a test organism.

The present study showed that the health of fish population at Loskop Dam was more affected than the population at Flag Boshielo Dam during summer. In contrast, Flag Boshielo Dam exhibited higher HAI value than Loskop Dam during winter. The population HAI value was higher at Loskop Dam than Flag Boshielo Dam during summer and higher at Flag Boshielo Dam than Loskop Dam during winter. The seasonal mean HAI values were 37.65 at Flag Boshielo Dam and 58.50 at Loskop Dam. Loskop Dam was characterised by elevated concentration of nutrients and ions. Low parasite diversity was recorded at Loskop Dam whereby one endoparasite and one ectoparasite species were recorded throughout the study. Based on the HSI no drastic deviation from the normal range was observed between the two localities. High concentration of nutrients and ions might have had an effect on lowering the diversity of parasites at Loskop Dam. The levels of metal and non-toxic constituents were higher at Flag Boshielo Dam than at Loskop Dam but the overall health of fish population at Flag Boshielo Dam was fairly better as compared to Loskop Dam.

CHAPTER 6

HISTOPATHOLOGY

6.1 INTRODUCTION

A variety of approaches have been used to evaluate the ecological integrity of aquatic ecosystems and most of them focused on higher level of biological organization, e.g. on individual, population or community level. The use of biomarkers has emerged during the last few decades whereby the lower levels of biological organizations are being used to determine prior exposure to toxic pollutants and the magnitude of organism's response (McCarthy & Shugart 1990). Cells respond to the metabolic demand of the body, energy supply and various physiological and pathological stimuli by adaptation. Cellular adaptation is a reversible adjustment to environmental conditions that includes changes in cell function, morphology or both (Damjanov 1996). Furthermore, Van Dyk (2003a) reported that the early toxic effects of pollution may however, be evident on cellular or tissue level before the significant changes can be identified in fish behaviour or external appearance.

The study of the structure of abnormal, diseased tissues is termed histopathology (Marchand 2008). Histopathological analysis appears to be very sensitive in measuring parameters that could be crucial in determining cellular changes that may occur in target organs, such as gills and livers (Munshi & Dutta 1996; Van Dyk 2003a). Apart from being a biomarker of prior exposure, histopathology has proved to be a cost-effective tool to determine the health of fish, hence reflecting the health of the entire freshwater ecosystem (Van Dyk 2003a).

The advantages of using histopathology as a biomarker as suggested by Hinton & Laurén (1990) and Van der Oost *et al.* (2003) include the following:

- Different organs can be assessed.
- Histological responses can indicate potential problems before the effects appear at higher organization levels.
- No geographical or ecosystem limitations.
- Histology sections retain *in situ* relationships of different cell types and tissues in organs.
- Many alterations persist even after exposure to a toxicant has ceased so that host response to prior toxicity can also be used to determine effects.

- Acute changes are seen when contact levels are sufficiently high, while chronic duration is required to determine sub lethal aspects of change.

The disadvantage is that the ability to detect alterations depends on the investigators expertise i.e. experience in recognition and interpretation of different alterations in different tissues and lack of specificity of certain detected lesions in reference to causality (Hinton & Laurén 1990).

The first effects of contaminants usually occur at the cellular or subcellular level. After the cellular changes, a sequence of pathologies is observed in the tissues, compromising survival and consequently the structure of the population, affecting the ecosystem. Therefore, it is important to study the effect of pollutants at all levels, taking into account that in the natural environment many different factors interact to affect ecological health (Sindermann 1979).

Gills

Teleosts have four pairs of respiratory gill arches. Each gill arch comprised of filaments, the primary and secondary lamella. Gills are in direct contact with the external water environment and the main functions include gaseous exchange, ion regulation, maintenance of acid-base balance, and excretion of nitrogenous wastes (Roberts 2012). Yasutake & Wales (1983) reported that the gill is a system for bringing the blood haemoglobin into close contact with the water, so that oxygen can be absorbed and carbon dioxide released. The normal histological structures of primary and secondary lamellae are illustrated in Figure 6.1a.

The vulnerability of gills is considerable because their external location means that they are liable to damage by any irritant material, whether suspended or dissolved in the water (Roberts 2012). The most frequently observed alterations reflect changes in membrane permeability at the cellular and tissue level in terms of swelling of epithelial cells, vacuolation, or as oedema of the interepithelial space. If the irritant stimulus is more severe there may be an occurrence of lamellar oedema, hyperplasia, fusion and necrosis of pillar cells leading to telangiectasia (Figs 6.1b-f) (Ackermann 2008).

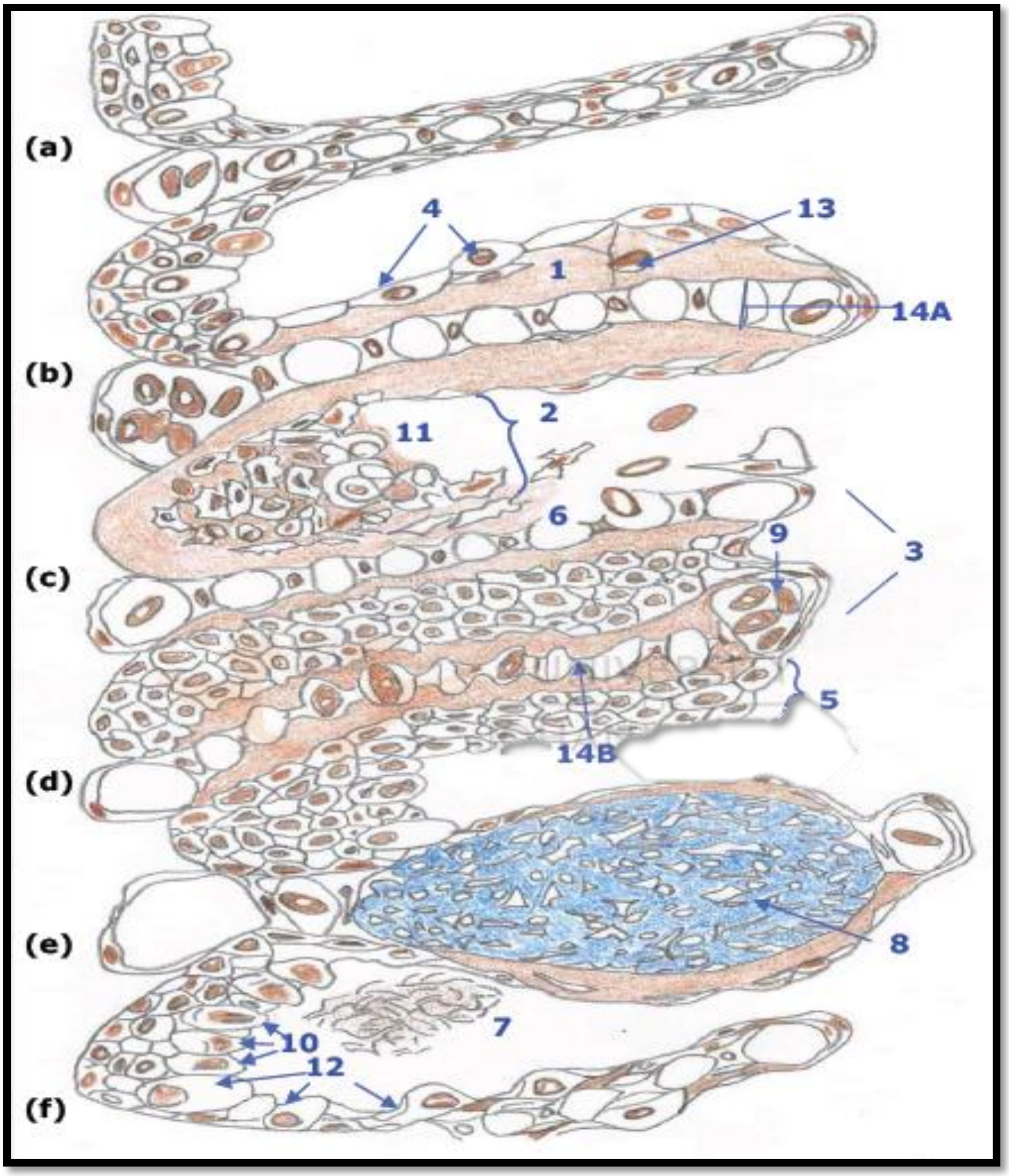

Figure 6.1 Diagrammatic representations of gill lamellae with different lesions. Six lamellae are shown with (a) as the normal lamella and (b-f) showing possible alterations. 1=epithelial lifting; 2=necrosis; 3=lamellar fusion; 4=hypertrophy; 5=hyperplasia; 6=epithelial rupture; 7=mucus secretion; 8=aneurism; 9=congestion; 10=mucus cell proliferation; 11=chloride cell damage; 12=chloride cell proliferation; 13=leucocyte infiltration; 14A=dilated blood sinus; 14B=constricted blood sinus (Ackermann 2008).

Liver

The liver is the largest mass of glandular tissues in the body and is unique among organs (Van Dyk 2003b). The colour of a fish liver is usually reddish brown in carnivores and lighter brown on herbivores (Roberts 2012). Fish liver comprises two tissue compartments, parenchyma and non-parenchyma or stroma (Hinton & Laurén 1990). Parenchyma tissue comprised of various cells i.e. hepatocytes, biliary epithelial, endothelial, ito cells, macrophages. In association with hepatocytes are extracellular spaces, sinusoids and space of disse (Fig. 6.1). Non-parenchyma or stroma comprises the blood vessels and connective tissues (Hinton & Laurén 1990; Van Dyk 2003b; Ackermann 2008).

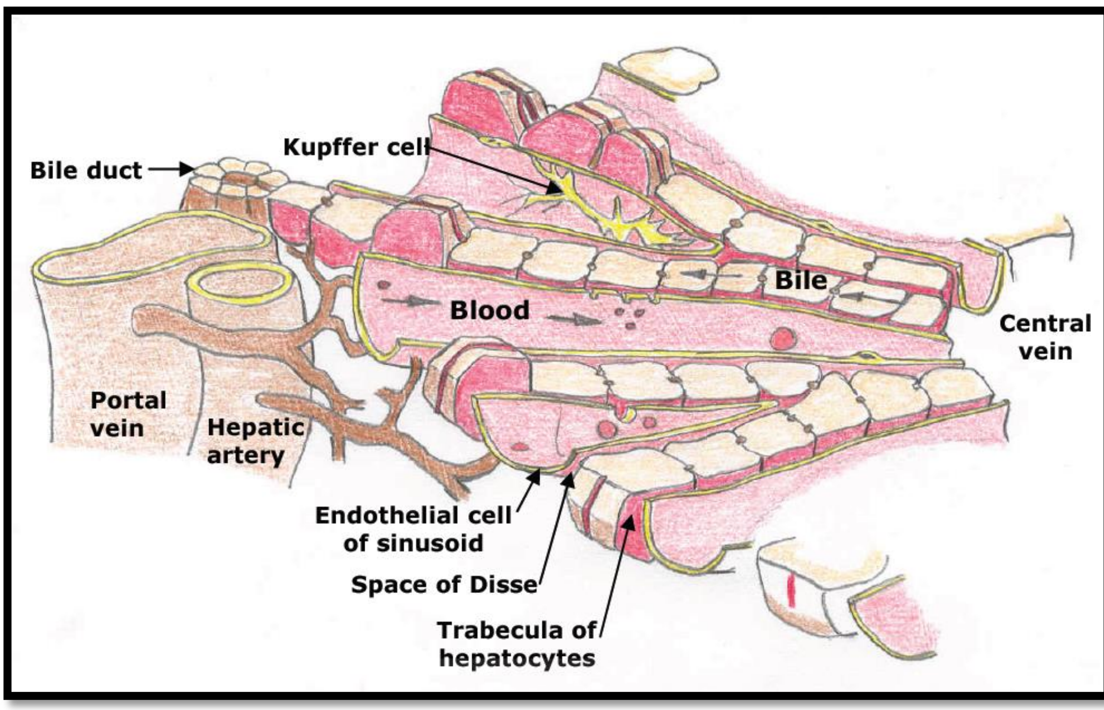

Figure 6.2 Classical vertebrate liver. Arrangement of hepatocytes and sinusoids in the classical liver lobe (Ross *et al.* 1989; Ackermann 2008).

Hepatocytes constitute about 80% of the cell population in the liver and perform most of the functions of the liver (Ackermann 2008). These cells are larger in size with distinctive centrally situated nucleus (Roberts 2012). The liver serves as a storage and central metabolizing organ that plays a dual role of secretion of digestive enzymes and as storage organ for nutrients as well as detoxification of metals (Reddy 2012). Although liver has the ability to degrade toxic compounds, it may be overwhelmed by elevated levels of these compounds and subsequently get damaged (Ross *et al.* 1989; Van Dyk 2003b). The alterations in liver structure may be useful as biomarker that indicates prior exposure to environmental stressors (Hinton & Laurén 1990).

6.2 RESULTS AND DISCUSSION

6.2.1 Gills

Fish gills are dominant site for gaseous exchange, ionic-regulation, acid-base balance, and nitrogenous waste excretion of fishes. The epithelium of fish gills is comparable to the total area of the skin and in many species is considerably larger, making its structure a major consideration in the homeostasis of the body of a fish (Roberts 2012). Epithelial lifting and oedema on the secondary lamellae was common at both localities with occasional pillar cell rupture (Figs 6.3a&b). According to Bhagwant and Elahee (2002), epithelial lifting is often accompanied by oedema.

The primary lamella showed some hyperplasia at both Loskop and Flag Boshielo dams (Fig. 6.3d). These alterations might be attributed to the concentration of metals and nutrients at both Loskop and Flag Boshielo dams. According to Arellano *et al.* (1999), the lifting, oedema, swelling, and hyperplasia of the lamellar epithelium serve as a defence function, because these histological changes could increase the distance across which waterborne irritants must diffuse to reach the bloodstream.

Fusion of primary and secondary lamella was recorded at both localities (Figs 6.3c&d) but it was observed more often at Flag Boshielo Dam than at Loskop Dam. The fusion of lamella may impair respiratory functions, especially at higher temperature when DO is lower and metabolic oxygen demand is higher (Roberts 2012). The gills from Flag Boshielo Dam exhibited more haemorrhage (aneurism) (Fig. 6.3e) than the gills from Loskop Dam and these aneurisms were observed on both primary and secondary lamella. According to Salamat and Zarie (2012), aneurism in secondary lamella is related to pillar cell rupturing as a result of an increased blood flow or direct effects of chemicals on the cells. Moreover, Figueiredo-Fernandes *et al.* (2007) noticed lamellar epithelium lifting, epithelium proliferation, lamellar axis vasodilation, oedema in the filament, fusion of lamellae and lamellar aneurisms on *Oreochromis niloticus* exposed to copper. In the present study, the concentrations of Al, Pb, Fe and Cu were above the TWQR for aquatic ecosystems, with Flag Boshielo Dam showing higher concentrations than Loskop Dam. Therefore, the higher magnitude of aneurism at Flag Boshielo Dam might be attributed to the elevated concentration of these metals.

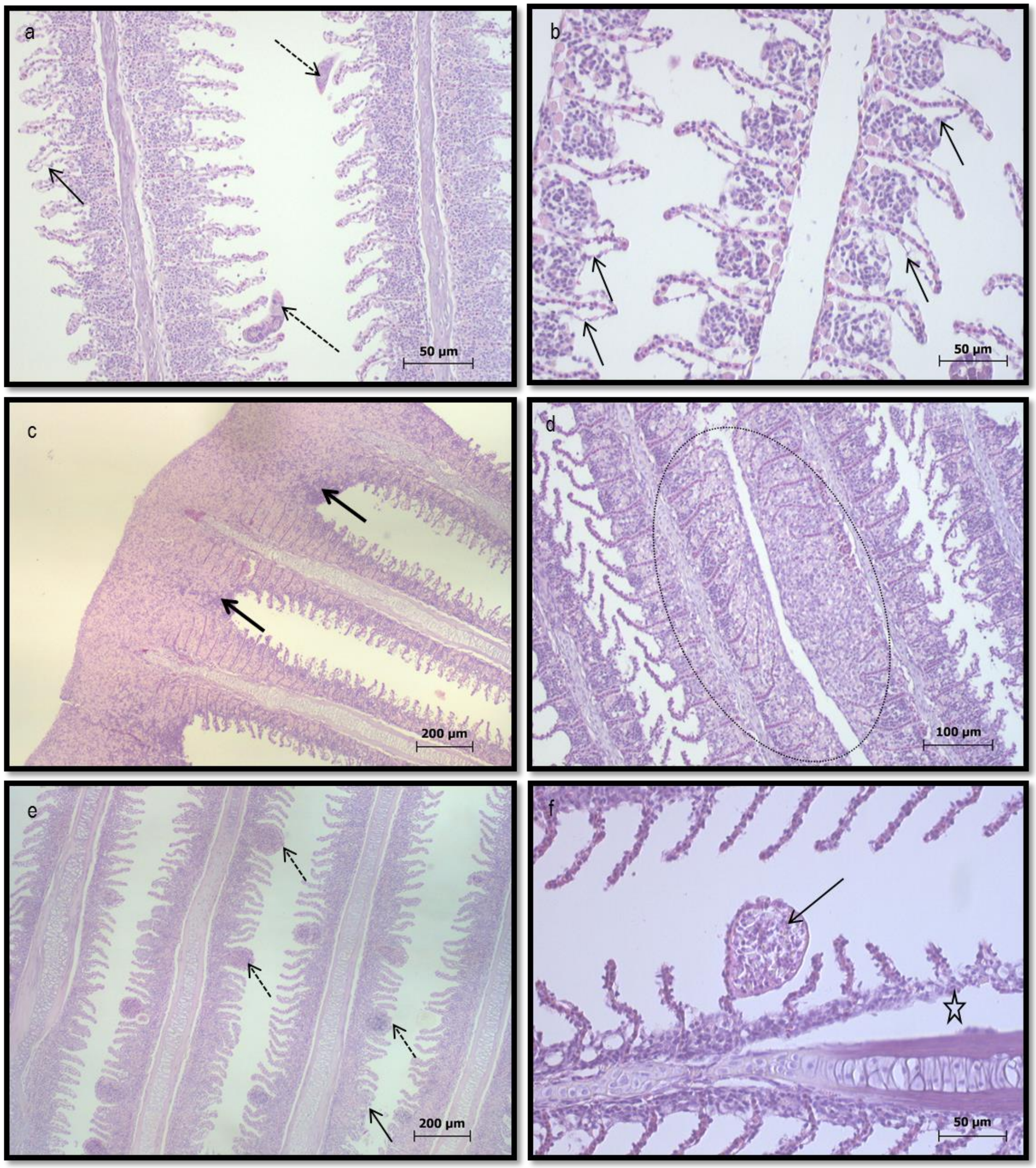

Figure 6.3 Gill alterations of *Labeo rosae* from Loskop and Flag Boshielo dams: a. epithelial lifting (solid arrow), monogenean parasites (dotted arrow); b. epithelial lifting; c. primary lamella fusion; d. secondary lamella fusion & hyperplasia on primary lamella (encircled); e. Haemorrhage (aneurism) (dotted-lined arrow), primary lamella fusion (solid-lined arrow); f. protozoan parasites infection (arrow), interstitial oedema (star).

Some cell necrosis and nuclear alterations were noticed at both localities. Most of the cells that showed necrosis were in the supporting cells. Some protozoan infections were recorded from both primary and secondary lamella at Flag Boshielo Dam (Fig. 6.3F). No protozoan infections were recorded at Loskop Dam; however, some ectoparasites such as *Dactylogyrus pienaari* were recorded (6.3a). According to Roberts (2012), metazoan parasites may induce severe lesions on the gills of a fish. Among them, monogeneans can cause severe damage with their hooks which provide a suitable milieu for protozoans.

6.2.2 Liver

The liver is comprised of a large mass of glandular tissues. In polluted water bodies, fish liver is a target organ for metals due to its detoxification function and bioaccumulation capabilities (Roberts 2012; Van Dyk 2003a). When the concentration of metals exceeds the threshold of the liver, metal-related pathological conditions occur (Hinton and Laurén 1990). The histopathological alterations of the liver of *L. rosae* were more or less similar at both localities. They included degenerative fatty vacuolization, necrosis, sinusoidal congestion (haemorrhage), lymphocytic infiltration, hepatocellular pleomorphism and hypertrophy, nuclear change, hyaline droplet and, hydropic (glycogen) degeneration, lipofuscin and haemosiderin pigment accumulation (Figs 6.4a-f).

All these alterations were observed at both localities with hyaline droplet and hydropic degeneration only recorded at Loskop Dam. Van Dyk (2003b) observed hyalinization, vacuolization, congestion of red blood cells and cellular swelling (hydropic degeneration) in *Clarias gariepinus* exposed to cadmium and zinc. Figueiredo-Fernandes *et al.* (2007) observed vacuolization and degenerative necrotic conditions in the liver of *Oreochromis niloticus* which was exposed to copper and Mohamed (2008) reported degeneration and necrosis in the liver of *O. niloticus* and *Lates niloticus* which were exposed to Fe, Zn, Cu, Pb, Cd and Co. In the present study, the concentrations of Al, Pb, Cu and Zn were above TWQR for aquatic ecosystem at both localities. Therefore, the histopathological alterations at both Loskop and Flag Boshielo dams might be attributed to the elevated concentration of metals in the water bodies and the metals cumulative effects in the liver.

The hepatocytes were normal during winter. Similar trends were observed for hepatocellular arrangement, differentiation as well as irregular and non-homogenous cell sizes during summer at both Loskop and Flag Boshielo dams. The hepatocytes were hypertrophied during summer and might be due to the fact that the fish were actively feeding.

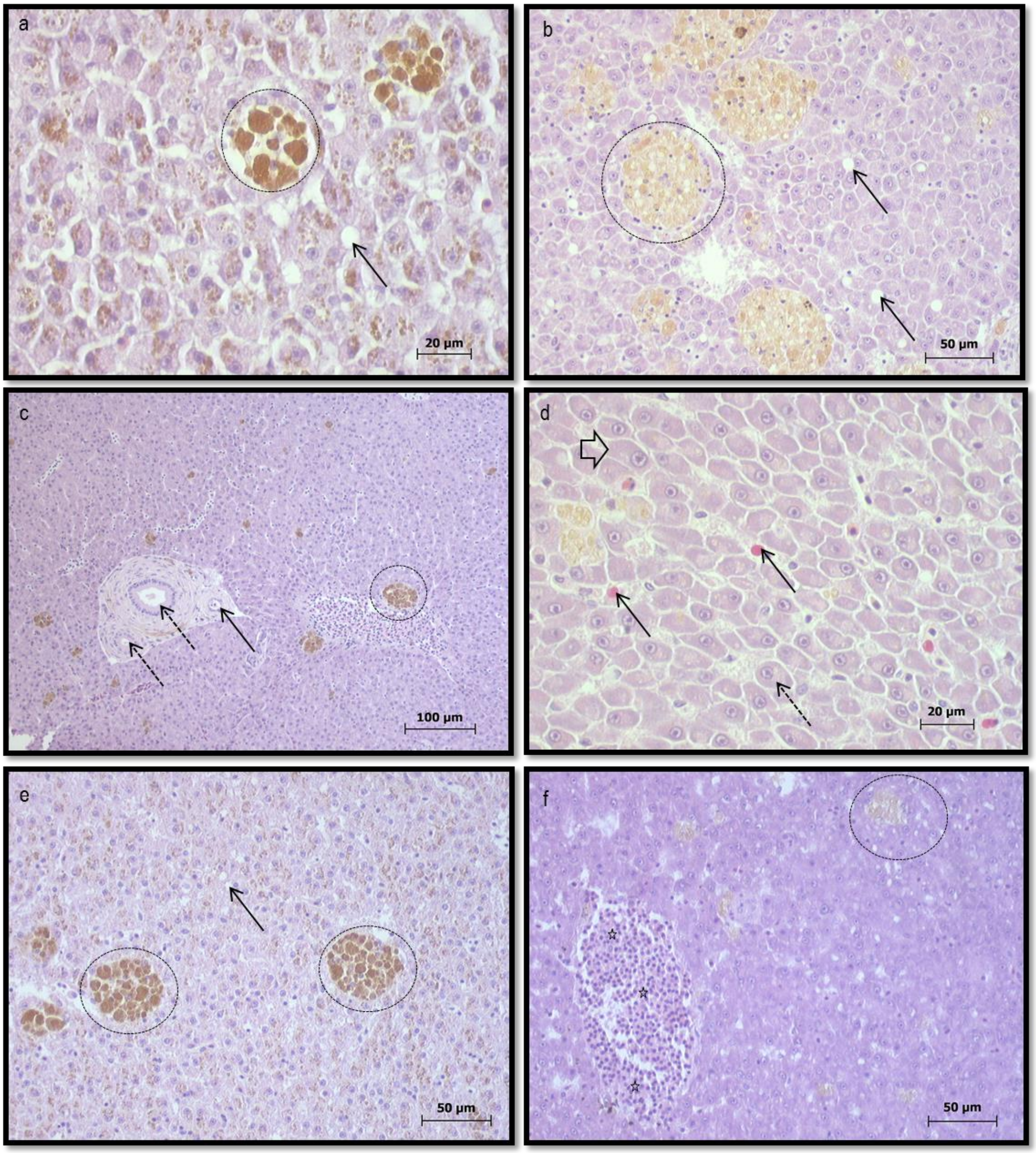

Figure 6.4 Liver histology observed during this study: a. lipofuscin and haemosiderin pigment (encircled), fatty vacuolization (arrow); b. lipofuscin (encircled), fatty vacuolization (arrows); c. bile ducts (dotted-line arrows), portal artery (solid-lined arrow); lipofuscin and haemosiderin pigment (encircled); d. hypertrophied hepatocyte (thick arrow), hyaline droplets (solid-lined arrows), hydropic/glycogen droplet degeneration (dotted-lined arrow); e. severe pigment (haemosiderin and lipofuscin) accumulation (FBD): lipofuscin and haemosiderin pigment (encircled), fatty vacuolization (arrow); f. scant pigment (haemosiderin and lipofuscin) accumulation(LD): central vein (star), lipofuscin (encircled).

Fish from Loskop Dam exhibited plasma alterations such as hyaline droplets degeneration and hepatocellular hydropic (glycogen) which were not evident at Flag Boshielo Dam. Van Dyk (2003b) reported that hyaline droplets are the results of disturbances of protein synthesis and were observed increasingly as the short-term exposure period progressed, but were mostly absent in fish exposed over the long-term exposure period in *O. mossambicus.*

Degeneration of hepatocytes such as non-homogeneity of size and peripheral situated nuclei were observed in most specimens at both localities. Damjanov (1996) reported that the move of nuclei to the periphery of the hepatocytes is often accompanied by large vacuoles as well as nuclear atrophy in the cells. However, Roberts (2012) reported that hepatocytes are often swollen with glycogen or neutral fat when nutrition is even marginally less than ideal and during cyclical starvation phases, the cells may be shrunken and the entire liver loaded with yellow ceroid pigments.

The liver of fish at Flag Boshielo Dam showed some yellow to yellow-brown granular pigments called melano-macrophage centre pigments which were comprised of haemosiderin and lipofuscin. Within the macrophages, lipofuscin generally appears to be the most abundant pigment and haemosiderin can be present in considerable quantities under certain conditions such as haemolytic anaemia. Haemosiderin pigmentation was only observed at Flag Boshielo Dam with lipofuscin being observed at both localities. Agius & Agbede (1984) reported that haemosiderin may either be the results of increased catabolism of damaged erythrocytes or increased retention of iron within melanomacrophage centres as a protective mechanism. Therefore, haemosiderin pigmentation at Flag Boshielo Dam might be attributed to the higher concentration of iron in the water.

Lipofuscin is formed as the cells die as well as when the age of a fish increases. It has been known as wear and tear pigment. Lipofuscin has been recorded in the liver of fish from both Loskop and Flag Boshielo dams. According to Van Dyk (2003a), lipofuscin itself does not signify cell damage, but increased amounts are the results of abnormalities or deaths of other cells. Furthermore, lipofuscin deposition has been observed in fish displaying a wide variety of pathological conditions, including nutritional deficiencies, bacterial and viral disease and disturbances caused by toxic material (Agius & Roberts 2003). Higher amounts of lipofuscin pigment were observed from fish at Flag Boshielo Dam than at Loskop Dam. The concentrations of metals in the water, sediment and tissues were higher at Flag Boshielo Dam than at

Loskop Dam, therefore the higher amount of lipofuscin at Flag Boshielo Dam might be due to the elevated concentration of metals.

6.2.3 Lesion indices

Alterations such as degenerative fatty vacuolization, necrosis, haemorrhage, lymphocytic infiltration, non-homogeneity of hepatocytes/hypertrophy, nuclear change, hyaline droplet, hydropic (glycogen) degeneration, lipofuscin pigment accumulation were common at both localities but they differed with their severity. In the present study, the alterations were weighed based on the extent, how it affects the organ function and the ability of the fish to survive. Four indices were calculated according to the protocol by Bernet *et al.* (1999) (refer to chapter 2) and the results are presented in table 6.1.

Table 6.1 Lesion indices calculated for gills and liver of *Labeo rosae* from Loskop and Flag Boshielo dams.

Organ	**CD**		**RC**		**PC**		**I**		**T**		$\mathbf{I_{org.}}$	
	LD	FBD	LD	FBD	LD	FBD	LD	FBD	LD	FBD	LD	FBD
Gills	3.19	3.27	20.34	14.45	6.58	4.35	2.69	2.86	0	0	**32.80**	**20.93**
Liver	2.48	2.08	15.39	14.37	0.65	0.57	3.39	3.12	0	0	**21.91**	**20.13**
$\mathbf{I_{.rp}}$	**5.67**	**5.35**	**35.73**	**28.82**	**7.23**	**4.92**	**6.08**	**5.98**	**0**	**0**	**54.71**	**41.06**

Abbreviations: (CD) circulatory disturbances; (RC) regressive changes; (PC) progressive changes; (I) inflammation; (T) tumour; ($I_{org.}$) organ index; ($I_{.rp}$) total reaction index; (FBD) Flag Boshielo Dam; (LD) Loskop Dam.

The most prominent reaction pattern observed throughout the study was regressive alterations at both localities. However, there were no significant difference between Loskop and Flag Boshielo dams ($p>0.05$). Despite this, the gills from Loskop Dam showed to have undergone more alterations than the gills from Flag Boshielo Dam. Gill indices of 32.80 and 20.98 were recorded at Loskop and Flag Boshielo dams respectively (Table 6.1). Although the study showed insignificant differences between the lesion indices of the localities, the gills showed a vast difference in regressive change with the index value of 20.34 and 14.45 recorded at Loskop and Flag Boshielo dams respectively (Table 6.1 & Fig. 6.5a). The higher gill index value at Loskop Dam was caused by necrosis which was observed on the supporting cells and epithelial lifting of the secondary lamella. No tumours were observed in the gills at both localities throughout the study.

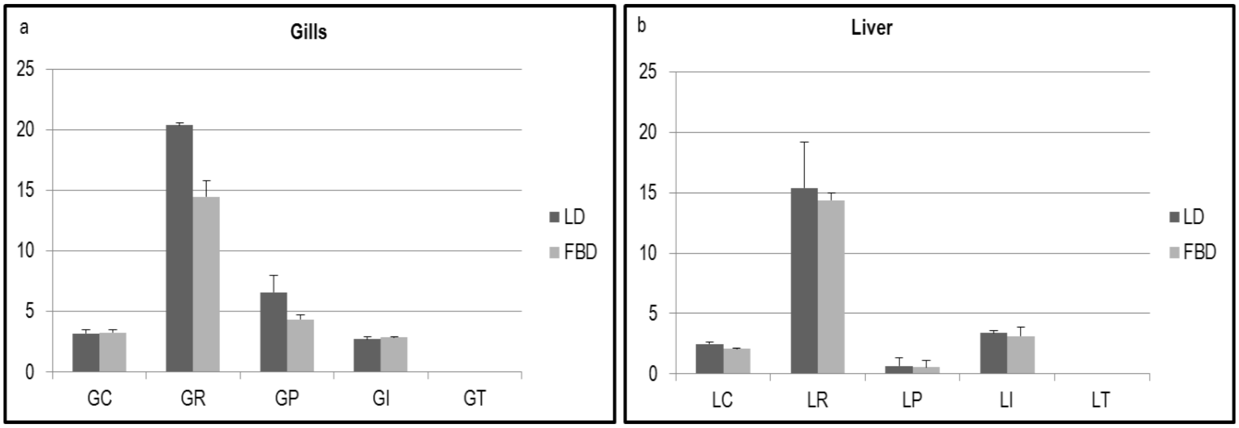

Figure 6.5 Lesion indices for the gills (a) and liver (b) of *Labeo rosae* from Loskop and Flag Boshielo dams.

As mentioned earlier, liver is known as a detoxification organ; hence the primary target organ for metals, but during the present study gills sustained more and severe alterations than the liver. Higher lesion indices were recorded for the gills at both localities (Figs 6.5a&b). This might be due to the intimate contact with the external water environment of the gills (Roberts 2012). However, there were no significant differences between all indices calculated between the localities (p>0). No tumours were observed in the liver at both localities throughout the study.

6.3 CONCLUSION

This study proved the effectiveness of gills and liver histopathology as a biomarker which can be used in any ecological study. There were no significant differences in histopathological alterations recorded on the gills and liver from both localities. Although, the pollutant concentrations were dissimilar between the localities, the difference was not significant as well. Therefore, the histopathological alterations might be attributed to the pollutant concentrations at both Loskop and Flag Boshielo dams. The pollutant concentrations at Loskop and Flag Boshielo dams showed to have had almost similar effects on the gills as well as on the liver. Secondary lamella epithelia of gills have shown to be more sensitive due to its intimate contact with the external water environment. This has been witnessed in the present study whereby 100% prevalence of oedema and epithelial lifting was recorded at both localities. However, chondrocytes are not in a direct contact with water but they showed to have undergone some architectural alterations as well.

Roberts (2012) reported that nuclei of the hepatocytes are situated at the centre of the cell. Coinciding with Roberts (2012), Reddy (2012) observed nuclei being situated at the centre of the hepatocyte in the control group. During the present study, some hepatocytes have undergone hypertrophy, which led to the non-homogeneity of cell size in the liver (at both localities) with nuclei being situated on the periphery of the hepatocytes. The concentration of Al, Pb, Cu and Zn were above TWQR for aquatic ecosystem at both localities with Flag Boshielo Dam showing to have higher concentration than Loskop Dam. Hepatocytes of the liver seemed to respond quickly when exposed to elevated concentrations of pollutants because the livers of fish from Loskop Dam showed to be macroscopically normal but some histopathological alterations were observed at cellular and tissue levels. In the present study, the components at the lower levels of biological organizations (cells and tissues) proved to be suitable tools to be used as biomarkers of prior exposure to toxic pollutants even at low concentration. Due to the fact that both Loskop and Flag Boshielo dams are polluted, histopathological alterations could not distinguish between the two localities.

CHAPTER 7

GENERAL CONCLUSION AND RECOMMENDATIONS

Freshwater ecosystems are the most severely degraded ecosystems in South Africa, suffering from over extraction of water, pollution as well as flow and channel modifications. Pollution has been a great concern in the Olifants River System over the past decade. The river has experienced massive fish and crocodile mortalities, particularly in the upper and lower catchment (Van Vuuren 2010). Van Vuuren (2009) reported that the on-going pollution of the Olifants River System would eventually result in some kind of ecological disaster. The uncertainty on the cause of deaths of aquatic biota in the Olifants River resulted in the establishment of the “Consortium for the Restoration of the Olifants Catchment” (De Villiers & Mkwelo 2009; Van Vuuren 2009).

Loskop Dam has been described as a repository for contaminants from the upper Olifants River catchment. In the present study, no drastic difference in water quality constituents was recorded between Loskop and Flag Boshielo dams. The pH at both localities was alkaline with values ranging from 7.81-10.1. A slight difference in water temperature was observed between the two localities with a higher temperature recorded at Loskop Dam. Lower water temperature at Flag Boshielo Dam resulted in DO being higher at Flag Boshielo Dam than Loskop Dam. The mean concentrations for EC, salinity and TDS were higher at Loskop Dam with alkalinity and turbidity being higher at Flag Boshielo Dam. According to DWAF (1996a), alkalinity, EC, salinity, TDS and turbidity cause toxic effects only at extreme concentrations.

For the ions, Cl^-, F^- and Na concentrations were higher at Flag Boshielo Dam with Mg, K and Ca being higher at Loskop Dam. Jooste *et al.* (2005) reported that mine pollution may in terms of salts increase the conductivity and salinity of the water. Higher concentrations of NO_2, NO_3, NH_3, N_2, and SO_4 were recorded at Loskop Dam than Flag Boshielo Dam. In the present study, N_2 nutrients categorise Loskop Dam as mesotrophic and Flag Boshielo Dam as oligotrophic. Although nutrient levels were lower at Flag Boshielo Dam, NH_3 was above the TWQR stipulated for aquatic ecosystems at both localities. There are no TWQR values for NO_2, NO_3, N_2, and SO_4 set for aquatic ecosystem. DWAF (1996a) reported that the chronic effects of NH_3 include a reduction in hatching success and reduction in growth rate and morphological development, and pathological changes in gill, hepatic and renal tissues.

Several toxic constituents (i.e. Al, Cu, Fe, Pb and Sr) were higher at Flag Boshielo Dam than at Loskop Dam even though most of them were below detection value during summer at both localities. The concentrations of Se, Pb, Cu and Al were above the TWQR stipulated for aquatic ecosystems at both localities. The concentration of Mn at Flag Boshielo Dam was within the TWQR suggested for aquatic ecosystem but above at Loskop Dam. The availability, solubility and toxicity of metals depend on pH, temperature, TDS as well as interaction between them and other constituents. For example, the toxicity of copper is reduced in the presence of Zn, Mo and SO^{2+} (Dallas & Day 2004). Therefore, the effect of water quality parameter cannot be described on its own. The present study showed that the water quality is in acceptable conditions at both localities.

Some of the metals could not be detected in the water but they were recorded in the fish tissues and in sediments. Fish are found near or at the top of the food chain and are thus known to accumulate metals within their tissues (Barbour *et al.* 1999). Selenium was below detection value in the water at Flag Boshielo Dam throughout the study but an elevated concentration was recorded in the fish tissues and sediment. The concentration of metals in the tissues and sediment did not differ much between the two localities. Higher concentration of metals (i.e. Cu, Fe, Mn, Pb, Se, and Zn) accumulated in the liver, followed by gills and muscle respectively. Bioaccumulation patterns of metals which has dominated throughout the study is liver > gills> muscle. This has resulted in higher BAF_w for liver followed by gills and muscle. The only metals that deviated from this pattern are Mn and Sr which were high in the gills but followed by liver and muscle respectively.

Due to the considerable high concentration of metals in sediment, the ratio of metals in fish organs to sediment (BAF_s) was low. The general trend of accumulation in sediment was as follows: Fe > Al > Si > Mn > Zn > Cu > Sb > Sr > Pb > Se at Loskop Dam whereas at Flag Boshielo Dam was as follows: Fe > Al > Mn > Si > Zn > Cu > Sr > Pb > Sb > Se. Coetzee *et al.* (2002) reported that metals can be reintroduced into the water column from the sediment in a bioavailable form and organisms such as fish may absorb these metals from the water through gills or epithelial tissues, potentially leading to accumulation in body tissues. Therefore, the sediment at Loskop and Flag Boshielo dams might be categorised among ecological stressors.

Based on the HAI, fish from Flag Boshielo Dam were in better health condition than the fish from Loskop Dam. Most of the abnormalities were common at both localities and were prevalent in the gills and liver. Gills are in direct contact with the external water environment and liver is known for its detoxification function, therefore gills and liver are the target organs for pathological metal interaction in an aquatic ecosystem (Osman & Kloas 2010). No abnormalities were observed for the kidney and eyes of fish at both localities. The fish from Loskop Dam showed more than 50% of their caeca being covered by mesenteric fats with less than 50% of the caeca being covered in fish from Flag Boshielo Dam. No pansteatitis were recorded for *L. rosae* throughout the study. Loskop Dam exhibited higher concentrations of ions (Mg, K, Ca) and nutrients (N_2) therefore there might be a correlation between the fish health and nutrients and/or between the fish health and ions.

Parasites (*L. cyprinacea*) induced lesions on the skin and erosion of fins were only observed at Flag Boshielo Dam. Five ectoparasite species (*Dactylogyrus pienaari*, *Paradiplozoon* sp., *Ergasilus* sp., *Lamproglena* sp. and *Lernaea cyprinacea*) and two endoparasites species (*Nematobothrium* sp. and *Paracamallanus cyathopharynx*) were recorded at Flag Boshielo Dam with only one ectoparasite species (*Dactylogyrus pienaari*) and one endoparasite (*Nematobothrium* sp.) being recorded at Loskop Dam. As mentioned earlier, several metals were higher at Flag Boshielo Dam than Loskop Dam. However, the nutrients levels exhibited Loskop Dam as mesotrophic with Flag Boshielo Dam being oligotrophic; therefore there might be a correlation between nutrients and metazoan parasites as a lower parasite species diversity was recorded at Loskop Dam.

The condition factor did not differ much between the two localities. The factors ranged from 1.10 to 1.71 at Loskop Dam with the range from 0.79 to 1.18 being recorded at Flag Boshielo Dam. Condition factor of 1.60 indicate an excellent condition (trophy class fish) while 0.80 indicate extremely poor fish condition with a big head and narrow, thin body (Barnham & Baxter 1998). The higher condition factor of fish from Loskop Dam might be indicative of abnormally high availability of food (eutrophic conditions causing abnormal algal blooms) in comparison to Flag Boshielo Dam, or it may indicate relative lack of food for fish at Flag Boshielo Dam. However, hepatosomatic index showed no drastic deviation from the normal range at both localities. The values ranged from 0.57% to 1.19% at Loskop Dam and 0.65% to 1.8% at Flag Boshielo Dam. The normal range of HSI values are from 1% to 2%; therefore this index showed that the health status of fish at both localities were not bad. The present study proved that HAI, HSI and CF are useful tools that can be used to detect gross change in the health of fish rapidly.

Similar histopathological alterations were recorded at both localities but with different magnitude and severity. The organ index showed that the fish at Flag Boshielo Dam were in a more favourable health state than the fish at Loskop Dam, especially with respect to the gill index. Scant abnormalities were observed for the gill during macroscopic assessment. However, histological examination showed that many significant lesions were present at both localities with regressive changes dominating at Loskop Dam. Prevalence of 100% for epithelial lifting and intercellular oedema were recorded at both localities. The fusion of primary and secondary lamella, necrosis in supporting tissues and haemorrhage were observed at both localities. In the present study, regressive changes dominated the alterations in the gills of fish from Loskop Dam. Regressive changes recorded included architectural and structural alterations, plasma alterations, nuclear alterations and necrosis. Some protozoan infections were recorded from both primary and secondary lamellae at Flag Boshielo Dam. No protozoan infections were recorded at Loskop Dam. The absence of protozoan infections in the gills of fish from Loskop Dam might be attributed to the historical distribution of the parasites or high concentration of nutrients and ions in the dam. However, some ectoparasites such as *Dactylogyrus pienaari* were recorded at Loskop Dam.

For the liver, similar histopathological alterations were recorded at both localities but hepatocellular hydropic (glycogen) and hyaline droplet degeneration were more prominent in the fish from Loskop Dam during summer. No macroscopic abnormalities were observed for the liver of fish from Loskop Dam during winter but histopathological alterations such as infiltration of lymphocytes and nuclear alterations were prevalent. Therefore, the lower levels of biological organisation such as tissues and cells respond rapidly to increased concentration of contaminants. It is evident that with some significant histopathological alterations, no abnormalities will be observed during gross examination (like HAI). Histopathology can thus be used as a useful tool to indicate and/or monitor prior or low grade exposure to harmful substances. This study has proven the effectiveness of gill and liver histopathology as biomonitoring tool for use in aquatic ecological studies. Little work on histopathology has been done in the Olifants River System; therefore, this study will provide a baseline data for *L.* rosae for upcoming histopathology studies in this river system.

This study has shown that elevated concentration of toxic constituents in fish organ can result in structural alterations in the lower level of biological organisation such as tissues and cells. Therefore, the incorporation of histopathological assessment with macroscopic HAI and bioaccumulation in biomonitoring can provide a more expanded view of the ecological state of a river system and/or catchment as a whole. The present study has shown that the overall health of fish population were better at Flag Boshielo Dam as compared to Loskop Dam.

For future studies, aging of fish should be carried out to find out if the difference in fish size between the two localities was pollution induced or the smaller size of fish at Flag Boshielo Dam is because the sample only included younger fish. Muscle tissues seem to accumulate low levels of metals; however, due to the fact that they are consumed by humans, risk assessment should be carried out to evaluate the potential risks of humans upon consuming fish from these localities.

CHAPTER 8

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Table 1. Water quality variables recorded at Loskop and Flag Boshielo dams during winter (July 2011) and summer (November 2011).

Variables	Loskop Dam	Flag Boshielo Dam	Loskop Dam	Flag Boshielo Dam
	Winter		Summer	
Temperature (˚C)	16.25	13.76	26.09	25.27
Dissolved Oxygen (%)	77.47	118.43	57.47	55.07
Dissolved oxygen (mg/ℓ)	8.17	12.35	4.17	4.62
pH	7.81 – 9.49	8.98 – 9.63	8.07 – 10.10	9.04 – 9.58
Total dissolved solids (mg/ℓ)	255.66	274.95	345.80	310.70
Alkalinity (mg/ℓ)	44.00	58.00	42.67	73.30
Electrical conductivity (mS/m	39.33	40.80	53.20	47.80
Salinity (‰)	0.19	0.20	0.26	0.23
Turbidity (NTU)	4.93	6.50	7.93	14.67
Ammonia (mg/ℓ)	1.00	0.27	1.10	0.20
Nitrite (mg/ℓ)	< 0.1	< 0.1	0.10	< 0.1
Nitrate (mg/ℓ)	0.56	0.40	0.60	0.30
Total nitrogen (mg/ℓ)	0.90	0.40	1.00	0.37
Sulphate (mg/ℓ)	119.00	96.00	141.77	93.30
Chloride (mg/ℓ)	14.33	24.00	16.67	26.67
Calcium (mg/ℓ)	35.30	31.53	38.00	26.33
Magnesium (mg/ℓ)	21.61	20.79	19.00	16.67
Potassium (mg/ℓ)	5.87	5.45	6.50	4.00
Sodium (mg/ℓ)	25.81	34.47	26.00	32.00
Aluminium (mg/ℓ)	0.0387	0.0543	< 0.1	0.1110
Antimony (mg/ℓ)	0.0047	0.0050	< 0.01	< 0.01
Copper (mg/ℓ)	0.0020	0.0023	< 0.025	< 0.025
Iron (mg/ℓ)	0.0767	0.1187	< 0.025	0.1130
Manganese (mg/ℓ)	0.0867	0.0343	0.2190	0.0250
Lead (mg/ℓ)	0.0100	0.0110	< 0.02	< 0.02
Selenium (mg/ℓ)	0.0100	< 0.01	< 0.02	< 0.02
Silica (mg/ℓ)	2.0677	0.8333	6.2267	5.2000
Strontium (mg/ℓ)	0.1860	0.1807	0.1730	0.1490
Zinc (mg/ℓ)	0.0040	0.0040	< 0.025	< 0.025

Table 1. Accumulation of selected toxic constituents in the muscle, gills, and liver of *Labeo rosae* and in sediment from Loskop and Flag Boshielo dams (mean concentration ± standard deviation). Units in mg/g.

Metals	**Muscle**		**Gills**		**Liver**		**Sediment**	
	Loskop	**Flag Boshielo**	**Loskop**	**Flag Boshielo**	**Loskop**	**Flag Boshielo**	**Loskop**	**Flag Boshielo**
Al	0.1051 ± 0.02	0.0779 ± 0.01	0.2334 ± 0.28	0.4335 ± 0.58	1.0604 ± 1.58	2.3902 ± 3.97	7.8381 ± 6.14	19.1379 ± 9.67
Cu	0.0018 ± 0.002	0.0119 ± 0.01	0.0001 ± 0.0004	0.0494 ± 0.07	2.0442 ± 2.55	0.4635 ± 0.42	0.0248 ± 0.02	0.0448 ± 0.01
Fe	0.0901 ± 0.07	0.0818 ± 0.05	0.3243 ± 0.34	0.4583 ± 0.66	7.0999 ± 12.22	10.8468 ± 7.37	14.7137 ± 11.92	35.4402 ± 10.30
Mn	0.0038 ± 0.003	0.0041 ± 0.002	0.0761 ± 0.09	0.1164 ± 0.15	0.0731 ± 0.12	0.0388 ± 0.04	0.8081 ± 0.70	2.6068 ± 2.98
Pb	0.0039 ± 0.01	0.0019 ± 0.002	0.0069 ± 0.005	0.0059 ± 0.01	0.0308 ± 0.07	0.1425 ± 0.22	0.0073 ± 0.002	0.018 ± 0.01
Sb	0.1194 ± 0.02	0.0069 ± 0.008	0.2470 ± 0.31	0.0212 ± 0.03	1.2692 ± 1.89	1.5564 ± 2.23	0.0236 ± 0.005	0.0106 ± 0.004
Se	0.0186 ± 0.01	0.0075 ± 0.005	0.0336 ± 0.05	0.0204 ± 0.30	0.2749 ± 0.36	0.1528 ± 0.24	0.0057 ± 0.0005	0.0034 ± 0.0009
Si	0.1247 ± 0.10	0.0785 ± 0.04	0.5759 ± 0.86	1.0068 ± 1.23	1.1019 ± 1.52	3.6450 ± 4.71	0.8855 ± 0.33	1.2559 ± 0.58
Sr	0.0088 ± 0.004	0.0097 ± 0.004	0.3605 ± 0.49	0.5231 ± 0.67	0.0362 ± 0.008	0.0433 ± 0.06	0.0131 ± 0.008	0.0213 ± 0.006
Zn	0.4873 ± 0.13	0.3052 ± 0.03	1.0356 ± 1.19	1.3606 ± 1.65	5.0261 ± 7.30	6.4553 ± 8.85	0.5827 ± 0.08	0.547 ± 0.04

Table 1. Health Assessment Index of *Labeo rosae* from Loskop Dam during winter (July 2011).

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Endo-PI	Ecto-PI	Ecto-IPI	HAI Total	HAI IPI
	TL	SL																	
1	45.8	38.1	1415.4	M	0	0	0	0	0	0	0	0	0	10	0	10	20	20	30
2	46.1	39	1438.8	M	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30
3	47.9	39.8	1463.8	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
4	43.6	37	1057.7	M	0	0	0	0	0	0	0	0	0	0	10	10	20	20	30
5	43.5	37.5	1037	F	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30
6	46.8	39	1433.4	F	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30
7	47.5	39.7	1435.1	F	0	0	0	0	0	0	0	0	0	20	0	0	30	20	40
8	44.7	36.6	1074.2	M	0	0	0	0	0	0	0	0	0	0	10	0	30	10	40
9	40	36	835.8	F	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
10	51.3	41.9	2080.9	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
11	49.5	43.5	1628.2	M	0	0	0	0	0	0	0	0	0	30	10	0	30	40	70
12	47.6	39.5	1620.8	M	0	0	0	0	0	0	0	0	0	20	0	10	20	30	40
13	47.1	39.4	1414.7	M	0	0	0	0	0	0	0	0	0	0	10	0	30	10	40
14	44.2	38.6	1059.5	M	0	0	0	0	0	0	0	0	0	30	0	0	30	30	60
15	44	37.3	1207.1	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
16	43.4	36.7	1130.2	M	0	0	0	0	0	0	0	0	0	0	10	0	30	10	40
17	44.1	37.9	1097.5	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
18	40.3	33.1	766.2	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
19	38.1	31.2	746.6	M	0	0	0	0	0	0	0	0	0	0	10	0	30	10	40
20	55.1	44.5	2068.4	M	0	0	0	0	0	0	0	0	0	20	10	10	20	40	50
															Total HAI			360	870
															Mean HAI			18	43.5

Table 2. Health Assessment Index of *Labeo rosae* from Loskop Dam during summer (November 2011).

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Endo-PI	Ecto-PI	Ecto-IPI	HAI Total	HAI IPI
	TL	SL																	
1	55.5	43.8	2030.4	F	0	0	0	0	0	30	0	0	0	20	0	0	30	50	80
2	50.6	42.5	1632.2	F	0	0	0	0	30	0	0	0	0	0	0	20	10	50	40
3	55	42.3	1833.7	F	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30
4	46.7	39.2	1315.2	F	0	30	0	0	30	30	0	0	0	20	0	10	20	120	130
5	44.5	37	1283.7	M	0	0	0	0	30	30	0	0	0	20	0	0	30	80	110
6	48.1	40.5	1518.7	M	0	0	0	0	30	0	0	0	0	0	0	0	30	30	60
7	41.3	34.7	1039.2	M	0	0	0	0	0	0	0	0	0	0	10	0	30	10	40
8	39.5	33.5	780.1	M	0	0	0	0	30	30	0	0	0	20	0	0	30	80	110
9	45	37.8	1333.3	F	0	0	0	0	0	30	0	0	0	20	10	20	10	80	70
10	45.5	37	1127.5	F	0	0	0	0	30	30	0	0	0	0	10	0	30	70	100
11	45.5	37.9	1313.5	F	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
12	47.5	39.5	1271.2	F	0	0	0	0	30	0	0	0	0	30	0	10	20	70	80
13	47.3	39.9	1526.8	F	0	0	0	0	0	0	0	0	0	20	10	0	30	30	60
14	48.8	41.2	1658.2	F	0	0	0	0	30	0	0	0	0	0	0	0	30	30	60
15	46.1	38.6	1471.8	F	0	0	0	0	0	0	0	0	0	20	10	0	30	30	60
16	47.1	40.3	1460.1	F	0	0	0	0	30	0	0	0	0	0	0	0	30	30	60
17	44	40.2	1070.1	M	0	0	0	0	0	30	0	0	0	20	0	10	20	60	70
18	48.7	40.7	1513.4	F	0	0	0	0	30	30	0	0	0	20	0	0	30	80	110
19	49	37.3	2012.2	F	0	0	0	0	30	0	0	0	0	20	0	0	30	50	80
20	44.5	38.1	1212.9	F	0	0	0	0	0	30	0	0	0	20	0	10	20	60	70
															Total HAI			1030	1470
															Mean HAI			51.5	73.5

Table 3. Health Assessment Index of *Labeo rosae* from Flag Boshielo Dam during summer (November 2011).

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Endo-PI	Ecto-PI	Ecto-IPI	HAI Total	HAI IPI
	TL	SL																	
1	32.5	25.9	346.2	M	0	0	0	0	30	0	0	0	0	0	10	10	20	50	60
2	30.2	23.4	297.3	M	0	0	0	0	0	0	0	0	0	10	10	20	10	40	30
3	35.4	28.4	475.5	F	0	0	0	0	0	0	0	0	0	20	10	10	20	40	50
4	30.5	24.6	295.2	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
5	33.9	27.5	434.9	F	0	0	0	0	0	0	0	0	0	0	0	10	20	10	20
6	29.5	23.4	240.4	F	0	0	0	0	0	30	0	0	0	0	10	0	30	40	70
7	29.4	23.2	233.7	F	0	0	0	0	0	0	0	0	0	30	0	30	0	60	30
8	31.9	26	341.0	F	0	0	0	0	0	0	0	0	0	0	10	10	20	20	30
9	30.5	23.4	290.1	F	0	0	0	0	0	0	0	0	0	0	0	30	0	30	0
10	29.1	22.6	226.4	M	0	0	0	0	0	0	0	0	0	20	0	20	10	40	30
11	28.3	22.7	225.6	F	0	0	0	0	0	0	0	0	0	0	0	30	0	30	0
12	28.3	22.9	219.1	M	0	0	0	0	0	0	0	0	0	0	0	30	0	30	0
13	22.0	17.1	93.1	M	0	0	0	0	0	0	0	0	0	0	10	10	20	20	30
14	21.3	17.0	81.9	M	0	0	0	0	0	0	0	0	0	0	0	10	20	10	20
15	20.0	15.3	62.8	M	0	0	0	0	0	0	0	0	0	0	10	10	20	20	30
															Total HAI			460	450
															Mean HAI			30.7	20

Table 4. Health Assessment Index of *Labeo rosae* from Flag Boshielo Dam during winter (July 2011).

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Endo-PI	Ecto-PI	Ecto-IPI	HAI Total	HAI IPI
	TL	SL																	
1	33.7	26.6	416.7	F	0	0	0	0	30	0	0	0	0	20	0	10	20	60	70
2	34.9	27.3	463.2	F	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
3	30.3	23.8	289.6	M	0	0	0	0	0	0	0	0	0	0	0	10	20	10	20
4	34	27	406.2	M	0	0	10	0	0	0	0	0	0	20	10	0	30	40	70
5	36.4	29	546.6	M	0	0	0	0	0	30	0	0	0	0	10	0	30	40	70
6	27	21.7	206.8	M	0	0	0	0	0	0	0	0	0	20	0	0	30	20	50
7	26.9	22	230.5	M	0	0	0	0	0	0	0	0	0	20	0	10	20	30	40
8	28.5	23	242.8	F	0	10	10	0	0	0	0	0	0	0	0	10	20	30	40
9	27.6	22.3	220.3	F	0	0	0	0	0	0	0	0	0	30	10	0	30	40	70
10	33.2	27	338.4	M	0	0	0	0	0	0	0	0	0	0	0	10	20	10	20
11	31	24.5	295.5	F	0	0	0	0	0	30	0	0	0	10	0	10	20	50	60
12	29.8	24.1	265.3	F	0	0	10	0	0	30	0	0	0	30	10	0	30	80	110
13	29.9	23.5	232.5	M	0	30	0	0	0	0	0	0	0	20	0	10	20	60	70
14	28.6	23.1	225.6	F	0	0	0	0	0	0	0	0	0	10	10	10	20	30	40
15	35.4	23.1	536.6	M	0	10	0	0	0	0	0	0	0	20	0	10	20	40	50
															Total HAI			560	830
															Mean HAI			37.3	55.3

Table 5. Hepatosomatic index of *Labeo rosae* from Loskop and Flag Boshielo dams during winter (July 2011).

Locality	Fish #	Sex	TL (cm)	SL (cm)	Mass (g)	Liver Mass (g)	HIS
Loskop Dam	1	M	45.8	38.1	1415.4	10.8	0.76
	2	M	46.1	39	1438.8	9.7	0.67
	3	M	47.9	39.8	1463.8	11.1	0.76
	4	M	43.6	37	1075.7	6.7	0.62
	5	F	43.5	37.5	1037	6.9	0.67
	6	F	46.8	39	1433.4	9.8	0.68
	7	F	47.5	39.7	1435.1	9.9	0.69
	8	M	44.7	36.6	1074.2	9	0.84
	9	F	40	36	835.8	9	1.08
	10	M	51.3	41.9	2080.9	13	0.62
	11	M	49.5	43.5	1628.2	10.5	0.64
	12	M	47.6	39.5	1620.8	10.6	0.65
	13	M	47.1	39.4	1414.7	8	0.57
	14	M	44.2	38.6	1059.5	8.7	0.82
	15	F	44	37.3	1207.1	9.3	0.77
	16	M	43.4	36.7	1130.2	10.3	0.85
	17	M	44.1	37.9	1097.5	8.1	0.74
	18	M	40.3	33.1	766.2	7.3	0.95
	19	M	38.1	31.2	746.6	5.8	0.78
	20	M	55.1	44.5	2068.4	15.4	0.74
Average ± SD	**N/A**	**N/A**	**45.53 ± 3.92**	**38.92 ± 3.06**	**1301.67 ± 372.25**	**9.49 ± 2.21**	**0.75 ± 0.12**
Locality	**Fish #**	**Sex**	**TL (cm)**	**SL (cm)**	**Mass (g)**	**Liver Mass (g)**	**HIS**
Flag Boshielo Dam	1	F	33.7	26.6	416.7	2.8	0.67
	2	F	34.9	27.3	463.2	4.2	0.91
	3	M	30.3	23.8	289.6	2.5	0.86
	4	M	34	27	406.2	4.9	1.21
	5	M	36.4	29	546.6	5.8	1.06
	6	M	27	21.7	206.8	2.1	1.02
	7	M	26.9	22	230.5	2.3	1
	8	F	28.5	23	242.8	1.5	0.62
	9	F	27.6	22.3	220.3	1.7	0.77
	10	M	33.2	27	338.4	3.6	1.06
	11	F	31	24.5	295.5	3.1	1.05
	12	F	29.8	24.1	265.3	1.7	0.64
	13	M	29.9	23.5	232.5	1.8	0.77
	14	F	28.6	23.1	225.6	-	-
	15	M	35.4	23.1	536.6	-	-
Average ± SD	**N/A**	**N/A**	**31.15 ± 3.15**	**24.53 ± 2.36**	**327.77 ± 107.19**	**2.92 ± 1.35**	**0.9 ± 0.19**

Table 6. Hepatosomatic index of *Labeo rosae* from Loskop and Flag Boshielo dams during summer (November 2011).

Locality	Fish #	Sex	TL (cm)	SL (cm)	Mass (g)	Liver Mass (g)	HSI
Loskop Dam	1	F	55.5	43.8	2030.4	22.1	1.09
	2	F	50.6	42.5	1632.2	15.3	0.94
	3	F	55	42.3	1833.7	10.7	0.59
	4	F	46.7	39.2	1315.2	11.1	0.84
	5	M	44.5	37	1283.7	9.5	0.74
	6	M	48.1	40.5	1518.7	14.9	0.98
	7	M	41.3	34.7	1039.2	6.8	0.65
	8	M	39.5	33.5	780.1	6.5	0.83
	9	F	45	37.8	1333.3	9.8	0.74
	10	F	45.5	37	1127.5	8.5	0.75
	11	F	45.5	37.9	1313.5	13.9	1.06
	12	F	47.5	39.5	1271.2	14.2	1.12
	13	F	47.3	39.9	1526.8	18.2	1.19
	14	F	48.8	41.2	1658.2	13.2	0.8
	15	F	46.1	38.6	1471.8	11.9	0.81
	16	F	47.1	40.3	1460.1	16.1	1.1
	17	M	44	40.2	1070.1	7.8	0.73
	18	F	48.7	40.7	1513.4	15.1	0.99
	19	F	49	37.3	2012.2	17.1	0.85
	20	F	44.5	38.1	1212.9	12.4	1.03
Average ± SD	**N/A**	**N/A**	**47.01 ± 3.75**	**39.1 ± 2.48**	**1420.21 ± 308.73**	**12.75 ± 3.95**	**0.91 ± 0.17**
Locality	**Fish #**	**Sex**	**TL (cm)**	**SL (cm)**	**Mass (g)**	**Liver Mass (g)**	**HSI**
Flag Boshielo Dam	1	M	32.5	25.9	346.2	4.9	1.415367
	2	M	30.2	23.4	297.3	4.3	1.44635
	3	F	35.4	28.4	475.5	5.8	1.219769
	4	M	30.5	24.6	295.2	3.1	1.050136
	5	F	33.9	27.5	434.9	4.3	0.988733
	6	F	29.5	23.4	240.4	2.1	0.873544
	7	F	29.4	23.2	233.7	3.1	1.326487
	8	F	31.9	26	341	2.7	0.791789
	9	F	30.5	23.4	290.1	3.2	1.103068
	10	M	29.1	22.6	226.4	2.3	1.015901
	11	F	28.3	22.7	225.6	1.8	0.797872
	12	M	28.3	22.9	219.1	2.8	1.277955
	13	M	22	17.1	93.1	1.4	1.503759
	14	M	21.3	17	81.9	1.3	1.587302
	15	M	20	15.3	62.8	1.1	1.751592
Average ± SD	**N/A**	**N/A**	**28.9 ± 4.3**	**22.9 ± 3.6**	**257.5 ± 114.9**	**2.9 ± 1.3**	**1.2 ± 0.3**