# THE IMPACT OF WATER AND SEDIMENT QUALITY ON THE HEALTH OF *SCHILBE INTERMEDIUS* RÜPPEL, 1832 AND *LABEO ROSAE* STEINDACHNER, 1894 AT FLAG BOSHIELO DAM, OLIFANTS RIVER SYSTEM, LIMPOPO PROVINCE

BY

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# DECLARATION

I declare that the **dissertation** hereby submitted to the University of Limpopo, for the degree of **Master 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 in execution, and that all material contained herein has been duly acknowledged.

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Kekana, M.B. (Ms) Date

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# EXTENDED ABSTRACT

Major anthropogenic activities such as, mining, coal-fired power stations and intensive agricultural practices in the upper catchment area of the Olifants River, have a dramatic impact on the water quality downstream. As a result the river is presently the third most polluted river in South Africa. The aim of this study was to assess the impact of water and sediment quality on the health of *Labeo rosae* and *Schilbe intermedius*. The aim was accomplished by applying the Fish Health Assessment Index (HAI) which includes the Parasite Index (PI) at Flag Boshielo Dam in the Middle Olifants River, Limpopo Province. This was achieved through; assessing the water quality of the dam by determining the levels of physical and chemical constituents in the water at three sampling sites, determining the bioaccumulation of selected metals in the muscle tissue of the two fish species, assessing the fish health (including the fish condition factor) and the fish parasites in the dam by using the fish HAI and PI, and ascertaining the Human Health risk factor upon consumption of fish contaminated with metals from the dam.

The water and sediment quality were seasonally sampled at three sites in the dam: inflow, middle and wall. Dorsal muscle tissues from both fish species were collected for metal bioaccumulation analyses. The water, sediment and fish muscle tissue samples were analysed by an accredited laboratory by means of ICP-OES spectrometry. For the fish health and parasites, ten fish per species were collected seasonally (July 2009 to April 2010) by means of gill nets and examined at a field laboratory using the HAI and PI protocol.

Generally the water quality of Flag Boshielo Dam was acceptable for aquatic ecosystems according to the SAWQG with the exception of phosphorus and some metals at the inflow area. The pH ranged between slightly acidic to alkaline values; water temperature: 15°C to 26°C; water hardness medium soft, salinity within the freshwater range; turbidity in the clear water range. The TDS and major ions (salts) were acceptable for the duration of the study. The highest concentrations of nutrients (specifically phosphorus) as well as metals (aluminium, cadmium, copper, iron and lead) were recorded at the inflow area of the dam. The nutrients were very low except the eutrophic range phosphorus concentrations recorded at the inflow

whereby, the Elands River may be an additional source of nutrients into Flag Boshielo Dam. The metals that were recorded above TWQR are; aluminium, cadmium, copper, iron and lead, of which were mostly recorded at the inflow. However, statistically there was no significant difference among the three sampling sites. The metal concentrations at the inflow were recorded only slightly higher than the middle and the dam wall. The main source of the metals may be the water coming from catchment area of the dam given the intensive agricultural activities taking place between Loskop Dam and Flag Boshielo Dam.

Sediment and bioaccumulation: All the metals were recorded at higher concentrations in the sediment than in the water and fish muscle tissue, except antimony, selenium and strontium. The most abundant metals recorded in the sediment were iron and aluminium. However, the concentrations above the TEL were cadmium, nickel and zinc. The elevated metal concentrations in the sediment are indicating that the metal load in the sediment of Flag Boshielo Dam could be a potential risk for the aquatic biota if they become bioavailable. Antimony, selenium and strontium metal concentrations were recorded at higher concentrations in the muscle tissue of both fish species than in the sediment and water. Iron was the most accumulated metal in the muscle tissue of both fish species. In terms of numbers, more metals were recorded in the muscle tissue of *S. intermedius* than in *L. rosae* however the metal concentrations were higher in the latter. This can be attributed to their different trophic levels in the food chain; *L. rosae* is a primary consumer while *S. intermedius* is a tertiary consumer. However, the metals that accumulated in the fish muscle tissue were indicative of bio-availability of the toxic metals in the dam and not water/sediment pollution.
According to a Human Health risk assessment (Chapter 3), metals that may have risks upon consumption of *L. rosae* are; antimony, arsenic, chromium, iron and vanadium; for *S. intermedius* are; antimony, chromium, iron, vanadium and arsenic (in descending order). These metals may pose toxic and carcinogenic risks to humans. Therefore, the rednose labeo (*L. rosae*) and to a lesser extend the silver catfish (*S. intermedius*) fish species from Flag Boshielo Dam may not be suitable for humans if consumed above 350 g per week.

Fish health and parasites: The Health Assessment Index (HAI) values of the two fish species differed significantly with higher index values recorded for *S. intermedius* than *L. rosae*. Besides the Parasite Index (PI), abnormal haematocrit readings, liver conditions, skin lesions and clubbed gills are the necropsy anomalies that contributed predominantly to the HAI. Overall, the PI contributed mostly to the total HAI value.

The parasite load and therefore also the mean intensity, mean abundance and prevalence of *S. intermedius* were higher during all seasons than that of *L. rosae*. The dominant ectoparasites for both species were from the Class Monogenea and the dominant endoparasites were nematodes. Out of 40 *L. rosae* sampled, 139 parasites were retrieved; five parasite species were ectoparasites and two endoparasites. From the 40 *S. intermedius* sampled, 2473 parasites were retrieved, from which two species (one genus) were ectoparasites and three species were endoparasites.

The condition factor is used to compare the “condition”, “fatness” or wellbeing of fish and it is based on the hypothesis that heavier fish of a particular length are in a better physiological condition. The *L. rosae* had a better condition factor, recorded at values less than (2) as compared to *S. intermedius* (>2).

The cause of the HAI necropsy anomalies may have been also from parasite load other than the metals in the water and sediment. However, the HAI alone cannot be used for metal pollution, unless it is done in conjunction with a histopathological study of the tissues/organs. Therefore, the cause of the recorded anomalies from both fish species is inconclusive. On the hand, fish can be used as bioindicators because the accumulated metals in the fish tissues are indicative of the bioavailability of metals in Flag Boshielo Dam.

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# GENERAL INTRODUCTION AND PURPOSE OF THE STUDY

## 1.1 INTRODUCTION

Water is one of the most important natural resources on earth, and creates a wide variety of benefits to humans, including fisheries, wildlife, agriculture, urban, industrial and social development (Kamika & Momba 2011). Due to urbanisation and modernisation, anthropogenic activities such as settlements, mining, industrialisation and deforestation have led to pollution of this important resource. These activities have resulted in changes of the physical, chemical and biological components of aquatic systems, which may in turn threaten the sustainability of water quality (Adams *et al.* 1993). The adverse effect of anthropogenic activities is therefore often harmful to the watercourses, and usually results in a degradation of the natural aquatic ecosystem (Karr *et al.* 1986; Schleiger 2000).

Water has been identified as South Africa's most limited natural resource (Ashton & Turton 2008). Thus monitoring water usage should be a priority in South Africa. The South African National Water Act (Act 36 of 1998) recognises that water should be used in an environmentally sustainable manner, and has the long-term protection and sustainable management of water resources as one of its main objectives (Palmer *et al*. 2005). A major aim was to ensure a better balance between efficiency, sustainability and equity needs in water allocations (Lévite *et al.* 2002). Water quality is a term used to describe the chemical, physical, and biological characteristics of water, usually in respect to its suitability for an intended purpose. Although scientific measurements are used to define the quality of water, it's not simple to just classify water as good or bad without knowledge of its intended use. The quality of water that is required for irrigation is not the same quality that is required for drinking. Rivers in South Africa are deteriorating as a result of industrial, mining and agricultural activities and the Olifants River is no exception (De Villiers & Mkwelo 2009). Therefore, when talking water quality, it should be known if the water is within the criteria for its intended use, be it for domestic, farming, mining or industrial purposes, or its suitability to maintain a healthy ecosystem. The South African Department of Water Affairs and Forestry (DWAF 1996a) produced a seven-volume Water Quality Guideline for domestic, industrial, livestock watering and agricultural (aquaculture and irrigation) water use, as well as for aquatic ecosystems. The DWAF guidelines are sets of water quality criteria for safeguarding freshwater ecosystems in South Africa (DWAF 1996b). The water quality criteria include the Target Water Quality Range (TWQR), the Chronic Effect Values (CEV) and the Acute Effect Values (AEV), which can be used to evaluate specific water quality constituents.

Metal pollution in South African rivers and especially in the Upper Olifants River catchment area, is mainly attributed to afforestation, mining and power generation, irrigation as well as domestic and industrial activities (Coetzee *et al.* 2002). Over the last few decades, contamination of freshwater ecosystems by metals and pesticides has become a matter of concern. In the aquatic ecosystem, fishes are the inhabitants that are mostly affected by the detrimental effects of these pollutants (Clarkson 1998; Dickman & Leung 1998; Olaifa *et al.* 2004), since fishes are mostly at the top of the aquatic food chain. They may ultimately absorb large amounts of metals and pesticides which tend to accumulate in their organs when they are exposed to high levels in a contaminated environment (Seymore *et al.* 1996; Kotze *et al.* 1999; Nussey *et al.* 2000; Barker 2006; Crafford & Avenant-Oldewage 2010). Fishes are widely used to evaluate the health of aquatic ecosystems (Nussey *et al.* 2000; Farkas *et al*. 2002; Barker 2006; Crafford & Avenant-Oldewage 2010) as they respond to any changes in the aquatic environment, and are therefore a valuable biomonitoring tool.

In the freshwater environment, toxic metals are potentially accumulated in sediment and aquatic biota and subsequently transferred to humans through the food chain. Metals are persistent and tend to accumulate in the sediment (Coetzee *et al.* 2002). As a result the sediment is considered to be a sink for pollutants and thus pose the highest risk to the aquatic environment (Salomons *et al*. 1987; Wepener & Vermeulen 2005). Metals bound in sediment have no direct danger to the system as long as they remain there. Dangers arise when there are changes in pH, water hardness, salinity, temperature or redox potential (Barker 2006). This allows bounded metals to be released back into the water (Van Vuren *et al*. 1999).

Depending on the metals bioavailability they will then accumulate in the fish muscle tissues and subsequently to humans upon consumption. Additionally, most metals can be carcinogenic, mutagenic and teratogenic.

Furthermore, fish parasites are indicative of many biological aspects of their hosts (Williams *et al.* 1992) and they may also be good direct indicators of environmental quality status (Khan & Thulin 1991; Sures *et al*. 1994; MacKenzie *et al.* 1995; Marcogliese & Cone 1997). For example, Galli *et al.* (2001), studying lakes with different trophic levels, found differences between ecto- and endoparasite populations whereby, ectoparasites such as monogeneans increased proportionally with the eutrophication level while endoparasites were restricted to unpolluted lakes. Furthermore, if has been found that ectoparasites are just as exposed to the environment as their hosts whereby it was then assumed that poor water quality will adversely affect ectoparasites to a greater degree than it would endoparasites (Avenant-Oldewage 1994). However, in spite of the great number of papers showing evidence of the possible use of parasites as indicators of freshwater quality, there are many factors that may have an impact on parasite populations; such as host resistance, fish species, parasite species, type of pollution, etc.

In South Africa there are several aquatic biomonitoring indices (e.g. South African Scoring System 5, Riparian Vegetation Index, Index of Habitat Integrity and Health Assessment Index) in use, each one with its independent advantages and disadvantages, depending on the monitoring method and organisms used. In the present study, the fish Health Assessment Index (HAI) protocol (Goede 1992; Adams *et al.* 1993) was followed (see chapter 4). The HAI protocol was followed because it is not specially designed to assess river health like the fore mentioned indices. The HAI is utilized to assess the health of fish and consequently the health of an aquatic ecosysytem (Avenant-Oldewage 2001; Jooste *et al.* 2004). When applying the index, a numerical value is awarded to examine fish tissue or organs depending on the degree of stressors-induced abnormalities. The total sum of values awarded is the index value for that fish and the mean for all sampled fish is the index value for that locality. An increase in the index value correlates with decreased water quality (Crafford & Avenant-Oldewage 2009). It is a method that has been intensively used in South African aquatic ecosystems (Avenant-Oldewage *et al*. 1995; Marx 1996; Robinson 1996; Luus-Powell 1997; Bertasso 2004; Jooste *et al*. 2004;

Crafford & Avenant-Oldewage 2009). In the original HAI (Adams *et al.* 1993), parasites were recorded as being present or absent (Crafford & Avenant-Oldewage 2009) and did not include the Parasite Index (PI). The PI is now incorporated in the HAI whereby the HAI uses fish as an indicator species (Avenant-Oldewage 2001; Jooste *et al*. 2004) and PI uses the presence of parasites found on/in fish as indicator species (Crafford & Avenant-Oldewage 2009). Crafford & Avenant-Oldewage (2009) motivated that the PI is a more comprehensive index that can be used to great effect in environmental management.

## 1.2 OVERVIEW OF THE STUDY AREA

The Olifants River has several impoundments in the Limpopo Province, two of which is Flag Boshielo Dam and the Phalaborwa Barrage. Sixty kilometers upstream from the Flag Boshielo Dam is the Loskop Dam, situated in the Mpumalanga Province. The Loskop Dam catchment area is dominated by coal mines which are concentrated mainly in the Olifants and Klein Olifants River catchments upstream of the Witbank and Middelburg dams respectively. These regions have intensive mining (coal, platinum, phosphate and copper) and industrial activities which have considerable impacts on the Olifants River (Oberholster *et al.* 2010). The mine and industrial effluents contain a complex of chemicals, many of which may have deleterious effects on aquatic systems (Van Vuren *et al.* 1999).

Major crocodile kills in the Olifants River in the Kruger National Park (KNP) and Loskop Dam have been reported since 2007 (Ashton 2010; Heath *et al.* 2010; Botha *et al*. 2011), the deaths were linked to eating rancid fish in the river. Post-mortem results showed that the crocodiles died of pansteatitis; a disease which results in the general hardening of the body fat, mostly as a result of inadequate antioxidant levels (e.g. vitamin E). The hardened fat causes the crocodiles to become stiff which results in reduction in mobility and the inability to swim (Heath *et al.* 2010). These kills were most probably caused by water pollution in the upper catchment area of the Olifants River from the mines and industries in the Mpumalanga Province (Ashton 2010; Heath *et al.* 2010; Botha *et al*. 2011). Claassen *et al.* (2005) also reported that coal mining and industries in the Witbank-Middelburg complex (eMalahleni) and Phalaborwa areas have a significant impact on the Olifants River. A further cause for concern is the decline in numbers of piscivorous bird, especially herons, which is also most likely to be linked to the deterioration of the "health" of this river (Myburgh & Botha 2009). The herons post mortems revealed excessive abdominal deposits of yellow fat (i.e. pansteatitis). These deaths have been observed in Loskop Dam, KNP and Massingir Dam (Myburgh & Botha 2009).

### 1.2.1 DESCRIPTION OF THE OLIFANTS RIVER SYSTEM

The Olifants River originates from the east of Gauteng Province (Breyton) and initially flows northwards before curving eastwards towards the KNP where it is joined by the Letaba River before flowing into Mozambique (Heath *et al.* 2010) (Figure 1.1). The Olifants River has several impoundments in the Limpopo Province; Flag Boshielo Dam (middle Olifants) and the Phalaborwa Barrage (lower Olifants). The basin of the Olifants River is divided into five hydrological areas generally regrouped into four sub-catchments, namely; Upper, Middle (upper middle and lower middle), Steelpoort and Lower Olifants sub-catchments (De Lange *et al.* 2003; Heath *et al.* 2010).

#### a) Upper Olifants sub-catchment

The upper Olifants sub-catchment originates in Gauteng near the town of Breyton and flows through the Highveld grasslands of Gauteng and Mpumalanga. Several tributaries namely the Klein Olifants, Klip, Wilge, Bronkhorstspruit and Brugspruit rivers join the main Olifants River before it flows into Loskop Dam. Loskop Dam, the largest major impoundment in Mpumalanga, is located 60 km upstream from Flag Boshielo Dam. This part of the river has a higher rainfall than the Middleveld (average 682 mm and 621 mm respectively), and is characterised by extensive cropping and livestock farming, coal mining and coal-fired power plants. Although the impact of these activities is significant, the strategic importance is clear: 55% of South Africa's electricity generated from coal is produced here. Annually, around 200 million cubic meters of water is imported from the neighbouring Vaal River basin for water-cooling the power plants (De Lange *et al.* 2003). This part of the sub-catchment includes the highly impacted Loskop Dam where declining crocodile populations and sporadic fish kills have been reported (Ashton 2010; Botha *et al.* 2011).

### b) Middle Olifants sub-catchment

The Middle Olifants sub-catchment is divided into the upper middle and lower middle Olifants. It comprises the portion of the Olifants basin between Loskop Dam and the junction of the Steelpoort and Olifants rivers.The Middle Olifants stretches for about 300 km along the Olifants River from below Loskop Dam to the dramatic drop down the Drakensberg escarpment. Below Loskop Dam (before Flag Boshielo Dam) are 28,800 hectares of intensively irrigated areas growing a variety of high value crops for export market (De Lange *et al.* 2003; Heath *et al.* 2010). The two main tributaries of the Olifants River are: the Elands River which pours into the Olifants River at the inflow area of Flag Boshielo, and the Moses River which joins the Olifants River between the towns of Groblersdal and Marble Hall (Jooste *et al.* 2012). After the Flag Boshielo Dam, the Olifants River flows through the semi-arid Sekhukuneland to the confluence with the Steelpoort River northeast of Burgersfort town. Agriculture, both dryland and irrigated, is the most important land use in the Middle Olifants sub-catchment (Heath *et al.* 2010). The highly erodible soils of Sekhukuneland contribute to the high silt load of the Olifants River in this region (Jooste *et al.* 2012).

### c) Steelpoort sub-catchment

It is a mountainous area and is composed of Steelpoort River and its tributaries (Klip, Dwars, Waterval and Spekboom rivers) in the Steelpoort sub-basin. The Steelpoort sub-basin is composed of small scale irrigation, extensive mining activities in the valley, and medium- and large-scale cattle farming. The mining and industrial activities include chrome, coal, granite, magnesite, alluvial gold, platinum and vanadium mines concentrated in the upper sub-catchment (Stimie *et al.* 2001). Expansion of platinum mining near the Steelpoort-Olifants confluence has resulted in construction of another major impoundment De Hoop Dam in Steelpoort River. Construction of the dam started in 2007 and is aimed to be completed in 2012/13 (Waterwheel 2011).

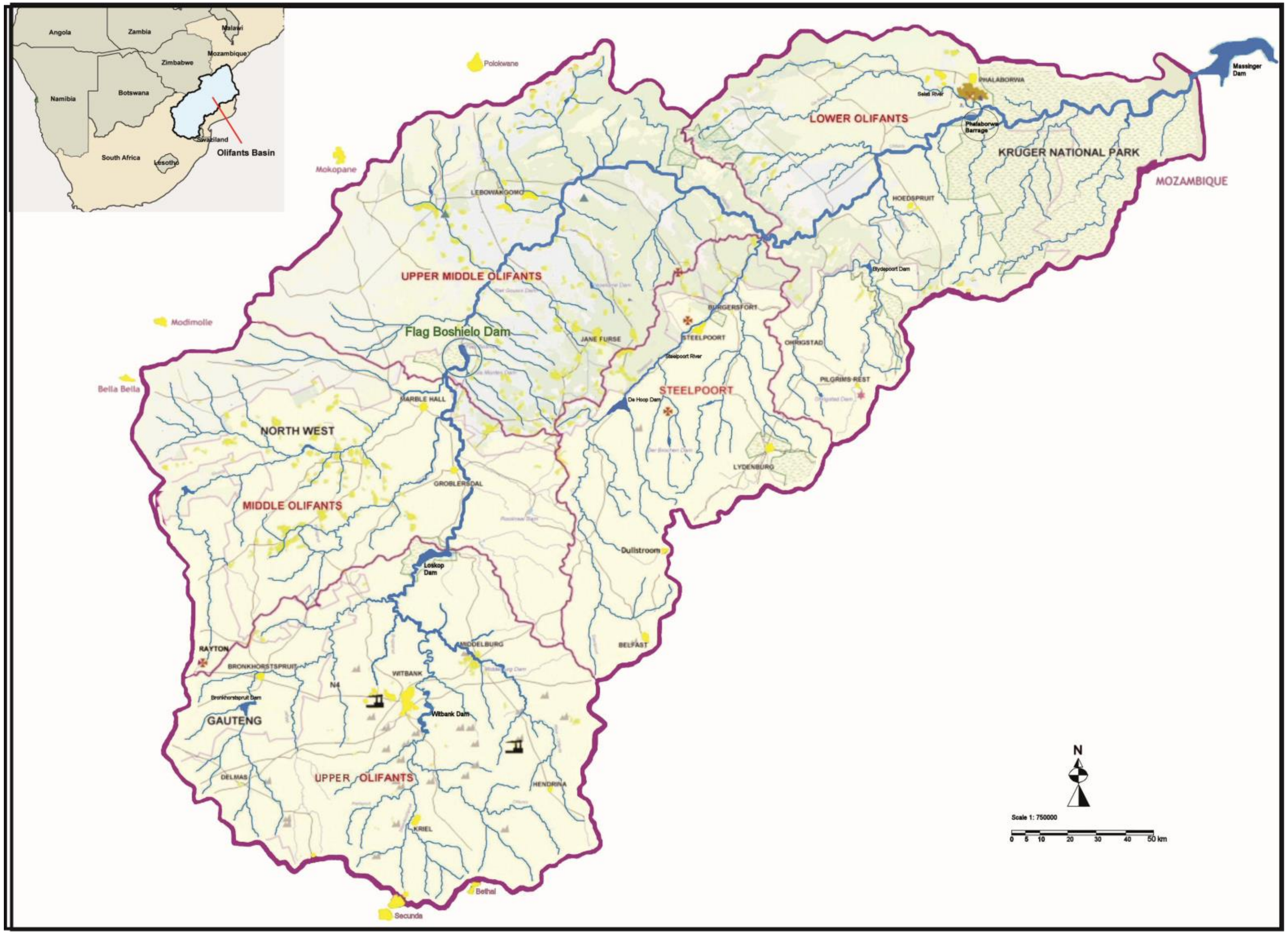


Figure 1.1: The Olifants River basin (after the Olifants River Forum – ORF)

#### d) Lower Olifants sub-catchment

The lower Olifants sub-catchment stretches from the Drakensberg escarpment through the KNP to Massingir Dam in Mozambique. Except for the upper part of this area (Blyde River sub-basin), there is little irrigation along the Olifants River below the escarpment**,** possibly because of poor soils (De Lange *et al.* 2003). This region is characterised by game farms and industrial activity concentrated at the town of Phalaborwa, on the border of the KNP conservation area (Coetzee *et al.* 2002). The improvement of water quality is due to better water from the Blyde River, but the Ga-Selati River, which joins the Olifants River before it enters the KNP, is negatively impacted by agricultural and mining activities. However, the Blyde and Mohlapitse rivers maintain the water quality in the lower Olifants River (DWAF 2004). The impact of the industrial effluents on the quality of the water entering the KNP is of a major concern to conservationists, since there have been reports on fish kills downstream of the Phalaborwa Barrage (Ashton 2010; Botha *et al.* 2011).

### 1.2.2 DESCRIPTION OF FLAG BOSHIELO DAM

Flag Boshielo Dam is located in the Middle Olifants sub-catchment and is a mainstream reservoir in the Olifants River. Flag Boshielo Dam provides water supplies to numerous small towns and settlements in the area, as well as large volumes of water for irrigation schemes below the dam (ARC-ILI 1999; De Villiers & Mkwelo 2009). This region is perhaps the most economically important agricultural area in the sub-catchment. Several towns (e.g. Marble Hall, Roedtan and Lebowakgomo) and numerous smaller settlements and farming communities are present in the upper and middle reaches of the sub-catchment. Population numbers and density are greatest in the upper and middle reaches of the Middle Olifants sub-catchment and decline somewhat towards the downstream reaches. Some light industry is present in the towns of Marble Hall and Roedtan and these industries are geared specifically to meet the needs of the extensive agricultural activities in the Middle Olifants sub-catchment (De Lange *et al.* 2003).

Agricultural pesticides are regularly applied (aerial application) to crops in the Groblersdal and Marble Hall areas and no regulation is taking place with regard to pesticide application in the area (Bollmohr *et al.* 2008). Hence toxic runoffs from the agricultural lands end up in the river. The following activities can be expected to have an impact on water resources in the Middle Olifants sub-catchment: landfills and solid waste disposal sites at all towns and larger settlements; disposal of liquid (domestic, light and heavy industrial) effluent at all towns; moderate volumes of runoff from towns, as well as all other urbanized areas; non-point domestic effluent from numerous small settlements and farms; minor non-point impact from non-intensive commercial or subsistence agriculture; non-point impact of agricultural return flows from intensive irrigation areas; and litter and domestic garbage discarded alongside the many roads that traverses the sub-catchment (De Lange *et al.* 2003).

Figure 1.2: The dam wall of Flag Boshielo Dam

The Flag Boshielo Dam is situated approximately 35 km north of Marble Hall in the Limpopo Province. The dam was constructed at the confluence of the Elands and the Olifants River and was completed in 1986, then named Arabie Dam. It supplies water to the rural villages in the Marble Hall and Fetakgomo municipal area. The sub-catchment covers an area of 4 213 $km^2$, or about 8% of the total Olifants River catchment of approximately 54 000 $km^2$ (ARC-ILI 1999). In response to increasing water demand and managing the risk of water shortages, the dam wall was raised in 2007/8 by five meters. Subsequently the storage capacity increased from 100 million $m^3$ to 188 million $m^3$ and the yield of 56 million $m^3$/a to 72 million $m^3$/a. Presently the Full Storage Capacity of the dam is 181.1 million $m^3$ and raw water consumption of 2920 mega liters per annum (Ml/a). The consumption of the water is 2515 Ml/a according to Lepelle Northern water board (LNW 2010).

## 1.3 FISH SPECIES

The selected fish species for this study were *Schilbe intermedius* Rüppell, 1832 (silver catfish or butter barbel) and *Labeo rosae* Steindachner, 1894 (rednose labeo). These two species were chosen because they occupy a variety of habitats, have different feeding habits and feed on diversity of food particles.

### 1.3.1 *Schilbe intermedius*

The common name for *S. intermedius* is silver catfish, falling under the Order Siluriformes, and Family Schilbeidae. The body of *S. intermedius* is elongated, compressed and tapered towards the caudal region (Skelton 2001). It has a smooth skin, dorsal and pectoral fins with slender and sharp spines. The base of the anal fin is extended but separated from the caudal fin. They usually occur in standing or slow moving waters with emergent or submerged vegetation. Generally they are more active at night or in subdued light. *Schilbe intermedius* is an important subsistence fisheries target species and serve as an angling species, but is often regarded as a nuisance. It is an opportunistic predator with a diet consisting largely of fish (41%), aquatic insect larvae (25%), terrestrial insects (14%), aquatic insects (7%) and crustaceans (5%) (Winemiller & Kelso-Winemiller 1996), making them secondary and tertiary consumers. The juveniles predominantly feed on insect larvae, while adults are largely piscivorous (Winemiller & Kelso-Winemiller 1996). The silver catfish breed during the rainy season and may be either a single or a multiple spawner in different localities, laying eggs on vegetation. They reach maturity at about 160 mm standard length (SL), and usually live for six to seven years (Skelton 2001).

Figure 1.3: The silver catfish – *Schilbe intermedius* Rüppell, 1832

### 1.3.2 *Labeo rosae*

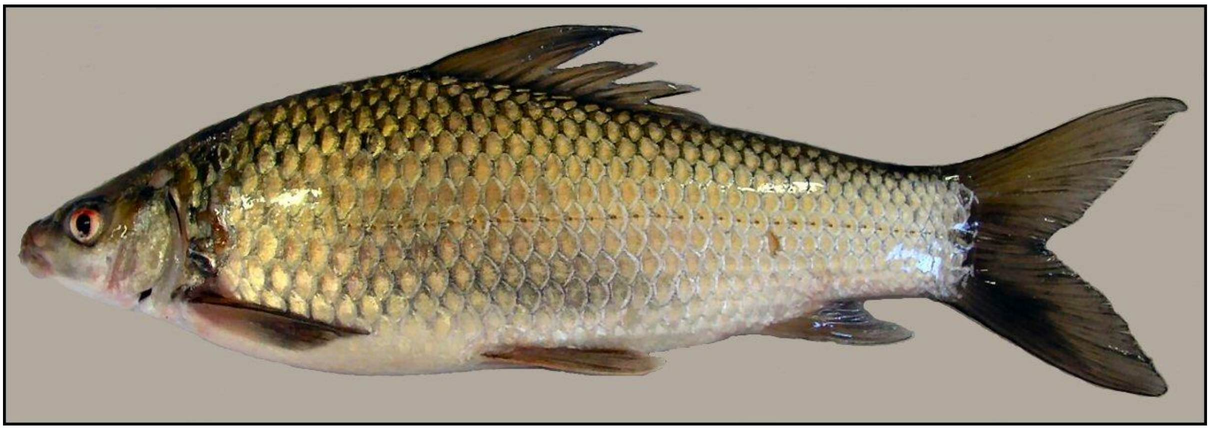

Figure 1.4: The rednose labeo – *Labeo rosae* Steindachner, 1894

*Labeo rosae* falls under Order Cypriniformes and Family Cyprinidae. Its common name is rednose labeo. The body of this fish species is moderately deep and compressed towards the dorsal fin with a concave posterior edge. The head is small, the mouth with papillose lips and a single pair of short barbels. *Labeo rosae* prefers sandy stretches of larger perennial and intermittent rivers. It is an active fish leaping at barriers when migrating upstream in swollen rivers in summer to breed. They are also a good angling species. They normally feed on detritus, algae and small invertebrates (Skelton 2001), making them primary and secondary consumers. *Labeo* species generally spawn on newly flooded ground, usually leaving the main river channel. Spawning may or may not be preceded by a longitudinal migration. They usually attain sexual maturity at about 150 mm total length (TL) (Skelton 2001).

## 1.4 PURPOSE OF THE STUDY

People living around the Olifants River make use of the river and impoundments in the system for daily living purposes. Water quality plays an important role in the wellbeing of all users. The Upper Olifants River catchment area is dominated by mines (coal, platinum, phosphate and copper), coal-fired power plants and industrial and agricultural activities, which have a considerable impact on the Olifants River system (De Villiers & Mkwelo 2009). Loskop Dam is the last impoundment before the river enters the Middle catchment area and is extensively impacted by the above mentioned anthropogenic activities (Kotze *et al*. 1999). Flag Boshielo Dam is downstream Loskop Dam, and apart from the paper of Bollmohr *et al.* (2008) very little has been published on pollutants in the dam and/or the Middle Olifants River. Pesticides like organophosphates and carbamates are regularly applied (aerial application) to crops in the Groblersdal area and Marble Hall (nearest towns to Flag Boshielo Dam), and no regulation is taking place with regard to pesticide application in the area (Bollmohr *et al.* 2008). Hence toxic runoffs from the agricultural lands end up in the river. This study would assist in the management of water quality and fish health in Flag Boshielo Dam. It would also provide knowledge of the state of the impoundment regarding water quality and the fish communities. This study was conducted to examine pollution in the dam using fish as bio-indicators, and wether upper catchment pollution has any impact on the water quality downstream.

### 1.4.1 PROBLEM STATEMENT

The Olifants River is one of the most polluted rivers in South Africa caused by mining, industrial and agricultural activities in the upper catchment areas (Highveld of the Mpumalanga and Gauteng provinces) (Ashton 2010). The Olifants River in the Limpopo province is subsequently receiving pollutants from the upper catchment, which can adversely impact the functions of the aquatic ecosystem in the province. Therefore the project was identified to assess the impact of water quality on fish health in the Olifants River of the Limpopo province.

### 1.4.2 HYPOTHESES

It is hypothesised that;

- Water and sediment quality of the dam can have adverse effects on fish health.
- Metals in the water and sediments can accumulate in fish tissues.
- Fish parasites and fish health can be used as bio-indicators of pollution.

### 1.4.3 AIM

The aim of the study was to assess the impact of water and sediment quality and bioaccumulation of metals on the health of *Labeo rosae* and *Schilbe intermedius* at Flag Boshielo Dam (Olifants River).

### 1.4.4 OBJECTIVES

The objectives of the study were to:

a) Assess the water quality composition of the dam by determining the level of physical and chemical substances in the water at various sites (dam wall, middle of the dam and inflow).

b) Determine accumulation of selected metals in the muscle tissue of the two selected fish species.

c) Assess the fish health and the fish parasites in the dam by using the fish Health Assessment Index (HAI) and Parasite Index (PI).

d) Ascertain the potential Human Health risk upon consumption of metal contaminated fish from the dam.

## 1.5 DISSERTATION LAYOUT

**Chapter 1**: General introduction and purpose of the study – introduces the title and fish species studied, includes a literature review on the Olifants River and Flag Boshielo Dam. It outlines the purpose of the study, problem statement, hypotheses, and lastly aims and objectives.

**Chapter 2**: Water quality – discusses water quality constituents in Flag Boshielo Dam.

**Chapter 3**: Bioaccumulation – discusses the metal concentrations in the fish muscle tissue as well as in the sediment.

**Chapter 4**: Health Assessment Index (HAI) and Parasite Index (PI) – discusses the fish health and parasite numbers in/on fish hosts, using the HAI and PI. The parasite infestation statistics; prevalence, mean intensity and mean abundance are included in this chapter.

**Chapter 5**: Includes the final conclusions of the study.

Material and methods of each aspect are included in every relevant chapter.

The journal of African Zoology referencing method was followed.

# CHAPTER 2

# WATER QUALITY

## 2.1 INTRODUCTION

Water quality is a term used to describe the chemical, physical, and biological characteristics of water, usually in respect to its suitability for an intended purpose. These characteristics are controlled or influenced by substances, which are either dissolved or suspended in water (DWAF 1996e). Although scientific measurements are used to define the quality of water, it's not simple to just classify water as good or bad without knowledge of its intended use. The quality of water that is required for irrigation is not the same quality that is required for drinking. Therefore, when talking water quality, it should be known if the water is within the criteria for its intended use, be it for domestic, farming, mining or industrial purposes, or its suitability to maintain a healthy ecosystem. In South Africa the Department of Water Affairs and Forestry (DWAF) now Department of Water Affairs (DWA) developed a series of South African Water Quality Guidelines (SAWQG). The guidelines are the primary source of information and decision-support to judge the fitness of water for use and for other water quality management purposes. These guidelines contain similar information to what is available in the international literature (DWAF 1996e), but with more detailed background information to help users of the guidelines make informed judgment about the fitness of water for its intended use.

The SAWQGs are scientifically based and have technical information provided for a particular water quality constituent in the form of numerical data and/or narrative descriptions of its effects on the fitness of the water for a particular use or on the health of aquatic ecosystems (Hols *et al.* 2002). The water quality criteria include the Target Water Quality Range (TWQR), the Chronic Effect Values (CEV) and the Acute Effect Values (AEV), which can be used to evaluate specific water quality constituents. The TWQR is the range of concentrations or concentrations within which no measurable adverse effects are expected on the health of aquatic ecosystems and should therefore ensure their protection (DWAF 1996e). The CEV is defined as that concentration or concentration of a constituent at which there is expected to be a significant probability of measurable chronic effects to up to 5% of the species in the aquatic community. The AEV is defined as that concentration or concentration of a constituent above which there is expected to be a significant probability of acute toxic effects to up to 5% of the species in the aquatic community.

South Africa is a semi-arid country, making water one of the most limited and precious resources considering that the average yearly rainfall in South Africa is about 500 mm, compared to the world average of 860 mm (Cowan 1995). Freshwater has been identified as South Africa's most limited natural resource (Ashton & Turton 2008). Due to the growing population, its upliftment and urbanisation, the total water demand for agriculture, domestic use, industrialisation and mining has increased rapidly. Estimates of the current patterns of use and anticipated future uses of South Africa's water resources indicate that the demands for water will increase (Ashton & Haasbroek 2001). Thus monitoring and the rehabilitation of water bodies should be a priority in South Africa. Research has become even more important by the realisation that water demand for domestic, industrial and agricultural use, coupled with pollution, is increasing at such a rate that in a few years' time there would be no sufficient water supply to meet the demand. With such a high demand for freshwater resources, aquatic biota usually suffers; sometimes even disappear, because they are exposed to all the effects of anthropogenic activities on the aquatic environment (Davies & Day 1998). In this study, water quality was examined in conjunction with fish health in order to determine the impact of water quality on fish health. This was done with the notion that, the better the fish health the better the water quality or vice versa (Chapter 4 discusses the fish health in detail).

## 2.2 MATERIAL AND METHODS

### 2.2.1 Field work

The water samples were collected seasonally (July 2009 – April 2010) from three sampling sites in the dam: dam wall, middle of the dam and inflow (Figure 2.1), with acid pre-treated sampling bottles and immediately refrigerated for laboratory analysis. A 1000 mℓ surface water sample was collected from each sampling site. Water variables, such as dissolved oxygen (DO), pH, water temperature, salinity, and electrical conductivity (EC) were determined *in situ* by means of a handheld multi parameter instrument (YSI model 54 Combometer) at the selected sampling sites.

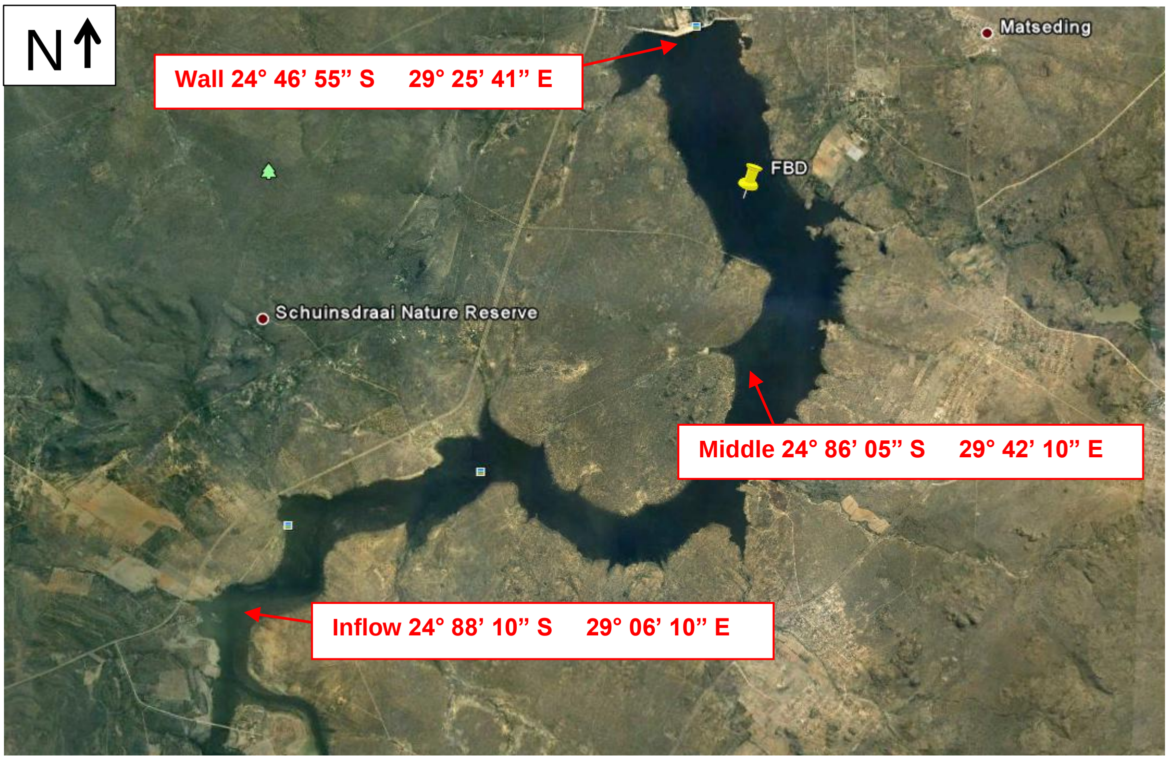


Figure 2.1: Satellite image of Flag Boshielo Dam and the three sampling sites (Google Earth)

### 2.2.2 Laboratory work

Water quality constituents namely; alkalinity, total water hardness, turbidity, nitrate-, nitrite-, and ammonia-nitrogen, phosphate, chloride, fluoride, and sulphate were analysed by an accredited chemical laboratory (ISO 17025) in Pretoria (WATERLAB (PTY) LTD). A broad spectrum of the other elements like calcium, magnesium, potassium and sodium as well as the metalloids and metals were also determined by the laboratory by means of an ICP-OES scan.

### 2.2.3 Results analysis

Water quality results from the chemical laboratory were interpreted using the South African Water Quality Guidelines (SAWQG) (DWAF 1996a to e). These results were compared with the TWQR, AEV and CEV for different water uses, where applicable and available. The US Environmental Protection Agency (USEPA) guidelines were used for metals that did not have South African water quality criteria for aquatic ecosystems. One-way analysis of variance (ANOVA) and other statistical methods in SPSS/STATIX were used. ANOVA was used to compare the seasonal water quality of the three different sites.

## 2.3 RESULTS AND DISCUSSIONS

There are many possible water quality variables that can be measured, however, the initial suite to be considered in South African waters includes: inorganic salts (sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium sulphate), nutrients (phosphate as $PO_4^{3-}$, and total inorganic nitrogen); physical variables (turbidity, pH, oxygen and temperature); and toxic substances listed in the SAWQG for aquatic ecosystems (DWAF 1996e; Palmer *et al.* 2005). However in this study the salts were tested in an elemental form (e.g. sodium, chloride, sulphate, etc.) not as compounds as stated (e.g. sodium chloride, magnesium chloride, sodium sulphate, etc.). A broad spectrum of metalloids and metals were tested and discussed amidst their absence in the list of SAWQG for aquatic ecosystems.

### 2.3.1 PHYSICO-CHEMICAL PARAMETERS

The physico-chemical parameters include temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), salinity, total dissolved solids (TDS), total alkalinity and turbidity. Aquatic ecosystems go through changes along with normal climatic fluctuation. These variations occur seasonally and in some systems over a period of 24 hours. The climatic fluctuations influence the physico-chemical parameters and which then influence the essential ecosystem processes (DWAF 1996e).

**Water Temperature** ranged from 15.13 to 29.1°C (Appendix A: Table 1) with lower temperatures recorded in winter and highest temperature in summer as it is expected (Table 2.1). Winter temperatures at all sites of the dam did not exceed 17°C, with the average temperature of 15.7°C. Summer temperatures were all above

25°C at all sites of the dam, with an average temperature of 28.1°C. The two fish species studied are warm water species. *Labeo rosae* appears in subtropical regions and breeds in summer (Skelton 2001). *Schilbe intermedius* appears in tropical regions and breeds in rainy seasons (Skelton 2001). Thus, no adverse health effects from the two fish species were recorded due to seasonal temperature changes.

Table 2.1: The average seasonal chemo-physical parameters in the water column recorded from the three sampling sites in Flag Boshielo Dam (n=3)

| **PARAMETERS** | **Winter 2009** | **Spring 2009** | **Summer 2010** | **Autumn 2010** | **P#** |
|---|---|---|---|---|---|
| Water temperature °C | 15.68 ±0.59 | 25.54 ±0.06 | 28.11 ±0.74 | 25.45 ±0.76 | 0.000 |
| Dissolved oxygen (mg/ℓ) | 6.70 ±0.77 | 9.19 ±0.19 | 6.53 ±1.62 | 7.64 ±0.82 | 0.094 |
| Dissolved oxygen (%) | 72 ±2.69 | 112.50 ±2.65 | 85.53 ±22.20 | 94.27 ±9.33 | 0.056 |
| pH | 7.9–8.2 ±0.12 | 8.2– 8.8 ±0.30 | 7.4–8.3 ±0.38 | 7.1–7.4 ±0.15 | 0.006 |
| EC (mS/m)* | 41.27 ±0.87 | 46.87 ±1.19 | 42.30 ±0.36 | 38.30 ±2.34 | 0.002 |
| Salinity ‰ | 0.18 | 0.22 | 0.20 | 0.18 ±0.01 | 0.000 |
| TDS (mg/ℓ) | 241.58 ±0.31 | 304.63 ±7.73 | 277.12 ±0.81 | 248.95 ±15.23 | 0.000 |
| Total Alkalinity (mg/ℓ) | 58 ±14.51 | 82.67 ±3.77 | 60 ±0 | 64 ±1.63 | 0.040 |
| Turbidity (NTU) | 6.50 ±1.85 | 1.47 ±0.63 | 2.30 ±1.04 | 2.10 ±0.57 | 0.008 |

*EC=Electrical Conductivity, #P=significance

Table 2.2: The average chemo-physical parameters recorded at the three sampling sites from Flag Boshielo Dam during the study period (n=4)

| **PARAMETERS** | **Inflow** | **Middle** | **Wall** | **P#** |
|---|---|---|---|---|
| Water temperature (°C) | 23.46 ±4.71 | 24.39 ±4.75 | 23.24 ±4.83 | 0.95 |
| Dissolved oxygen (mg/ℓ) | 6.73 ±1.71 | 7.35 ±1.16 | 8.47 ±0.65 | 0.28 |
| Dissolved oxygen (%) | 79.85 ±20.43 | 92.25 ±14.64 | 101.13 ±15.27 | 0.35 |
| pH | 7.1 – 8.2 ±0.42 | 7.4 – 8.8 ±0.48 | 7.2 – 8.8±0.61 | 0.45 |
| EC (mS/m)* | 42.13 ±4.79 | 42.48 ±2.44 | 41.95 ±2.29 | 0.98 |
| Salinity (‰) | 0.20 ±0.02 | 0.20 ±0.01 | 0.20 ±0.01 | 0.98 |
| TDS (mg/ℓ) | 265.20 ±33.90 | 270.24 ±22.10 | 268.78 ±20.80 | 1.00 |
| Total Alkalinity (mg/ℓ) | 62.50 ±17.74 | 68.50 ±8.05 | 67.50 ±7.53 | 0.81 |
| Turbidity (NTU) | 3.93 ±2.93 | 2.88 ±2.29 | 2.48 ±0.95 | 0.72 |

*EC=Electrical Conductivity, #P=significance

The highest water temperatures were recorded in the middle of the dam as compared to other sampling sites within the dam (Table 2.2). The lowest water temperatures were recorded at the dam wall. This could be due to the water current at different sites of the dam. At the inflow and dam wall the water flow could be faster, inducing mixing of warmer surface water with cooler water from the bottom. Water in the middle could be warmer due to slower water current or fairly stagnant water allowing surface water to be heated during the day without mixing with cooler water at the bottom. Statistically, there was a significant variance of water temperatures between different seasons ($p<0.001$) (Table 2.1) but there wasn't any significant variance between the three sampling sites ($p>0.05$) (Table 2.2). The latter is evident in Table 2.2, whereby water temperature at the inflow and the dam wall were somewhat similar but differed by approximately 1°C to water temperature recorded in the middle of the dam.

**Dissolved oxygen** ranged from 4.35 to 9.42 mg/ℓ i.e. 55.3% to 116.2% saturation (Appendix A: Table 1). The dissolved oxygen was below the TWQR of aquatic ecosystems (80 – 120%) in winter at all sites, and also during summer at the inflow (Appendix A: Table 1). There was no dissolved oxygen concentration recorded above the TWQR throughout the survey. On average when comparing sites, dissolved oxygen below the TWQR for aquatic ecosystem was recorded at the inflow (Table 2.2), whilst among seasons it was during winter (Table 2.1). There was no significant variance of the dissolved oxygen ($p>0.05$) between the three sampling sites (Table 2.2) and the four seasons (Table 2.1). During winter dissolved oxygen saturation was even below the SAWQG Minimum Allowable Values (MAV) for aquatic ecosystems. The MAV is meant to protect sensitive life stages which may last for only a few days and takes into account the resilience of aquatic organisms to short duration oxygen depletion (DWAF 1996e). Within the MAV there is sub-lethal (>60) and lethal (>40) value. The sub-lethal condition of the MAV has a mean minimum of seven days while the lethal condition has a mean minimum of 1 day; violation of these days is likely to cause acute toxic effects on aquatic biota (DWAF 1996e). Dissolved oxygen recorded during winter was within the sub-lethal condition. The low oxygen recorded during this survey can be attributed to several aspects such as; re-suspension of anoxic sediments, as a result of river floods or dredging activities, turnover or release of anoxic bottom water, presence of oxidisable organic matter, either of natural origin (detritus) or waste discharges (DWAF 1996e; Dallas & Day 2004).

The **pH** ranged from 6.24 to 8.83, that is, slightly acidic to alkaline. These records are in agreement with findings from Heath *et al.* (2010), where it is mentioned that the pH across Witbank Dam to Phalaborwa Barrage in the Olifants River catchment is neutral to slightly alkaline. Most freshwaters, in South Africa, are relatively well buffered and more or less neutral, with pH ranges between six and eight (DWAF 1996e). Lowest pH values were recorded at the inflow (ranging between 7.01 and 8.17). Highest pH values were recorded in the middle of the dam (ranging between 7.44 and 8.78). The pH values recorded during spring at all sites were the highest (>8) as compared to other seasons. The slightly increased pH values recorded in spring could be due to increased biological activity more especially due to the warm temperatures (DWAF 1996e). The pH between different sampling sites shows no significant variance between the three sampling sites ($p>0.05$), however seasonally there was a significant variance ($p=0.006$).

**Total Alkalinity** is a measure of the capacity of water to neutralise strong acid or a summary measure of the ionic character of water. The total alkalinity ranged from 38 to 88 mg/ℓ. Highest total alkalinity was recorded during spring (Table 2.1) ranging between 80 and 88 mg/ℓ (Appendix A: Table 1), with the lowest records during winter (between 38 and 72 mg/ℓ). However these concentrations were within the South African TWQR for aquaculture (20 – 100 mg/ℓ). Total alkalinity was lowest at the inflow as compared to other sites (Table 2.2) which might suggest that the water upstream may be acidic. According to DWAF (1996c) the chemical composition of rocks and soils strongly influences the natural alkalinity of water, which can range from very low values to several hundred mg/ℓ. Flag Boshielo Dam is situated in a region that is said to be containing a very large proportion of the region's mineral wealth. The alkalinity in Flag Boshielo Dam might be influenced by the rock formations in its region. Statistically, total alkalinity did not show significant variance ($p>0.05$) among the three sampling sites (Table 2.2), however, there was significant variance between the seasons ($p<0.05$) (Table 2.1).

**Turbidity** is a measure of the light-scattering ability of water and is indicative of the concentration of suspended matter in water (measured in NTU) (DWAF 1996a). Turbidity records in Flag Boshielo Dam ranged from 0.6 to 8.6 NTU. British Columbia Environmental Protection Division Water Quality Guidelines state that clear flow is equal to eight and turbid is <50 NTU. The highest values were recorded at the inflow (3.93 NTU) and the lowest at the dam wall (2.48 NTU) (Figure 2.6). The SAWQG of turbidity for aquatic ecosystems are not available however, aquaculture guidelines indicate that <25 NTU is an acceptable turbidity for clear water fish species (DWAF 1996d). Turbidity >25 NTU was never recorded from this study. There was no significant variance of turbidity between the three sampling sites ($p>0.05$), but between seasons ($p=0.008$).

The **electrical conductivity (EC)** refers to the number of charged particles and is a measure of the ability of the water to conduct electricity (DWAF 1996a, b). This highly depends on the concentration of ions, temperature and the nature of ions in the water. The EC records ranged from 35 to 48.5 but did not vary much between the sampling sites and this was also evident after statistical analysis ($p>0.05$) (Table 2.2). Highest records were observed in the middle of the dam (42.48 mS/m) and lowest records at the dam wall (41.95 mS/m). Seasonally the lowest records were observed during autumn (38.30 mS/m) and the highest records during spring (46.87 mS/m), and there was a significant variance among the seasons ($p=0.002$) (Table 2.1). The SAWQG of EC for aquatic ecosystems is not available. In the SAWQG, EC is discussed under Total Dissolved Salts (TDS) guidelines since it (EC) is a surrogate measure of TDS. This type of measure is rapid and useful when the organic content of the water is low (DWAF 1996e). However, the effect of EC on aquatic biota is not well known (Dallas and Day 2004).

The **Total Dissolved Solids (TDS)** is a composite measure of the total amount of material dissolved in it, which includes charged organic and inorganic compounds in water (DWAF 1996e). The TDS concentrations ranged from 227.5 to 315.25 mg/ℓ (Appendix A: Table 1). The highest values were recorded in the middle of the dam (270.24 mg/ℓ) but the values did not vary much among sites ($p>0.05$) (Table 2.2). Seasonally, highest TDS concentrations were recorded during spring (304.63 mg/ℓ) with the lowest during winter (241.58 mg/ℓ), with a significant variance among seasons ($p>0.001$). The SAWQG of TDS are site specific because most aquatic biota possess a wide range of physiological mechanisms and adaptations to maintain the necessary balance of water and dissolved ions in cells and tissues. Sudden changes in the concentrations of TDS can affect individual species adaptations, the community structure, and the microbial and ecological processes such as rates of metabolism and nutrient cycling in an aquatic ecosystem (DWAF 1996d). However, the *Labeo rosae* and *Schilbe intermedius* populations did not show any significant decline or increase throughout the survey.

**Salinity** measures only the dissolved inorganic content of water (also known as saltiness) in contrast with the TDS which measure both organic and inorganic contents (DWAF 1996d). Salinity ranged from 0.17 to 0.23‰, with highest values recorded during spring. There was no variation in salinity concentrations among sites ($p>0.05$), the concentrations were all 0.20‰ at all sites (Table 2.2); however, there was a significant variation among seasons (Table 2.1). The tolerance of fish species to variations in salinity is dependent on their physiological adaptation. Species capable of tolerating wide salinity ranges defined as euryhaline while those tolerating only limited ranges are referred to as stenohaline (DWAF 1996c; Skelton 2001). Specific TWQRs of the fish species studied were not available, so closely related species (that were appearing in the SAWQG list) tolerances were followed; *L. rosae* with *Labeo umbratus* and *S. intermedius* with *Clarias gariepinus*. They were both within the SAWQG TWQR of the selected closely related fish species. However, it should be noted that every fish species have different salinity tolerance limits. The limits are influenced by acclimation, life history stage and temperature (DWAF 1996d).

### 2.3.2 NUTRIENTS

Nutrients include all major inorganic nitrogen compounds (i.e. ammonia, ammonium, nitrate and nitrite) and phosphorus. Nitrogen and phosphorus are essential macronutrients and are required by all living organisms (DWAF 1996d). Both nitrogen and phosphorus are essential constituents of DNA and proteins, which include the enzymes that catalyse all biochemical processes and are therefore a major component of all living organisms (Dallas & Day 2004).

Total nitrogen concentrations were very low ranging from less than 0.2 to 0.7 mg/ℓ (Appendix A: Table 1). Nitrite was recorded at concentrations below the detection level (<0.2 mg/ℓ) during all seasons at all sampling sites (Table 2.3 and 2.4). Ammonia was also recorded at concentrations below the detection level (<0.2 mg/ℓ) during spring and summer. Nitrate was detectable throughout the survey with a maximum of 0.4 mg/ℓ (Table 2.4). Overall, the total nitrogen concentrations recorded in this study were very low (<0.5 mg/ℓ); these concentrations were within the TWQR (<0.5 mg/ℓ nitrogen) for aquatic ecosystems (DWAF 1996a), indicating oligotrophic conditions. The ortho-phosphate was recorded below 0.2 mg/ℓ.

Table 2.3: The average seasonal nutrients recorded from the three sampling sites in Flag Boshielo Dam (n=3)

| **Nutrients (mg/l)** | **Winter 2009** | **Spring 2009** | **Summer 2010** | **Autumn 2010** | **P** |
|---|---|---|---|---|---|
| Nitrate | 0.40 ±0 | 0.20 ±0 | 0.20 ±0 | 0.20 ±0 | 0.441 |
| Nitrite | <0.1 | <0.2 | <0.2 | <0.2 | - |
| Ammonia | 0.27 ±0.05 | 0 | 0 | 0.20 ±0 | 0.052 |
| Total Nitrogen | 0.40 ±0.22 | 0.20 ±0 | 0.13 ±0.09 | 0.27 ±0.09 | - |
| Phosphorus | 0 | 0.05 ±0 | 0.04 ±0 | 0.05 ±0 | 0.768 |
| Ortho-Phosphate | <0.2 | <0.2 | <0.2 | <0.2 | - |

Table 2.4: The average nutrient concentrations recorded from the three sampling sites in Flag Boshielo Dam during the study period (n=4)

| **Nutrients (mg/ℓ)** | **Inflow** | **Middle** | **Wall** | **P** |
|---|---|---|---|---|
| Nitrate | 0.2 ±0 | 0.25 ±0.09 | 0.2 ±0 | 0.405 |
| Nitrite | <0.2 | <0.2 | <0.2 | 1 |
| Ammonia | 0.3 ±0 | 0.25 ±0.05 | 0.2 ±0 | 0.622 |
| Nitrogen | 0.5 ±0.04 | 0.5 ±0.02 | 0.4 ±0.09 | - |
| Phosphorus | 0.036 ±0.02 | 0 | 0 | 0.009 |
| Ortho-Phosphate | <0.2 | <0.2 | <0.2 | - |

Phosphorus concentrations were also very low ranging from non-detectable values less than 0.001 to 0.052 mg/ℓ. However the phosphorus concentrations recorded at the inflow during all seasons, were above SAWQG TWQR of <0.005 mg/ℓ indicating eutrophic conditions (i.e. phosphorus concentrations between 0.025 – 0.25 mg/ℓ

(DWAF 1996e). The Elands River confluence with Olifants River at the inflow of Flag Boshielo Dam and is characterised with elevated nitrate and phosphate levels (Heath *et al.* 2010), which could be the main source of elevated nutrients levels at the inflow. Therefore, this poses a potential eutrophication source to Flag Boshielo Dam.

Nitrogen is seldom present in high concentrations in un-impacted surface waters (DWAF 1996e). This is because inorganic nitrogen is rapidly taken up by aquatic plants and converted into proteins and other organic forms of nitrogen in plant cells. The recorded nitrogen levels are to be expected as dams act as “sinks”, collecting and reducing the nutrient content via utilisation by microphytes and micro-organisms present in the dams (Heath *et al.* 2010). In South Africa, inorganic nitrogen concentrations in un-impacted, aerobic surface waters are usually below 0.5 mg/ℓ (DWAF 1996e). On the other hand, in un-impacted and well oxygenated waters (dissolved oxygen concentration 80 – 120% saturation), most (>80%) of the inorganic nitrogen should be present as nitrate; typically, ammonia concentrations will be below 0.1 mg/ℓ, or less than 20% of the inorganic nitrogen present (DWAF 1996e). These finding are in line with records from this study.

### 2.3.3 MAJOR IONS

Ions have a major contribution to TDS. The concentrations and proportions of the individual major ion also affect water quality (DWAF 1996d). There are two major categories of ions; anions (negatively charged element) and cations (positively charged element). The cations mostly found in natural waters are; calcium, magnesium, sodium and potassium. The anions mostly found in natural waters are; bicarbonate, carbonate, chloride, fluoride and sulphate. Although the major ions are not toxic at low concentrations, they may induce toxic effects on aquatic biota and water users at high concentrations (Dallas and Day 2004). The major ions of inland waters are derived from the rocks with which they are in contact, and from the atmosphere (Dallas & Day 2004). Depending on the relative influences of these sources, different ions predominate in different systems. In South Africa the waters of the Highveld (including the Olifants River) tend to be dominated by calcium, magnesium and bicarbonate ions, whereas those in the coastal regions and the arid west tend to be dominated by sodium and chloride ions (Day & King 1995). In this study the anions analysed include: chloride, fluoride and sulphate, and the cations namely; calcium, magnesium, sodium, potassium and lithium (Tables 2.5 & 2.6).

**Chloride** concentrations ranged from 18 mg/ℓ to 33 mg/ℓ (Appendix A: Table 1). Highest concentrations were recorded at the inflow with lowest concentrations at the dam wall (Table 2.6). However, the variation was too low among sites ($p>0.05$), ranging between 24.25 mg/ℓ and 25.25 mg/ℓ. Seasonally, highest concentrations were recorded during spring with lowest records during summer (Table 2.5). There was a significant variance among seasons ($p<0.01$). The SAWQG for Aquaculture indicates that 600 mg/ℓ $Cl^-$ is acceptable for freshwater fish species (DWAF 1996c). Chlorides are widely distributed in nature as salts of sodium (NaCl), potassium (KCl), and calcium ($CaCl_2$). Chloride anthropogenic inputs to surface waters can arise from irrigation return flows, sewage effluent discharges and various industrial processes (Dallas & Day 2004). Chloride exhibits no toxic effects on living systems, except where it has an effect by increasing the TDS (DWAF 1996e). Chloride is common constituent in water, highly soluble and tends to accumulate when in solution. On the other hand, chloride is the major anion in many inland waters, particularly in South Africa (Dallas & Day 2004).

Table 2.5: The average seasonal concentrations of the major ions recorded in the water samples from the three sampling site in Flag Boshielo Dam (n=3)

| Ions (mg/ℓ) | Winter 2009 | Spring 2009 | Summer 2010 | Autumn 2010 | P |
|---|---|---|---|---|---|
| Sulphate | 96 ±2.16 | 106 ±0.82 | 115.33 ±0.94 | 112 ±1.63 | 0.000 |
| Chloride | 24 ±0.82 | 30.67 ±1.70 | 19.67 ±1.25 | 25 ±0.82 | 0.000 |
| Fluoride | 0.40 ±0 | 0.60 ±0 | 0.40 ±0 | 0.40 ±0 | - |
| Calcium | 31.53 ±2.37 | 22.50 ±1.04 | 23.33 ±1.48 | 22.71 ±1.64 | 0.002 |
| Magnesium | 20.79 ±0.78 | 16.37 ±1.76 | 15.47 ±2.46 | 14.49 ±2.47 | 0.055 |
| Sodium | 34.47 ±1.72 | 7.69 ±8.99 | 6.82 ±8.23 | 7.17 ±8.90 | 0.016 |
| Lithium | 0.009 ±0 | 0.002 ±0.001 | 0.004 ±0 | 0.005 ±0.001 | 0.000 |

**Fluoride** concentrations ranged from 0.4 mg/ℓ to 0.6 mg/ℓ (Appendix A: Table 1). Fluoride concentrations in this study did not show any variation seasonally or among sites ($p>0.05$) and were all within the TWQR for aquatic ecosystems (DWAF 1996e) of 0.75 mg/ℓ. Seasonally, the highest concentrations were recorded during spring

(0.6 mg/ℓ) at all three sites. During all the seasons the fluoride concentrations were the same (0.4 mg/ℓ) at all sites (Appendix A: Table 1). Fluoride is a halogen gas which is highly reactive with a variety of substances. It is seldom found as free fluorine gas in nature, but occurs either as the fluoride ion or in combination with calcium, potassium and phosphates (DWAF 1996e). Natural fluorides occur in rocks in some areas. Anthropogenic source of fluoride in aquatic ecosystems include releases from sewage treatment plants, since most public water supplies add fluoride to drinking water to reduce dental decay (DWAF 1996c).

Table 2.6: The average concentrations of the major ions recorded in the water column of Flag Boshielo Dam at different sites during the study period (n=4)

| Ions (mg/ℓ) | Inflow | Middle | Wall | P |
|---|---|---|---|---|
| Sulphate | 107.75 ±5.85 | 106.75 ±8.04 | 107.50 ±8.32 | 0.986 |
| Chloride | 25.25 ±5.31 | 25.00 ±3.61 | 24.25 ±2.95 | 0.953 |
| Fluoride | 0.45 ±0.09 | 0.45 ±0.09 | 0.45 ±0.09 | 1.000 |
| Calcium | 24.92 ±5.80 | 24.34 ±3.24 | 25.80 ±2.48 | 0.909 |
| Magnesium | 14.72 ±4.28 | 17.98 ±1.43 | 17.63 ±1.59 | 0.329 |
| Sodium | 23.86 ±7.53 | 8.94 ±14.20 | 9.31 ±13.61 | 0.282 |
| Lithium | 0.004 ±0.003 | 0.006 ±0.002 | 0.006 ±0.002 | 0.788 |

**Sulphate** concentrations ranged from 94 mg/ℓ to 116 mg/ℓ during this study. The highest concentrations were recorded during summer with lowest concentrations in winter (Table 2.5). Although a typical concentration of sulphate in surface water is 5 mg/ℓ, 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 (DWAF 1996a; d). There are no SAWQG of sulphate available for aquatic ecosystems. Sulphate is a common constituent of water and arises from the dissolution of mineral sulphates in soil and rock, particularly calcium sulphate (gypsum) and other partially soluble sulphate minerals. The interactions of sulphate are governed by the associated cations, usually magnesium and sodium (DWAF 1996d). Sulphates are discharged into the aquatic environment in wastes from industries that use sulphates and sulphuric acid, such as mining and smelting operations, kraft pulp and paper mills, textile mills and tanneries. Sulphates are also released during blasting and the deposition of waste rock in dumps at metal mines. This is known as acid rock drainage and is a significant source of sulphate generation (DWAF 1996d). Sulphate ions tend to occur in lower concentrations and sulphates themselves are not toxic (DWAF 1996e; Dallas & Day 2004). However, it has been reported by de Villiers and Mkwelo (2009), that Olifants River suffers from high levels of sulphate caused by acid mine drainage from the Upper Olifants sub-catchment.

**Calcium** concentrations ranged from 20.6 mg/ℓ to 34.88 mg/ℓ (Appendix A: Table 1). During winter calcium was recorded the highest at 31.53 mg/ℓ and the lowest during spring at 22.50 mg/ℓ (Table 2.5). There are no SWAQG of calcium for aquatic ecosystems (DWAF 1996c). There was no significant variance among the sites ($p>0.05$) but among seasons. Highest concentrations were recorded at the dam wall during all seasons (Table 2.5), the source of this elevated concentrations could be the dam wall itself since calcium is present in various construction materials such as cement, brick lime and concrete. Calcium ions are often the major cations in inland waters, whereby soft waters contain low calcium concentration and hard waters high calcium concentration (DWAF 1996a; c). Calcium is one of the major elements essential for living organisms found as a structural material in bones, teeth, shells and exoskeletons (Dalesman & Lukowiak 2010). It is a divalent salt and it is one of the most common sources of water hardness. Although calcium is a vital element, very little is known about the actual effects of changes in its concentration on aquatic biotas (DWAF 1996c).

**Magnesium** concentrations recorded in this study ranged from 11 mg/ℓ to 21.89 mg/ℓ (Appendix A: Table 1). The highest magnesium concentrations were recorded during winter and lowest levels during autumn (Table 2.5). There were no significant variations between sites and seasons ($p>0.05$). Typical concentrations of magnesium in freshwater ecosystems are usually between 4 and 10 mg/ℓ (DWAF 1996a; d). Magnesium is usually found in relatively high concentrations in water, but it is unlikely to act as a toxin (DWAF 1996c). A common magnesium mineral is dolomite, the type of rock formation which is also found around Flag Boshielo Dam catchment area (Ashton *et al.* 2001). Magnesium is an essential element, being found in chlorophyll and in a variety of enzymes and is involved in the processes of muscle contraction and the transmission of nervous impulses. It is also a divalent salt and one of the most common sources of water hardness. Very little is known about its effects on aquatic organisms (Dallas & Day 2004).

**Sodium** concentrations recorded in this study ranged from 0.41 mg/ℓ to 36.8 mg/ℓ (Appendix A: Table 1). Highest concentrations of sodium were recorded during winter (34.47 mg/ℓ) with lowest concentrations during summer (6.82 mg/ℓ) (Table 2.5). The sodium concentrations were highest at the inflow (23.86 mg/ℓ); an indication that the water upstream may contain higher sodium concentrations. There is no sodium SAWQG available for aquatic ecosystems. Sodium concentrations are elevated in runoffs or leachates from irrigated soils (DWAF 1996b). Arid areas also often contain elevated concentrations of sodium, since sodium in surface waters are generally low in areas of high rainfall but high in arid areas with low rainfall (DWAF 1996b). Sodium is probably the least toxic metal cation (Hellawell 1986) and its important occurrence on aquatic systems is that, it's a major contributor to TDS. Sodium is everywhere in natural waters and is the major cation in many South African inland waters (DWAF 1996a; d). It is the major cation involved in ionic, osmotic and water balance in all organisms and is also involved in the transmission of nervous impulses and in muscle contraction.

**Potassium** concentrations recorded in this study ranged from 2.18 mg/ℓ to 7.02 mg/ℓ (Appendix A: Table 1). It was highest at the inflow (6.47 mg/ℓ) with lowest concentrations recorded at the wall (4.79 mgℓ); indicating that the water upstream may contain higher concentrations. There was a significant variance among sites ($p<0.01$), but the dam wall and the middle of the dam concentrations didn't vary much. Seasonally highest concentrations were recorded during winter (5.45 mg/ℓ), however, the concentrations did not vary much among the seasons ($p>0.05$). There are no SAWQG available, and its toxic effects to aquatic ecosystems are not known. Typical concentration of potassium in freshwater is within the range of 2 - 5 mg/ℓ. Potassium usually occurs in much lower concentrations than sodium, hence, it sometimes acts as a nutrient and the lack of it may limit plant growth (Hellgren *et al.* 2006). Potassium, like sodium, is involved in ionic balance in all organisms, and in transmission of nervous impulses and in muscle contraction in animals (DWAF 1996a). It has been shown that potassium may act as a limiting nutrient for animal communities as well as for plants (Hellgren *et al.* 2006).

**Lithium** concentrations ranged from 0.001 mg/ℓ to 0.009 mg/ℓ (Appendix A: Table 2). Highest concentrations were recorded during winter (0.009 mg/ℓ) with lowest concentration recorded in spring at 0.002 mg/ℓ (Table 2.8). There was no significant difference of lithium concentrations between sites ($p>0.05$) but among seasons ($p<0.05$). Lithium is the 27th most abundant element in nature and is found as the pearly-coloured spodumene (lithium aluminium silicate) and amblygonite (lithium aluminium fluorophosphate) in granite pegmatites. It is most concentrated in the earth's core and is the lightest of all the metals; also lighter than water (DWAF 1996b). Lithium is also derived from a variety of manufactured goods such as pharmaceuticals and electronic devices (Aral & Vecchio-Sadus 2008). The recommended maximum acceptable lithium concentration by BC-EPD (2003) is 0.870 mg/ℓ. The recorded concentrations of lithium during the survey were acceptable; hence it can be assumed that its presence in the water may be from natural sources such as the granites surrounding Flag Boshielo Dam.

## 2.4 METALLOIDS AND METALS

Aluminium concentrations were recorded above the TWQRs for aquatic ecosystems (0 - 0.01 mg/ℓ at pH>6.5). The concentrations ranged between 0.018 mg/ℓ and 0.283 mg/ℓ (Appendix A: Table 2). High concentrations were recorded at the inflow (0.199 mg/ℓ) and low concentrations at the dam wall (0.088 mg/ℓ) (Table 2.7). These concentrations were also above the CEV (0.02 mg/ℓ at pH>6.5) according to the SAWQG. The concentrations recorded during spring were above the AEV (0.15 mg/ℓ) (Table 2.8). Elevated concentrations of aluminium can be linked to acid mine drainage and /or acid rain because it is one of the principal particulates emitted from combustion of coal (DWAF 1996e). This could mean that the water upstream may be contaminated with acid mine drainage or acid rain. This is also visible in Table 2.8, whereby the highest concentrations were recorded at the inflow during all seasons. Seasonally, the highest concentrations were recorded during spring (0.206 mg/ℓ) with the lowest concentrations during winter (0.054 mg/ℓ) (Table 2.8). There was no significant difference of aluminium concentrations among either the sites or the seasons ($p>0.05$).

Aluminium is the third most abundant element in the earth's crust. It occurs primarily as alumina-silicate minerals which are too insoluble to participate readily in bio-geochemical reactions. The solubility of aluminium in water is strongly pH dependent (Dickson 1983; DWAF 1996e). Under acid conditions, it occurs as soluble, available and toxic (Buergel & Soltero 1983; Norrgren *et al.* 1991). At intermediate pH values, it is partially soluble while at alkaline pH values, it is present as soluble but biologically unavailable (DWAF 1996e). In this study the pH was recorded at levels greater than 6.5 (that is intermediate to alkaline). Hence even though aluminium was recorded at concentrations above the TWQR, it was not biologically available and the aquatic biota may not be affected.

Table 2.7: The average seasonal metalloid and metal concentrations recorded in the water column of Flag Boshielo Dam (n=3)

| METALS (mg/ℓ) | Winter 2009 | Spring 2009 | Summer 2010 | Autumn 2010 | P |
|---|---|---|---|---|---|
| Aluminium | 0.054 ±0.034 | 0.206 ±0.063 | 0.137 ±0.044 | 0.148 ±0.047 | 0.074 |
| Antimony | 0.005 ±0.001 | <0.001 | <0.001 | <0.001 | 0.000 |
| Arsenic | 0.003 ±0.002 | <0.001 | 0.002 ±0 | 0.003 ±0 | 0.653 |
| Barium | 0.058 ±0.003 | 0.057 ±0.016 | 0.047 ±0.010 | 0.049 ±0.006 | 0.664 |
| Boron | 0.077 ±0.012 | 0.004 | 0.009 ±0 | 0.007 ±0 | 0.000 |
| Cadmium | 0.001 ±0 | <0.001 | <0.001 | <0.001 | - |
| Chromium | 0.001 ±0 | <0.001 | <0.001 | <0.001 | 0.441 |
| Cobalt | 0 | <0.001 | 0.001 ±0 | 0.001 ±0 | - |
| Copper | 0.002 ±0.001 | <0.001 | <0.001 | <0.001 | 0.156 |
| Iron | 0.119 ±0.074 | 0.109 ±0.050 | 0.195 ±0.155 | 0.140 ±0.119 | 0.856 |
| Lead | 0.011 ±0.001 | <0.001 | <0.001 | <0.001 | 0.000 |
| Manganese | 0.034 ±0.004 | 0.030 ±0.013 | 0.025 ±0.006 | 0.020 ±0.007 | 0.405 |
| Nickel | 0 | <0.001 | <0.001 | 0.001 ±0 | - |
| Silver | 0.001 ±0.001 | 0.007 ±0.005 | 0.010 ±0 | 0.004 ±0 | 0.650 |
| Strontium | 0.173 ±0.007 | 0.139 ±0.012 | 0.149 ±0.001 | 0.139 ±0.005 | 0.006 |
| Tin | 0 | 0.004 ±0.002 | 0.005 ±0.003 | 0.005 ±0 | 0.255 |
| Titanium | 0 | 0.170 ±0 | 0.026 ±0 | 0.022 ±0 | 0.517 |
| Vanadium | 0.002 ±0 | <0.001 | <0.001 | 0.002 ±0 | 0.012 |
| Zinc | 0.002 ±0.003 | <0.001 | <0.001 | <0.001 | 0.683 |

**Antimony** was one of the metals that were detectable during winter only, at an average value of 0.005 mg/ℓ (Table 2.8). The highest levels were recorded at the inflow (0.006 mg/ℓ) with the lowest levels at the dam wall (0.004 mg/ℓ) (Table 2.7).

There was no significant difference of antimony concentrations among sites ($p>0.05$), but between seasons ($p<0.05$). The acceptable concentration of boron in water is 0.01 mg/ℓ (USEPA 2012). Therefore antimony concentrations were acceptable throughout the survey period.

Table 2.8: The average metalloid and metal concentrations recorded at the three sampling sites in Flag Boshielo Dam during the study period (n=4)

| **METALS(mg/ℓ)** | **Inflow** | **Middle** | **Wall** | **P** |
|---|---|---|---|---|
| Aluminium | 0.199 ±0.066 | 0.123 ±0.057 | 0.088 ±0.042 | 0.095 |
| Antimony | 0.006 ±0 | 0.005 ±0 | 0.004 ±0 | 0.942 |
| Arsenic | 0 | 0.002 ±0 | 0.004 ±0.002 | 0.087 |
| Barium | 0.065 ±0.007 | 0.045 ±0.007 | 0.049 ±0.007 | 0.018 |
| Boron | 0.028 ±0.037 | 0.072 ±0 | 0.065 ±0 | 0.901 |
| Cadmium | 0.001 ±0 | 0.001 ±0 | 0.001 ±0 | - |
| Chromium | 0.001 ±0 | 0.001 ±0 | 0 | 0.405 |
| Cobalt | 0 | 0.001 ±0 | 0 | 1.000 |
| Copper | 0.001 ±0 | 0.004 ±0 | 0.002 ±0 | 0.522 |
| Iron | 0.279 ±0.091 | 0.074 ±0.020 | 0.069 ±0.023 | 0.002 |
| Lead | 0.013 ±0 | 0.010 ±0 | 0.010 ±0 | 0.971 |
| Manganese | 0.037 ±0.006 | 0.022 ±0.007 | 0.023 ±0.009 | 0.060 |
| Nickel | 0 | 0.001 ±0.001 | 0 | 1.000 |
| Silver | 0.007 ±0.004 | 0.002 ±0.001 | 0 | 0.017 |
| Strontium | 0.148 ±0.021 | 0.150 ±0.014 | 0.152 ±0.009 | 0.950 |
| Tin | 0.002 ±0.002 | 0.004 ±0.003 | 0.003 ±0.002 | 0.488 |
| Titanium | 0.055 ±0.067 | 0 | 0 | 0.200 |
| Vanadium | 0.003 ±0 | 0.002 ±0 | 0.002 ±0 | 0.856 |
| Zinc | 0.001 ±0 | 0.006 ±0 | 0 | 0.347 |

However, antimony is not likely to occur at significantly higher concentrations in natural waters, except in those areas affected by acid mine drainage (WHO 2004). The upper Olifants catchment is characterised by coal mining and coal-fired power generation which could be the source of antimony in Flag Boshielo Dam and these concentrations could highly impact aquatic biota. Antimony is a toxic metalloid and also suspected to be carcinogenic (Zhiyou *et al.* 2011). It has been considered to be a major pollutant and also listed as a priority pollutant in both the United States and the European Union (USEPA 1999). Effects of antimony are not well known. Like arsenic, antimony is omnipresent in the environment, especially in the ground (Filella *et al.* 2002). The environmental behaviour of antimony has received less attention than other toxic metals such as arsenic, mercury, lead and cadmium, because its abundance in the Earth's crust is generally low (Zhiyou *et al.* 2011).

Table 2.9: Water quality guidelines for the metals and the references used.

| **Metals** | **Water quality guidelines (mg/ℓ)** |
|---|---|
| Aluminium | 0.001* (pH > 6.5)[1] |
| Antimony | 0.01[2] |
| Arsenic | 0.01[1] |
| Barium | 0.7[2] |
| Boron | No guidelines |
| Cadmium | 0.00015 - 0.004[1] |
| Chromium | Cr III: 0.012*[1] |
| Cobalt | No guidelines |
| Copper | 0.0003 – 0.0014[1] |
| Iron | Fe vary <10% background concentration.[1] |
| Lead | 0.0002-0.0012[1] |
| Manganese | 0.18[1] |
| Nickel | < 0.47[2] |
| Selenium | 0.002[1] |
| Silver | No guidelines |
| Strontium | 4.0[2] |
| Tin | No guidelines |
| Titanium | No guidelines |
| Vanadium | No guidelines |
| Zinc | 0.002[1] ; <0.12[2] |

1 - DWAF (1996) South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.
2 - US-EPA (2012) – United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life.

**Arsenic** concentrations ranged between non-detectable concentrations <0.001 mg/ℓ and detectable concentrations 0.006 mg/ℓ (Appendix A: Table 2). Detectable concentrations were recorded during winter at all sites, except the inflow, with an average of 0.003 mg/ℓ. These concentrations are within the TWQR for aquatic ecosystems. There was no significant difference of arsenic concentrations between sites and seasons ($p>0.05$). Arsenic may occur at high concentrations in water bodies subject to industrial pollution, or in the vicinity of industrial activities utilising or discharging arsenic or arsenal compounds such as producers of pesticides and fertilizers (DWAF 1996e). Given the fact that Flag Boshielo Dam catchment area is characterised by agricultural activities, it can be assumed that some of the arsenic concentrations may be from these activities.

Arsenic is a metalloid element which is toxic to marine and freshwater aquatic life and is a known carcinogen (USEPA 2007). It is also one of the Endocrine Disruptive Metals (EDM). The aquatic chemistry of arsenic is complex. Redox values and pH levels play a major role in determining the form of arsenic in freshwater, and thus its toxic effects (DWAF 1996e). The presence of dissolved and particulate organic matter, suspended solids and sediments are also important, since arsenic adsorbs readily to suspended material and combines with dissolved organic carbon (DWAF 1996e; Foata *et al.* 2009).

**Barium** concentrations ranged between 0.039 mg/ℓ and 0.077 mg/ℓ (Appendix A: Table 2). Highest concentrations were recorded at the inflow (0.065 mg/ℓ) (Table 2.7) and seasonally during winter (0.058 mg/ℓ) (Table 2.8). There was no significant difference of barium concentrations among seasons ($p>0.05$) but between sites ($p<0.05$). There are also no SAWQG for barium, but barium concentrations recorded were within the World Health Organisation (WHO 2004) drinking water guidelines (0.7 mg/ℓ). Barium in water originates primarily from natural sources and is present as a trace element in both igneous and sedimentary rocks (WHO 2004). This could explain the presence of barium in the dam, because Flag Boshielo Dam is characterised by igneous and sedimentary rock formations (Ashton 2000). Although barium is not found free in nature (USEPA 1985), it occurs in a number of compounds, most commonly barium sulfate (barite) and, to a lesser extent, barium carbonate (witherite). The solubility of barium compounds increases as the pH level decreases (USEPA 1985). In this study the pH levels ranged between neutral to alkaline, hence barium toxicity may not be expected. Although, toxic effects of barium concentrations recorded in this study are not known, given that it was within WHO drinking water guidelines, it can be assumed that the recorded concentrations are acceptable for aquatic ecosystems.

**Boron** was recorded only at the inflow during all seasons except winter; autumn (0.007 mg/ℓ), spring (0.004 mg/ℓ) and summer (0.009 mg/ℓ) (Appendix A: Table 2). However, during winter it was detectable at all sites at an average of 0.077 mg/ℓ (Table 2.8). There was a significant difference of boron concentrations among seasons ($p<0.05$) but not between sites ($p>0.05$). Boron is a metalloid that usually occurs in combined forms such as borax and is rare in the earth crust but is found throughout the environment (USEPA 2008). Representative species of aquatic organisms, such as plants, invertebrates, fishes, and amphibians, usually tolerate up to 10 mg/ℓ of boron for extended periods without harm (Eisler 1990; USEPA 2008). However, boron criteria recommended for the protection of sensitive species in aquatic ecosystems is less than 1 mg/ℓ (USEPA 2008). On the other hand, recommended guidelines for aquatic ecosystems by BC-EPD (2003) are 1.2 mg/ℓ. The recorded values from Flag Boshielo Dam are within the recommended levels. Boron concentrations were recorded the highest concentrations at the inflow as compared to other sampling sites within the dam during all seasons, which could suggest that the water upstream may have high boron concentrations. Anthropogenic sources of boron in aquatic environment include coal-fired plants, mine drainage waters, municipal wastes, and agricultural drainage waters (Eisler 1990). All these activities are present in either the upper or middle catchment of the Olifants River, but their concentrations are still acceptable for aquatic ecosystems.

**Cadmium** was recorded at undetectable concentrations (<0.001 mg/ℓ) during all seasons except winter at 0.001 mg/ℓ at all sites (Table 2.8). These concentrations were above DWAF's TWQR (0.00025 mg/ℓ) and the CEV (0.0005 mg/ℓ) for aquatic ecosystems. Its presence during this season maybe due to turnover since it is mainly found in bottom sediments and suspended particles (Friberg *et al.* 1986). To add on that, other sources of cadmium in Flag Boshielo Dam may be from the dominant agricultural activities in its vicinity, since agricultural use of sludges, fertilizers and pesticides containing cadmium is mentioned as one of the contributors of the cadmium presence in natural waters.

Cadmium is the most toxic and non-essential metal which has wide distribution in the earth's crust and aquatic environments. Due to its uses in aquaculture, metal (zinc, lead and copper) smelters, and industries involved in manufacturing alloys, paints, batteries and plastics; agricultural use of sludges, fertilizers and pesticides containing cadmium and burning of fossil fuels (very limited effect), its concentration is increasing in the aquatic system (DWAF 1996e; Muthukumaravel *et al.* 2007). Due to its abundance, large quantities of cadmium also enter the global environment annually as a result of natural weathering processes. It is found at trace concentrations in freshwaters and mostly a result of industrial activity. The solubility of cadmium in water is influenced to a large degree by its acidity; suspended or sediment-bound cadmium may dissolve when there is an increase in acidity (CCME

1999). In natural waters, cadmium is found mainly in bottom sediments and suspended particles (Friberg *et al.* 1986).

Cadmium like arsenic is also one of the EDM and is toxic at very low concentrations. It is a metal element which is highly toxic to marine and freshwater aquatic life. It is known to inhibit bone repair mechanisms, and is teratogenic (causing embryo/foetus malformations), mutagenic (increase frequency of mutation) and carcinogenic (DWAF 1996e). Elemental cadmium is insoluble in water though many of its organic and inorganic salts are highly soluble. It occurs primarily in freshwaters as divalent forms including free cadmium (II) ion, cadmium chloride and cadmium carbonate, as well as a variety of other inorganic and organic compounds. Presence of cadmium is usually in association with zinc, lead and copper sulphide ore bodies (DWAF 1996e). Bioavailable cadmium may be accumulated by macrophytes, phytoplankton, zooplankton, invertebrates and fish (CCME 1999)

**Chromium** also was recorded at concentrations below the detection level (<0.001 mg/ℓ) during all seasons at all sites except winter at the inflow and the middle of the dam, both at 0.001 mg/ℓ (Table 2.7 & 2.8). The recorded concentrations during winter were within TWQR for aquatic ecosystems (DWAF 1996e), 0.007 mg/ℓ (chromium VI) and 0.012 mg/ℓ (chromium III). There was no significant difference of chromium concentrations among sites and seasons ($p>0.05$).

Chromium is a relatively scarce metal, and the occurrence and amounts thereof in aquatic ecosystems are usually very low (DWAF 1996e). Chromium ions occur in a variety of forms:

- Chromium (II) - chromous ion ($Cr^{2+}$),
- Chromium (III) - chromic ion ($Cr^{3+}$, trivalent),
- Chromium (III) - chromite ion ($CrO_3^{3-}$, trivalent),
- Chromium (VI) - chromate ion ($CrO_4^{2-}$, hexavalent),
- Chromium (VI) - dichromate ion ($Cr_2O_7^{2-}$, hexavalent).

Chromium (VI) is a highly oxidized state and occurs as the yellow dichromate salt in neutral or alkaline media, and as the orange chromate salt in acid medium. Both of these chromium (VI) salts are highly soluble at all pH values. The reduced forms, chromium (II) and chromium (III) are reported as being less toxic and therefore less hazardous than chromium (VI).

**Cobalt** was below the detection level during the whole survey except during autumn and summer in the middle of the dam both at 0.001 mg/ℓ (Table 2.7 & 2.8). There was no significant difference of cobalt concentrations between sites and seasons ($p>0.05$). To protect aquatic life in freshwater environments, an interim acute (maximum) guideline of 0.11 mg/ℓ and an interim chronic (30-day average) guideline of 0.0044 mg/ℓ are recommended cobalt, based on a literature review and toxicity testing by Nagpal (2004). The recorded cobalt concentrations were below the recommended guidelines by Nagpal (2004).

Cobalt is a naturally-occurring element that has properties similar to those of iron and nickel (ATSDR 2004a). Cobalt occurs naturally in soil, rock, air, water, plants, and animals. It may enter air and water, and settle on land from windblown dust, seawater spray, volcanic eruptions, and forest fires and may additionally get into surface water from runoff and leaching when rainwater washes through soil and rock containing cobalt (WHO 2006). Soils near ore deposits, phosphate rocks, or ore smelting facilities, and soils contaminated by airport traffic, highway traffic, or other industrial pollution may contain high concentrations of cobalt (WHO 2006).

**Copper** concentrations were also recorded below detection level (<0.001 mg/ℓ) during all seasons at all sites except during winter with highest record in the middle of the dam (0.004 mg/ℓ) and lowest at the inflow (0.001 mg/ℓ) (Tables 2.7 & 2.8). These concentrations were above the DWAF's TWQR (0.0008 mg/ℓ) for aquatic ecosystems (DWAF 1996e). There was no significant difference of copper concentrations between sites and seasons ($p>0.05$). Copper is a micronutrient and an essential component of enzymes involved in redox reactions (Sorensen 1991; Galvin 1996) and is rapidly accumulated by plants and animals (DWAF 1996e). It is toxic at low concentrations in water (DWAF 1996e; Welsh *et al.* 1996; Wepener *et al.* 2001). It occurs in four oxidation states, namely, 0, I, II and III (DWAF 1996e). The two most common forms are; cuprous copper (I) and cupric copper (II) (DWAF 1996e).

The occurrence of natural sources of copper in the aquatic environment is due to weathering processes or from the dissolution of copper minerals and native copper

(DWAF 1996e). The main anthropogenic sources of copper in the aquatic environment include: sewage treatment plant effluents; copper compounds used as aquatic algaecides; runoff of the use of copper as fungicides and pesticides in the treatment of soils; and liquid effluents and atmospheric fallout from industrial sources such as mining, smelting and refining industries, coal-burning, and iron- and steel-producing industries (DWAF 1996e). Given that, there could be several sources of copper into Flag Boshielo Dam which may include run-offs from the agricultural activities which are using copper containing pesticides in the treatment of soils for crops, coal burning, and iron and steel producing industries within the catchment or in the upper catchment.

**Iron** concentrations ranged from 0.030 mg/ℓ to 0.414 mg/ℓ (Appendix A: Table 2), with the highest concentrations recorded at the inflow with an average of 0.279 mg/ℓ (Table 2.7). Seasonally highest concentrations were recorded during summer with an average of 0.195 mg/ℓ (Table 2.8). There was no significant difference of iron concentrations between seasons ($p>0.05$) but among sites ($p<0.05$). There are no SAWQG available for iron, it is only mentioned that its concentrations are site specific and should not exceed 10% of the background dissolved iron concentration (DWAF 1996e). According to the British Columbian (BC-EPD 2008) guidelines, to protect freshwater aquatic life 1 mg/ℓ is acceptable for total iron and 0.35 mg/ℓ for dissolved iron. To add on that, iron concentration of 0.3 mg/ℓ is acceptable according to the WHO (2003b) drinking water guidelines. The recorded concentrations in this study were within the British Columbian (BC-EPD 2008) guidelines and WHO (2003b) drinking water guidelines, except for the summer and autumn values at the inflow (Appendix A: Table 2).

Iron is the fourth most abundant element in the earth's crust and may be present in natural waters in varying quantities depending on the geology of the area and other chemical properties of the water body (DWAF 1996e). It is naturally released into the environment from weathering of sulphide ores (pyrite, $FeS_2$) and igneous, sedimentary and metamorphic rocks. Leaching from sandstones releases iron oxides and iron hydroxides to the environment (DWAF 1996e). Iron is also released into the environment by human activities, mainly from the burning of coke and coal, acid mine drainage, mineral processing, sewage, landfill leachates and the corrosion of iron and steel (BC-EPD 2008). Among all the detected metals, iron was the third most abundant concentrations recorded during the survey. The presence of iron in Flag Boshielo Dam may be linked to the igneous rock formations in its vicinity; however, anthropogenic activities in the upper catchment might have contributed to its concentration. Iron is not known to be hazardous to aquatic biota, except for animals that need small concentrations to transport oxygen in the blood.

**Lead** was recorded at below detectable concentrations (<0.001 mg/ℓ) during all seasons except during winter (Appendix A: Table 2). During winter it was recorded at an average of 0.011 mg/ℓ (Table 2.8). These concentrations were significantly higher than the SAWQG TWQR (0.0005 mg/ℓ), the CEV (0.001 mg/ℓ) and AEV (0.007 mg/ℓ) for aquatic ecosystems. There was no significant difference among sites ($p>0.05$) but between seasons ($p<0.05$). Lead is a common and toxic trace metal which readily accumulates in living tissue and is potentially hazardous to most forms of life, and is considered carcinogenic, an EDM and relatively accessible to aquatic organisms (DWAF 1996e). Like chromium lead exists in several oxidation states, that is, 0, I, II and IV, all of which are of environmental importance (DWAF 1996e).

Decreasing pH concentrations increases the bioavailability of divalent lead, which is accumulated by aquatic biota (Latif *et al*. 1982; Dallas & Day 2004; DWAF 1996e). Most of the lead entering aquatic ecosystems besides natural weathering of sulphide ores can be associated with suspended sediments (DWAF 1996e). Lead might have been released to surface water during turnover when temperatures started to change in winter. It might have accumulated in the sediments over time from anthropogenic sources such as acid rain, fallout of lead dust and street runoff (associated with lead emissions from gasoline-powered motor vehicles); industrial and municipal wastewater discharge; mining, milling and smelting of lead and metals associated with lead, e.g. zinc, copper, silver, arsenic and antimony; and combustion of fossil fuels (DWAF 1996e).

**Manganese** concentrations recorded during the survey ranged from 0.013 mg/ℓ to 0.046 mg/ℓ (Appendix A: Table 2). Seasonally the highest concentrations were recorded during winter at 0.034 mg/ℓ and lowest concentrations recorded during autumn at 0.020 mg/ℓ (Table 2.8). When comparing sites, the highest concentrations were recorded at the inflow (0.037 mg/ℓ) (Table 2.7). These concentrations are within the TWQR for aquatic ecosystems (0.18 mg/ℓ) (DWAF 1996e). There was no significant difference of manganese concentrations between sites and seasons ($p>0.05$). Manganese is the eighth most abundant metal in nature, and occurs in a number of ores. In aquatic ecosystems, manganese does not occur naturally as a metal but is found in various salts and minerals, frequently in association with iron compounds (DWAF 1996e).

Soils, sediments and metamorphic and sedimentary rocks are significant natural sources of manganese. Industrial discharges such as dry cell batteries manufacture, chemical industry in paints, dyes, glass, ceramics, matches and fireworks, fertilizer industry whereby manganese is used as a micro-nutrient fertilizer additive, also account for elevated concentrations of manganese in receiving waters (Howe *et al.* 2005). Acid mine drainage also releases a large amount of manganese. Manganese is similar to iron in its chemical behaviour, and is frequently found in association with iron. Toxicity effects of manganese to aquatic biota are very limited. High concentrations of manganese are toxic, and may lead to disturbances in various metabolic pathways, in particular disturbances of the central nervous system caused by the inhibition of the formation of a neurotransmitter called dopamine (USEPA 2004).

**Nickel** was below the detection level throughout the survey except only once during autumn in the middle of the dam at 0.001 mg/ℓ. There are no SAWQGs available for nickel. Nickel is widespread in the environment, with a slightly higher occurrence than copper in the earth's crust and tends to be concentrated in particles of manganese oxide in soils (USEPA 2005). It occurs together with iron as a major constituent of most meteorites (WHO 2007). Most of it is released globally from the burning of fossil fuels (WHO 2007). Nickel is insoluble in water and other common solvents and most soils tend to tie up relatively large quantities of it. Nickel is considered an essential trace element in animal nutrition and is thought to be involved in nucleic acid metabolism (ATSDR 2005b).

**Strontium** was one of the most abundant records during the survey. The concentrations ranged from 0.132 mg/ℓ to 0.183 mg/ℓ (Appendix A: Table 2). The concentrations did not differ much between sites ($p>0.05$) with highest concentrations recorded at the dam wall (0.152 mg/ℓ) and the lowest concentrations in the middle of the dam (0.120 mg/ℓ) (Table 2.7). Seasonally, the highest concentrations were recorded during winter (0.173 mg/ℓ) and the lowest concentrations during spring and autumn both at 0.139 mg/ℓ (Table 2.8). There was a significant difference of strontium concentrations between seasons ($p<0.05$) but not among sites ($p>0.05$). There are no SAWQG available for strontium. However, the recorded strontium concentrations are within the USEPA (2012) guidelines (4.0 mg/ℓ).

Strontium is a naturally occurring element found in rocks, soil, dust, coal, and oil. Naturally occurring strontium is not radioactive and in the environment it exists in four stable isotopes, 84Sr (read as strontium eighty-four), 86Sr, 87Sr, 88Sr (ATSDR 2004b). Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and medicines. Strontium commonly occurs in nature, forming about 0.034% of all igneous rock and in the form of the sulfate mineral celestite ($SrSO_4$) and the carbonate strontianite ($SrCO_3$). Celestite occurs frequently in sedimentary deposits of sufficient size (Irwin *et al.* 1997). Neighboring Flag Boshielo Dam, there are protrusions of igneous rocks, which can also attest to the presence of strontium in the water at such abundant concentrations as compared to other metals.

**Zinc** is also one of the metals which were recorded below detection level in the water column during all seasons except during winter (Appendix A: Table 2). During winter it was recorded only at the inflow with a concentration of 0.001 mg/ℓ and middle of the dam at 0.006 mg/ℓ (Table 2.8). The concentration recorded in the middle of the dam was above the SAWQG TWQR (0.002mg/ℓ) for aquatic ecosystems (Table 2.8). There was no significant difference of zinc concentrations among sites and among seasons ($p>0.05$).

Zinc is a metallic element and also an essential micronutrient for all organisms as it forms the active site in various metallo-enzymes (Galvin 1996; Dallas & Day 2004). It occurs in two oxidation states in aquatic ecosystems, namely as the metal, and as zinc (II) (DWAF 1996e). In aquatic ecosystems the zinc (II) ion is toxic to fish and aquatic organisms at relatively low concentrations (DWAF 1996e). The highest dissolved zinc concentrations will occur in water with low pH, low alkalinity and high ionic strength. Sources of zinc into the aquatic environment besides natural processes such as weathering and erosion from rocks and ores can be associated with industrial wastes, pharmaceuticals, fertilizers and insecticides (DWAF 1996e).

## 2.5 CONCLUSIONS

Water **temperature** levels were normal, with highest records in summer and lowest in winter. **Dissolved oxygen** was within the TWQR during all seasons except during winter with records below MAV. The recorded **pH** levels ranged from slightly acidic to alkaline levels (6.24 – 8.83). **Total alkalinity, turbidity, EC, TDS and salinity** were acceptable for aquatic ecosystems. The **total nitrogen concentrations** were very low, indicative of oligotrophic conditions. However, **phosphorus** concentrations at the inflow were above the TWQR (<0.005 mg/ℓ) indicating eutrophic conditions (i.e. phosphorus concentrations between 0.025 – 0.25 mg/ℓ). Hence phosphorus, if it becomes bioavailable, is a potential risk for eutrophication in the dam. Chloride, fluoride and sulphate concentrations were all within the TWQRs. Calcium, magnesium, sodium and potassium did not have the SAWQG for aquatic ecosystems.

The **metalloids and metals** that were detectable included; aluminium, barium, boron, copper, iron, lead, lithium, selenium, silicon, silver, tin and zinc. The metalloids and metals recorded above the TWQRs for aquatic ecosystems are aluminium, cadmium, copper, iron and lead, mostly at the inflow. Aluminium, cadmium and lead were recorded at concentrations above CEV, an indication that these metals may pose adverse effects to the aquatic ecosystem in Flag Boshielo Dam if exposed to these concentrations over a period of time. Additionally, aluminium and lead were recorded above AEV, indicating that these metals may have acute effects to the aquatic ecosystem in Flag Boshielo Dam. The majority of metal concentrations were below 0.001 mg/ℓ or not detectable at all, during all seasons except winter (Appendix A: Table 2). Although most metals were detectable during winter; cobalt, nickel, selenium and tin were not detected. Among the metals that were detectable during winter only (Table 2.8); cadmium, copper, lead and zinc were recorded at concentrations above TWQR's for aquatic ecosystems. The metalloids and metals that were detectable during all seasons at all sites include;

aluminium, barium, iron, lithium, manganese and strontium. Among them only aluminium and iron were recorded at concentrations above TWQR's. Strontium was the highest metal recorded during the whole survey; however the recorded concentrations were within the USEPA (2012) guidelines.

The hypothesis that Flag Boshielo Dam is polluted due to the high levels of pollution in the upper Olifants sub-catchment as well as between Loskop and Flag Boshielo dams is not fully supported. With exception of some metals and phosphorus concentrations at the inflow area, the water quality in Flag Boshielo Dam was acceptable for aquatic ecosystems during the study period. However the elevated levels of phosphorus and some metals at the inflow support the hypothesis fully.

# CHAPTER 3
# BIOACCUMULATION AND SEDIMENT

## 3.1 INTRODUCTION

Bioaccumulation means an increase in the concentration of a chemical (mostly toxic) in the tissues of organism over time, compared to the chemical's concentration in the environment. Toxic substances to the environment are mostly metals. However, all metals are natural constituents of the environment and are found in varying levels in all ground and surface waters. Some are essential, required for the normal metabolism of aquatic organisms, while others are non-essential and play no significant biological roles (Coetzee *et al.* 2002). The presence of metals in aquatic ecosystems is the result of two main sources of contamination; natural processes or natural occurring deposits and anthropogenic activities (Barker 2006). The main source of metal pollution to life forms is regularly the result of anthropogenic activities. In the freshwater environment, toxic metals are potentially accumulated in sediment and aquatic biota and subsequently to humans through the food chain (biomagnification). Metal concentration in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediment and biota (Camusso *et al.* 1995) which generally exist in low levels in water and attain considerable concentration in sediment and biota (Ekeanyanwu *et al.* 2011).

There is an increasing awareness of the potential hazards that exist due to the contamination of freshwater ecosystems by toxic metals associated with the mining industry (Du Preez *et al*. 2003). The reason is the world demand for food and minerals, which has intensified the exploitation of natural resources. Agriculture and mining contribute tremendously to metals contamination of aquatic ecosystems (Heath & Claassen 1999). A large amount of industrial developments has taken place within South Africa over the past two decades and has had a major impact on water resources. Pollution and impoundments has changed the biodiversity in many of the river systems whilst increased agricultural developments have amplified sediment load and pesticides in rivers like Olifants River in the Limpopo Province

(Dallas & Day 2004). Additionally, several fish kills have been reported within the river since 2007 (Heath *et al.* 2010;) and declining populations of Nile crocodiles, terrapins and African fish eagles have been reported with regard to pollution within the river (Myburg & Botha 2009; Ashton 2010; Botha *et al*. 2011).

In order to obtain a completely reliable assessment of pollution within aquatic ecosystems, physical and chemical monitoring should be supported by some means of biological monitoring. This is important because living organisms are indicative of the chemical condition of the water not only at present times but as they have experienced it throughout their lives (Abel 1989). Fish are considered to be suitable monitoring organisms in aquatic systems because as consumers they are high in the aquatic food chain and thus reflect any environmental changes that occur over time (Heath & Claassen 1999). Fish are often used in biological monitoring and bioaccumulation due to the fact that they are easily identifiable, can be sampled easily and quantitatively and have a cosmopolitan distribution often found throughout more than one river system. They are also known to accumulate pollutants in their organs and tissues (Hellawell 1986).

Metals are persistent and tend to accumulate in the environment, especially in the sediment (Coetzee *et al.* 2002). Excessively high concentrations of metals can be toxic to aquatic organisms resulting in a reduction of species richness and diversity and ultimately a change in ecosystem composition (Wepener *et al.* 2001; Dallas & Day 2004). However, usually the metals are attached to the suspended sediment and are trapped in the bottom sediment thereby eliminating pollutants from the water column and reducing the toxicity to aquatic organisms. As a result the sediment is considered to be a sink for pollutants and thus pose the highest risk to the aquatic environment (Salomons *et al*. 1987; Wepener and Vermeulen 2005). Metals bound in sediment have no direct danger to the aquatic ecosystem as long as they remain bio-unavailable. Dangers arise when there are changes in pH, water hardness, salinity, temperature or redox potential (Barker 2006). This allows bounded metals to be released back into the water (Van Vuren *et al*. 1994).

Metal pollution in South Africa and especially in the Upper Olifants River catchment area, is mainly attributed to water use for afforestation, mining and power generation, irrigation as well as domestic and industrial activities (Coetzee *et al.* 2002). A variety of studies have been carried out on the Olifants River with regards to metal bioaccumulation (Grobler *et al.* 1994; Seymore *et al*. 1994; Van Vuren *et al.* 1994; Seymore *et al.* 1995; Robinson & Avenant-Oldewage 1997; Kotze *et al.* 1999; Avenant-Oldewage & Marx 2000a, b; Coetzee *et al.* 2002), to name a few. However no research was done in the Flag Boshielo Dam with regard to metals accumulation in fish muscle tissue. Flag Boshielo Dam (study area) receives water from the upper Olifants River catchment area and with it also all the accompanying pollutants from households, mines, factories and farms. In this study, metalloids and metals were determined in the water, sediment and the muscle tissue of two fish species (*Labeo rosae* and *Schilbe intermedius*) from the dam. This chapter therefore covers bioaccumulation of metals in the two fish species, as well as their concentration levels in the sediment. The protocol of Heath *et al.* (2004) was followed whereby only the fish muscle tissue was tested for metals, not the other fish tissues. The fish muscle tissue forms a major edible portion of the whole fish; hence is a good indicator for human health risk assessment upon consumption of the fish.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Field work

Sediment samples of 500 ml were collected biannually at the inflow, middle of the dam and the dam wall using a Friedlinger mud grab (225 $cm^3$). The samples were put in acid treated sampling bottles and immediately refrigerated for laboratory analysis. Fish were collected by means of gill nets with different mesh sizes. The nets were set at various sites in the dam depending on the depth, vegetation and substratum. The live fish were kept in large holding tanks filled with dam water. Fish was then sacrificed by severing the spinal cord behind the head and dissected on a polyethylene work surface using stainless steel dissecting tools; care was taken to prevent contamination. Ten grams of muscle tissue was removed and placed in aluminium foil. The muscle samples were then frozen for laboratory analysis of metals. The muscle samples were collected from five fishes per survey from the two fish species.

### 3.2.2 Laboratory work

All the samples were analysed for metals by an accredited chemical laboratory in Pretoria (WATERLAB (PTY) LTD). At the water laboratory, the fish muscle tissue samples were thawed and dried, then digested with nitric acid and hydrogen peroxide. Inductive coupled plasma optical emission spectrometry (ICP-OES) was used for the detection of metals present in the sediment and muscle tissue samples.

### 3.2.3 Results analysis

The sediment quality results were interpreted following Sediment Quality Guidelines (SQGs) compiled by Burton (2002) for North America. The guidelines are described at three levels; the Threshold Effect Level (TEL), Probable Effect Level (PEL) and Toxic Effect Threshold (TET). However comparisons were checked against the TEL because it is the level at which no negative effects are be expected. A Human Health Risk assessment was carried out to determine whether consumption of fish from Flag Boshielo Dam might result in adverse human health effects. The health risk assessment was carried out according to the methodology as described by the USEPA (1988, 1996) and the WHO (2002) as stated in Jooste *et al.* (2012) report. For metals that cause non-cancer toxic effects, a Hazard Quotient (HQ) was calculated. The HQ is a value comparing the expected exposure to an exposure that is assumed not to be associated with toxic effects (Jooste *et al.* 2012). Any HQ less than one is considered to be safe for a lifetime exposure. The HQ for barium, cadmium, cobalt, copper, lead, silver, tin and titanium were not calculated because their levels in the fish muscle tissue were below the “safe” daily limit for human consumption (Jooste *et al.* 2012). Potential human health risks are illustrated in Table 3.2 as HQ. Highlighted cells indicate risks that are considered to be “unacceptable” by the USEPA and WHO.

## 3.3 RESULTS AND DISCUSSION

The 20 metal concentrations recorded from the sediment and the muscle tissue of the *Labeo rosae* and *Schilbe intermedius* are summarised in Table 3.1. Metals such as beryllium, bismuth, molybdenum and wolfram (tungsten) were analysed but were not included in the table because they were below the detection limits, either in the sediment or the muscle tissue of the two fish species.

Table 3.1: Average metalloids and metal concentrations and the standard deviations recorded in the sediment and the muscle tissue of *Labeo rosae* and *Schilbe intermedius* collected from Flag Boshielo Dam.

| METALS (mg/kg) | Sediment (n=3) | *Labeo rosae* (n=15) | *Schilbe intermedius* (n=8) |
|---|---|---|---|
| Aluminium | 37719 ±34976 | 63 ±6.6 | 93 ±41 |
| Antimony | 7.2 ±5.4 | 16 ±36 | 12 ±13 |
| Arsenic | 1.8 ±3.7 | 0.8 ±0.6 | 0.7 ±1.0 |
| Barium | 497 ±239 | 27 ±3.0 | 33 ±4.5 |
| Boron | 241 ±96 | 120 ±23 | 121 ±17 |
| Cadmium | 0.8 ±1.0 | 0.3 ±0.9 | 0 |
| Chromium | 87 ±52 | 45 ±5.6 | 48 ±3.6 |
| Cobalt | 16 ±11 | 2.7 ±5.5 | 1.7 ±1.8 |
| Copper | 48 ±20 | 5.8 ±1.7 | 9.6 ±3.4 |
| Iron | 42834 ±14693 | 1068 ±576 | 1545 ±517 |
| Lead | 25 ±15 | 3.3 ±1.1 | 3.2 ±1.1 |
| Manganese | 2027 ±2437 | 9.7 ±4.6 | 36 ±26 |
| Nickel | 47 ±25 | 2.5 ±0.8 | 3.2 ±1.1 |
| Selenium | 2.1 ±1.8 | 13 ±39 | 2.0 ±0.8 |
| Silver | 704 ±451 | 0 | 0.1 ±0.3 |
| Strontium | 27 ±10 | 12 ±4.6 | 88 ±72 |
| Tin | 6.3 ±5.5 | 3.5 ±2.4 | 5.3 ±2.2 |
| Titanium | 1353 ±739 | 1.4 ±0.1 | 1.7 ±0.4 |
| Vanadium | 70 ±31 | 32 ±4.3 | 29 ±2.5 |
| Zinc | 373 ±217 | 50 ±26 | 164 ±92 |

**Aluminium**

Aluminium concentrations were recorded at an average of 37719 mg/kg in the sediment. Aluminium in the muscle tissue of *S. intermedius* (93 mg/kg) was higher than in *L. rosae* (63 mg/kg) (Table 3.1). The recorded levels in the muscle tissue of both fish species were higher compared to previous studies done in South African waters (Coetzee *et al.* 2002; Crafford & Avenant-Oldewage 2010); however, records from the sediment were lower. Aluminium was the second most abundant (to iron) record in the sediment and the two fish muscle tissue samples. There are no SQGs for aluminium available; however its concentration in the water was recorded above the TWQR for aquatic ecosystems (see Chapter 2). Probably the sediment might be the source of the undesirable concentrations in the water.

Even though aluminium was recorded at concentrations above the TWQR in the water and in the sediment, it was seemingly not biologically available as it did not bioaccumulate in the fish muscle tissue (see Chapter 2). Some studies have shown that aluminium does not appear to accumulate to any significant degree in many aquatic organisms; including fish (Rosseland *et al.* 1990). This could also bare evidence with HQ calculation below one in both fish muscle tissue. Hence even though Al accumulated in high concentrations in the fish muscle tissue, the levels may not pose any adverse health effects upon human consumption. With regard to humans, prolonged exposure to toxic levels of aluminium has been implicated in chronic neurological disorders such as dialysis dementia and Alzheimer's disease (ATSDR 2008a). It is, however, not clear whether the presence of aluminium causes such conditions or is an indicator of other factors. Therefore, the link between aluminium in water and adverse effects on human health remains to be inconclusive.

Table 3.2: Calculated Hazard Quotients for the consumption of *Labeo rosae* and *Schilbe intermedius* from Flag Boshielo Dam (adapted from Jooste *et al.* 2012)

| **Elements** | ***Labeo rosae*** | ***Schilbe intermedius*** |
|---|---|---|
| Aluminium | 0.0429 | 0.0646 |
| Antimony | 37.5 | 17.857 |
| Arsenic | 19.048 | 1.9048 |
| Boron | 0.425 | 0.4464 |
| Chromium | 10.238 | 11.667 |
| Iron | 2.331 | 3.7714 |
| Manganese | 0.049 | 0.1888 |
| Nickel | 0.0714 | 0.1107 |
| Selenium | 0.2714 | 0.0243 |
| Strontium | 0.016 | 0.1083 |
| Vanadium | 2.3214 | 2.1429 |
| Zinc | 0.1095 | 0.4262 |

### Antimony

The highest antimony concentration was recorded in the muscle tissue of *L. rosae* (16 mg/kg) followed by *S. intermedius* muscle tissue (12 mg/kg) and the lowest in the sediment (7.2 mg/kg) (Table 3.1). Compared to most elements, little is known about the environmental behaviour of antimony, especially with respect to its mobility in sediments and soils (Krupka & Serne 2002). Additionally, antimony is not well studied in South African waters; this may be due to its low abundance in the sediment, hence regarded as unimportant. The muscle tissue of the two fish species had higher antimony levels as compared to the sediment (Figure 3.1). This can be an indication of biomagnification of the antimony in the food web, however it has been reported that high antimony concentrations are usually found in the upper trophic levels of the aquatic food chain (Zhiyou *et al.* 2011).

The calculated HQ value for antimony in both fish muscle tissue was above one, indicating that antimony intoxication might prevail upon consumption of these fishes. *Labeo rosae's* HQ (35.7) was the extremely higher than that of *S. intermedius* (17.9) (Table 3.2). In general antimony (III) is more toxic than antimony (V) (WHO 2003a), and the inorganic compounds are more toxic than the organic compounds (ATSDR 1992), with stibin ($SbH_3$), a lipophilic gas, being most toxic (by inhalation). However, it does not bioaccumulate in human tissues, so exposure to naturally occurring antimony through food is very low (WHO 2003a). Expected adverse effects in humans are from soluble antimony salts, whereby after oral uptake, it exerts a strong irritating effect on the gastrointestinal mucosa and trigger sustained vomiting. Other effects include abdominal cramps, diarrhea and cardiac toxicity (WHO 2003a). On the other hand, its toxicity to fish depends on water solubility and oxidation state of the antimony species under consideration (WHO 2003a).

### Arsenic

Arsenic was recorded at 1.8 mg/kg in the sediment (Table 3.1). In the muscle tissue arsenic was recorded at 0.8 mg/kg in *L. rosae* and 0.7 mg/kg in *S. intermedius*. Previous studies in South African waters (Botes & van Staden 2005) recorded arsenic at concentrations higher than levels recorded during this study. Arsenic has been classified as a carcinogen (USEPA 2007) and it is also one of the endocrine disruptive metals (EDMs) which can cause cancer. Exposure to arsenic results in reduced growth and reproduction in both fish and invertebrate populations. It can also cause fish behavioural changes such as reduced migration (DWAF 1996b). On contrary to humans, aquatic organisms have been shown to develop tolerance and reduction in adverse effects when duration of exposure to arsenic at a given concentration is prolonged (DWAF 1996c). The response of aquatic organisms to arsenic is reduced by pre-exposure, and organisms may become gradually acclimated to high concentrations in aquatic ecosystems (DWAF 1996c). Although inorganic arsenic does not accumulate in aquatic organisms, various forms of arsenic are lipid-soluble and therefore may accumulate in fatty tissue (DWAF 1996c). Even though bottom-feeding fish are most likely to accumulate arsenic, its accumulation is usually higher in algae and invertebrates than in fish (Foata *et al.* 2009). This may explain the presence of higher levels of arsenic in the muscle tissue of *L. rosae* as compared to *S. intermedius*, because the former feed mostly on plant materials.

The level of arsenic recorded in the muscle tissue of both fish species had an HQ value above one, an indication that adverse health effects may be probable upon fish consumption. The HQ value for arsenic in *L. rosae* muscle tissue was extremely high (19.05) as compared to that of *S. intermedius* (1.91) (Table 3.2). Given the many sources of arsenic to humans, food is usually the largest source of exposure (ATSDR 2007a). The predominant dietary source of arsenic is seafood, followed by rice/rice cereal, mushrooms and poultry. While seafood (fish and shellfish) contain the greatest levels of arsenic, this is mostly in an organic form called arsenobetaine that is much less harmful (ATSDR 2007a; DWAF 1996c). Contrary to that, humans are more sensitive to arsenic than are aquatic organisms (DWAF 1996c), and it has been reported to be slowly excreted from the human body, hence it can easily accumulate or stored in different tissues (Kapaj *et al.* 2006). Human chronic exposure to arsenic has been shown to increase the risk of skin, lungs, urinary bladder, and possibly kidney, liver and prostate cancer (Kapaj *et al.* 2006). It can also affect the peripheral nervous system and the blood count. Although some studies suggest that arsenic may also contribute to poor circulation, high blood pressure, heart disease, liver toxicity, diabetes, and reproductive effects, its role in these illnesses has not been clearly defined (DWAF 1996a). Hence, given the HQ far above recommended levels (>1), consumption of fish collected from Flag Boshielo Dam can cause adverse health (Jooste *et al.* 2012).

**Barium**

Barium concentrations were the highest in the sediment (497 mg/kg) followed by 33 mg/kg in *S. intermedius* muscle tissue and the lowest in *L. rosae* muscle tissue at 27 mg/kg (Table 3.1). Barium in sediment is found largely in the form of barium sulphate (barite). The level of barium in the sediment recorded in this study was higher as compared to the levels recorded from the study done by Botes and van Staden (2005) in a tributary of the Olifants River near Ohrigstad. The dominant agricultural activities in the catchment of Flag Boshielo Dam maybe the main anthropogenic source of barium in the sediment because, barium concentrations in fertilizers and soil amendments range from <0.2 to 669 µg/g (ATSDR 2007b). Hence, continued use of fertilizers and soil amendments may result in accumulation of barium in sediment from agricultural run offs.

The barium levels recorded from the two fish species had an HQ below one (Table 3.2), indicating that barium adverse health effects may not be expected upon human consumption of the two studied fish species. Direct toxicity of barium to fish is not known, however from studies done on daphnids it appears that barium may pose certain risks to some aquatic populations (ATSDR 2007b). However, some studies have reported that fish and other freshwater and marine life can accumulate barium (ATSDR 2007b).

**Boron**

Higher boron levels were recorded in the sediment (241 mg/kg) than in *S. intermedius* (121 mg/kg) and *L. rosae* (120 mg/kg). Boron cannot be destroyed in the environment. It can only change its form or become attached or separated from particles in soil, sediment, and water (ATSDR 2010). Boron can exist in various forms but the most important one biologically is boric acid (Ronald 1990). It is an essential trace element for the growth and development of higher plants (Ronald 1990); however its purpose in animals and humans is not well established. Boric acid is the main form in which boron is present in biological fluids in plants and animals, and only has a low ability to move into fat. Laboratory experiments have confirmed that boric acid does not bioaccumulate in animals which live in water (USEPA 2008). In humans boron is readily absorbed from the gastrointestinal tract (USEPA 2008). However, given the HQ level below one recorded during this study, adverse health effects may not be expected from the boron levels in the muscle tissue of the two fish species.

**Cadmium**

Cadmium was not detected in *S. intermedius* muscle tissue. In the sediment it was recorded at 0.8 mg/kg and in *L. rosae* at 0.3 mg/kg (Table 3.1). Similar levels as recorded in *L. rosae* muscle tissue were recorded in the muscle tissue of *L. capensis* (~0.3 µg/g) collected from Mooi River (van Aardt & Erdmann 2004). However, cadmium levels in the sediment were recorded at very low concentrations as compared to other studies done within the South African rivers (Grobler 1994; van Aardt & Erdmann 2004; Botes & van Staden 2005). The levels recorded from Flag Boshielo Dam in the sediment were above TEL (0.6 mg/kg) of Sediment Quality Guidelines (SQGs) (Burton 2002). In natural waters, cadmium is found mainly in bottom sediment and suspended particles (WHO 2011). However, the cadmium levels in the fish muscle tissue had HQ value less than one (Table 3.2). Even though the fish muscle may not cause any adverse health effect upon consumption, the levels of cadmium in the sediment stay a potential risk to other aquatic biota including fish if it dissociate from the sediments into the water and become bio-available Cadmium is an EDM, toxic and carcinogenic to humans (ATSDR 2008b). Small amounts of cadmium in food and water enters the human body through the digestive tract. Cadmium is more likely to be retained from food in human's body if there is no enough iron or other nutrients in the diet (ATSDR 2008b). Once absorbed, it gets retained in the human body, in which it accumulates throughout life. Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells, the main site of accumulation (ATSDR 2008b). It can also cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction (ATSDR 2008b).

**Chromium**

Chromium levels in the sediment had an average of 87 mg/kg (Table 3.1). In the muscle tissue of *L. rosae* it was recorded at 45 mg/kg and of *S. intermedius* at 48 mg/kg (Table 3.1). The recorded levels in the sediment were much lower than previous studies done in the Olifants River (Robinson & Avenant-Oldewage 1997; Avenant-Oldewage & Marx 2000a; Nussey *et al.* 2000), except from the study done by Botes and van Staden (2005) that recorded higher levels of chromium. However, the levels recorded from the fish muscles were higher as compared to previous studies (du Preez *et al.* 1997; Robinson & Avenant-Oldewage 1997; Avenant-Oldewage & Marx 2000a; Nussey *et al.* 2000; Coetzee *et al.* 2002). The recorded level in the sediments was above the TEL of SQGs (37.3 mg/kg) (Burton 2002).

Chromium is a naturally occurring element predominantly in its insoluble and nontoxic trivalent form [Chromium (III)] (See chapter 2 for different forms of chromium)]. However, the most biologically important form is the soluble and toxic hexavalent chromium (VI). Chromium (VI) is thermodynamically unstable in anoxic (without oxygen) sediment (Berry *et al.* 2004). Hence, aquatic biota toxicity may be expected because usually sediments are anoxic. Hexavalent chromium is persistence in the environment and has the ability to induce a variety of adverse effects in biological systems, including fish. It can also pose a significant threat to aquatic ecosystems (Velma *et al.* 2009).

The HQ values for chromium were above one for both fish species, whereby the HQ value for Cr in *S. intermedius* (11.7) was higher than that of *L. rosae* (10.2) (Table 3.2), indicating possible risks upon consumption of the two fish species. At elevated concentrations cadmium is acutely toxic and can cause severe renal damage with renal failure in humans. It also causes acute gastroenteritis which closely mimics the gastroenteritis caused by micro-organisms. The half-life of chromium in the body is several decades; hence, it is important to avoid exposure (DWAF 1996a). Absorption depends on chromium speciation; chromium (VI) appears to be absorbed from the gastrointestinal tract to a greater extent than chromium (III) (WHO 2011). The hexavalent chromium is regarded as carcinogenic to humans through inhalation (Berry *et al.* 2004).

**Cobalt**

Cobalt was recorded at an average of 16 mg/kg in the sediment, 2.7 mg/kg in *L. rosae* and 1.7 mg/kg in *S. intermedius* (Table 3.1). The recorded levels in the sediment were higher than Botes and van Staden (2005) results recorded from

Crocodile, Olifants and Bronkhorstspruit Rivers. The adsorption of cobalt on sediment and soils is largely controlled by the presence of iron and manganese oxide and clay minerals (Krupka & Serne 2002). Cobalt released into water may stick to particles in the water column or to the sediment at the bottom of the body of water into which it was released, or remain in the water column in ionic form. Both in soil and sediment, the amount of cobalt that is mobile will increase under more acidic conditions. Ultimately, most cobalt ends up in the soil or sediment (WHO 2006).

**Copper**

Sediment had an average of 48 mg/kg copper concentration; with *L. rosae* at 5.8 mg/kg and *S. intermedius* at 9.6 mg/kg. The concentrations recorded in fish tissues were in line with previous findings recorded from Mooi River (van Aardt & Erdmann 2004). However there are studies in the Olifants River which recorded levels lower than the recent study (du Preez *et al*. 1997; Kotze *et al.* 1999; Avenant-Oldewage & Marx 2000a). The levels in the sediments were higher than previous records (Avenant-Oldewage & Marx 2000a; Botes & van Staden 2005). The level of copper in the sediments was recorded above TEL of SQGs 35 mg/kg (Burton 2002). Copper is moderately soluble in water and binds easily to sediment and organic matter. It has the ability to bioaccumulate (DWAF 1996c). The most bioavailable and therefore most toxic form of copper is the cupric ion ($Cu^{2+}$). Fish and crustaceans are 10 to 100 times more sensitive to the toxic effects of copper than are mammals. Also aquatic animals are more sensitive than aquatic plants to the toxic effects of metals (Francis-Floyd 2009). In addition, the use of copper to kill algae, fungi and mollusks demonstrate that it is highly toxic to aquatic organisms (DWAF 1996b). Hence, the recorded levels may pose adverse effects upon the aquatic ecosystem, and the sediment is the potential source of unacceptable concentrations in water. The recorded copper levels in the muscle tissue of both studied fishes fall within the required range for human copper intake. Given the HQ value below one it can be assumed that no copper toxicity can be expected upon human consumption.

**Iron**

Iron was the highest metal recorded in the sediment and both fish species. In the sediment it was recorded at 42834 mg/kg, *S. intermedius* at 1545 mg/kg and *L. rosae* at 1068 mg/kg (Table 3.1). The recorded levels of iron in sediment and fish tissues were higher than records from previous studies (Avenant-Oldewage & Marx 2000a; Botes & van Staden 2005) except for results from Robinson & Avenant-Oldewage (1997). Iron is naturally released into the environment from weathering of sulphide ores and igneous, sedimentary and metamorphic rocks (DWAF 1996c). Sources from anthropogenic activities include burning of coke and coal, acid mine drainage, mineral processing, sewage, landfill leachates and the corrosion of iron and steel (DWAF 1996c), to name a few. Within the Olifants River catchment area, coal mining, acid mine drainage (Claassen *et al.* 2005) and sewage spills have been reported. However, the sources of iron in the sediments may be from either natural or anthropogenic activities because surrounding Flag Boshielo Dam is the Bushveld Igneous Complex type of rock formations which is a natural iron source (Ashton *et al.* 2001).

Iron can exist in aquatic systems (natural waters and their sediments) in several oxidation states: metallic iron (iron metal), ferrous iron (Fe II), and ferric iron (Fe III) (Javed 2005). It usually exists in the sediment in the reduced form, ferrous iron (Fe II) because sediments are commonly anoxic (without oxygen) (Phippen *et al.* 2008). In the anoxic sediment environment, the ferric hydroxide is reduced to ferrous (Javed & Saeed 2010). In aquatic systems where undisturbed sediments are in contact with the oxic water column, the surface of the sediment can have a reddish brown colour characteristic of oxidized, ferric hydroxide. This layer is underlain by the black ferrous sulfide/polysulfide of anoxic sediments (Jones-Lee & Lee 2005). The black layer can be mistaken for “organics” if the aqueous environmental chemistry of iron is not understood (Jones-Lee & Lee 2005). The possible toxic effects to fish from iron include fish gill damage by iron corrosive effects, asphyxiating the eggs laid in sediment and reduce visibility in water which can affect feeding success and other behaviour (Phippen *et al.* 2008).

Iron in the muscle tissue of both fish species was recorded at an HQ above one, indicating that iron toxic effects are probable upon consumption of the fish species by humans. Humans usually absorb iron from animal products than from plants. Even though it is essential for human blood (haemoglobin), if consumed at toxic levels it can affect the eyes (conjunctivitis, choroiditis and retinitis) and can remain in the tissues (WHO 2003b). When iron remains in the tissues it is usually accumulated in the liver and heart which can result in haemochromatosis (WHO 2003b).

Haemochromatosis is a genetic disease which results from a build-up of excess iron (because the individuals can absorb iron efficiently) and causes liver cirrhosis and heart failure.

**Lead**

Average lead concentration in the sediment was 25 mg/kg (Table 3.1). In the fish muscle tissue it was recorded at 3.3 mg/kg in *L. rosae* and 3.2 mg/kg in *S. intermedius*. The recorded levels in the sediment are lower than previous records in South African rivers (Grobler 1994; Nussey *et al.* 2000; Botes & van Staden 2005; Crafford & Avenant-Oldewage 2010). Contrary to sediments, previous records of lead in fish muscle tissue exceeded the present study (Seymore *et al.* 1995; du Preez *et al.* 1997; Nussey *et al.* 2000; Coetzee *et al.* 2002; Aardt & Erdmann 2004; Crafford & Avenant-Oldewage 2010). Lead poisoning to fish result in haematological (e.g. higher blood glucose levels), neuronal, muscular and other effects like black tails, lordoscoliosis, pigment alterations and coagulation of surface mucus (ATSDR 2007c). It can also result in a delay in larval development (ATSDR 2007c). Toxicity of lead is reduced in hard water (Galvin 1996) because calcium (major contributor to hard waters) has the ability to antagonize some of lead toxic effects (DWAF 1996c). The recorded levels in the muscle tissue did not exceed the maximum allowable concentrations for human consumption of lead in fish flesh (8 µg/g dry mass) (ATSDR 2007c), hence toxicity of lead from consumption of these fish may not be expected.

**Manganese**

The levels of manganese were much higher in the sediment samples than in the fish muscle tissue; sediment 2027 mg/kg, *L. rosae* 9.7 mg/kg, *S. intermedius* 36 mg/kg (Table 3.1). The recorded levels in the sediment and fish muscle tissue are higher than previous records in South African waters (du Preez *et al.* 1997; Robinson & Avenant-Oldewage 1997; Avenant-Oldewage & Marx 2000b; Nussey *et al.* 2000; Coetzee *et al.* 2002; Botes & van Staden 2005), except in the study of Seymore *et al.* (1995) where similar concentrations were recorded.

Manganese is not found naturally in its pure (elemental) form, but is a component of over 100 minerals (Howe *et al.* 2005). The recorded differences between sediment and fish tissue are not surprising because often manganese in water will settle into suspended sediment. The ability of manganese compounds to adsorb to sediment is reliant upon the cation exchange capacity and organic content of the sediment (ATSDR, 2008c). Approximately 91% of environmental manganese is released to soil (USEPA 2004). The main source of this release is land disposal of manganese-containing wastes. Manganese can bio-accumulate in lower organisms (e.g. phytoplankton, algae, mollusks, and some fish), but not in higher organisms, and bio-magnification in food-chains is not expected to be significant (ATSDR 2008c). Uptake of manganese by aquatic invertebrates and fish significantly increases with temperature and decreases with pH (USEPA 2004). The uptake by organisms has been found to increase with decreasing salinity (USEPA 2004).From this study, an HQ below one was recorded (Table 3.2); hence manganese toxicity may not be expected upon human consumption of these fish species.

**Nickel**

Nickel was recorded in the sediment at 47 mg/kg, in the muscle tissue of *L. rosae* at 2.5 mg/kg and *S. intermedius* at 3.2 mg/kg. These levels are much higher than records from previous studies (du Preez et al. 1997; Avenant-Oldewage & Marx 2000a; Botes & van Staden 2005; Crafford & Avanant-Oldewage 2010) except for the Nussey *et al.* (2000) records which were higher than recent records. Nickel was recorded at levels above TEL (18 mg/kg) in the sediment. Much of nickel released into the environment ends up in soil or sediment where it strongly attaches to particles containing iron or manganese. Under acidic conditions, nickel is more mobile in soil and might seep into groundwater. Nickel does not appear to accumulate in fish tissues (ATSDR 2005b). However, ATSDR (2005b) states that some plants can take up and accumulate nickel. This is also evident with the recorded HQ below one (Table 3.2).

**Selenium**

Selenium was recorded at higher levels in *L. rosae* (13 mg/kg) muscle tissue, as compared to the sediment (2.1 mg/kg) and *S. intermedius* muscle tissue (2.0 mg/kg) (Table 3.1). This may be an indication of biomagnification of selenium in *L. rosae* muscle tissue. Selenium may enter surface water from irrigation drainage waters (DWAF 1996c). Selenium is an essential nutrient for animals, humans, and microorganisms (Ellis & Salt 2003). Some plants take up selenium and sometimes accumulate it to an extent which it can be accumulated or toxic to animals feeding on plant materials. With the recorded levels in the herbivorous *L. rosae* it can be assumed that selenium biomagnified through the food chain. Some evidence indicates that selenium can be taken up in tissues of aquatic organisms and possibly increase in concentration as the selenium is passed through the food chain (ATSDR 2003). However, selenium toxicity may not be expected upon consumption of *L. rosae* muscle tissue because the HQ was below one (Table 3.2).

**Silver**

Silver was not detected in *L. rosae* muscle tissue; this finding is in line with Rodgers *et al*. (1997) as they have found that fathead minnows were not sensitive to silver and did not accumulate it. It was only detectable in the muscle tissue of *S. intermedius* at an average of 0.1 mg/kg and in the sediment at 704 mg/kg (Table 3.1). Silver can disperse as dissolved or colloidal species, but ultimately it accumulates in bottom sediment, hence, higher levels in the sediments than in the fish tissues. Bioavailable silver (especially silver nitrate) is relatively toxic to fish and other aquatic organisms (USEPA 1980; USEPA 2005) and it has affinity for particulate matter and sediment (Rodgers *et al.* 1997).

**Strontium**

Levels of strontium in the sediment were recorded at an average of 27 mg/kg, in the muscle tissue of *L. rosae* at 12 mg/kg and of *S. intermedius* at 88 mg/kg. The recorded levels in the fish muscle tissue were higher than results from Avenant-Oldewage & Marx (2000a) but lower than Crafford & Avenant-Oldewage (2010) findings in the Vaal Dam. Strontium is of little biological interest however; its chemistry and its biological cycling are similar enough to those of other alkaline earths, particularly calcium and magnesium (Vitousek *et al.* 1999). In localities where strontium is elevated, it is an important freshwater quality ion which contributes to water hardness (Chowdhury & Blust 2002). As is the case for barium and calcium, there are relatively few organometallic compounds of strontium; their industrial uses are few and their toxicology is of limited concern. Although pure strontium does not appear to be very toxic, many strontium compounds are hazardous to fish and wildlife (Mellado *et al.* 2002). Strontium toxicity from the studied fish may not be probable because the HQ was below one (Table 3.2).

**Tin**

Tin concentration in the sediment was recorded at 6.3 mg/kg and in the muscle tissue *L. rosae* at 3.5 mg/kg and of *S. intermedius* at 5.3 mg/kg (Table 3.1). Tin occurs naturally in the Earth's crust, with an average concentration of approximately 2–3 mg/kg (Budavari 2001). Tin binds to soils and to sediment in water and is generally regarded as being relatively immobile in the environment. Its compounds may also settle out of the water into sediment and may remain unchanged for years (ATSDR 2005c). Organic tin compounds may be taken up into the tissues of aquatic animals. It was estimated that the bioaccumulation factors of inorganic tin were 100, 1000, and 3000 for marine and freshwater plants, invertebrates, and fish, respectively (WHO 2005).

**Titanium**

Titanium was recorded at significantly higher levels in the sediment (1353 mg/kg) as compared to the fish muscle tissue; *L. rosae* 1.4 mg/kg and *S. intermedius* 1.7 mg/kg. Although it is not found unbound to other elements in nature, titanium is the ninth most abundant element in the Earth's crust (0.63% by mass) and is present in most igneous rocks and in sediment derived from them (ATSDR 1997). The presence of high concentrations of titanium in the sediment can be explained by igneous rock formation surrounding Flag Boshielo Dam. There is no known biological role for titanium (NIOSH 2011). Some of the titanium compounds may settle out to soil or water. In water, they sink into the bottom sediment. They may remain for a long time in the soil or sediment. No environmental effects have been reported.

**Vanadium**

Vanadium was recorded in the sediment at 70 mg/kg, *L. rosae* muscle tissue at 32 mg/kg, and *S. intermedius* muscle tissue at 29 mg/kg (Table 3.1). Vanadium levels in the sediment were higher as compared to the levels recorded in the previous studies done in the Olifants River (Grobler 1994; Botes & van Staden 2005). Vanadium had a HQ above one for both fish species (*L. rosae* = 2.3 and *S. intermedius* = 2.1)

(Table 3.2), indicating that vanadium toxicity is possible upon human consumption of the two fish species.

Some studies suggested that the potential for bioaccumulation or bioconcentration of vanadium is low or limited for mammals, birds, and fish. It has a natural affinity for fats and oils (Irwin *et al.* 1997). The major anthropogenic sources of vanadium pentoxide are the burning of certain fossil fuels, which is one of the anthropogenic activities in the upper catchment of Flag Boshielo Dam. The element is found only in chemically combined form in nature. Vanadium occurs naturally in about 65 different minerals and in fossil fuel deposits (Irwin *et al.* 1997). Large amounts of vanadium ions are found in some organisms, possibly as a toxin. The oxide and some other salts of vanadium have moderate toxicity. Vanadium is probably a micronutrient in mammals, including humans, but its precise role in this regard is unknown.

Information on the toxic threshold of vanadium in humans is rather sparse and animal studies suggest that there is a strong interaction between dietary composition and susceptibility to symptoms of vanadium poisoning (Irwin *et al.* 1997). Vanadium affects the metabolism of the amino acid cytosine; resulting in a reduction of the concentration of coenzyme A. Symptoms of vanadium toxicity include conjunctivitis, rhinitis, a sore throat and a persistent cough (DWAF 1996c). Other sources report it to be in diabetes medications since it can act as insulin (Irwin *et al.* 1997).

**Zinc**

Zinc was recorded at an average of 373 mg/kg in the sediment, 50 mg/kg in *L. rosae* and 164 mg/kg in *S. intermedius* muscle tissue (Table 3.1). Previous sediment studies done in the Olifants River recorded zinc at very low concentrations or levels below detection (Grobler 1994; Kotze *et al*. 1998; Coetzee *et al.* 2002; van Aardt & Erdmann 2004; Botes & Staden 2005; Bollmohr *et al.* 2008) as compared to the current study. Zinc levels in the sediment were above TEL (123 mg/kg) according to Burton (2002) sediment quality guidelines. This could have adverse health effects on the fish in Flag Boshielo Dam. However, from the human risk assessment results (HQ below one); the levels recorded from both fish species may not pose any risk upon human consumption (Table 3.2).

## 3.4 CONCLUSIONS

### Sediment

Most metals were recorded at higher levels in the sediment than in water and the fish muscle tissue. The detection of more metals at higher concentrations in the sediment is not surprising as the sediment serves as a sink of metal contamination in aquatic ecosystems (Milenkovic *et al.* 2005). Furthermore, in most aquatic systems, the concentrations of metals associated with sediment are far greater than the concentrations dissolved in the water column. Sediment accumulates contaminants and serves as sources of pollution to the ecosystems that are associated with it (Milenkovic *et al.* 2005). Iron (42834 mg/kg) and aluminium (37719 mg/kg) was the most abundant metal recorded in the sediment (Table 3.1), followed by manganese at 2027 mg/kg. The concentration levels of cadmium, nickel and zinc were above the TEL of sediment quality guidelines. When comparing metal concentrations recorded in the sediment and the two fish species, the sediment accumulated metals at higher concentrations with the exception of antimony, boron, chromium, selenium, strontium, tin, vanadium and zinc. This means that the other metals were not bio-available as bioaccumulation is an indication of the bio-availability of metals.

### *Schilbe intermedius*

The most abundant metal recorded in the muscle tissue of *S. intermedius* was iron (1545 mg/kg). Second highest to iron was zinc (164 mg/kg), followed by boron (121 mg/kg), strontium (88 mg/kg), aluminium (93 mg/kg), and chromium (48 mg/kg), in descending order. Cadmium was not detected in the muscle tissue of *S. intermedius*. Similar to *L. rosae*, most metal concentrations were detected below "safe" daily limit for human consumption in *S. intermedius*. Antimony, arsenic, chromium, and to a lesser extend iron and vanadium were the metals that may cause risks because the HQ is above one.

### *Labeo rosae*

The most abundant metal recorded in the muscle tissue of *L. rosae* was iron (1068 mg/kg). Second highest to iron was boron (241 mg/kg), followed by aluminium (63 mg/kg), zinc (50 mg/kg), chromium (45 mg/kg) and barium (27 mg/kg) in a descending order. Silver and cadmium were not detected in the muscle tissue of *L. rosae*. Most of the tested metal concentrations were detected below "safe" daily limit for human consumption in *L. rosae* muscle tissue. However, the HQ calculated values for antimony and arsenic were extremely high (>1). Therefore, antimony, arsenic, chromium, and to a lesser extend iron and vanadium may be the metals that may cause risks upon human consumption of *L. rosae*.

The hypothesis that metals can accumulate in fish tissues was supported. On overall comparison between the two fish species, *S. intermedius* accumulated more metals in terms of numbers higher concentrations than *L. rosae*. This may have been due to different food items they feed on. *Schilbe intermedius* is an opportunistic predator with a diet consisting largely of fish, aquatic insect larvae, terrestrial insects, aquatic insects and crustaceans, in decreasing order (Winemiller & Kelso-Winemiller 1996). Given such a wide variety diet, it increases its potential to accumulate more metals also at higher concentrations (biomagnification). Tertiary trophic level consumers, like *S. intermedius*, are more exposed to metals through biomagnification. On the other hand, *L. rosae* normally feed on detritus, algae and small invertebrates (Skelton 2001) and are therefore primary and secondary trophic level consumers. Primary and secondary trophic level consumers like the rednose labeo are less exposed to biomagnification than tertiary consumers.

# CHAPTER 4

# FISH HEALTH AND FISH PARASITES

## 4.1 INTRODUCTION

Healthy ecosystems are comprised of balanced populations of indigenous organisms with diverse structural and functional organisations (Landsberg *et al.* 1998), and also have complex trophic structures with many species forming the food web. In a freshwater ecosystem, fish are on or near the top of that food web and are one of the most common and useful bio-indicators of aquatic ecosystems. They are great bio-indicators because they spend all of their lives in water and they are constantly surrounded by it. They are common in most freshwater ecosystem and easy to catch and identify. Also, different fish species have different tolerances to pollution. No other aquatic organism is suitable for the application of so many different methods, which allow the evaluation of the severity of toxic impacts by determining the accumulation of toxicants in tissues, by using histological and haematological approaches, by studying their parasites or by detecting morphological anomalies. Thus bio-monitoring using fish represents a good monitoring tool especially with regard to pollution aspects (Grabarkiewicz & Davis 2008).

Fish and other aquatic animals are subject to a broad variety of stressors because their homeostatic mechanisms are highly dependent on prevailing conditions in their immediate surroundings. Examples of stressors for fish include fluctuations in water salinity, pH, water hardness, alkalinity, dissolved solids, water level or current, and exposure to waterborne pathogens or toxicants (Harper & Wolf 2009). The response of fish to stress may be hormonal or physical, depending on the type of stress or period of exposure to stress. This study focuses mainly on physical indicators of stress. Physical stress responses are detectable with the naked eye or through microscopic examination, for examples organs or tissues such as the gills, liver, skin, and components of the digestive tract. The response of gills to stress may be gill epithelial hypertrophy and hyperplasia, goblet cell proliferation with increased mucus secretion, hemorrhage, oedema, telangiectasis, etc. (Francis-Floyd 2009; Harper & Wolf 2009). The skins visible responses to stressors include inflammations, dermal ulcerations, lesions, epithelial erosion and ulcers that primarily affect the fins and/or hyper- or hypo-secretion of mucus (Francis-Floyd 2009). The liver size and coloration, detection of hepatic necrosis, hemorrhaging or lipidosis can also indicate fish stress response (Harper & Wolf 2009), just to name a few.

Most of the human impacts on the aquatic environment affect the health of the resident fish fauna, eventually causing parasitic infections, disease and associated mortalities (Poulin 1992). Furthermore, parasitic diseases of fish are very common all over the world (Roberts & Janovy 2000), and are of particular importance (prevalence and/or abundance) with regard to water quality. Parasites, just like fish are also an indigenous component of food webs. The parasites of fish reflect the life habits of the fish, including their interactions with the benthic, planktonic, and fish communities. Evaluation of parasitic fauna of selected fish species within a particular freshwater environment may provide both qualitative and quantitative bioindicators indicative of, and to changes in, the overall health of that aquatic environment. Parasites are indicative of many biological aspects of their hosts, including diet, migration, recruitment and phylogeny (Williams *et al*. 1992). They are also good direct indicators of environmental quality status (Marcogliese & Cone 1997). There have been a vast growing number of reasonable successful attempts to use parasites in environmental impact studies, such as investigating effects of pollution on parasite communities, mean intensity, abundance and prevalence, and their fish hosts (Williams *et al.* 1992; Mosquera *et al.* 2003; Bayoumy 2008). To add on that, parasites with complex life cycles may provide information about the biological properties of different biotopes within an ecosystem by synthetically recording the presence of intermediate, paratenic and definitive hosts (Cone *et al*. 1993). Parasites can thus be considered complementary to fish health assessment, chemical analysis or traditional biological surveys (bacteria counts or invertebrate assessments) as indicators of dysfunctional ecosystems.

The increasing interest on fish parasites as bioindicators can be related to the high number of parasite species commonly found in or on fish (Sures 2001) and the variety of ways in which they respond to anthropogenic pollution. The majority of recent investigations have examined the effects of various forms of pollution on the abundance and distribution of parasites. There are several rules derived from trends of how different parasites respond to pollution. Several hundred papers have been published since 1980 that are directly concerned with the relationship between pollution and parasitism in the aquatic environment (reviewed by Khan & Thulin 1991; Poulin 1992; Vethaak & Rheinallt 1992; Overstreet 1993; MacKenzie *et al*. 1995; Lafferty 1997; Kennedy 1997; Sures *et al*. 1997; Valtonen *et al*. 1997; Sures *et al*. 1999; Sures 2001; Madanire-Moyo *et al.* 2012).

### 4.1.1 Fish HAI and PI

In this study, the fish health is assessed by using the Health Assessment Index (HAI) with the Parasite Index (PI) incorporated. This HAI was initially proposed in USA by Adams *et al*. (1993). It is a quantitative index that allows statistical comparison of fish among datasets. The index was based on modifications of the autopsy-based system developed by Goede and Barton (1990). When applying the index, a numerical value is awarded to examined fish tissue or organs depending on the degree of stressors-induced abnormalities. The total sum of values awarded is the index value for that fish and the mean for all sampled fish is the index value for that locality. An increase in the index value correlates with decreased water quality (Crafford & Avenant-Oldewage 2009). In the original HAI by Adams *et al*. (1993), parasites were merely recorded as being present or absent. 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 in the Olifants River system (e.g. Avenant-Oldewage *et al.* 1995; Watson 1998; Marx 1996; Luus-Powell 1997; Jooste *et al.* 2004 & 2005; Crafford & Avenant-Oldewage 2009; Madanire-Moyo *et al.* 2012). The HAI has proven to be a simple and inexpensive means of rapidly assessing general fish health in field situations.

Given the relevance of parasite data in environmental monitoring, the original HAI notation was thus expanded and developed into a Parasite Index tested at the Lower Olifants River in conjunction with the HAI (Marx 1996; Robinson 1996; Luus-Powell 1997). The PI was developed to use as an index to detect pollution effects on parasites. It can also reflect the effect of the number of parasites on the HAI i.e. fish health. The parasite assemblage of fish can have a potential role as response indicators to environmental stress in relation to the PI. Therefore the index based on the parasite assemblage of fish could be integrated with other indices of biological integrity (such as the HAI). Because of the way parasites respond to pollution, the parasites of fish could be more sensitive indicators of environmental stressors than the fish themselves.

### 4.1.2 Condition factor

Knowledge of quantitative aspects such as weight-length relationship, condition factor, growth, recruitment, and mortality of fishes is important tools for the study of fish biology, mainly when the species lies at the base of the higher food web. The fish condition factor (K) reflects, through its variations, information on the physiological state of the fish in relation to its welfare. In fisheries science, the condition factor is used to compare the “condition”, “fatness” or wellbeing of fish (Williams 2000), and it is based on the hypothesis that heavier fish of a particular length are in a better physiological condition. It is also a useful index for the monitoring of feeding intensity, age, and growth rates in fish (Abowei 2009). It is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (Gislaine *et al.* 2011). In this study, the condition factor was used to assess the fish condition and fish population welfare in conjunction with the HAI.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Field work

Fish were collected by means of gill nets with different mesh sizes (30-120 mm). Ten specimens of each fish species were assessed seasonally. As soon as a fish is removed from the gill nets, macroscopic examinations were done on the boat for mobile ectoparasites. All mobile ectoparasites were collected and recorded and kept in small glass containers filled with water from the respective site for further processing in the field laboratory. Sampled fish were kept in large aerated holding tanks filled with dam water to minimise stress.

In the field laboratory, one fish was selected at a time to determine the HAI and PI. Two skin smears were made with a glass slide and examined with the aid of a stereo microscope. Blood was drawn from the dorsal aorta of the fish as quickly as possible before the fish dies. Capillary tubes were filled with blood and plugged at one end using commercial Critoseal$^{TM}$ clay, for centrifugation. After centrifugation (15 000 rpm for 5 minutes) the haematocrit values was read with a haematocrit reader and recorded. The fish was then sacrificed by severing the spinal cord. The mass of each fish was determined in grams using a Mettler$^{TM}$ balance, and recorded. The total length, fork length and standard lengths of each fish was measured using a measuring board calibrated in millimetres (mm).

The fish was then opened ventrally so that the body cavity and mesenteries can be examined for parasites. Different organs such as eyes, swim bladder and guts were placed in separate petri-dishes containing saline solution and examined for parasites with the aid of a stereo microscope. The gills were placed in dam water to prevent dehydration and also examined for ectoparasites. All the organs removed from the fish were examined for parasites using a Leica Stereo microscope.  The fish was examined externally and internally by using the revised HAI method and recorded on a HAI data sheet (indicating any abnormalities of the skin, eyes, fins and gills – See Appendix C, Table 1). The liver, spleen and bile colour were assessed with the aid of a colour chart developed by Watson (2001).

### 4.2.2 Preserving and mounting parasite specimens

The monogeneans used for measurements of the hamuli (anchors) and marginal hooks were mounted in glycerine jelly and sealed with a clear nail varnish and labelled. Digeneans were placed in saline solution and shaken vigorously from time to time to dislodge debris; sometimes a fine brush was used. They were fixed flat between two slides in hot AFA for approximately 30 minutes and preserved in 70% ethanol. Nematodes were removed by a brush and fixed in glacial acetic acid for approximately 2 minutes and preserved in 70% ethanol. Copepods were fixed by adding 70% ethanol to the water in small quantities over a period of approximately one hour and preserved in 70% ethanol. The parasites which were not used for whole mounts were fixed and preserved in 4% formaldehyde. Preparation of whole mounts (monogeneans and digeneans) and identification of different parasites were done at the laboratory where specimens were stained either with Horen's Trichome™ or Aceto Carmine™ solution. Parasites were cleared in lactophenol or clove oil for 10 minutes or overnight if necessary. Specimens were mounted on pre-cleaned glass slides with Canada balsam™ or Entellan™ and labelled. Nematodes were cleared with lactophenol and mounted without staining (temporary mounts). Parasite photographs were taken with the aid of a Wild™ stereo microscope or Olympus™ compound microscope which has an attached Olympus™ digital camera adapter and an Olympus™ digital camera (C50-50 Zoom).

### 4.2.3 Results analysis

Original field designations of all variables from the necropsy-based system (e.g. normal, clubbed gills, missing eye, etc.) were substituted with comparable numerical values into the HAI. All variables of the HAI were represented by a value ranging from 0 – 30, depending on the condition of the organs tested. Normal conditions are indicated by zero. To calculate an index value for each fish within a sample, numerical values for all variables are summed. To calculate the HAI for a sampled population, all individual HAI values were summed and divided by the total number of fish examined for that sample.

The inverted PI was followed whereby endo- and ectoparasites are incorporated as a separate variable in the HAI tested in South Africa (Crafford & Avenant-Oldewage 2009). Contaminants have different influences on endo- and ectoparasites (Avenant-Oldewage 2001). However, the score points for ecto- and endoparasites are different, since endoparasites are usually occurring in higher numbers as compared to ectoparasites (Table 4.1). More than 1000 endoparasites can be observed in a single host. Hence, the ranging values for endoparasites will be less than hundred to greater than a thousand. Ectoparasites ranging values will be from one to thirty (Table 4.1). 'Zero parasites observed' are indicated by 0. Good water quality correlates with a low HAI value. In view of this, there is a discrepancy to be found in the refined parasite index classification. Increased numbers of ectoparasites are allocated increasing numerical scores, thereby causing an increase in the HAI value. Larger numbers of ectoparasites, however, are indicative of better water quality (Crafford & Avenant-Oldewage 2009) and should be given a lower score for this correlation to be reflected in the HAI value. The zero is indicative of good water quality while the 30 is representing poor water quality. The resulting Inverted Parasite Index (IPI) classification is given in Table 4.1. The HAI including both the PI and IPI were followed to evaluate the IPI impact on the results. Furthermore, the mean intensity, mean abundance and prevalence were calculated for parasite populations according to Bush *et al.* (2001);

• Prevalence = number of infested individuals of a host species divided by the number of hosts examined, expressed in percentage.

• Mean Intensity = total number of a particular parasite species divided by the number of infested hosts.

• Mean Abundance = total number of particular parasite species divided by the total number of hosts in a sample.

Table 4.1: The numerical scoring system of Parasite Index (PI) and Inverted Parasite Index (IPI)

| **Ectoparasites** | **PI** | **IPI** | **Endoparasites** | **PI** |
|---|---|---|---|---|
| 0 | 0 | 30 | <100 | 0 |
| 1-10 | 10 | 20 | 100-500 | 10 |
| 11-20 | 20 | 10 | 501-1000 | 20 |
| >20 | 30 | 0 | >1000 | 30 |

The condition factor (K) for each fish was calculated using the formula $K = 10^5 W/L^3$, where W = weight in g, and L = standard length in mm (Bagenal & Tesch 1978).

## 4.3 RESULTS AND DISCUSSIONS

### 4.3.1 Health Assessment Index (HAI)

The HAI for both fish species was recorded the highest during summer as compared to other seasons; *Schilbe intermedius* (72) and *Labeo rosae* (41) (Figure 4.2). During spring the HAI was equal (25) for both fish species. *Labeo rosae* was more affected in terms of necropsy related anomalies as compared to *S. intermedius* regarding the overall HAI. However, the HAI of *S. intermedius* was higher than the HAI of *L. rosae* during all seasons except spring (Figure 4.2). The PI contributed mostly (>50%) to the HAI of *S. intermedius*, whereas in *L. rosae,* the necropsy related anomalies contributed more to the HAI value than the PI. There was a significant difference ($p<0.05$) of HAI value among the two fish species and also among the seasons (Table 4.2). The recorded high HAI values during summer may be because higher temperatures reduce the solubility of dissolved oxygen, on the other hand, increase the metabolism, respiration and oxygen demand of fish and other aquatic life. As a result, available oxygen will be reduced in the water (Harper & Wolf 2009). The decrease in oxygen availability to fish tissues can lead to necrotic or apoptotic lesions in organs (Geng 2003; van der Meer *et al.* 2005). To add on that, higher temperatures also intensifies the solubility of many toxic substances (Harper & Wolf 2009). Hence, fish are subject to a broad variety of stressors because their homeostatic mechanisms are highly dependent on prevailing conditions in their environment.

Table 4.2: Statistical comparison of significant differences of the Health Assessment Index, Parasite Index, Inverted Parasite Index of *Schilbe intermedius* and *Labeo rosae* among seasons and among both fish species

| **Index** | ***Schilbe intermedius*** | ***Labeo rosae*** | **Between both species** |
|---|---|---|---|
| HAI | 0.000* | 0.002* | 0.000* |
| PI | 0.000* | 0.359 | 0.000* |
| IPI | 0.057 | 0.208 | 0.726 |

***p≤ 0.05 significant difference p>0.05 no significant difference**

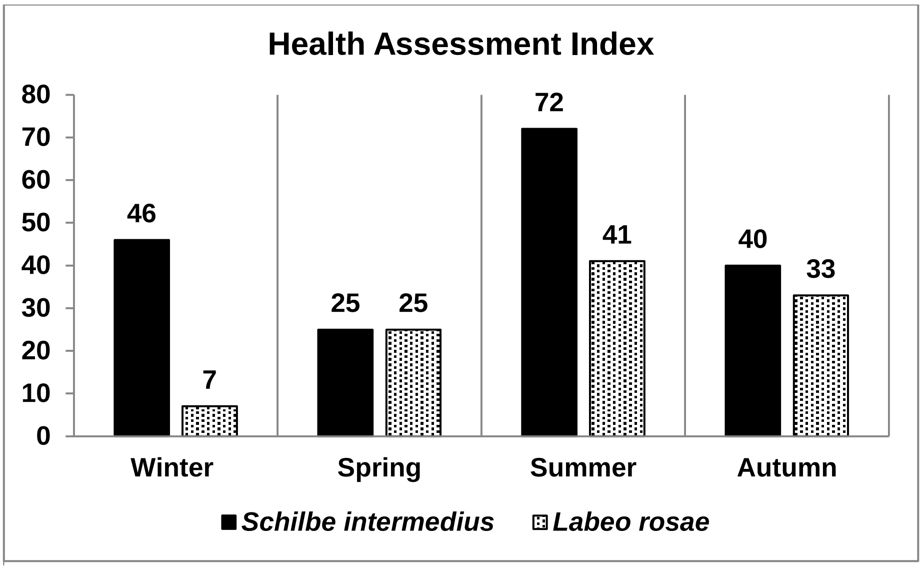


Figure 4.1: Seasonal comparison of the Health Assessment Index between *Schilbe intermedius* and *Labeo rosae* at Flag Boshielo Dam

When fish encounter stress their susceptibility to diseases and parasite infections increase. With this altered immune system physiological changes may occur in the fish depending on the stressor. The necropsy-related abnormalities observed from the two fish species were similar, of which include discoloured liver, haematocrit readings above or below normal range, skin lesions (*L. rosae*) and gills covered in mucus and/or clubbed or frayed gills (Figure 4.1).

- **Liver** – A fatty liver was recorded from both fish species. Fatty liver is a pathological state attributable to excessive accumulation of fat in cellular cytoplasm. A fatty liver is one of the conditions evident that in wild freshwater fish were exposed to mixed contaminants (Adams *et al.* 1993; Harper & Wolf 2009). The recorded discoloured fatty liver in this survey may be attributable to the metals recorded above the TWQR and some detected in the fish muscle tissue and sediment at unacceptable levels (see chapter 2 and chapter 3 respectively).
- **Haematocrit** – Two specimens of *L. rosae* and five specimens of *S. intermedius* had a haematocrit value below normal range (Appendix B: Table 3). The normal haematocrit range for fish is between 30 to 45%, and haematocrit values below normal range usually indicate severe anaemia,

stress, loss of appetite, or haemodilution due to gill damage. Loss of appetite normally happens when fish have a decrease in metabolic activity or when fish are diseased (Blaxhall 1972). Five specimens of *S. intermedius* were recorded with a haematocrit above normal range. A rise in haematocrit is associated with increased red blood cells mostly resulting from hypoxia (low oxygen) to maximise oxygen transport, and to minimise the work required for cardiac pumping (Wells & Weber 1991). These records were visible during warmer seasons which are characterised by reduced oxygen in the waters.

- **Skin lesions** – Mild skin aberrations were recorded from *L. rosae* during summer. The skin aberrations were lesions caused by *Lernaea cyprinacaea* (Figure 4.3).
- **Gills** – Gills of *L. rosae* were mostly clubbed or covered in mucus during summer and autumn. It was only in winter and autumn when *S. intermedius* gills were recorded to be pale or covered in mucus. *Labeo rosae* had a higher frequency record of gills covered in mucus as compared to *S. intermedius*, indicating to be more susceptible to the changing environment. Gills rapidly exhibit abnormal conditions (paleness and increased mucus production), in response to deteriorating water conditions (Overstreet 1997) because of their immediate contact with the environment. The cause of excessive mucus on both fish species gills could be from the fluctuating environment conditions such as temperature, pH, dissolved oxygen, alkalinity and presence of other metals at unacceptable concentrations.

### 4.3.2 Parasite Index (PI) and Inverted Parasite Index (IPI)

The PI of *S. intermedius* was higher than the PI of *L. rosae* during all seasons, whereby the highest PI was recorded in summer for *S. intermedius* (38), on the other hand, summer and autumn (10) for *L. rosae* (Figure 4.3). The high PI value during warm seasons could be because during summer the rain brings in a lot of organic debris from the catchment run-offs. This can serve as food to intermediate host of most indirect life cycle parasites, which in turn may serve as food for fish host, thus increasing infection potential to the final host. Out of 40 *L. rosae* sampled, 139 parasites were retrieved; five parasite species were ectoparasites and two endoparasites (Table 4.3).

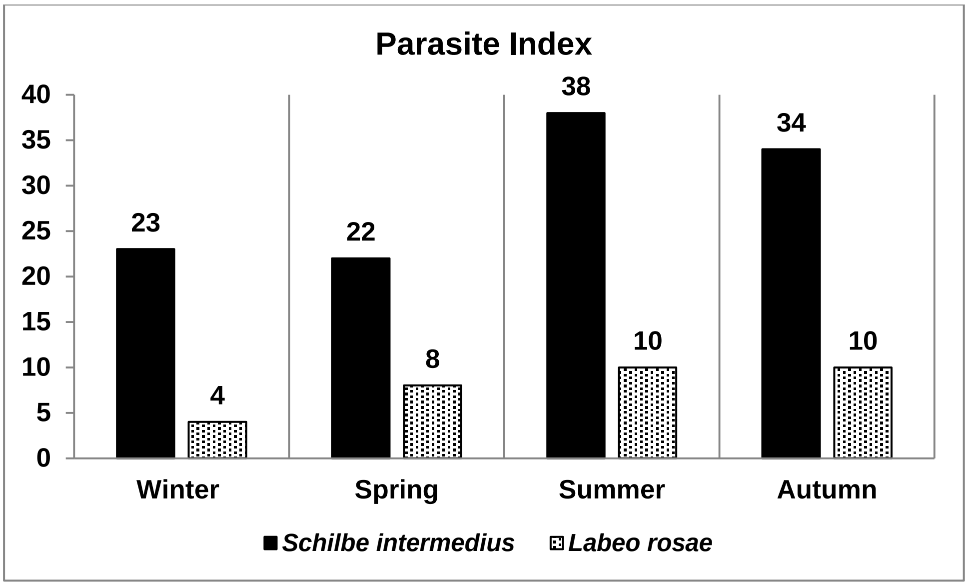


Figure 4.2: Seasonal comparison of the Parasite Index between *Schilbe intermedius* and *Labeo rosae* from Flag Boshielo Dam.

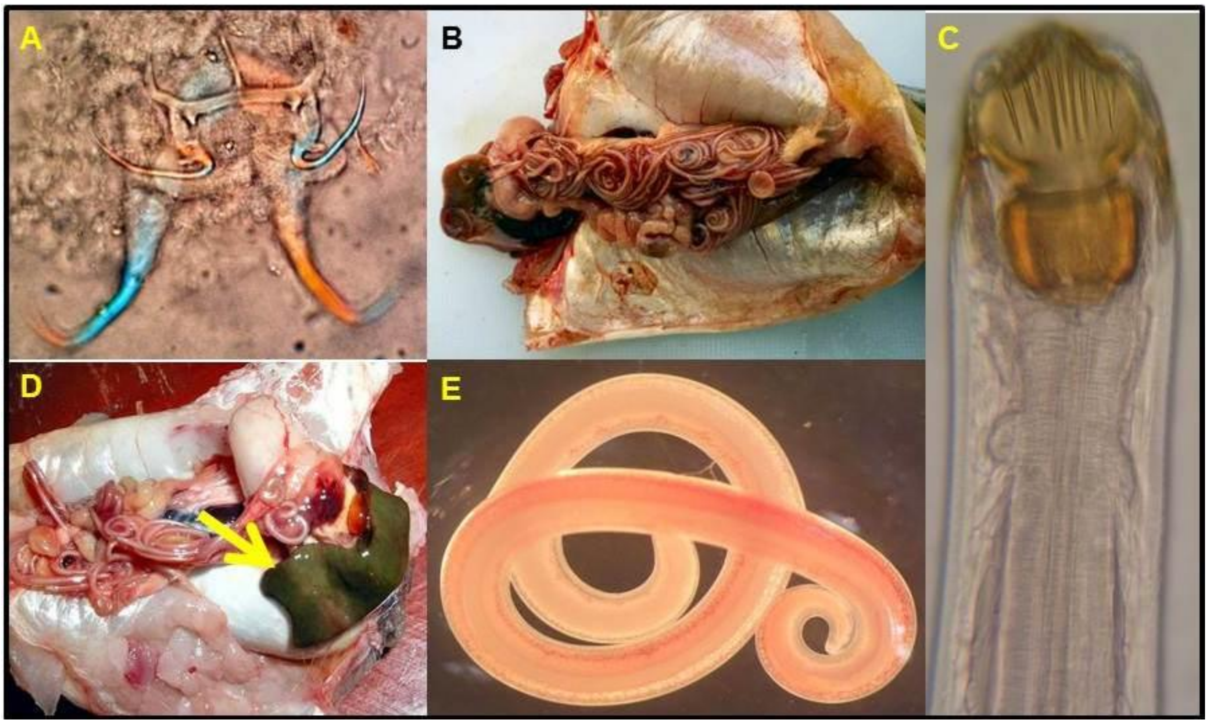


Figure 4.3: Parasites of *Schilbe intermedius*. Ectoparasites; **A.** Opistohaptoral features of *Schilbetrema* sp. showing the anchors, bars and hooklets. Endoparasites; **B** *Contracaecum* spp larvae in the body cavity. **C.** *Paracamallanus cyanthopharynx* from the intestine. **D.** A discoloured liver of *Schilbe intermedius* **E.** *Contracaecum* sp larva.

Ectoparasites recorded included two monogeneans (*Dactylogyrus* sp. and *Diplozoon* sp.) and three copepods (*Lamproglena* sp., *Lernaea cyprinacaea* and *Ergasilus* sp.) (Figures 4.6). The two endoparasites species retrieved include one nematode (*Paracamallanus cyanthopharynx*) and one digenean (*Nematobothrium* sp.) (Figure 4.6). All the ectoparasites were collected from the gills, except *Ergasilus* sp. which was collected from the skin (Table 4.3).

Endoparasites, *P. cyanthopharynx* were collected from the intestine and *Nematobothrium* sp. from the eye socket (orbit) (Table 4.4). Most parasites were not recorded during autumn as compared to parasites infestation during other seasons (Table 4.4). *Paracamallanus cyanthopharynx* was only recorded in winter. It was only the *Dactylogyrus* spp. that was recorded during all seasons. *Diplozoon* sp. and the *Ergasilus* sp. were recorded in winter and spring only, while *L. cyprinacaea* was recorded in winter and summer only. One *Dactylogyrus* sp., *Ergasilus* sp. and *Lamproglena* sp. are being investigated as a new species, whilst *Nematobothrium* sp. is the new record for southern Africa and for *L. rosae* as new host record.

From the 40 *S. intermedius* sampled, 2473 parasites were retrieved, from which two species (one genus) were ectoparasites and three species were endoparasites (Table 4.4). The ectoparasites include monogeneans i.e. *Schilbetrema* spp. and the endoparasites include nematodes *Contracaecum* larvae and *Paracamallanus cyanthopharynx* (Figure 4.5). *Schilbetrema* spp. are the only ectoparasites recorded and they were collected from the gills (Table 4.4). *Contracaecum* larvae were collected from the body cavity, and the *P. cyanthopharynx* from the intestine.

The PI was applied to determine the possible effect of water quality on ecto- and endoparasites recorded from the two fish species. The assumption is that the ectoparasite numbers should decrease in polluted water as they are directly exposed to the environment as much as their host, whilst endoparasites numbers should increase in polluted water as they are protected by the host’s body (Crafford & Avenant-Oldewage 2009). *Labeo rosae* harboured more ectoparasites than endoparasites, hence supporting the hypothesis, while *S. intermedius* harboured more endoparasites than ectoparasites. It should be noted that even though *S. intermedius* had more endoparasites than ectoparasites, it exhibited more ectoparasites than *L. rosae*, thus increasing its HAI value.


Figure 4.4: Parasites of *Labeo rosae*. Ectoparasites; **A.** *Dactylogyrus* sp. attached to the gills. **B.** *Diplozoon* sp. attached to the gills. **C.** *Diplozoon* sp. **D.** *Ergasilus* sp. **E.** *Lamproglena* sp. attached to the gills. **F.** *Lamproglena* sp. **G.** *Lernaea cyprinacaea* attached to the skin. **H.** *Lernaea cyprinacaea*. **I.** *Paracamallanus cyanthopharynx*. **J.** *Nematobothrium* sp. **K.** *Nematobothrium* sp. on fatty tissues of the eye ball.

The presence and/or absence of the parasites in/on both fish species may be due to certain pollution stress present in Flag Boshielo Dam, because different parasites and fish species are affected differently with differing types of stressors/pollution (Sures 2001). The dominating ectoparasites for both species were from the Class Monogenea. Monogenean parasites are considered as one of the most important sensitive parasites to any changes in water parameters, such as temperature (Bayoumy *et al.* 2008; Madanire-Moyo *et al.* 2012) and certain pollutants (Mackenzie 1999).

Table 4.3: Number of parasite recovered from the different organs of *Labeo rosae* and *Schilbe intermedius* from Flag Boshielo Dam

| ***Labeo rosae*** | **Name of parasite** | **Site/organ** | **Winter** | **Spring** | **Summer** | **Autumn** |
|---|---|---|---|---|---|---|
| **Ectoparasites** | | | | | | |
| Monogenea | *Dactylogyrus* sp. | gills | 10 | 15 | 37 | 24 |
| | *Diplozoon* sp. | gills | 1 | 3 | 0 | 0 |
| Copepoda | *Lamproglena* sp. | gills | 0 | 1 | 7 | 0 |
| | *Lernaea cyprinacaea* | gills | 9 | 0 | 1 | 0 |
| | *Ergasilus* sp. | skin | 1 | 0 | 0 | 3 |
| **Endoparasites** | | | | | | |
| Digenea | *Nematobothrium* sp. | eye socket | 0 | 5 | 4 | 17 |
| Nematoda | *Paracamallanus cyathopharynx* | intestine | 1 | 0 | 0 | 0 |
| ***Schilbe intermedius*** | | | | | | |
| **Ectoparasites** | | | | | | |
| Monogenea | *Schilbetrema* spp | gills | 90 | 115 | 241 | 248 |
| **Endoparasites** | | | | | | |
| Nematoda | *Contracaecum* sp. | Body cavity | 591 | 423 | 547 | 448 |
| | *Paracamallanus cyanthopharynx* | Intestine | 2 | 1 | 9 | 9 |

*Schilbe intermedius* IPI was the highest during all seasons except during summer as compared to the IPI of *L. rosae* (Figure 4.4). There was no significant difference ($p>0.05$) of IPI among the two fish species and also among the seasons (Table 4.2). This is also visible in the graph (Figure 4.4) where there is little difference between the IPI value of *S. intermedius* (28 – 35) and *L. rosae* (29 – 34). *Labeo rosae* parasites records are congruent with the PI premise; more ectoparasite records as compared to endoparasites. This is indicative of good water quality in Flag Boshielo Dam.

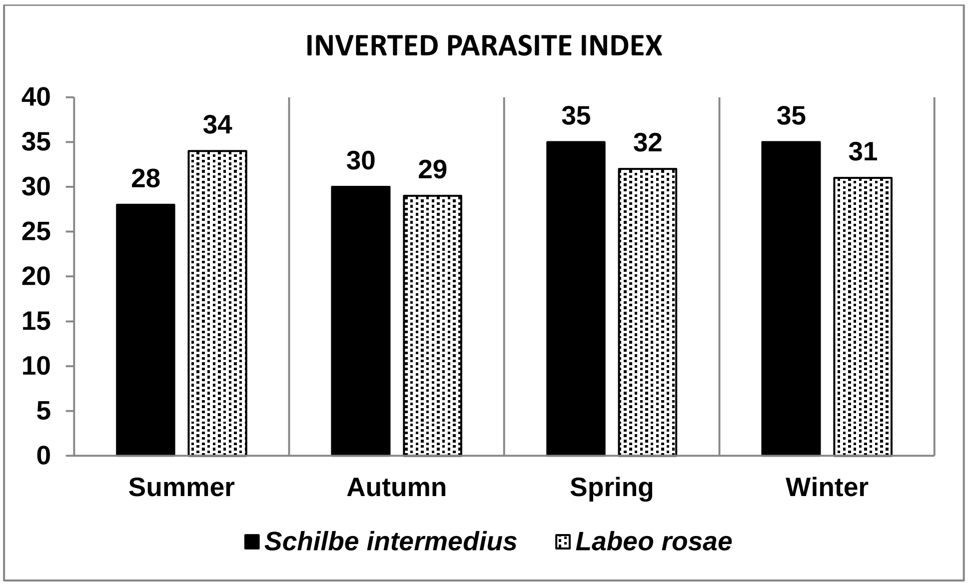


Figure 4.5: Seasonal comparison of Inverted Parasite Index between *Schilbe intermedius* and *Labeo rosae* from Flag Boshielo Dam.

### 4.3.3 Mean intensity, mean abundance and prevalence of parasites from *Schilbe intermedius* and *Labeo rosae*.

***Schilbe intermedius***

Seasonal fluctuation of mean intensity, mean abundance and prevalence of parasites found on/in *S. intermedius* are illustrated in Figure 4.7. The *Contracaecum* larvae had the highest parasites mean intensity, mean abundance and prevalence recorded, i.e. 59.1, 59.1 and 100% respectively. The lowest records were of the *P. cyathopharynx* with mean intensity of one, the mean abundance of 1.9 and prevalence of 10%. The prevalence of *Contracaecum* larvae and *Schilbetrema* spp. was 100% during all seasons.

Table 4.4: Infestation statistics of metazoan parasites for *Labeo rosae* and *Schilbe intermedius* from Flag Boshielo Dam.

| | | | Winter | Spring | Summer | Autumn |
|---|---|---|---|---|---|---|
| ***Schilbe intermedius*** | | | | | | |
| Endoparasites | *Contracaecum* spp. larvae | MI | 59.1 | 42.3 | 54.7 | 44.8 |
| | | MA | 59.1 | 42.3 | 54.7 | 44.8 |
| | | P (%) | 100 | 100 | 100 | 100 |
| | *Paracamalanus cyanthopharynx* | MI | 1 | 1 | 2.25 | 1.5 |
| | | MA | 0.2 | 0.1 | 0.9 | 0.9 |
| | | P (%) | 20 | 10 | 40 | 60 |
| Ectoparasites | *Schilbetrema* spp. | MI | 9 | 11.5 | 24.1 | 24.8 |
| | | MA | 9 | 11.5 | 24.1 | 24.8 |
| | | P (%) | 100 | 100 | 100 | 100 |
| ***Labeo rosae*** | | | | | | |
| Endoparasites | *Nematobothrium* sp. | MI | 0 | 1.3 | 2 | 3.4 |
| | | MA | 0 | 0.4 | 0.4 | 1.7 |
| | | P (%) | 0 | 30 | 20 | 50 |
| | *Paracamalanus cyanthopharynx* | MI | 1 | 0 | 0 | 0 |
| | | MA | 0.1 | 0 | 0 | 0 |
| | | P (%) | 10 | 0 | 0 | 0 |
| Ectoparasites | *Dactylogyrus* spp. | MI | 3.3 | 7.5 | 12.3 | 12 |
| | | MA | 1 | 1.5 | 3.7 | 2.4 |
| | | P (%) | 30 | 20 | 30 | 20 |
| | *Diplozoon* sp. | MI | 1 | 1.5 | 0 | 0 |
| | | MA | 0.1 | 0.3 | 0 | 0 |
| | | P (%) | 10 | 20 | 0 | 0 |
| | *Lamproglena* sp. | MI | 0 | 1 | 2.3 | 1.67 |
| | | MA | 0 | 0.1 | 0.7 | 0.5 |
| | | P (%) | 0 | 10 | 30 | 30 |
| | *Lernaea cyprinacaea* | MI | 9 | 0 | 1 | 0 |
| | | MA | 0.9 | 0 | 0.1 | 0 |
| | | P (%) | 10 | 0 | 10 | 0 |
| | *Ergasilus* sp. | MI | 1 | 1.5 | 0 | 0 |
| | | MA | 0.1 | 0.3 | 0 | 0 |
| | | P (%) | 10 | 20 | 0 | 0 |

MI = mean intensity, MA = mean abundance and P (%) = prevalence

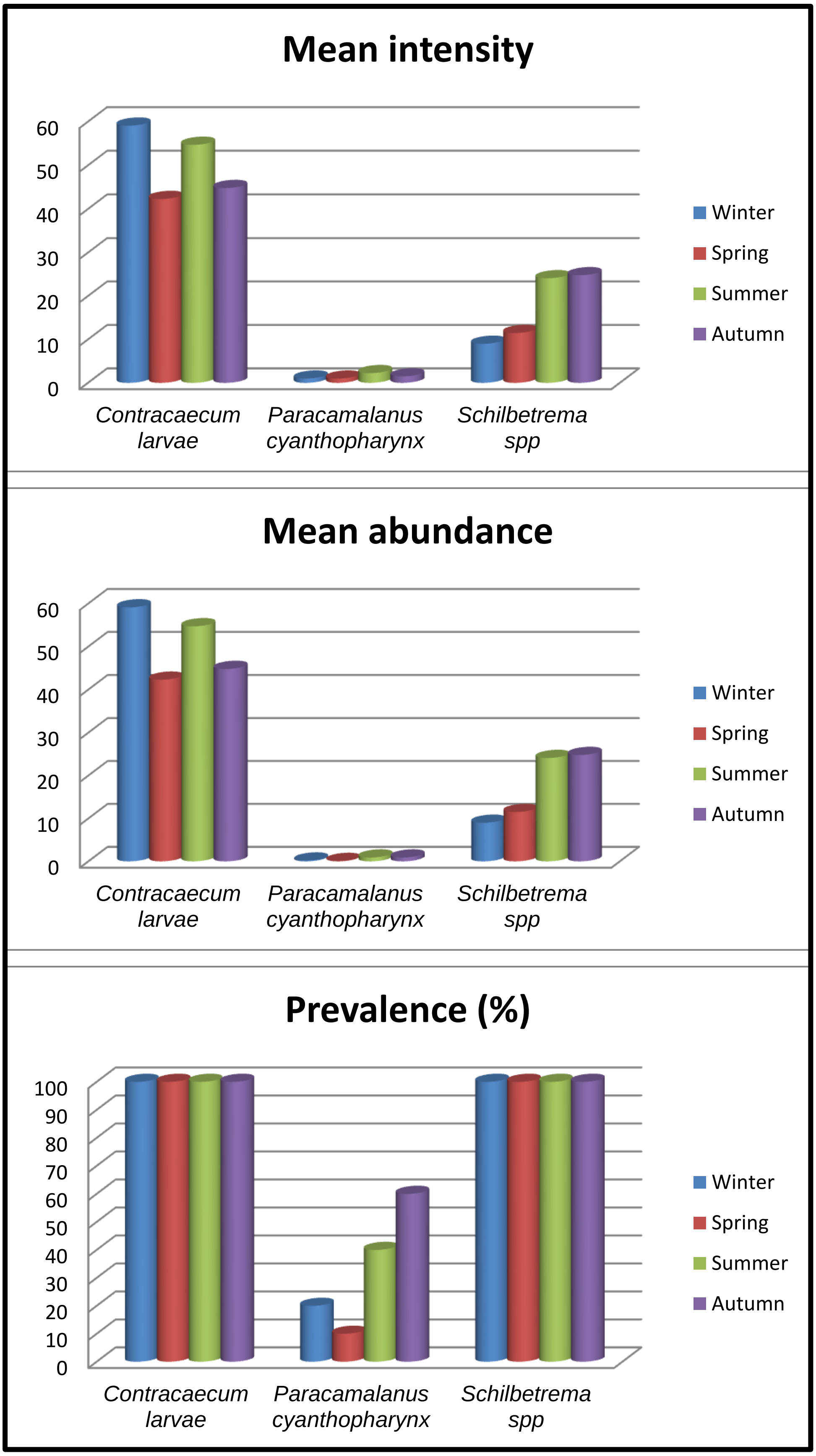


Figure 4.6: Seasonal comparison of mean intensity, mean abundance and prevalence of parasites found on and in *Schilbe intermedius* at Flag Boshielo Dam.

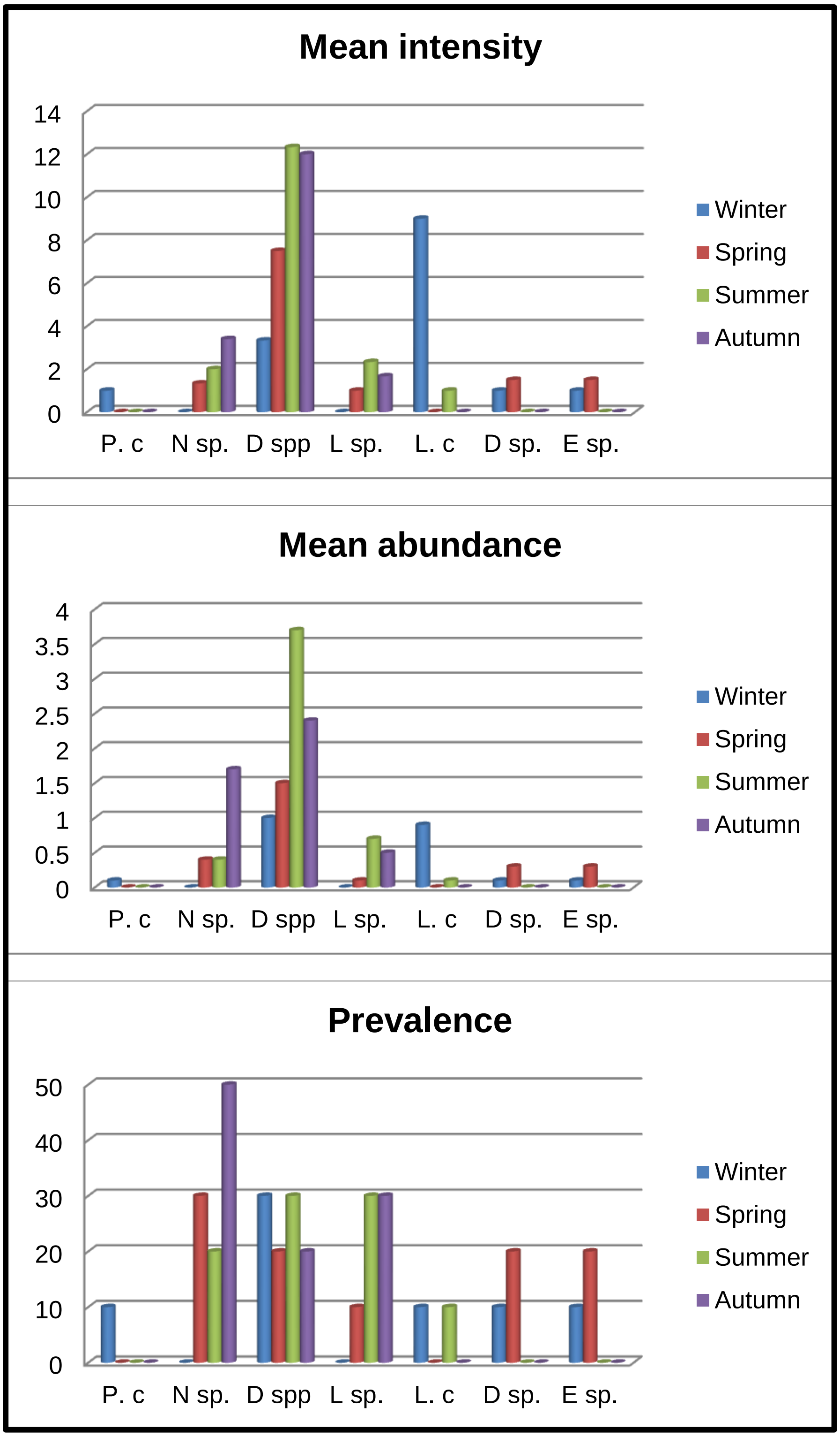


Figure 4.7: Seasonal comparison of mean intensity, mean abundance and prevalence of parasites (P. c – *Paracamallanus cyanthopharynx*, N sp. – *Nematobothrium* sp., D spp – *Dactylogyrus* spp, L sp. – *Lamproglena* sp., L. c – *Lernaea cyprinacea*, D sp. – *Diplozoon* sp. and E sp. – *Ergasilus* sp.) found on and in *Labeo rosae* at Flag Boshielo Dam.

### *Labeo rosae*

Seasonal fluctuation of mean intensity, mean abundance and prevalence of parasites found on/in *L. rosae* are illustrated in Figure 4.8. *Nematobothrium* sp. had the highest prevalence infestation of 50% during autumn. *Dactylogyrus* spp. had the highest mean intensity of 12.3 and mean abundance of 3.7 during summer, of which both were increasing gradually over the seasons from winter till summer and then decreasing slightly in autumn. However, the *Dactylogyrus* spp. prevalence of infestation was fluctuating between 20% and 30% consecutively in subsequent seasons. The lowest parasite prevalence recorded was 10% which includes *P. cyanthopharynx*, *L. cyprinacaea*, *Diplozoon* sp. and *Ergasilus* sp. *Paracamallanus cyanthopharynx* had the lowest mean intensity (1) and mean abundance (0.1).

## 4.3.4 Condition factor (K)

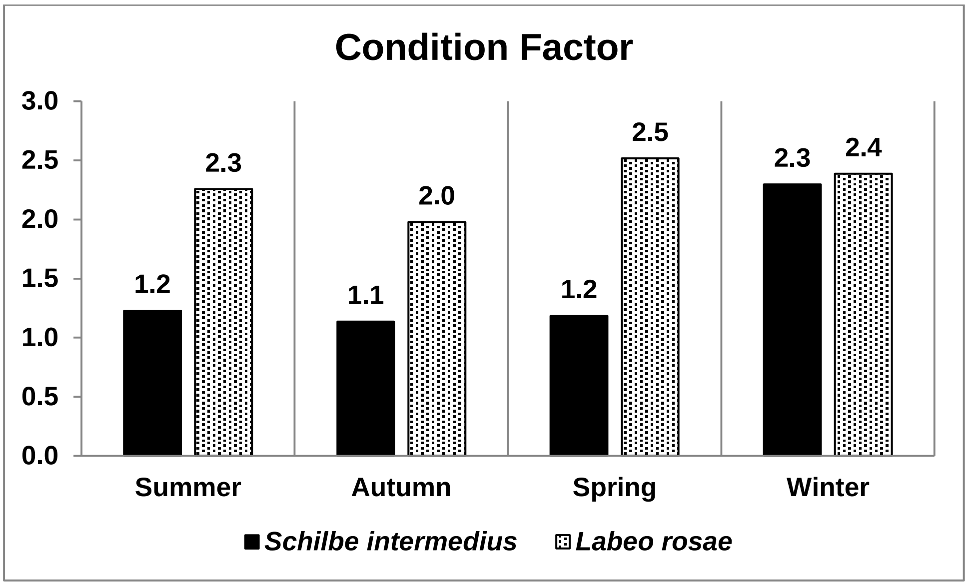


Figure 4.8: Seasonal condition factor of *Schilbe intermedius* and *Labeo rosae* from Flag Boshielo Dam.

The seasonal average condition factor fluctuated (Figure 4.8) whereby the condition factor of *L. rosae* was higher than that of *S. intermedius* during all seasons. Given the hypothesis that heavier fish of a given length indicate a better condition (Bagenal

& Tesch 1978), it can be assumed that *L. rosae* had a better condition as compared to *S. intermedius*. This is visible in the recorded masses of both species throughout the survey. The mass of *L. rosae* species were recorded between 102 g and 779 g, whilst *S. intermedius* was recorded between 16 g and 725 g (Appendix C: Table 2 & 3). It should be noted that there was only one *S. intermedius* that weighed 725 g, the second highest mass was 397 g. The parasite load might have impacted the health of *Schilbe intermedius* host population, since they harboured more parasites than *L. rosae*. There was a significant difference ($p<0.05$) between the condition factor among the two fish species and there was also seasonal significant difference for *L. rosae* but not for *S. intermedius* ($p>0.05$).

## 4.4 CONCLUSIONS

The HAI values for *S. intermedius* were higher than that of *L. rosae*. From this study the hypothesis that less ectoparasites are expected in polluted water than endoparasites, was supported in *L. rosae*, but not in *S. intermedius*. The condition or wellness of *L. rosae* was also better than that of *S. intermedius* during all seasons except autumn. Furthermore, the condition factor findings correlate with the HAI values of both fish species whereby *S. intermedius* had highest parasite numbers and highest HAI values, of which both might have impacted on the overall condition of its health as compared to *L. rosae*. *Labeo rosae* depicted opposite results to *S. intermedius*; less ectoparasites, lower HAI values and condition factor. The mean intensity, mean abundance and prevalence of *S. intermedius* were all higher during all seasons as compared to than that of *L. rosae*.

The hypothesis that water and sediment quality can have adverse effects on fish health was not supported. The accumulated metals in the fish muscle tissue might have affected the fish health because, several necropsy anomalies were recorded. However, there are several other factors such as parasite load that might have contributed. Hence the cause of the necropsy anomalies remains inconclusive.

This study lead to discovery of new records of two parasite species namely; *Nematobothrium* sp. and *Ergasilus* sp., both found in *L. rosae*. One of them was an endoparasite found in the orbit (*Nematobothrium* sp.) and the other, an ectoparasite found on the skin (*Ergasilus* sp.). Both of them were identified to genus level.

# CHAPTER 5

# GENERAL CONCLUSIONS

The main aim of the study was to assess the impact of water and sediment quality and bioaccumulation of metals on the health of *Labeo rosae* and *Schilbe intermedius* at Flag Boshielo Dam (Olifants River). This was achieved through; assessing the water quality of the dam by determining the level of physical and chemical constituents in the water at three sampling sites, determining the bioaccumulation of selected metals in the muscle tissue of the two fish species, assessing the fish health and the fish parasites in the dam by using the fish HAI and PI, and ascertaining the potential Human Health risk upon consumption of fish contaminated with metals from the dam.

**Water quality**

Generally the water quality of Flag Boshielo Dam was acceptable for aquatic ecosystems according to the SAWQG with the exception of phosphorus and some metals at the inflow area. The pH ranged between slightly acidic to alkaline values; water temperature: 15°C to 26°C; water hardness medium soft, salinity within the freshwater range; turbidity in the clear water range. The TDS and major ions (salts) were acceptable for the duration of the study. However, the water coming from upstream stays a potential risk of pollution to Flag Boshielo Dam because, the highest concentrations of metals such as aluminium, cadmium, copper, iron and lead, and nutrients such as phosphorus were recorded at the inflow area. The nutrients were very low except the eutrophic range phosphorus concentrations recorded at the inflow whereby, the Elands River may be an additional source of nutrients into Flag Boshielo Dam as it has been reported to be impacted with high nutrient concentrations. The hypothesis that Flag Boshielo Dam is polluted due to the high level of pollution in the upper catchment area and Loskop Dam was not fully supported. The metals that were recorded above TWQR are; aluminium, cadmium, copper, iron and lead, of which were mostly recorded at the inflow. However, statistically there was no significant difference among the three sampling sites because the metal concentrations at the inflow were only slightly higher. The source of the slightly elevated metal concentrations may be from the water coming from upstream given the intensive agricultural activities taking place between Loskop Dam and Flag Boshielo Dam. On the other hand, sediments might also have been the source through re-suspension.

**Sediment and bioaccumulation**

All the metals were recorded at higher concentrations in the sediment than in the water and fish muscle tissue, except antimony, selenium and strontium. The most abundant metals recorded in the sediment were iron and aluminium. However, metals with concentrations above the TEL were cadmium, nickel and zinc. The elevated metal concentrations in the sediment are indicating that the metal load in the sediment of Flag Boshielo Dam could be a potential risk for the aquatic biota if they become bioavailable. Antimony, selenium and strontium metal concentrations were recorded at higher concentrations in the muscle tissue of both fish species than in the sediment and water. Iron was the most accumulated metal in the muscle tissue of both fish species. More metals were recorded in the muscle tissue of *S. intermedius* than in *L. rosae*; however the metal concentrations were higher in the latter. This can be attributed to their different trophic levels in the food chain; *L. rosae* is a primary consumer while *S. intermedius* is a tertiary consumer. Therefore, the hypothesis that metals in the water and sediment can accumulate in fish tissues was supported. However, the metals that accumulated in the fish muscle tissue were indicative of bio-availability of the toxic metals in the dam and not water and/or sediment pollution.

According to a Human Health risk assessment (Chapter 3), metals that accumulated in the muscle tissue of *L. rosae* and may have risks upon consumption are; antimony, arsenic, chromium, iron and vanadium; for *S. intermedius* are; antimony, chromium, iron, vanadium and arsenic (in descending order). These metals may pose toxic and carcinogenic risks to humans. Therefore, the rednose labeo (*L. rosae*) and to a lesser extend the silver catfish (*S. intermedius*) fish species from Flag Boshielo Dam may not be suitable for humans if consumed above 150 g per week.

**Fish health and parasites**

The Health Assessment Index (HAI) values of the two fish species differed significantly with higher values recorded for *S. intermedius* than *L. rosae*. Besides the Parasite Index (PI), abnormal haematocrit readings, liver conditions, skin lesions and clubbed gills are the necropsy anomalies that contributed predominantly to the HAI. Overall, the PI contributed mostly to the total HAI value.

The parasite load and therefore also the mean intensity, mean abundance and prevalence of *S. intermedius* were higher during all seasons than that of *L. rosae*. The dominant ectoparasites for both species were from the Class Monogenea and the dominant endoparasites were nematodes. Out of 40 *L. rosae* sampled, 139 parasites were retrieved; five parasite species were ectoparasites and two endoparasites. From the 40 *S. intermedius* sampled, 2473 parasites were retrieved, from which two species (one genus) were ectoparasites and three species were endoparasites.

The condition factor is used to compare the “condition”, “fatness” or wellbeing of fish and it is based on the hypothesis that heavier fish of a particular length are in a better physiological condition. The *L. rosae* had a better condition factor, recorded at values less than (2) as compared to *S. intermedius* (>2).

The cause of the HAI necropsy anomalies may have been also from parasite load other than the metals in the water and sediment. However, the HAI alone cannot be used for metal pollution, unless it is done in conjunction with a histopathological study of the tissues/organs. Therefore, the cause of the recorded anomalies from both fish species is inconclusive. The hypothesis that water and sediment quality of the dam can have adverse effects on fish health was partially supported. On the other hand, fish can be used as bioindicators for pollutants because the accumulated metals in the fish tissues are indicative of the bioavailability of metals in the water and sediment of an aquatic ecosystem such as Flag Boshielo Dam.

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## APPENDIX A

Table 1: The seasonal water quality values for variables recorded from Flag Boshielo Dam

| Parameters | FBD April 2010 | | | FBD July 2009 | | | FBD October 2009 | | | FBD January 2010 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inflow | Middle | Wall | Inflow | Middle | Wall | Inflow | Middle | Wall | Inflow | Middle | Wall |
| Water temperature °C | 25.42 | 26.4 | 24.53 | 15.4 | 16.5 | 15.13 | 25.62 | 25.54 | 25.47 | 27.38 | 29.13 | 27.82 |
| Dissolved oxygen (mg/ℓ $O_2$) | 6.61 | 7.69 | 8.61 | 6.75 | 5.73 | 7.62 | 9.19 | 8.95 | 9.42 | 4.35 | 7.01 | 8.23 |
| Dissolved oxygen (%) | 82.0 | 96.2 | 104.6 | 70.9 | 69.4 | 75.7 | 111.2 | 110.1 | 116.2 | 55.3 | 93.3 | 108.0 |
| pH | 7.09 | 7.44 | 7.16 | 6.24 | 7.22 | 6.5 | 8.17 | 8.78 | 8.83 | 7.43 | 8.10 | 8.33 |
| Conductivity (EC) $mS/m^{-1}$ | 35.0 | 40.2 | 39.7 | 37.2 | 37.1 | 37.2 | 48.5 | 46.4 | 45.7 | 42.5 | 42.6 | 42.8 |
| Salinity % | 0.17 | 0.19 | 0.19 | 0.18 | 0.18 | 0.18 | 0.23 | 0.22 | 0.22 | 0.20 | 0.20 | 0.20 |
| TDS mg/ℓ | 227.5 | 261.3 | 258.05 | 241.8 | 241.15 | 241.8 | 315.25 | 301.6 | 297.05 | 276.25 | 276.9 | 278.2 |
| Alkalinity as $CaCO_3$ | 64 | 62 | 66 | 38 | 72 | 64 | 88 | 80 | 80 | 60 | 60 | 60 |
| Turbidity NTU | 2.8 | 1.4 | 2.1 | 8.6 | 6.8 | 4.1 | 0.6 | 2.1 | 1.7 | 3.7 | 1.2 | 2.0 |
| Nitrate (mg/ℓ $NO_3N$) | 0.2 | 0.2 | <0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | <0.2 |
| Nitrite (mg/ℓ $NO_2N$) | <0.2 | <0.2 | <0.2 | <0.1 | <0.1 | <0.1 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Ammonia (mg/ℓ $NH_3N$) | <0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Total Nitrogen | 0.2 | 0.4 | 0.2 | 0.5 | 0.7 | 0.4 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0 |
| Ortho-Phosphate (mg/ℓ $PO_4^{3-}$) | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Phosphorous (mg/ℓ P) | 0.046 | <0.001 | <0.001 | - | - | - | 0.052 | <0.001 | <0.001 | 0.044 | <0.001 | <0.001 |
| Sulphate (mg/ℓ $SO_4^{2-}$) | 112 | 110 | 114 | 99 | 94 | 95 | 106 | 107 | 105 | 114 | 116 | 116 |
| Chloride (mg/ℓ $Cl^-$) | 25 | 26 | 24 | 25 | 24 | 23 | 33 | 30 | 29 | 18 | 20 | 21 |
| Fluoride (mg/ℓ $F^-$) | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.6 | 0.6 | 0.6 | 0.4 | 0.4 | 0.4 |
| Calcium (mg/ℓ Ca) | 20.6 | 22.93 | 24.6 | 34.88 | 29.72 | 30 | 22.8 | 21.1 | 23.6 | 21.4 | 23.6 | 25 |
| Magnesium (mg/ℓ Mg) | 11 | 16.2 | 16.28 | 21.89 | 20.13 | 20.34 | 14 | 18.2 | 16.9 | 12 | 17.4 | 17 |
| Potassium (mg/ℓ K) | 6.45 | 4.184 | 4.205 | 5.591 | 5.51 | 5.255 | 6.82 | 4.944 | 4.702 | 7.022 | 5.065 | 4.988 |
| Sodium (mg/ℓ Na) | 19.75 | 0.412 | 1.343 | 36.85 | 33.69 | 32.87 | 20.4 | 1.089 | 1.566 | 18.44 | 0.566 | 1.443 |

Table 2: The seasonal metal constituents of the water at the Flag Boshielo Dam

| Parameters (mg/ℓ) | FBD April 2010 | | | FBD July 2009 | | | FBD October 2009 | | | FBD January 2010 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Inflow** | **Middle** | **Wall** | **Inflow** | **Inflow** | **Middle** | **Wall** | **Inflow** | **Inflow** | **Middle** | **Wall** | **Inflow** |
| Aluminium | 0.214 | 0.124 | 0.107 | 0.099 | 0.046 | 0.018 | 0.283 | 0.207 | 0.128 | 0.198 | 0.116 | 0.098 |
| Arsenic | <0.001 | <0.001 | 0.003 | 0 | 0.002 | 0.006 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 |
| Antimony | <0.001 | <0.001 | <0.001 | 0.006 | 0.005 | 0.004 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Boron | 0.007 | <0.001 | <0.001 | 0.093 | 0.072 | 0.065 | 0.004 | <0.001 | <0.001 | 0.009 | <0.001 | <0.001 |
| Barium | 0.058 | 0.045 | 0.045 | 0.062 | 0.057 | 0.055 | 0.077 | 0.038 | 0.055 | 0.062 | 0.041 | 0.039 |
| Cadmium | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Cobalt | <0.001 | 0.001 | <0.001 | 0 | 0 | 0 | <0.001 | <0.001 | <0.001 | <0.001 | 0.001 | <0.001 |
| Chromium | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Copper | <0.001 | <0.001 | <0.001 | 0.001 | 0.004 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Iron | 0.308 | 0.05 | 0.062 | 0.215 | 0.106 | 0.035 | 0.179 | 0.068 | 0.079 | 0.414 | 0.072 | 0.098 |
| Lead | <0.001 | <0.001 | <0.001 | 0.013 | 0.01 | 0.01 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Lithium | 0.004 | 0.006 | 0.006 | 0.009 | 0.009 | 0.009 | 0.001 | 0.003 | 0.003 | 0.003 | 0.004 | 0.004 |
| Manganese | 0.03 | 0.013 | 0.017 | 0.038 | 0.028 | 0.037 | 0.046 | 0.029 | 0.014 | 0.033 | 0.019 | 0.022 |
| Nickel | <0.001 | 0.001 | <0.001 | 0 | 0 | 0 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Selenium | <0.001 | <0.001 | <0.001 | 0 | 0 | 0 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Silicon | 1.85 | 1.784 | 1.408 | 6.313 | 6.189 | 6.178 | 2.02 | 1.941 | 1.47 | 1.92 | 1.777 | 1.509 |
| Silver | 0.004 | <0.001 | <0.001 | 0.002 | 0.001 | 0.000 | 0.011 | 0.002 | <0.001 | 0.01 | <0.001 | <0.001 |
| Tin | <0.001 | 0.005 | <0.001 | 0 | 0 | 0 | 0.003 | 0.002 | 0.006 | <0.001 | 0.007 | 0.002 |
| Strontium | 0.132 | 0.144 | 0.141 | 0.183 | 0.171 | 0.165 | 0.129 | 0.133 | 0.156 | 0.149 | 0.15 | 0.147 |
| Titanium | 0.022 | <0.001 | <0.001 | 0.001 | 0 | 0.000 | 0.17 | <0.001 | <0.001 | 0.026 | <0.001 | <0.001 |
| Vanadium | <0.001 | 0.002 | <0.001 | 0.003 | 0.002 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Zinc | <0.001 | <0.001 | <0.001 | 0.001 | 0.006 | 0 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

## APPENDIX B

Table 1: Metal concentrations in the sediment from Flag Boshielo Dam

| Sediment quality (mg/kg) | Flag Boshielo Dam Winter 2009 | | | Flag Boshielo Dam Summer 2010 | | |
|---|---|---|---|---|---|---|
| | Inflow | Middle | Wall | Inflow | Middle | Wall |
| Phosphorus | 170 | 250 | 270 | 1643 | - | 1728 |
| Aluminium | 17869 | 31568 | 7976 | 10588 | 6469 | 25300 |
| Antimony | 1.2 | 1.5 | 5 | <1 | <1 | <1 |
| Arsenic | <1 | 2 | 5 | <1 | 9 | 9 |
| Barium | 628 | 816 | 575 | 337 | 237 | 129 |
| Boron | 324 | 318 | 317 | 119 | 122 | 129 |
| Cadmium | <1 | <1 | <1 | 2 | 2 | 2 |
| Chromium | 59 | 72 | 23 | 179 | 135 | 102 |
| Cobalt | 11 | 10 | 4 | 34 | 29 | 20 |
| Copper | 48 | 58 | 29 | 79 | 47 | 25 |
| Iron | 35124 | 48212 | 22985 | 67175 | 55925 | 40675 |
| Lead | 19 | 31 | 5 | 50 | 35 | 21 |
| Manganese | 677 | 6817 | 326 | 1667 | 1135 | 649 |
| Nickel | 41 | 54 | 20 | 90 | 38 | 29 |
| Selenium | 5 | 3 | 3 | <1 | <1 | <1 |
| Silver | 558 | 480 | 1592 | 554 | 443 | 338 |
| Strontium | 22 | 28 | 14 | 45 | 37 | 24 |
| Tin | 9 | <1 | 16 | 4 | <1 | 2 |
| Titanium | 536 | 481 | 1701 | 2398 | 2135 | 1649 |
| Vanadium | 44 | 68 | 34 | 122 | 217 | 83 |
| Zinc | 568 | 578 | 495 | 152 | 123 | 69 |

Table 2: Metal concentrations in the muscle tissues of *Labeo rosae* at the Flag Boshielo Dam

| Specimen | Al mg/kg | Sb mg/kg | As mg/kg | Ba mg/kg | B mg/kg | Cd mg/kg | Cr mg/kg | Co mg/kg | Cu mg/kg | Fe mg/kg | Pb mg/kg | Mn mg/kg | Ni mg/kg | Se mg/kg | Ag mg/kg | Sr mg/kg | Sn mg/kg | Ti mg/kg | V mg/kg | Zn mg/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **LR1** | 48.6 | 144.1 | 0.6 | 25.6 | 68.2 | 0.0 | 32.6 | 21.6 | 4.2 | 163 | 6.2 | 3.2 | 1.0 | 0.0 | 0.0 | 17.2 | 0.0 | 1.6 | 25.4 | 12.6 |
| **LR2** | 55.3 | 18.2 | 0.8 | 27.2 | 89.5 | 0.0 | 34.7 | 1.6 | 4.4 | 316 | 4.6 | 3.8 | 1.0 | 0.0 | 0.0 | 14.8 | 0.0 | 1.4 | 26.6 | 18.0 |
| **LR3** | 58.4 | 1.8 | 0.8 | 22.0 | 114.4 | 0.0 | 43.2 | 0.0 | 5.2 | 1377 | 2.6 | 9.6 | 2.4 | 1.8 | 0.0 | 6.6 | 2.0 | 1.4 | 39.0 | 86.6 |
| **LR4** | 57.4 | 1.6 | 1.0 | 23.7 | 121.8 | 0.0 | 41.1 | 0.0 | 3.4 | 483 | 3.4 | 6.4 | 2.2 | 2.4 | 0.0 | 10.4 | 3.6 | 1.4 | 37.5 | 43.1 |
| **LR5** | 62.0 | 1.8 | 0.2 | 28.0 | 112.8 | 0.6 | 45.2 | 0.8 | 7.4 | 1876 | 3.4 | 18.8 | 3.4 | 2.4 | 0.0 | 18.4 | 6.2 | 1.4 | 31.2 | 33.0 |
| **LR6** | 65.9 | 1.2 | 1.2 | 28.9 | 176.5 | 3.6 | 53.7 | 0.0 | 6.8 | 1272 | 1.8 | 12.2 | 3.0 | 159.5 | 0.0 | 18.2 | 1.6 | 1.4 | 33.1 | 87.8 |
| **LR7** | 69.2 | 37.8 | 1.0 | 26.6 | 107.5 | 0.0 | 42.4 | 4.8 | 3.8 | 331 | 3.0 | 6.2 | 1.4 | 7.4 | 0.0 | 15.6 | 6.8 | 1.4 | 29.8 | 22.6 |
| **LR8** | 62.5 | 1.2 | 2.0 | 25.0 | 128.9 | 0.0 | 45.8 | 0.0 | 4.8 | 441 | 2.8 | 4.6 | 2.2 | 6.6 | 0.0 | 6.2 | 2.8 | 1.4 | 37.6 | 39.4 |
| **LR9** | 63.9 | 0.4 | 0.0 | 35.0 | 129.4 | 0.0 | 46.5 | 7.4 | 7.4 | 1842 | 2.2 | 18.4 | 3.4 | 4.8 | 0.0 | 17.8 | 3.0 | 1.2 | 32.0 | 58.3 |
| **LR10** | 61.4 | 0.8 | 0.0 | 26.0 | 130.4 | 0.0 | 45.8 | 0.0 | 7.0 | 1686 | 2.8 | 13.2 | 3.4 | 4.2 | 0.0 | 9.4 | 3.6 | 1.2 | 32.6 | 54.0 |
| **LR11** | 59.4 | 17.8 | 0.6 | 29.0 | 107.9 | 0.0 | 45.8 | 2.6 | 6.8 | 1133 | 4.4 | 9.6 | 2.4 | 1.0 | 0.0 | 13.6 | 6.2 | 1.6 | 25.8 | 86.1 |
| **LR12** | 63.5 | 2.2 | 2.2 | 28.4 | 138.2 | 0.0 | 49.1 | 0.0 | 7.0 | 1316 | 2.6 | 11.2 | 2.6 | 4.4 | 0.0 | 15.8 | 2.6 | 1.6 | 37.9 | 76.1 |
| **LR13** | 61.6 | 4.6 | 0.0 | 23.8 | 120.8 | 0.0 | 47.6 | 1.2 | 5.0 | 1303 | 3.4 | 9.4 | 3.0 | 2.4 | 0.0 | 6.6 | 6.6 | 1.2 | 29.6 | 29.4 |
| **LR14** | 77.9 | 1.8 | 0.6 | 24.6 | 124.6 | 0.0 | 47.9 | 0.0 | 4.0 | 816 | 3.4 | 6.8 | 3.0 | 1.0 | 0.0 | 5.4 | 6.6 | 1.2 | 30.2 | 32.3 |
| **LR15** | 70.6 | 1.4 | 0.4 | 27.3 | 125.5 | 0.0 | 53.5 | 0.0 | 9.2 | 1659 | 2.6 | 12.6 | 3.6 | 2.4 | 0.0 | 10.0 | 0.2 | 1.4 | 31.9 | 74.0 |

Table 3: Metal concentrations in the muscle tissues of *Schilbe intermedius* at the Flag Boshielo Dam

| Specimen | Al mg/kg | Sb mg/kg | As mg/kg | Ba mg/kg | B mg/kg | Cd mg/kg | Cr mg/kg | Co mg/kg | Cu mg/kg | Fe mg/kg | Pb mg/kg | Mn mg/kg | Ni mg/kg | Se mg/kg | Ag mg/kg | Sr mg/kg | Sn mg/kg | Ti mg/kg | V mg/kg | Zn mg/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SI1** | 80.8 | 26.2 | 0.6 | 28.2 | 106.3 | 0.0 | 45.8 | 3.8 | 4.2 | 1151 | 3.4 | 5.4 | 2.4 | 2.4 | 0.0 | 1.8 | 7.2 | 1.8 | 27.2 | 30.6 |
| **SI2** | 63.0 | 0.6 | 0.0 | 27.2 | 128.5 | 0.0 | 51.0 | 0.0 | 8.2 | 888 | 2.2 | 5.8 | 2.4 | 3.2 | 0.0 | 1.2 | 4.4 | 1.2 | 30.8 | 85.1 |
| **SI3** | 59.0 | 32.8 | 0.6 | 27.0 | 109.7 | 0.0 | 43.8 | 4.4 | 5.4 | 788 | 4.2 | 4.8 | 2.0 | 1.4 | 0.0 | 1.0 | 8.8 | 1.4 | 25.8 | 44.6 |
| **SI4** | 98.9 | 0.4 | 0.4 | 35.7 | 120.2 | 0.0 | 48.5 | 0.6 | 12.6 | 2377 | 2.2 | 64.9 | 4.0 | 2.8 | 0.0 | 152.4 | 3.8 | 1.8 | 31.6 | 258.4 |
| **SI5** | 64.1 | 6.2 | 0.2 | 32.1 | 123.2 | 0.0 | 50.1 | 0.8 | 11.4 | 1872 | 4.4 | 41.3 | 3.4 | 1.4 | 0.0 | 94.0 | 6.4 | 1.6 | 31.2 | 216.1 |
| **SI6** | 97.1 | 2.2 | 1.4 | 34.9 | 155.8 | 0.0 | 52.0 | 0.0 | 9.0 | 1609 | 2.0 | 40.3 | 3.0 | 2.0 | 0.0 | 115.2 | 2.2 | 1.8 | 31.5 | 264.0 |
| **SI7** | 194.9 | 24.4 | 0.8 | 36.6 | 94.1 | 0.0 | 41.4 | 3.6 | 10.6 | 1873 | 4.8 | 60.4 | 3.0 | 0.4 | 0.8 | 174.7 | 7.0 | 2.8 | 25.6 | 162.3 |
| **SI8** | 85.7 | 1.2 | 2.8 | 39.6 | 130.7 | 0.0 | 51.1 | 0.0 | 15.2 | 1803 | 2.2 | 66.5 | 5.6 | 2.4 | 0.0 | 165.6 | 3.0 | 1.6 | 31.0 | 250.9 |

## APPENDIX C

Table 1: Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy based system (adapted from Adams, Brown and Goede, 1993)

| 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 | No active erosion or previous erosion healed over | 0 | 0 |
| | Mild active erosion with no bleeding | 1 | 10 |
| | Severe active erosion with haemorrhage / secondary infection | 2 | 20 |
| Skin | Normal, no aberrations | 0 | 0 |
| | Mild skin aberrations – “black spot” < 50 | 1 | 10 |
| | Moderate skin aberrations – “black spot” > 50 | 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 |
| Thymus[a] | No haemorrhage | 0 | 0 |
| | Mild haemorrhage | 1 | 10 |
| | Moderate haemorrhage | 2 | 20 |
| | Severe haemorrhage | 3 | 30 |
| | **Internal variables (necropsy)** | | |
| Mesenteric fat | (Internal body fat expressed with regard to 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 | - |

Table 1 continued….

| Variables | Variable condition | | Original field designation | Substituted value for the HAI |
|---|---|---|---|---|
| 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 |
| Bile | 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 |
| Parasites | No observed parasites | | 0 | 0 |
| | Few observed parasites | | 1 | 10 |
| *Endoparasites[b] | No observed endoparasites | | 0 | 0 |
| | Observed endoparasites | < 100 | 0 | 10 |
| | | 101 -1000 | 1 | 20 |
| | | > 1000 | 3 | 30 |
| *Ectoparasites[b] | No observed ectoparasites | | 0 | 0 |
| | Observed ectoparasites | 1 - 10 | 1 | 10 |
| | | 11 - 20 | 2 | 20 |
| | | > 20 | 3 | 30 |

a - no values were assigned to these values in the original HAI
b - refinement of the HAI, variables inserted during previous studies

Table 2: Seasonal Health Assessment Index variables for *Labeo rosae* from Flag Boshielo Dam

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 27.1 | 33.7 | 471 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| 2 | 22 | 28 | 254 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 40 |
| 3 | 25 | 31.5 | 371 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 21.5 | 27.5 | 212 | F | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 40 |
| 5 | 28 | 35 | 498 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 6 | 21 | 26.5 | 196 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 7 | 22.6 | 27.1 | 195 | M | 0 | 0 | 0 | 0 | 30 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 60 |
| 8 | 22.1 | 28.4 | 233 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| 9 | 22 | 28 | 263 | M | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 0 | 50 |
| 10 | 26.8 | 34.4 | 497 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 20 | 0 | 10 | 60 |
| | | | | | | | | | | | | | | **Total** | 50 | 50 | 330 |
| | | | | | | | | | | | | | | **Mean** | 5 | 5 | 33 |

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 26.5 | 33.5 | 400 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 10 |
| 2 | 30 | 37 | 758 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| 3 | 30.5 | 37 | 664 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| 4 | 39 | 47 | 1530 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| 5 | 29 | 36 | 470 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 26.5 | 33.5 | 455 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 25.5 | 31.5 | 383 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 10 | 30 |
| 8 | 24 | 30 | 326 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 24 | 30 | 348 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 25 | 31 | 378 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | **Total** | 30 | 10 | 70 |
| | | | | | | | | | | | | | | **Mean** | 3 | 1 | 7 |

Table 2 continued…

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct(%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 27.5 | 34.5 | 521 | F | 30 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 70 |
| 2 | 30 | 36.5 | 635 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 24 | 30 | 310 | M | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 50 |
| 4 | 27.5 | 32.5 | 380 | | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 40 |
| 5 | 28 | 35.5 | 552 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 27 | 33 | 445 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 27 | 34 | 442 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 20 | 10 | 0 | 60 |
| 8 | 25 | 31.5 | 394 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 20 |
| 9 | 29 | 37 | 764 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 28 | 38 | 779 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| | | | | | | | | | | | | | | **Total** | 50 | 30 | 250 |
| | | | | | | | | | | | | | | **Mean** | 5 | 3 | 25 |

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct(%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 19.5 | 25 | 143 | F | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 50 |
| 2 | 23 | 29 | 255 | M | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 30 |
| 3 | 24 | 29.7 | 283 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 40 |
| 4 | 17 | 21.5 | 102 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 40 |
| 5 | 27 | 33 | 390 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| 6 | 18.5 | 23.3 | 118 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 50 |
| 7 | 25 | 31.5 | 258 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 10 | 0 | 60 |
| 8 | 19 | 24.5 | 130 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 40 |
| 9 | 20 | 25.5 | 148 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 40 |
| 10 | 26 | 32.5 | 422 | F | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| | | | | | | | | | | | | | | **Total** | 90 | 10 | 410 |
| | | | | | | | | | | | | | | **Mean** | 9 | 1 | 41 |

Table 3: Seasonal Health Assessment Index variables for *Schilbe intermedius* from Flag Boshielo Dam

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 23 | 27 | 134.5 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 40 |
| 2 | 20 | 23 | 85 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 30 | 10 | 50 |
| 3 | 17 | 20 | 62 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 40 |
| 4 | 13 | 15 | 20 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 5 | 19 | 22 | 77 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 40 |
| 6 | 13 | 15 | 21.5 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 7 | 21 | 25 | 128 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 40 |
| 8 | 13 | 16 | 22.5 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 10 | 10 | 70 |
| 9 | 24 | 27 | 252 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 40 |
| 10 | 14 | 17 | 28.2 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| | | | | | | | | | | | | | | **Total** | 230 | 110 | 400 |
| | | | | | | | | | | | | | | **Mean** | 23 | 11 | 40 |

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 31 | 35 | 397 | F | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 0 | 10 | 60 |
| 2 | 19 | 21 | 725 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 3 | 14 | 17 | 24.8 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 4 | 19 | 23 | 77.5 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 |
| 5 | 27 | 31 | 271 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 20 | 20 | 10 | 80 |
| 6 | 28 | 33 | 322 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 10 | 50 |
| 7 | 19 | 22 | 83.5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 8 | 27 | 31 | 253 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 20 | 10 | 60 |
| 9 | 20 | 24 | 84.3 | M | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 30 | 20 | 10 | 90 |
| 10 | 20 | 24 | 107 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 10 | 50 |
| | | | | | | | | | | | | | | **Total** | 130 | 100 | 460 |
| | | | | | | | | | | | | | | **Mean** | 13 | 10 | 46 |

Table 3 continued…

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 15 | 17 | 24.4 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 2 | 18 | 22 | 66.4 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 3 | 26 | 30 | 196.6 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 4 | 19 | 23 | 85.7 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 10 | 40 |
| 5 | 19 | 24 | 108.6 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 6 | 26 | 30 | 217 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| 7 | 29 | 29 | 202.9 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 |
| 8 | 28 | 32 | 266 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 20 |
| 9 | 27 | 32 | 266.1 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 |
| 10 | 17 | 21 | 64.5 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 30 |
| | | | | | | | | | | | | | | **Total** | 120 | 100 | 250 |
| | | | | | | | | | | | | | | **Mean** | 12 | 10 | 25 |

| Fish | Length | | Mass | Sex | Eyes | Skin | Fins | Oper-cula | Gills | Liver | Spleen | Hind gut | Kidney | Blood Hct (%) | Ecto PI | Endo PI | Total HAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | TL | | | | | | | | | | | | | | | |
| 1 | 21 | 25 | 113.8 | M | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 20 | 20 | 20 | 90 |
| 2 | 11 | 13 | 16 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 20 | 10 | 60 |
| 3 | 14 | 16 | 28.1 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 30 | 30 | 10 | 100 |
| 4 | 19 | 22 | 73.5 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 30 | 10 | 70 |
| 5 | 14 | 17 | 30.8 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 30 | 20 | 70 |
| 6 | 23 | 26 | 135 | F | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 20 | 30 | 20 | 100 |
| 7 | 27 | 32 | 197.2 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 10 | 50 |
| 8 | 14 | 17 | 25.6 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 10 | 50 |
| 9 | 19 | 21 | 84.4 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 20 | 10 | 60 |
| 10 | 19 | 22 | 79.6 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 30 | 20 | 70 |
| | | | | | | | | | | | | | | **Total** | 240 | 140 | 720 |
| | | | | | | | | | | | | | | **Mean** | 24 | 14 | 72 |