BIOASSESSING THE IMPACT OF WATER QUALITY ON THE HEALTH AND PARASITE COMPOSITION OF *OREOCHROMIS MOSSAMBICUS* AT THE PHALABORWA INDUSTRIAL COMPLEX (PIC) AND THE BARRAGE (OLIFANTS RIVER) IN THE LIMPOPO PROVINCE, SOUTH AFRICA.

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Declaration

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

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Abstract

Three sites at the Phalaborwa Industrial Complex (PIC) and one site at the Phalaborwa Barrage in the Olifants River were selected to illustrate the possible influence of different water quality parameters on the health and parasite composition of the Mozambique tilapia (*Oreochromis mossambicus*). Ten fish were collected seasonally at each site using gill nets of different mesh sizes. Selected water quality variables were determined at all the sites to establish possible differences in water quality between the sites. Hosts were examined for mobile ectoparasites, weighed and measured. Blood samples were drawn and skin smears were made. Fish were killed, dissected and all external and internal organs were examined as prescribed in the fish health assessment index (HAI). The condition factor was determined for each fish population from the different sites. All parasites were collected, fixed and preserved using standard methods. A parasite index (PI), abundance, prevalence and mean intensity of the parasite infestations were calculated.

Results obtained for the system variables (pH, water temperature and dissolved oxygen) indicated that the pH levels and water temperature fell within the target water quality range (TWQR) for aquatic ecosystems, but the dissolved oxygen recorded during most of the surveys were below 5mg O_2 l^{-1} which may adversely affect the functioning and survival of biological communities. The mean turbidity values were high at sites A, C and D (caused by fine particles such as silt, clay and organic matter). The total dissolved solids (TDS) and the electrical conductivity (EC) were very high at sites B and C throughout the study. The total water hardness and salinity were also very high at sites B and C which can be attributed to the mine tailings water as well as the geology of the region. The cations (calcium, magnesium and potassium) and anions (chloride, fluoride and sulphate) were above the TWQR for aquatic ecosystems at all sites. The fluoride and sodium levels were high at sites B and C. All the major ions contributed significantly to increased levels of TDS, salinity and EC at sites B and C.

The nitrogen and phosphate levels indicated that there was an influx of nutrients into the four sampling sites at varying degrees, which can have an effect on eutrophication conditions at the sampling sites. Trace and heavy metal concentrations differed significantly between all the sites. Aluminium, iron and manganese levels were within the

TWQR for aquatic ecosystems at all sites. Copper, lead and zinc levels were above the TWQR and sometimes above the chronic and acute effect values for aquatic ecosystems throughout the study (except for lead concentrations at site A). Thus, the mining activities do affect the water quality at sites B and C adversely in terms of the dissolved salts, nutrients and trace and heavy metals (with the water at site C more impacted than that of site B). The toxicity of some metals is however, dependant on the pH (if it changes to be more acidic, some metals may become toxic) and water hardness of the specific site.

The lowest population HAI values (indicating healthier fish populations) were mostly recorded from sites A and D (the sites with better water quality) and the highest at site C (the site with poorer water quality) for three surveys. The high HAI values at site C can mainly be attributed to liver discoloration and abnormal haematological parameters. The fish condition factor values ranged from 0.92 to 1.2 with the lowest mean value recorded at site A and the highest mean value recorded at site B. But, the condition factors indicated that the fish from all sites were generally in good health. The values attained for the haematological parameters, liver discolorations, fins (due to parasitic infestation), abnormal gills, as well as the type of parasites present in/on the fish, were the most indicative parameters in the HAI. No abnormalities in the kidneys, opercules and spleens of fish were observed at any of the four sampling sites during this study. Results from the HAI thus indicated that the fish population from site C was more affected by the water quality (with a higher HAI) compared to the fish populations from the other sites. Also, dissimilar water quality at the different sites affected the health of fish differently. The results recorded for the HAI of the different fish populations thus substantiate the results obtained from the water analysis, indicating that fish from site C (with the poorest water quality) was more affected by their environment.

The parasites recorded from *O. mossambicus* were all site-specific and seem to be moderately influenced by the water quality of the different sites. Some groups, e.g. monogeneans were more affected by the differences in water quality than other parasitic groups. The following ectoparasites were recorded: *Cichlidogyrus* sp. from the gills and *Lernaea cyprinacea* and *Argulus japonicus* from the skin. Endoparasites included digenean larvae from the skin ("black spot") and gills, *Neutraclinostomum* larvae in the branchial region, *Diplostomum* metacercariae from the eyes and swimbladder,

Diplostomum tregenna from the brain, dilepidid cestode larvae from the liver and outer surface of the intestine*, Contracaecum* larvae from the body cavity and sinus venosus of the heart, adult acanthocephalans from the intestine, and pentastomatid larvae of two genera (*Subtriquetra rileyi* and *Alofia* sp.) from the swimbladder.

The hypothesis that the number of ectoparasites will be lower in more polluted water and the number of endoparasites will be higher was well supported for *O*. *mossambicus* at all sites except at site C during Spring survey. The PI for endoparasites was higher at all the mine sites (except during Spring) but similar results were also obtained at sites A and D (the less impacted sites). However, all sites tested during this study were impacted to a lesser or higher degree and the PI for endoparasites can thus be higher at all sites. Some ectoparasites (i.e. *Lernaea cyprinacea*) were present in high numbers at the more polluted site (site C), but the abundance of monogeneans (also ectoparasites) was most of the time lower at sites B and C, suggesting that monogeneans have been strongly influenced by the poorer water quality at these sites. The specific water parameter/s that influenced the abundance of certain ectoparasites needs further investigation which would most probably best be tested under controlled laboratory conditions.

In conclusion, all sites sampled during this study were contaminated to some degree with sites B and C more impacted than the other two sites. The water quality results thus confirmed the results obtained using the HAI and to a lesser extent, the PI. The water quality differed between the four sites and had dissimilar impacts on the health of *O. mossambicus* and the prevalence of some parasites at the different sites.

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Argulus japonicus

Chapter 1
Introduction

Rivers belong to some of the most intensively human influenced ecosystems on earth. In highly industrialized countries, many rivers are now among the most severely degraded ecosystems, suffering from channel and bank modifications, flow regulation and fragmentation, as well as chemical pollution. According to Poff *et al*. (1997) these alterations have led to an extensive ecological degradation of many rivers making them no longer sustainable in providing goods and services (e.g. decline in water quality and availability, intense flooding, loss of species and changes in the distribution and structure of the aquatic biota). Recognition of these adverse effects on river systems has driven initiatives for biological monitoring and river restoration world wide. In South Africa, the River Health Progamme (RHP) was initiated by the Department of Water Affairs and Forestry (DWAF) in 1994 to monitor selected aquatic communities to characterize the response of the aquatic environment to multiple disturbances. However, the RHP focuses primarily on biological responses as an indicator of ecosystem health, with only a general assessment of the cause-and-effect relationship between the drivers and the biological responses (Kleynhans *et al.* 2005).

The health of a river system is influenced by a multitude of factors. The geomorphology and geological formations, the chemical and physical quality of the water, the hydrological regimes and the nature of instream and riparian habitats have all an influence on the river ecosystem and river health (Poff *et al*. 1997). Water quality is described as the physical, chemical and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems (DWAF 1996). Each aquatic ecosystem has some natural buffering capacity. The latter allows the ecosystem to adapt to and compensate for natural changes in the environment such as leaching from the

soil or the occasional heavy rain. Water pollution occurs when conditions exceed the water ecosystemôs ability to compensate for these changes (Dallas *et al.* 1998).

Point source pollution may be discharged deliberately and illegally, or even accidentally and are fairly easy to measure and control (Davies and Day 1998). Non-point source pollution occurs when toxic substances enter surface and underground water through runoff from urban and industrial areas, leaching from domestic and solid waste disposal sites and seepage from mines. These are very difficult to quantify and control and there is little or no data available in South Africa due to the irregular discharges of non-point source pollution (Roux 1994; Heath and Claassen 1999; Dallas and Day 2004).

In South Africa, the quality of water was primarily determined by carrying out chemical analysis of water and measuring physical variables (Roux *et al.* 1993). Chemical analysis can give very accurate measures of the amounts of individual substances in the water of a river but they only consider the water passing at the moment of collection (Davies and Day 1998) and are thus only accurate at the time the sample was taken (Abel 1989; Bertasso 2004). Furthermore, chemical and physical water analysis is expensive and requires skilled and trained personnel. With the above in mind, alternative methods of determining the quality of water sources are continuously being investigated. One of the alternative methods is biological monitoring. Biological monitoring provides a bigger picture of both the past and the present conditions in a river. This is because the organisms that are living in a river must have been able to survive whatever conditions the river has been subjected to in the recent past (Davies and Day 1998) and the integrity or health of the biota provides a direct and integrated measure of the health of the river as a whole.

Biomonitoring is increasingly being known as an important component in the overall monitoring and assessment of water resources. The use of biological field assessments of fish or macro-invertebrate communities, provide an integrated and

sensitive measurement of environmental problems and assists in the assessment of ecological impacts, and hence in the management of water resources (Roux 2001). There are essentially two distinct monitoring approaches that can be followed, namely stressor monitoring and environmental response monitoring. Stressor monitoring focuses on the stressors that are assumed to be associated with the cause of pollution and ecological change. Environmental response monitoring refers to the monitoring of biological or ecological indicators (including biomarkers) in order to characterize the response of the environment to a disturbance (Van Dyk 2003).

Biomonitoring programs may be qualitative, semi-quantitative or quantitative and involve the use of indicator species or indicator communities. Macro-invertebrates are frequently used for biomonitoring (Rosenberg and Resh 1993) and have for some time been used in South Africa. The most recent is the South African Scoring System 5 (SASS5) community index (Dickens and Graham 2002). Fish also received attention as indicator organisms in South Africa, especially the intolerance of certain species to particular environmental conditions (Kleynhans *et al*. 1992; Kleynhans 1999). In addition, certain aquatic plants have been used as indicator species for pollutants, including nutrient enrichment (Batiuk *et al*. 1992; Phillips and Rainbow 1993). Changes in the structure of riparian vegetation may have an influence on the entire ecosystem as it maintain channel form and serve as filters for light, nutrients and sediment. The Riparian Vegetation Index (RVI) is used to determine the status of riparian vegetation within river segments. Loss of habitats contributes to the loss in biodiversity and the Index of Habitat Integrity (IHI) is used to assess the impact of major disturbances on river ecosystems (Kleynhans 1996). Only a few biomonitoring programs and indexes used in South Africa are mentioned, and there are advantages as well as disadvantages to each type of monitoring method and organism used.

Physiological, morphological and genetic changes in certain organisms have been recognised to be related to particular environmental stressors and can also be

used as indicators of adverse conditions. Furthermore, aquatic organisms tend to bioaccumulate pollutants, especially metals, from the surrounding environment or from food sources and these are also important biomonitoring devices (Alabaster and Lloyd 1980; Phillips and Rainbow 1993; Seymore *et al*. 1996; Kotzé *et al*. 1999). The monitoring of metals in an aquatic system is not only important for indicators of temporal and spatial extent of metal accumulation in a system, but also for organism health and the potential impact on human health (fish consumed) (Heath *et al*. 2004).

Organisms in aquatic environments are considered biologically sensitive and respond to changes that occur in the water. The biotic integrity of an ecological system is therefore reflected in the health of its fauna (Robinson, 1996). Changes, occurring specifically in fish populations due to chemical stress, are manifestations of biochemical, histological and physical alterations, and can give a relatively rapid indication of how environmental conditions affect fish populations. Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution levels. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Furthermore, fish populations will adapt to environmental changes, migrate or move away from the source of pollution, or they may die a slow death. To manage healthy fish populations, it is necessary to identify early warning signs of damage on cellular level, before physiological and behavioural processes are affected (Van Dyk 2003).

Biological evaluation thus provides a means to quantify ecological changes that resulted from the combination of physical, chemical and biological stressors (Oberdorff and Hughes 1992). One such method is the Health Assessment Index (HAI) which is used to asses the health of fish in an ecosystem and consequently the health of the system. It was developed in the United States of America and introduced and tested in South Africa in the Olifants River (Avenant-Oldewage *et al*. 1995), the Vaal River System (Crafford 2000; Groenewald 2000; Crafford and Avenant-Oldewage 2001) and the lower reaches of the Ga-Selati River (Jooste *et*

al. 2004). The HAI is a quantitative index that allows statistical comparison between different water bodies and it gives a rapid indication of the health status of a selected environment. Although the exact cause of pollution cannot be assessed, the HAI is useful in assessing first level problems in the health profile of fishes (Heath *et al*. 2004). This method is based on scores, with higher scores indicating the polluted sites and lower scores the better sites (Avenant-Oldewage *et al*. 1995).

One of the categories in the HAI is the presence or absence of parasites. Most freshwater fish harbour parasites which are normally not harmful to the host. But, pollution can increase parasitism if the host defense mechanisms are negatively affected, thereby increasing host susceptibility (Sures 2006). Conversely, pollution can also decrease parasitism if the parasites are more susceptible to a particular pollutant than the host, or pollution levels eliminate the suitable intermediate host. The presence or absence of parasites can thus also reflect environmental conditions and possibly environmental health. When the HAI is supplemented by a Parasite Index (PI), the value of the HAI can only be enhanced. The HAI and associated PI will test differently in every aquatic system, depending primarily on the fish species used and type of pollution (Jooste *et al*. 2004).

1.2 Rationale

The HAI and associated PI were used during this study as biomonitoring tools to assess the impact of water quality on the health and parasite composition of a freshwater fish species. To accomplish this, four sites with dissimilar water quality were selected, i.e. two industrial sites, a mine site and a reference site (the latter is a site in the Olifants River where all the water used by the Phalaborwa Industrial Complex (PIC) originates from; see Chapter 2: Material and Methods). The reason for selecting different sites was to ascertain the possible effect that the PIC has on the water quality of the Olifants River (where the water originates) and on the

health and parasite composition of the Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852).

The Olifants River originates in the Highveld grasslands (to the east of Johannesburg) and flows in a north-easterly direction, ultimately flowing through the Kruger National Park to Mozambique where it flows into the Massingir Dam before joining the Limpopo River in Mozambique. According to Grobler *et al.* (1994) the Olifants River is degraded and contaminated with metals and other chemicals. The Olifants River has been systematically impaired because of an increase in agricultural and mining activities, industrial development and urbanization and Van Vuren *et al.* (1999) described the river as one of the most polluted systems in South Africa and called it ñthe Battered Riverò. Along the Olifants River there are intensive and subsistence agriculture practices as well as numerous point and diffuse sources of mining and industrial pollution (Heath and Claassen 1999).

A large number of mines, predominately coal mines, are located in the Loskop Dam catchment and are concentrated mainly in the Olifants and Klein Olifants River catchments upstream of the Witbank and Middelburg Dams respectively. The most extensive coal mining takes place at the Witbank Coalfields and Highveld Coalfields. Claassen *et al*. (2005) reported that coal mining and industries in the Witbank-Middelburg and Phalaborwa areas have a significant impact on the Olifants River. These mine effluents contain a complex of chemicals, many of which may have deleterious effects on aquatic systems (Van Vuren *et al.* 1999).

Water discharges from the mines can originate from various sources, including sewage treatment plants and seepage from opencast and underground mining operations. The return flows from sewage treatment plants are released into natural streams or re-used in mining operations. Return flows are also used for irrigation purposes (Van Vuren *et al.* 1999). Seepage and decanting from mines may result in serious water quality related problems.

Claassen *et al*. (2005) reported that return flows from the PIC have an effect on the water quality of the Ga-Selati River (Figure 2.1), which as a result impacts on the water quality of the lower Olifants River. To ascertain this, it was decided to apply the HAI and PI to test the impact of mining and industrial activities in the PIC on the health of fish and the ecosystem. Therefore, as mentioned above, three sites were selected for this study at the PIC and one in the Olifants River. These sites were selected because of the dissimilarity in water quality represented by different water constituent concentrations.

The HAI and PI were applied at these different sites using the same host, i.e. the Mozambique tilapia, to allow for accurate comparison between the different sites. This fish species occurs in all but fast flowing water but thrives in standing waters. It is an euryhaline species with a high tolerance for excessive salinity concentrations. They can survive in low temperatures (below about 15ºC) but prefers warm water temperatures (above 22ºC) (Skelton 2001). They can, however, tolerate water temperature up to 42ºC (Popma and Masser 1999). They feed on algae especially diatoms and detritus, but large individuals may also consume large aquatic invertebrates (Skelton 2001).

1.3 Aim and Objectives

Aim: to evaluate the impact of water quality on the health and parasite composition of the Mozambique tilapia (*Oreochromis mossambicus)* at the Phalaborwa Industrial Complex (PIC) and the Phalaborwa Barrage (Olifants River) in the Limpopo Province.

1.3.1 Specific objectives

- To determine the water quality at the different sites (selected water constituents).

- To determine if the water quality differ significantly between the four sites and whether the diverse conditions affect *O. mossambicus* populations differently.
- To determine if the mining and industrial activities in the PIC have an impact on the health of *O. mossambicus* by applying the HAI.
- To record, collect and identify the ecto- and endoparasites of *O. mossambicus*.
- To determine if the abundance, prevalence and mean intensity of parasites from *O. mossambicus* differ between the four sites.
- To ascertain the site preferences, specialization and host specificity of parasites of *O. mossambicus.*
- To determine the possible effects of parasite prevalence on the health of *O. mossambicus.*
- To determine the possible impact of different water constituents on the presence/absence of ecto- and endoparasites of *O. mossambicus*.

In order to achieve the aim and specific objectives of this study the results and findings are submitted as follows:

Chapter Two contains a general description of the study area, sampling sites and fish species used during this study. The material and methods used for water analysis, the Health Assessment Index determination and parasite collection are discussed. The fixation, preservation and staining methods used for parasites are listed.

Chapter Three describes the results obtained for the selected water constituents determined during seasonal surveys at the four sampling sites. Each of these variables and their possible influence on the aquatic environment is discussed. This is followed by the conclusions on the water quality at the different sites and references cited during this chapter.

Chapter Four focuses on the health and parasite composition of *O. mossambicus* by applying the Health Assessment Index and Parasite Index. A brief history on the development of the HAI and PI is given. A detailed discussion on external and internal variables of fish is included. This is followed by the conclusions on the health and PI of the fish populations at the different sites and the references.

Chapter Five deals with the ecto- and endoparasites recorded from *O. mossambicus* at the different sites. Infestation statistics (including prevalence, intensity and abundance) and host specificity is included. The conclusion and references bring this chapter to a close.

Chapter Six contains a general summary of results obtained and conclusions drawn and recommendations are provided at the end of the dissertation.

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Chapter 2
Materials and Methods

2.1 Sampling sites

Four surveys were undertaken at four selected sites to determine the water quality, health and parasite composition of the Mozambique tilapia (*Oreochromis mossambicus*). The surveys were conducted in June 2005 (Winter), October 2005 (Spring), January 2006 (Summer) and April 2006 (Autumn). Three sites selected for this study were at the Phalaborwa Industrial Complex (PIC) and one in the Olifants River (Figures 2.1 and 2.2).

The three sites in the PIC include a site at a fertilizer plant (Sasol Nitro), a site at a phosphate mine (Foskor Ltd) and a site at a copper mine (Palabora Mining Company). A fourth site was selected i.e. the Phalaborwa Barrage in the Olifants River, to represent a site that is less impacted by mining and industrial activities. The Phalaborwa Barrage is also the source water for the PIC. Four sites were selected for this study because of the dissimilarity in water quality at the sites, represented by different water constituents.

Furthermore, fish in aquatic ecosystems are considered biologically sensitive and respond to changes that occur in the water. Changes, occurring specifically in fish populations due to stress, are manifestations of biochemical, histological and physical alterations, and can give a relatively rapid indication of how environmental conditions affect fish populations (Heath and Claassen 1999). The four sites were thus selected to ascertain the possible effect that the PIC has on the water quality of the different sites and on the health and parasite composition of O. *mossambicus*.

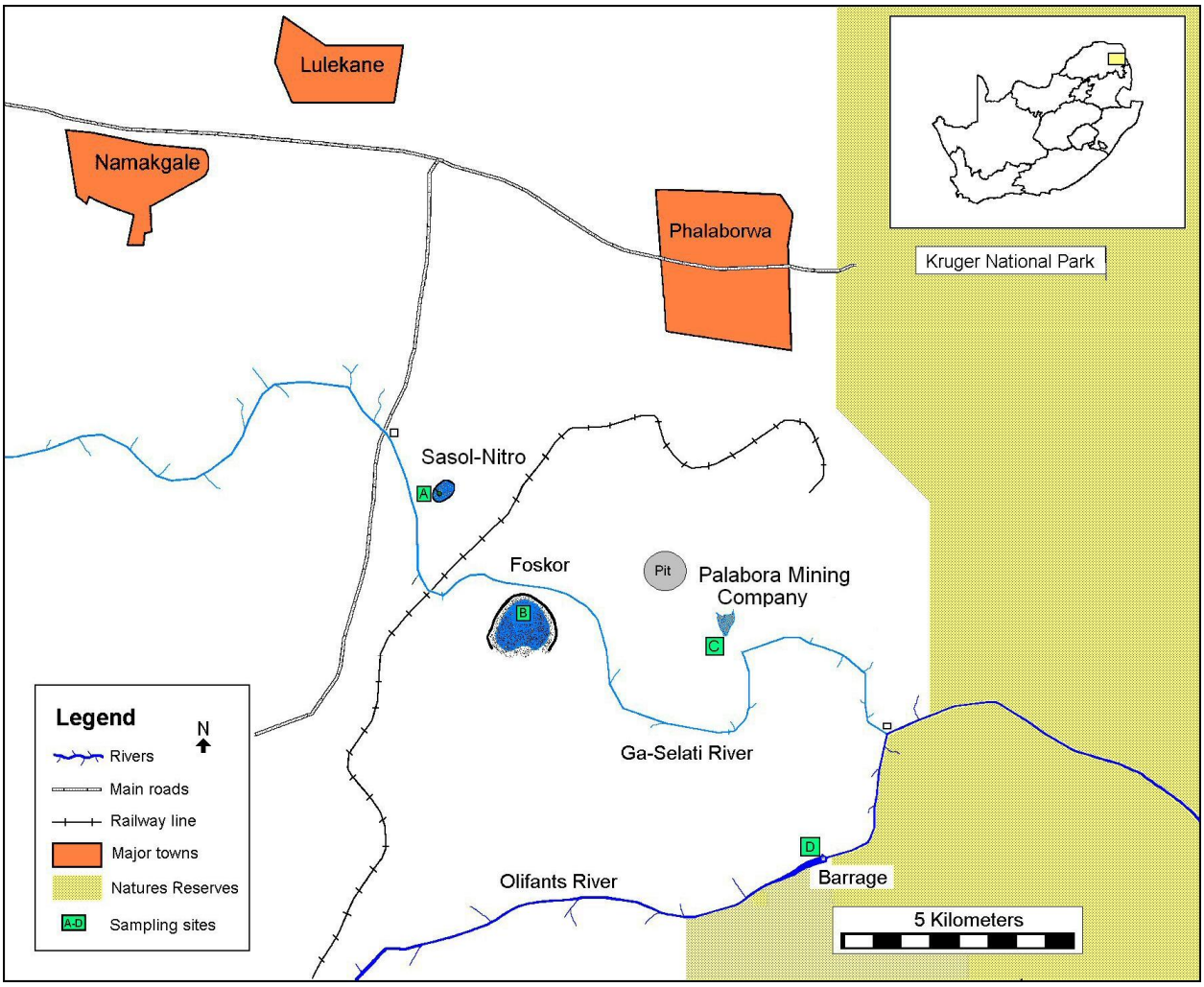

Figure 2.1: Sampling sites at the Phalaborwa Industrial Complex and Phalaborwa Barrage (Olifants River): A ï Sasol Nitro, B ï Foskor Limited, C - Palabora Mining Company, D ï Phalaborwa Barrage.

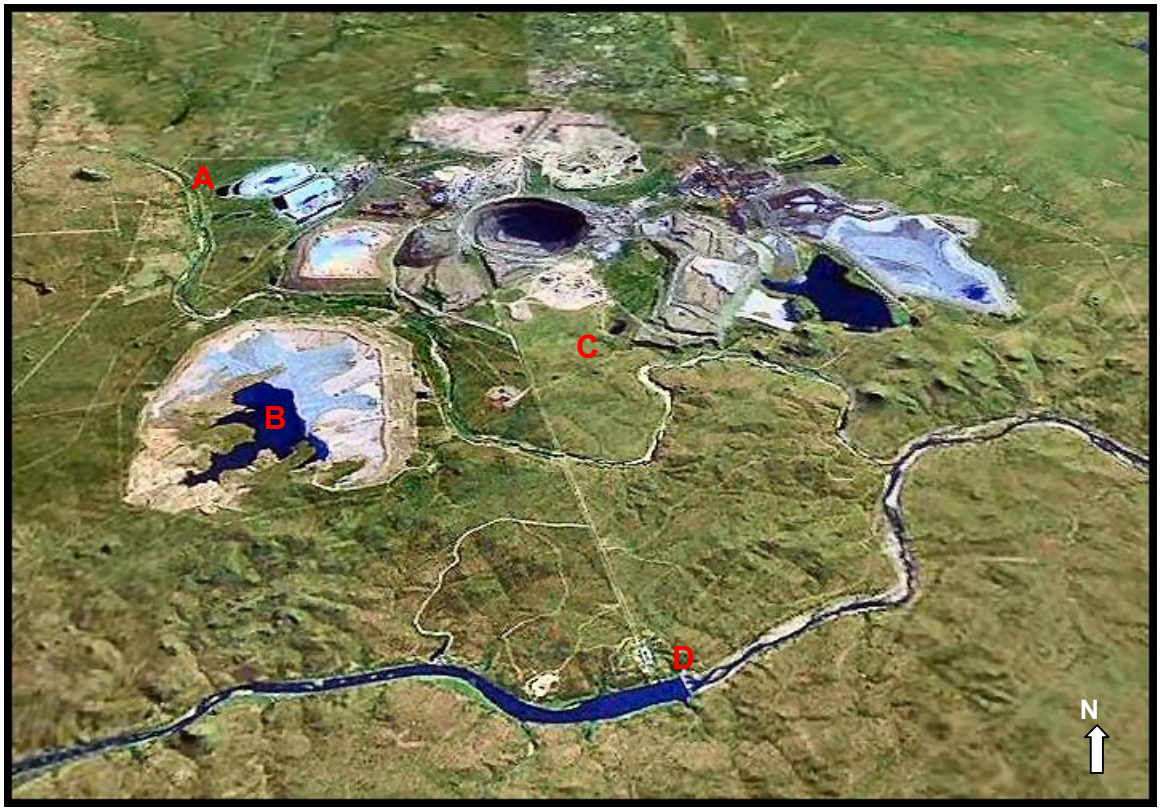

Figure 2.2: Satellite photo of Phalaborwa Industrial Complex (Google Earth 2006): A ï Sasol Dam at Sasol Nitro, B ï Tailings Dam at Foskor Ltd, C - Loole Dam at Palabora Mining Company, D ï Phalaborwa Barrage.

Site A: Sasol Dam **Co-ordinates**: 23º 59ô20.22õS 31º 05ô11.66õE

Sasol Dam (Figures 2.3A and B) is a recreational dam, located in the Sasol Game Reserve. It receives water from the Phalaborwa Barrage (Lepelle Water Board). The dam is bordered by large indigenous trees for example, wild fig trees and fever trees (*Acacia xanthophloea*) (Figure 2.3B). The banks of the dam are covered with grass and large reed beds. It serves as a recreational dam for Sasol staff and also provides water to the wildlife in the Game Reserve. The following fish species were encountered in the dam: Mozambique tilapia (*O. mossambicus*), sharptooth catfish (*Clarias gariepinus*), and alien fish species like the common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*). There are also several terrapins, crocodiles and Nile water monitor lizards in and around the dam.

Figure 2.3 A and B: Sampling site A (Sasol Dam): A - Setting of nets at Sasol Dam, B - Sasol Dam showing the large indigenous trees on the bank.

Site B: Tailings dam of Foskor Ltd **Co-ordinates**: 24º 01ô46.4ðS, 31º 05ô53.33ðE

Foskorôs main tailings dam (Figures 2.4A and B) is large (±1000 hectares) and situated south of the Selati River on the Foskor Ltd grounds (Figure 2.1). This dam was created to store the tailings from the phosphate plant which is pumped into this dam daily. Foskor Ltd is the largest, single production facility of phosphoric acid based products in the southern hemisphere and three million tons of phosphate rock is produced per annum. Foskor Ltd also receives industrial water from the Phalaborwa Barrage (Lepelle Water Board). This is used in the various processing plants, mainly as a transport medium for the mining residues (tailings) to the tailings dams. The tailings dam decants into the return water dam, from where the water is recycled back into the process. This site is home to crocodiles, hippopotami and many piscivorous birds which probably serve as final hosts for some helminth parasites. It is surrounded by indigenous trees such as acacias, marulas and mopane trees (Figure 2.4B). There are large stands of the pondweed (*Potamogeton* sp.) in the shallows of the dam. Despite the high salinity level of the water, water buck, elephants and other game frequent the dam and utilize it intensively as a permanent source of water.

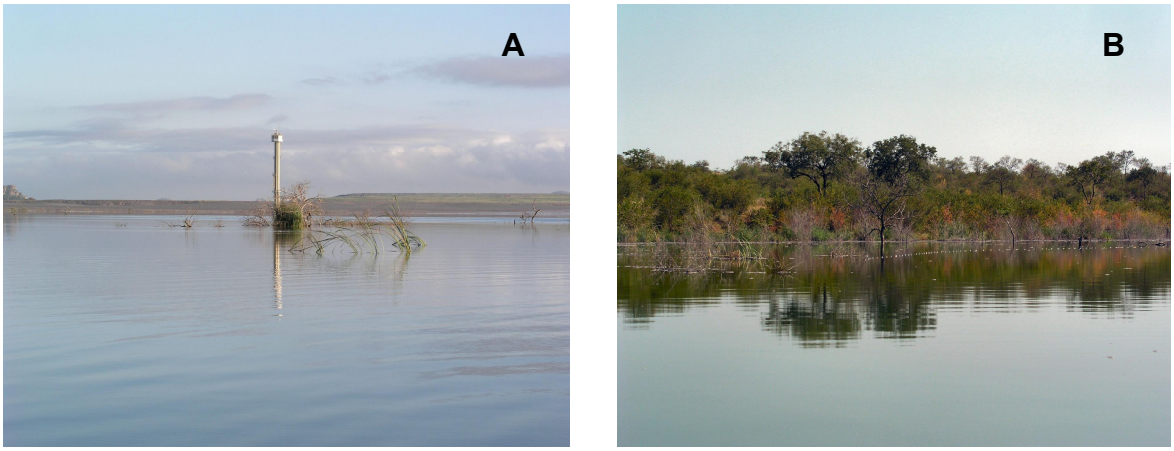

Figure 2.4 A and B: Sampling site B (Foskor tailings dam): A - Tower with outlet (also indicating the water level) in Foskor tailings dam, B ï Large trees on the banks of the tailings dam.

Site C: Loole Dam **Co-ordinates**: 24º 00ô56.2òS, 31º 05ô32.47òE

The Loole Dam (Figure 2.5) is located on the southeastern section of the copper mine (Palabora Mining Company) (Figure 2.1). Loole Dam was built in the Loole creek to store the treated effluent from the mine operations and to prevent water from flowing into the Selati River. The Loole Dam receives water from the Loole stream as well as storm water. Also, the water level is regulated by pumping water from and to the return water dam. Generally, water is pumped from the excavations (open pit mine) and the underground mine to maintain a dry, safe and efficient operating environment for the various mining operations. The Palabora Mining Company formerly extracted copper from a large open-pit mine but presently the copper is mined underground. It also mines phosphate and vermiculite through an open pit mine. Copper by-products include nickel sulphates, magnetite, sulphuric acid and anode slimes. The Palabora Mining Company also receives their industrial water from the Phalaborwa Barrage (Lepelle Water Board). Many piscivorous birds, crocodiles and a pod of hippopotami inhabit this dam permanently (Figure 2.5).

Figure 2.5: Sampling site C (Loole Dam) with the pod of hippopotami.

Site D: Phalaborwa Barrage **Co-ordinates**: 24º 04ô10.04ôS, 31º 08 ô39.58ôE

The Phalaborwa Barrage (Figures 2.6A and B) was built in the Olifants River and is situated approximately 10 km from the Phalaborwa town. It is a large but shallow Barrage (due to siltation). The Lepelle Water Board pumps water from the Phalaborwa Barrage, purifies and distributes potable and industrial water to the various users in the Phalaborwa region. The industrial water is used in the various processing plants, mainly as a transport medium for and to transport the mining residues to the tailings dams. The Barrage site is bordered by riparian vegetation of the river including large indigenous trees, reeds and grasses (Figure 2.6B). The Barrage provides habitat for many birds, crocodiles, Nile water monitor lizards and several hippopotami.

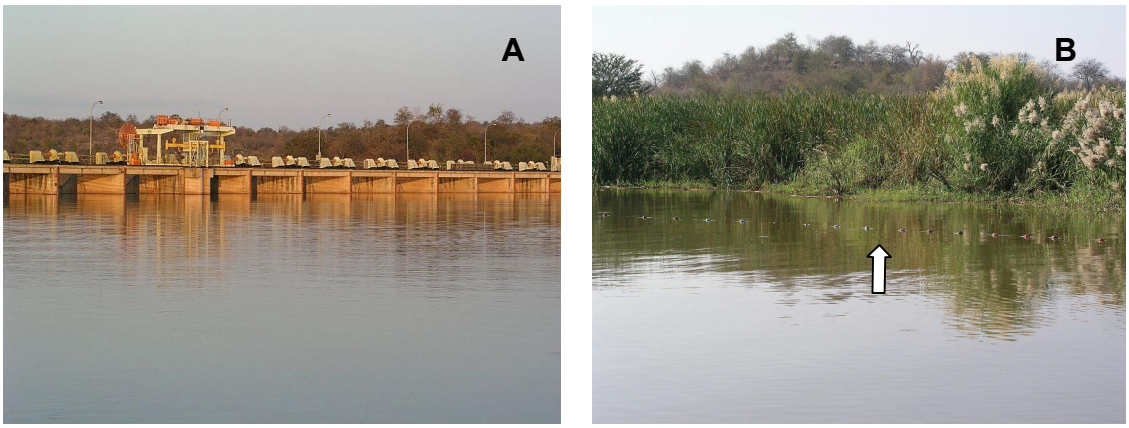

Figure 2.6 A and B: Sampling site D (Phalaborwa Barrage): A - Wall with sluices, B - Gill nets in the river.

2.2 Fish species

The Mozambique tilapia (O. *mossambicus*) (Figure 2.7) occurs in all but fast flowing waters but thrives in standing waters. This species is able to adapt to a wide range of salinities (Skelton 2001) and are also found in brackish and marine water.

Figure 2.7: The Mozambique tilapia; *Oreochromis mossambicus* (Peters 1852).

Tilapia may survive acute exposures to increased salinity levels by reducing branchial permeability; however, if salinity stress is high due to increased salinity levels or extended duration of exposure, this mechanism may not be sufficient, and more conventional strategies of osmoregulation, such as increased drinking rate and Na^+, K^+-ATPase activity, are then observed (Sardella *et al.* 2004). They can survive in low temperatures (below about 15^{o}C), but prefers warmer temperatures (above 22^{o}C). They can, however, tolerate water temperature up to 42^{o}C (Skelton 2001). They feed on algae, especially diatoms and detritus, but large individuals may also feed on large invertebrates. The Mozambique tilapia breeds during Summer and the females raise multiple broods every 3 ï 4 weeks during a breeding season. Males usually construct a saucer-shaped nest on sandy bottoms and guard the nests protectively. The female mouthbroods the eggs and after hatching, the larvae and small fry may seek protection in her mouth. The juveniles shoal in shallow water and grow rapidly. Under favourable conditions the young fish may mature and breed within a year, but they are prone to stunting under adverse or crowded conditions (Skelton 2001).

2.3 Materials and Methods

2.3.1 Water quality

The dissolved oxygen (DO), pH, water temperature, salinity and electrical conductivity (EC) of water were determined *in situ* by means of a handheld multi parameter instrument (YSI model 54 Combometer) at all sampling sites. Water samples were collected at all sites in acid treated sampling bottles and immediately refrigerated for laboratory analyses. The following parameters were analyzed by the Foskor Ltd chemical lab: total dissolved solids (TDS), alkalinity, total hardness, nitrate, phosphorus, sulphate, chloride, sodium, potassium, calcium, magnesium, fluoride, manganese and copper. The following parameters were determined by means of calibrated Merck tests kits with a Merck SQ 118 digital

photospectrometer in the Department of Biodiversity at the University of Limpopo (Turfloop campus): turbidity, dissolved nutrients (nitrite, ammonia and total nitrogen), and selected metals (lead, iron and zinc).

Water quality criteria

Water quality guidelines provide an objective means for judging the quality needed to maintain a particular environmental value. The South African Water Quality Guidelines for aquatic ecosystems (DWAF 1996) list a recommended target range (i.e. TWQR), a Chronic Effect Value (CEV) and an Acute Effect Value (AEV) for specific water quality variables (the AEV and CEV are only for toxic constituents). The Target Water Quality Range (TWQR) is the range of concentrations or levels within which no measurable adverse effects are expected on the health of aquatic ecosystems and should therefore ensure their protection (DWAF 1996). The CEV is defined as that 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 of a constituent above which there is expected to be a significant probability of acute effects to up to 5% of the species in the aquatic community. The TWQR is a management objective (rather than a water quality criterion) derived from quantitative and qualitative criteria. As a matter of policy the Department of Water Affairs and Forestry strives to protect South Africaôs water resources by maintaining water quality within the TWQR (DWAF 1996). The results obtained for water quality analyses during this study were compared with the TWQR, AEV and CEV for aquatic ecosystems where applicable and available.

2.3.2 Sampling of fish

Fish were collected by using gill nets with stretched mesh sizes of 50 mm, 70 mm, 90 mm and 110 mm. Ten *O. mossambicus* specimens were collected at each

sampling site during all four sampling periods. Only *O. mossambicus* and *Clarias gariepinus* (for another study) were kept, all other fish species were returned to the water.

2.3.3 Health Assessment Index

Fish were kept in large holding tanks filled with water from each site respectively to minimize stress. One fish was selected at a time to determine the Health Assessment Index (HAI) and Parasite Index (PI). Blood collection was done as quickly as possible before the fish dies. Blood was drawn and two blood smears per fish were made on dry pre-clean glass slides. Blood slides were air-dried for approximately ten minutes and fixed by dipping it in 96% methanol to avoid osmotic shock of cells. Blood smear slides were immersed in a GiemsaÊ solution for 10 minutes at a later stage in the laboratory (UL). When stained adequately, slides were removed and rinsed with tap water to remove excess stain. Slides were dried at room temperature for approximately 12 hours. Dried slides were covered with cover slips using EntellanÊ mounting medium before blood counts were made. Five places were randomly chosen on the slide (using 400x microscope magnification) and the mean percentage of white blood cell (WBC) counts was determined for each fish. Capillary tubes were filled with blood and plugged at one end using commercial critoseal clay. The haematocrit reading (Figure 2.8) was read and recorded after centrifugation of capillary tubes for five minutes. Blood samples were centrifuged in 5 ml VacutainersÊ for five minutes to separate the blood in plasma and red blood cells (Figure 2.8). Blood plasma (top layer) were drawn into a plastic dropper and frozen in small plastic containers for plasma protein determinations at the laboratory of the Department of Biodiversity (UL). Total blood protein concentration was determined using a Boehringer Mannheim test-Combination kit (Cat no 1553836. SYS 1).

Table 2.1: Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy-based system (adapted from Adams *et al*. 1993 and Jooste *et al*. 2004)

Variables	Variable condition	Original field designation	Substituted value for the HAI
External variables			
Length	Total length in millimeters	mm	-
Weight	Weight in gram	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[b]	No active erosion or previous erosion healed over	0	0
	Mild active erosion with no bleeding. >10 parasite cysts	1	10
	Severe active erosion with haemorrhage / secondary infection. > 50 parasite cysts	2	20
Skin[b]	Normal, no aberrations	0	0
	Mild skin aberrations. >10 parasite cysts	1	10
	Moderate skin aberrations. >50 parasite cysts	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	-
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Other	OT	30

Table 2.1 continued

Variables	Variable condition	Original field designation	Substituted value for the HAI
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
	ñFattyò liver, ñcoffee with creamò colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other	OT	30
Bile[a]	Yellow or straw colour, bladder empty or partially full	0	-
	Yellow or straw colour, bladder full, distended	1	-
	Light green to ñgrassò green	2	-
	Dark green to dark blue-green	3	-
Blood (haematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30
Blood (plasma protein)	Normal range	30-69mg/dL	0
	Above normal range	>70mg/dL	10
	Below normal range	<30mg/dL	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10
Endoparasites[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 ï 10	1	10
	11 ï 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 this study

The fish were examined externally by using the revised HAI method (Table 2.1) (Heath *et al.* 2004, Jooste *et al*. 2004) and recorded on a HAI data sheet. Fish were killed prior to dissection by severing the spinal cord. Fish were weighed and total and standard lengths were measured. The fish were dissected and all internal organs were assessed with the help of a colour chart developed by Watson (2001)

and values were assigned to each organ as indicated in the revised HAI table (Table 2.1).

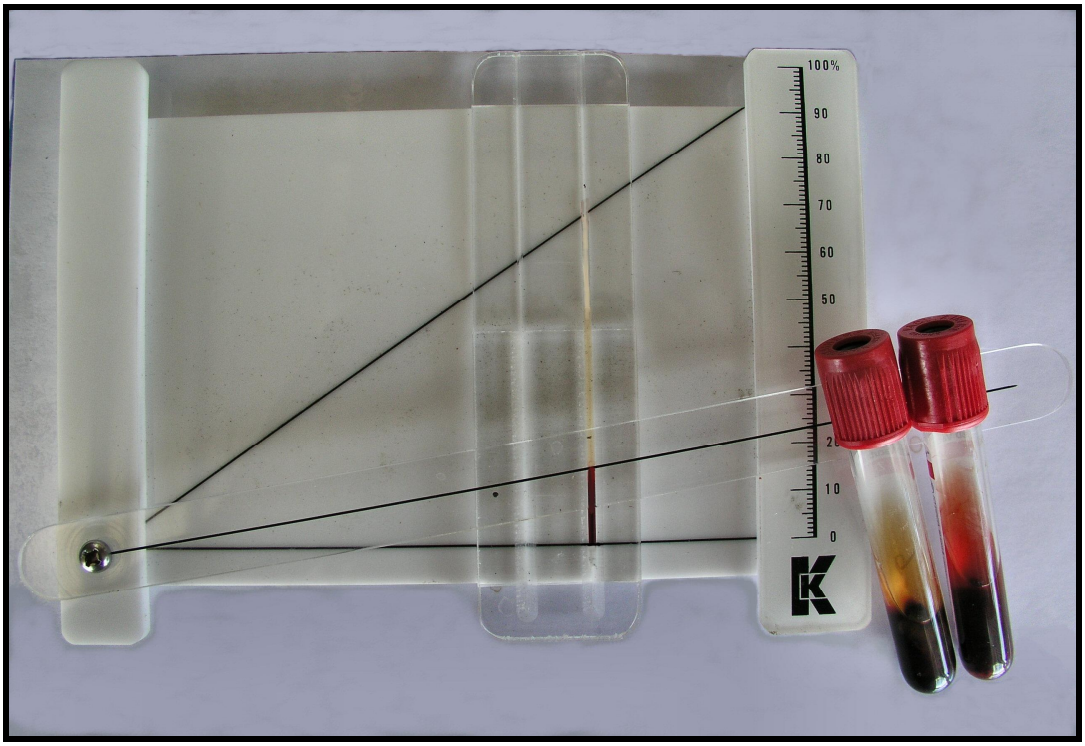

Figure 2.8: The heamatocrit reader with capillary tube and blood samples for protein determinations.

Calculation of the Health Assessment Index

Original field designations of all variables from the necropsy-based system were substituted with comparable numerical values into the HAI (Table 2.1). All the variables of the HAI were represented by a value ranging from 0 - 30, depending on the condition of the organs, etc. tested, with normal conditions indicated by 0. To calculate an index value for each fish within a sample, numerical values for all variables are summed. By adding all individual fish health index values and

dividing it by the total number of fish examined, the HAI for a sample population was calculated.

Condition factor (CF): Length- weight relationship

The condition factor (CF) of fish, based on the analysis of length-weight data, indicates the health of fish in a habitat. The CF was determined for the different fish populations to ascertain any differences in health of the fish between the different sampling sites.

The population condition factor was calculated according to Heath *et al*. (2004) where:

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

W = weight in g

L = total length in cm

2.3.4 Parasites

As soon as a fish was removed from the gill nets, macroscopic examinations were done on the boat for mobile ectoparasites. Mobile ectoparasites found were recorded and kept in small glass containers filled with water from the respective site for further processing in the field laboratory. Skin smears were made and scrutinized for parasites. After the fish were killed, they were opened ventrally and the body cavity and mesenteries were examined for parasites.

The different organs, e.g., eyes, gut (alimentary canal and associated organs), swimbladder and urinary bladder were placed in separate petri-dishes containing saline solution and examined for endoparasites with the aid of a stereo microscope

(gills were placed in water from the respective sites). The muscles were also thoroughly scrutinized for encysted parasites.

Monogeneans were fixed in hot (± 70°C) alcohol formalin-acetic acid (AFA) and preserved in 4% formaldehyde or 70% ethanol. Digeneans were fixed in hot AFA for 30 minutes (flat between microscopic slides) and stored in 70% ethanol. Cestodes from the intestinal tract and liver were swirled in saline until they are relaxed, fixed in AFA for 10 minutes and preserved in 70% ethanol. Nematodes were fixed in glacial acetic acid and preserved in 70% ethanol. Acanthocephalans, pentastomids, branchiurans and copepods were fixed and stored in 70% ethanol.

Preparation of whole mounts and identification of different parasites were done in the laboratory where specimens were stained either with Horen`s TrichromeÊ or Aceto CarmineÊ solution. If over-stained, they were placed in 2% hydrochloric acid (HCl) water 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 balsamÊ or EntellanÊ and labeled. Nematodes were cleared with lactophenol and mounted without staining (temporary mounts).

All parasites were micrographed with the aid of a WildÊ stereo microscope or OlympusÊ light microscope with an OlympusÊ digital camera adapter and an OlympusÊ digital camera (C50-50 Zoom).

Ecological terms used in infestation statistics

A variety of terms are used by parasitologists to describe the number of parasites in a host or the number of infected hosts in a sample. Examples of such terms are parasite burden; parasite load; level or extent of infection; degree of infection or infection rate. The terminology as suggested by the American Society of Parasitologists (Margolis *et al*. 1982) was used during this study and includes prevalence (expressed in percentage), intensity and abundance where:

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

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

Abundance (relative density) = total number of particular parasite species divided by the total number of hosts in a sample.

Parasite Index

Contaminants have different influences on ecto- and endoparasites respectively (see Chapter 5 on Fish Parasites) and therefore endo- and ectoparasites were incorporated as separate variables in the HAI tested in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001). Endoparasites are usually much higher in number than ectoparasites and more than 1000 trematode cysts or nematode larvae can be observed in a single host. Therefore endo- and ectoparasites were categorized as presented in Table 2.2 below:

Table 2.2: The Parasite Index (values used during the calculation of the PI) from Jooste *et al*. (2004)

ECTOPARASITES	PI	ENDOPARASITES	PI
Zero parasites observed	**0**	Zero parasites observed	**0**
1 ï 10	**10**	< 100	**10**
11 ï 20	**20**	101 ï 1000	**20**
> 20	**30**	> 1000	**30**

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Chapter 3
Water Quality

Introduction

Water is a vital natural resource, since it is fundamental to any form of life. Water quality is described as the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems (DWAF 1996c). It is much easier to describe what poor water quality is than to describe what conditions are considered to be good water quality. Many of the ranks between good and poor water quality are stream-specific and each aquatic ecosystem has some natural buffering capacity. The latter allows the aquatic ecosystem to adapt and compensate for normal changes in the environment such as leaching from the soil or the occasional heavy rain (Chapman and Kimstach 1996). Water pollution occurs when conditions exceed the aquatic ecosystemôs ability to compensate for the changes.

Polluted water may be discoloured, possess a coating on the bottom of the stream, or may have no visible sign at all. According to Davies and Day (1998) there are two kinds of water pollution, i.e. point source and non-point source pollution. Point source pollution occurs when the contaminants are emitted directly (discharged deliberately and illegally) into the waterway while pollution from non-point sources occurs when substances enter the water body through runoff from urban and industrial areas, seepage from mines and leaching from domestic and solid waste disposal sites (Heath and Claassen 1999). The latter type of pollution is difficult to quantify and control due to irregular discharges (Dallas and Day 2004).

Good water quality is the key to flourishing fish health since all of a fishôs life processes occur in its watery environment. Their total dependence on water means that all their metabolic processes take place in water, therefore they breathe in the same substance

where they excrete their wastes (Novotny 2003). Novotny (2003) also stated that poor water quality can kill fish directly; moreover, it is a major contributing factor to disease outbreaks since the fishôs immune system is lowered in response to the poor conditions.

As mentioned in Chapter 2, the Olifants River originates to the east of Johannesburg and initially flows northwards before gently curving eastwards towards the Kruger National Park (KNP), where it is joined by the Letaba River and other rivers before flowing into Mozambique. Both the upper and lower Olifants River catchments experience water quality problems due to the Highveld coal mines, and the Phalaborwa Mining and Industrial Complex, respectively (DWAF 2005). Agricultural activities furthermore contribute to water quality problems in both catchments. In addition, the sediment from upstream activities, through agricultural (including overgrazing), industrial and mining activities, accumulates in the Phalaborwa Barrage (RHP 2001). The water quality of the Olifants River, in the KNP, is thus influenced by siltation (due to injudicious agriculture practices) and mining effluent (Venter and Deacon 1992; Wepener *et al*. 2000). When the Barrage is scoured from time to time, large quantities of sediment are released into the Olifants River inside the KNP (Ashton *et al.* 2001). In the past, this increased sediment load below the Barrage caused severe fish kills due to silt clogging their gills.

Metals are not homogeneously distributed in sediment and the fine grain (silt) is generally enriched while the course grain fractions are depleted of metals (Müller *et al*. 2001). According to Mason and Macdonald (1988) heavy metals attach to clay particles in silt. The silt loads in the Olifants River inside the KNP is usually high during summer with generally lower silt loads during the dry seasons (Buermann *et al.* 1995). According to Kotzé *et al.* (1999), the high silt loads in the Phalaborwa Barrage resulted in bioaccumulation of metals in the tissues and organs of fish, and subsequently lead to higher concentrations of metals in mostly the gills and livers. The bioaccumulation of metals in fish can reduce their survival and may disrupt their development, growth and reproductive potential (Buermann *et al.* 1995, Venter and Deacon 1995, Marx and Avenant-Oldewage 1998). Furthermore, by consuming fish with these higher metal

concentrations, an organism may build up very high concentrations of metals in its tissues (Carbonell *et al*. 2000). This process (biomagnification) may be repeated several times through the food chain, leaving the top predator with very high and sometimes lethal concentrations of metals (Carbonell *et al*. 2000).

Although the water quality of the Olifants River is negatively affected by agricultural, mining and industrial activities in the catchment area, the water quality improves before flowing into the KNP and neighbouring private game reserves (RHP 2001). This improvement in water quality is due to better water from the Steelpoort and Blyde tributaries, and the Selati River, which join the Olifants River before it enters the KNP (RHP 2001). The water emanating from the Blyde River (Lowveld) is of good quality and, together with the good quality water from the Mohlapitse River (Middleveld), maintains the water quality in the lower Olifants River in the KNP at an acceptable quality (DWAF 2004).

The catchment geology of an aquatic ecosystem plays also a major role in the water quality of the system. The catchment geology of the Phalabora Igneous Complex is underlain by igneous rocks. Volcanic rocks that are mined in the region comprise different rock types which host various metals and inorganic salts. These rocks are mainly composed of ultramafic rocks (dunite and pyroxenite) with a core of carbonatite and phoscorite. The core of such a composite meddling typically shows a concentric arrangement of phoscorite around the margin and a core of banded carbonatite (Otto *et al.* 2007). Both these rock types were intruded by the central transgressive carbonatite. The banded carbonatite consists largely of magnetite-rich calcite-carbonatite, with minor amounts of apatite, olivine, phlogopite and biotite. The transgressive carbonatite is mineralogically similar to the banded carbonatite, but lacks the banding and represents a younger crosscutting intrusive rock (Fontana 2006).

The underground rocks of Foskor Limited and Palabora Mining Company also contain phoscorite. The phoscorite formation is composed of olivine, magnetite, apatite and phlogopite (Otto *et al.* 2007). Minor rock types include glimmerite, syenite and fenite.

Phoscorite (Mg, $Fe)_2SiO_4$, is composed of olivine (a magnesium iron silicate) with apatite and phlogopite in variable proportions. Patches of calcite occur in addition to the carbonatite veining from later intrusives. Apatite and baddeleyite are abundant and economic viable, but the magnetite is too high in titanium to be explored (Groves and Vielreicher 2001b). The main chemical processes which releases ions into solutions are hydrolysis, reduction, oxidation or chelation (Stallard and Edmond 1983). Rocks can be dissolved by strong acids in the presence of oxygen and the solubility is affected by heat circulation. They react with water, gases and solutions (acids) which add or remove elements from minerals (Grochau and Johaness 1997). Water chemistry may thus change as water passes from one geological environment to another because of characterization of extensive rocks and the geology of the catchment (Lee ***et al.*** 2003).

Phlogopite hosts the following elements: $K(Mg,Fe,Mn)_3Si_3AlO_{10}(F,OH)_2$; and Magnetite ($Fe_3o_4$) is one of several iron oxides which hosts iron. Chalcopyrite dissociates to form copper, sulphur and iron (Deer ***et al***. 1963). Fluoroapatite ($Ca_5F_2(PO_4)_6$) is a mineral and carbonatite, if composed entirely of carbonate minerals, is extremely unusual in its major element composition as compared to silicate igneous rocks, obviously because it is composed primarily of Na_2O and CaO plus CO_2 (Lee ***et al.*** 2003). Carbonatites may contain anomalous concentrations of rare earth elements namely, phosphorus, niobium, uranium, thotium, copper, iron, titanium, barium, fluorine, zirconium and other rare or incompatible elements (Duncan and Willett 1990). The Phalaborwa carbonatites are unusual and geochemically different from others, and are especially high in copper content (Groves and Vielreicher 2001a).

Results and Discussion

3.1 System variables: regulate essential ecosystem processes such as spawning and migration of fish species. According to DWAF (1996c), the biota of aquatic ecosystems is adapted to the natural changes in water quality during seasonal cycles (which characterize most systems). If the amplitude, frequency and duration of these cycles

change, it may cause severe disruptions to the ecological and physiological functions of aquatic organisms and hence the ecology of the system. Acceptable and non-acceptable criteria are given as numerical ranges for system variables such as temperature, pH and dissolved oxygen (DWAF 1996c).

3.1.1 Temperature

Water bodies undergo temperature variations along with normal climatic fluctuations. These variations occur seasonally and in most water bodies, also over periods of 24 hours. The temperature of surface waters is influenced by latitude, altitude, seasons, time of day, air circulation, cloud cover as well as the flow and depth of the water body (Chapman and Kimstach 1996). In turn, temperature affects physical, chemical and biological processes in water bodies as well as the concentration of many variables. The metabolic rate of aquatic organisms is also related to temperature, and in warm waters, respiration rates increase leading to increased oxygen consumption and increased decomposition of organic matter (Bartram and Ballance 1996).

Many of the physical, biological, and chemical characteristics of a water body are directly affected by temperature. The temperature of water influences the amount of oxygen that can be dissolved in the water. Warmer water cannot hold as much oxygen as cooler water. Water temperature also influences the sensitivity of organisms to toxic material, parasites and diseases. Warmer water temperatures may stress organisms and they become less resilient to other stressors (Bartram and Ballance 1996). Most aquatic organisms have a narrow temperature range in which they are able to function effectively. Therefore, lowering the temperature may also affect the organisms (Michaud 1995). The temperatures of inland waters in South Africa generally range from 5 - 30°C (DWAF 1996c). According to Dallas and Day (2004) the water quality guidelines for aquatic ecosystems in South Africa specify a target water quality range (TWQR) whereby water temperature should not be allowed to vary from the background daily average water temperature considered to be normal for that specific site and time of day, by >2°C or by >10%.

Table 3.1: Seasonal water temperatures (ºC) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	18.03	19.73	18.91	17.56
Spring	24.54	24.01	22.28	26.35
Summer	28.85	29.64	28.7	26.18
Autumn	22.26	23	22.78	22.37
Mean	23.42	24.1	23.17	23.12

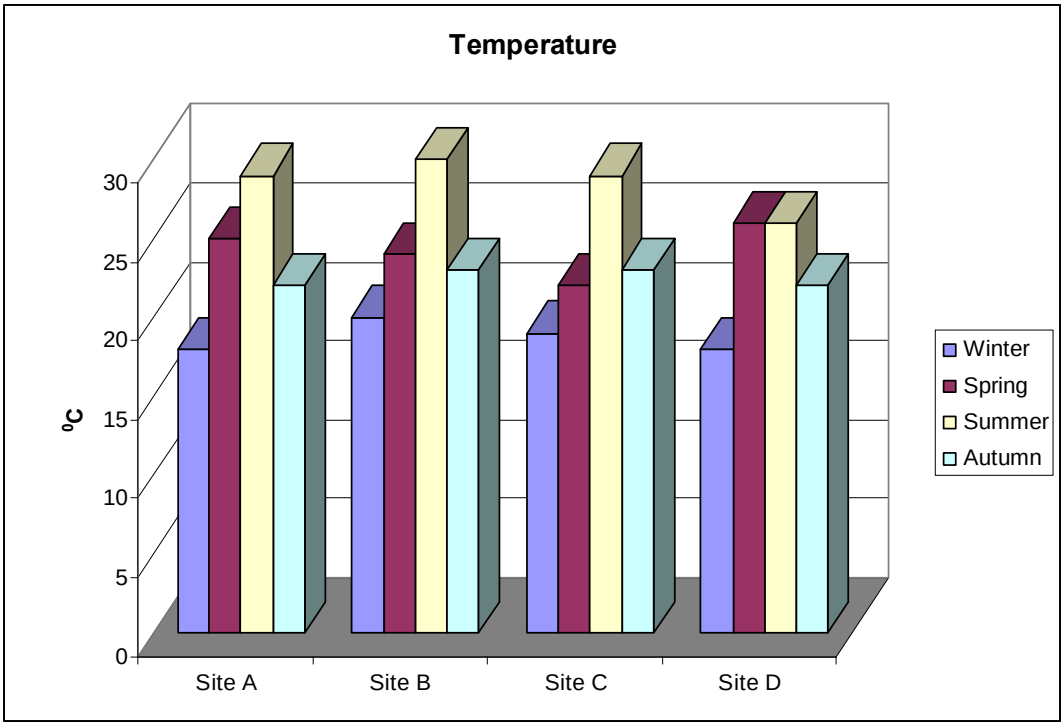

Figure 3.1: Water temperatures in degrees Celsius (ºC) of the four sampling sites

The water temperature did not exceed 20ºC at all the sampling sites during the Winter survey, which is expected for the region; it ranged from 17.56ºC to 19.73ºC (Table 3.1 and Figure 3.1). The highest water temperature recorded during this study was at site B, i.e. 29.64ºC during the Summer survey, with a mean of 24.1ºC recorded for this site. The maximum water temperature recorded for Autumn was 23ºC (also at site B) and the lowest 22.26ºC (at site A). A slight difference in mean values was recorded for the four sites. The highest mean water temperature was recorded at site B (24.1ºC) and the lowest at site D (23.12ºC) (Table 3.1).

The temperatures recorded during this study fell in the range suitable for *Oreochromis mossambicus* (according to Skelton 2001) and had thus no adverse effects on the host.

3.1.2 Dissolved Oxygen

Dissolved oxygen (DO) is the amount of oxygen that is dissolved in the water at given atmospheric pressure, water temperature and salinity. The oxygen is vital to fish, other aquatic animals, microorganisms and plants, which depend upon it for the process of respiration (Chapman and Kimstach 1996). Maintenance of healthy and diverse aquatic ecosystems depends on oxygen levels being maintained at consistently high levels. Waters with low amounts of DO can support only limited amounts and types of aquatic organisms. Reductions in DO levels may result in loss of the more sensitive species and at extremely low levels, very few species may be present in the system. Human activities have great potential to influence DO levels because the latter are closely linked to temperature and nutrient levels in the system (Michaud 1991).

According to Dallas and Day (2004), variations in DO can occur seasonally, or even over 24 hour periods, in relation to temperature and biological activity (i.e. photosynthesis and respiration). Biological respiration can be related to decomposition processes which reduce DO concentrations (Chapman and Kimstach 1996). Oxygen that is dissolved in the water is in much smaller quantities compared to oxygen in the air. Aquatic organisms use the dissolved oxygen and if more oxygen is consumed than are produced, DO levels decline and some sensitive species may move away, weaken, or die (DWAF 1996c).

Atmospheric pressure, temperature and salinity affect the concentrations of DO in an aquatic ecosystem, as cold freshwater holds more oxygen than warmer and salty water (Bartram and Ballance 1996). Low DO levels are also caused by fertilizer and manure runoff from farms. Furthermore, fertilizers and fecal matter encourage the growth of algae in aquatic ecosystems, which may then use the oxygen more rapidly. Also, when plants and animals die, they are decayed by bacteria, which also use a great deal of oxygen (Dallas and Day 2004).

As mentioned above, higher temperatures reduce the solubility of DO in water, decreasing its concentration and thus its availability to aquatic organisms (Chapman and Kimstach 1996). If the organic load of a system is high, oxygen depletion is further accelerated by greater microbial activity at higher temperature (Dallas and Day 2004). Dissolved oxygen of water may be measured as milligrams per litre (mg l^{-1}) or as a percentage of the saturation concentration at the time of sampling. Concentrations below 5mg O_2 l^{-1} may adversely affect the functioning and survival of biological communities and below 2mg O_2 l^{-1} may lead to the death of most fish (DWAF 1996c).

Table 3.2: Seasonal dissolved oxygen values (mg O_2 l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	4.75	2.73	5.02	4.65
Spring	6.15	4.55	4.73	3.51
Summer	2.68	2.87	3.78	3.27
Autumn	1.84	2.05	1.99	1.68
Mean	3.86	3.05	3.88	3.28

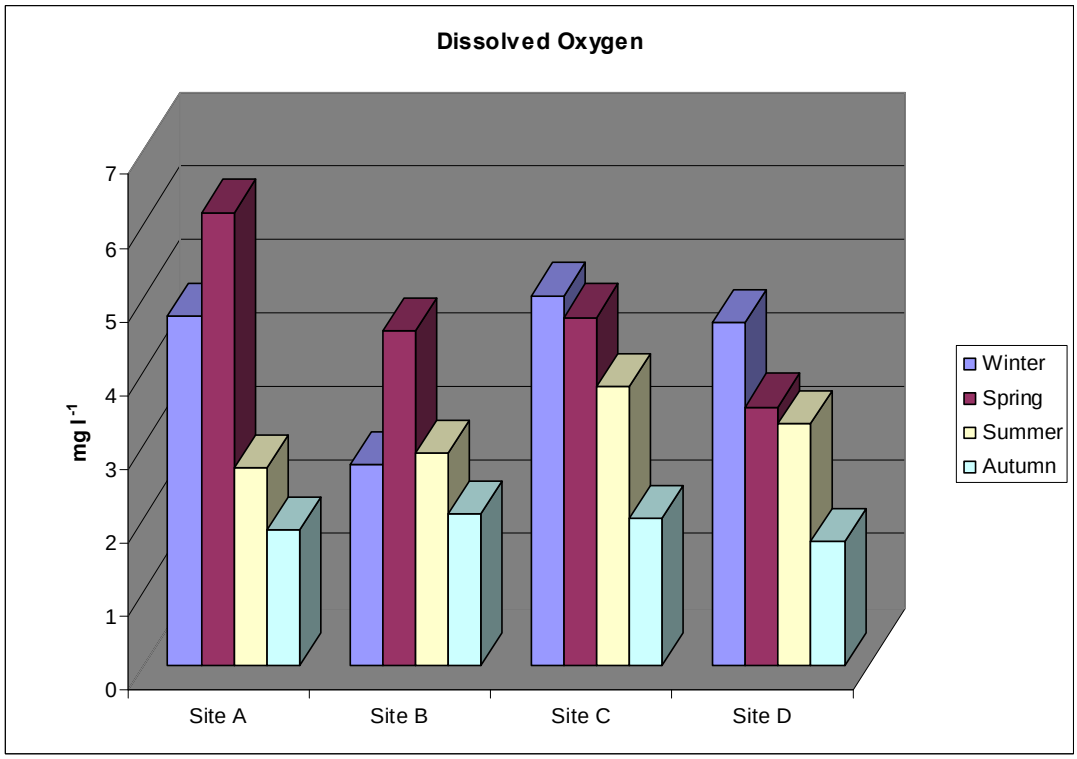

Figure 3.2: Dissolved Oxygen concentrations of the four sampling sites

The mean DO concentrations recorded during this study indicated only slight differences between the four sites with site B the lowest mean DO concentration (Table 3.2 and Figure 3.2). The maximum DO concentration of 6.15mg l^{-1} was recorded during Spring at site A which might be attributed to an algal bloom at the time of the survey. The minimum value recorded was 1.68mg O_2 l^{-1} at site D in Autumn (Table 3.2). Values during Autumn were constantly lower compared to Summer which was unexpected. These lower DO concentrations might be due to the high inflow of organic material from the preceding rains (January to March 2006). The highest mean of 3.88mg O_2 l^{-1} was recorded at site C which received additional nutrients through the effluent water (as well as the hippopotami defecating in the water). This can lead to algal blooms in Summer, which may result in very low DO levels during the night and over-saturated DO levels during the daylight hours. The lowest DO concentration recorded during this study can be attributed to increased levels of organic matter. If the organic load is high, oxygen depletion is further accelerated by greater microbial activity at higher temperatures (Dallas and Day 2004). The mean DO concentrations recorded during this study were all below 5mg O_2 l^{-1} (Table 3.2) which may, according to DWAF (1996c) adversely affect the functioning and survival of the biological communities.

3.1.3 pH

The pH of an aquatic ecosystem is a measure of the concentration of hydrogen ions in the water (Davies and Day 1998). This measurement indicates the acidity or alkalinity of the water. On the pH scale of 0 ï 14, a reading of 7 is considered to be "neutral". Readings below 7 indicate acidic conditions, while readings above 7 indicate the water is alkaline, or basic. The pH is an important variable in water quality assessment as it influences many biological and chemical processes within a water body (Michaud 1991). At a given temperature, the pH (or the hydrogen ion activity) indicates the intensity of the acidic or basic character of a solution and is controlled by the dissolved chemical compounds and biochemical processes in the solution (Dallas and Day 2004). In unpolluted waters, the pH is principally controlled by the balance between the carbon dioxide, carbonate and bicarbonate ions as well as other natural compounds such as humic and fulvic acids (Bartram and Ballance 1996).

The most common cause of unnatural changes to the pH occurs in catchments where certain types of soils have been disturbed and exposed to the atmosphere. But it can also be affected by a range of other factors including industrial run-off, mining and agricultural practices or urban development, as well as atmospheric deposition of acid-forming substances (Chapman and Kimstach 1996). An increase or decrease in the pH outside the normal range of a water body will cause sequential loss of the species depending on their sensitivity.

Extremely high or low pH values will lead to the death of all aquatic life (Bartram and Ballance 1996). Dallas and Day (2004) stated that a change in the pH from what is normally encountered in unpolluted streams may have severe effects upon aquatic biota, but that the severity of the effects depends on the magnitude of change. Some streams are naturally more acidic than others and their biota are adapted to these conditions. Natural waters has a pH range between 6.0 and 9.0 (DWAF 1996c), but wide variations may occur because of the catchment geology (Faniran *et al.* 2001). Davies and Day (1998) reported that biological activities play an important role in the pH of natural waters.

Table 3.3: Seasonal pH values of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	8.82	8.33	8.61	7.7
Spring	8.8	8.72	8.54	7.83
Summer	8.14	8.74	8.53	7.83
Autumn	7.72	8.47	8.15	8.52

Alkaline pH values were recorded throughout the study period with the pH values of sites B and C consistently above 8 (Table 3.3 and Figure 3.3). The highest value recorded was 8.82 (during Winter at site A) and the lowest value was 7.7 (during Winter at site D) (Table 3.3 and Figure 3.3). The pH varied from 7.72 to 8.52 at all sites during Autumn with no value recorded below 7. An increase in pH values may be affected by an increase in the biological activity and photosynthetic activity of algae and

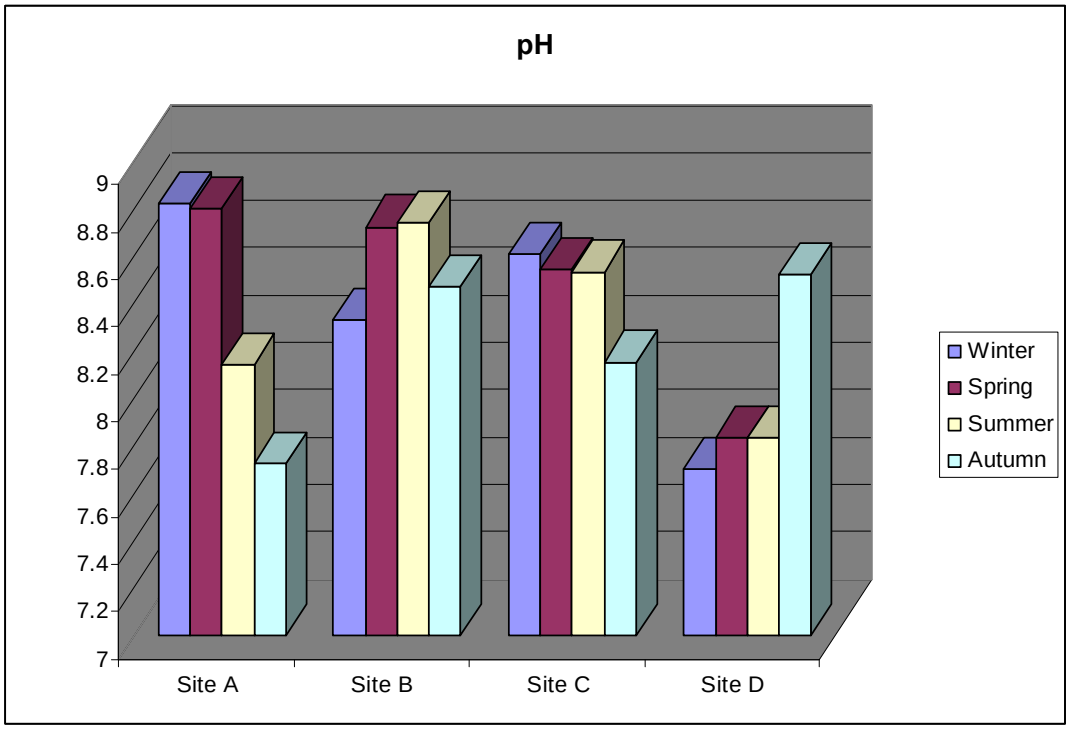

Figure 3.3: pH values of the four sampling sites

higher plants (Hellawell 1986). In this study the pH increased in association with the total water hardness (Tables 3.3 & 3.8). Also, the elevated levels of sulphates (Table 3.16) and TDS (Table 3.5) during this study could have been a major contributing factor to the increase in pH values.

3.1.4 Turbidity

Turbidity refers to the "cloudiness" of water caused by suspended particles and is measured in nephelometric turbidity units (NTU) (DWAF 1996c). The turbidity of water is caused by particles such as silt, clay, fine organic matter and microscopic organisms that are suspended in the water (Michaud 1995). Thus, the higher the turbidity of water, the murkier the water will be (Chapman and Kimstach 1996). During dry weather, undisturbed streams usually have low levels of suspended particles and the water looks clear. According to Dallas and Day (2004), an increase in turbidity reduces the transmission of light, therefore photosynthesis will decrease if less light penetrates the water, resulting in even further drops in oxygen levels. Furthermore, water becomes warmer as suspended particles absorb heat from the sunlight and cause oxygen levels

to deplete and as mentioned before, warm water holds less oxygen than cooler water (Dallas and Day 2004). The combination of warmer water, less light and oxygen depletion can make it impossible for some forms of aquatic life to survive.

Suspended solids affect aquatic life in other ways as well; it can clog fish gills, reduce growth rates and decrease resistance to diseases (Davies and Day 1998). Thus, with very high levels of turbidity, water might loses its ability to support a diversity of aquatic organisms. Values over 35 ï 40 NTU might have a negative influence on predatory fish, which hunt on sight (Davies and Day 1998). There is no target water quality range for turbidity for aquatic ecosystems.

Table 3.4: Seasonal turbidity values in NTU at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	13	6	39	19
Spring	27	8	35	44
Summer	51	50	48	84
Autumn	35	6	25	16
Mean	31.5	17.5	36.8	40.8

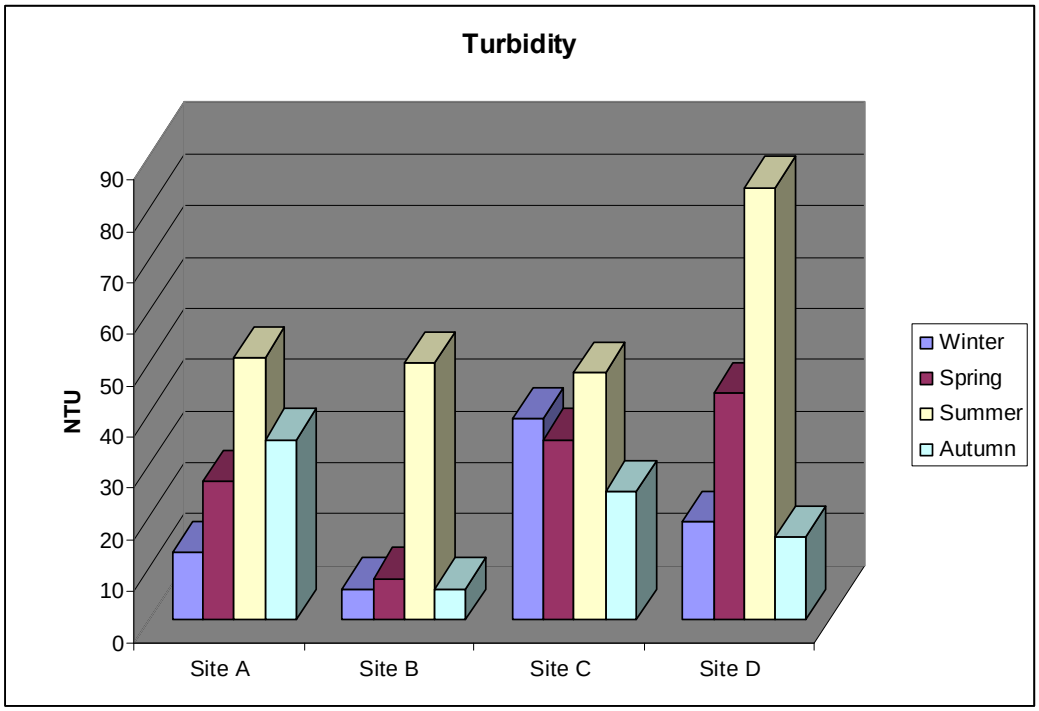

Figure 3.4: Seasonal turbidity values at the four sampling sites

The turbidity levels varied between the different seasons and ranged from 6 to 84 NTU throughout the study (Table 3.4 and Figure 3.4). All sites had high turbidity levels during the Summer survey with a maximum value of 84 NTU recorded at site D (Table 3.4). This high level might be due to the preceding rain period (January ï March 2006) that caused higher silt loads in the Olifants River. The lowest levels were recorded during Winter and Autumn as expected, with the lowest level of 6 NTU recorded at site B (Table 3.4). The highest mean value for turbidity was recorded at site D (40.8 NTU) followed by site C (36.8 NTU) and the lowest at site B (17.5 NTU) (Table 3.4).

Natural turbidity in rivers often increases with rainfall, as spates wash particles from surface soils into the rivers (Dallas and Day 2004). The character of the catchment geology can thus contribute towards high or low turbidity levels at a specific site. Furthermore, various anthropogenic activities such as release of sewage and industrial discharges can be implicated in the increasing turbidity levels (Davies and Day 1998). The high turbidity levels recorded at site D can be attributed to high silt levels in the Barrage which originated from upstream sediments caused by mining, weathering and agricultural activities.

3.2 Non-toxic inorganic constituents are defined as those water parameters which may have a toxic effect on aquatic life at extreme concentrations (DWAF 1996c), but which are generally system variables because their natural concentrations depend on localized geochemical, physical and hydrological processes, as well as geological formations (Leske and Buckley 2003). Criteria are given as numerical ranges or as proportional changes from local background conditions for these constituents which include total dissolved solids (TDS), electrical conductivity (EC), salinity, total suspended solids (TSS) and total water hardness (DWAF 1996c).

3.2.1 Total dissolved solids

Total dissolved solids (TDS) are the total amount of material dissolved in a water sample (Davies and Day 1998). Suspended solids are varied, ranging from clay, silt and plankton, to industrial wastes and sewage. Geological weathering and atmospheric conditions contribute to the TDS of natural aquatic ecosystems (DWAF 1996c).

Furthermore, domestic and industrial discharges and surface runoff from urban and agricultural areas, together with evaporation can also increase the TDS levels (DWAF 1996c). Natural fluctuations in TDS could be the result of dissolution of rocks and/or soils as well as decomposing plant material (DWAF 1996c). According to Dallas and Day (2004) changes in the amounts of dissolved solids can be harmful because the density of TDS determines the flow of water in and out of an organism's cells. A concentration of TDS that is too high or too low may limit growth and may lead to the death of many aquatic organisms (Dallas and Day 2004). Ions that are related to TDS that are commonly found in natural waters include the cations; calcium, magnesium, sodium and potassium, and the anions; bicarbonate, carbonate, chloride and sulphate (Dallas and Day 2004).

High concentrations of TDS may reduce water clarity, which contributes to a decrease in photosynthesis and lead to an increase in water temperature (DWAF 1996c). Many aquatic organisms cannot survive in high temperatures that are not in their normal temperature range. It is possible for dissolved ions to affect the pH of a water body, which in turn may influence the overall health of many aquatic species (Chapman and Kimstach 1996). According to DWAF (1996c) the TWQR of TDS concentrations in all inland waters should not be changed by more than 15% from the normal cycle of the water body under unimpacted conditions at any time of the year. Also, the amplitude and frequency of natural cycles in TDS concentrations should not be changed.

Table 3.5: Seasonal TDS values (in mg l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	444	3764	3445	473
Spring	337	2961	2620	204
Summer	355	3060	2730	150
Autumn	362	2609	3434	258
Mean	374.5	3098.5	3057.25	271.25

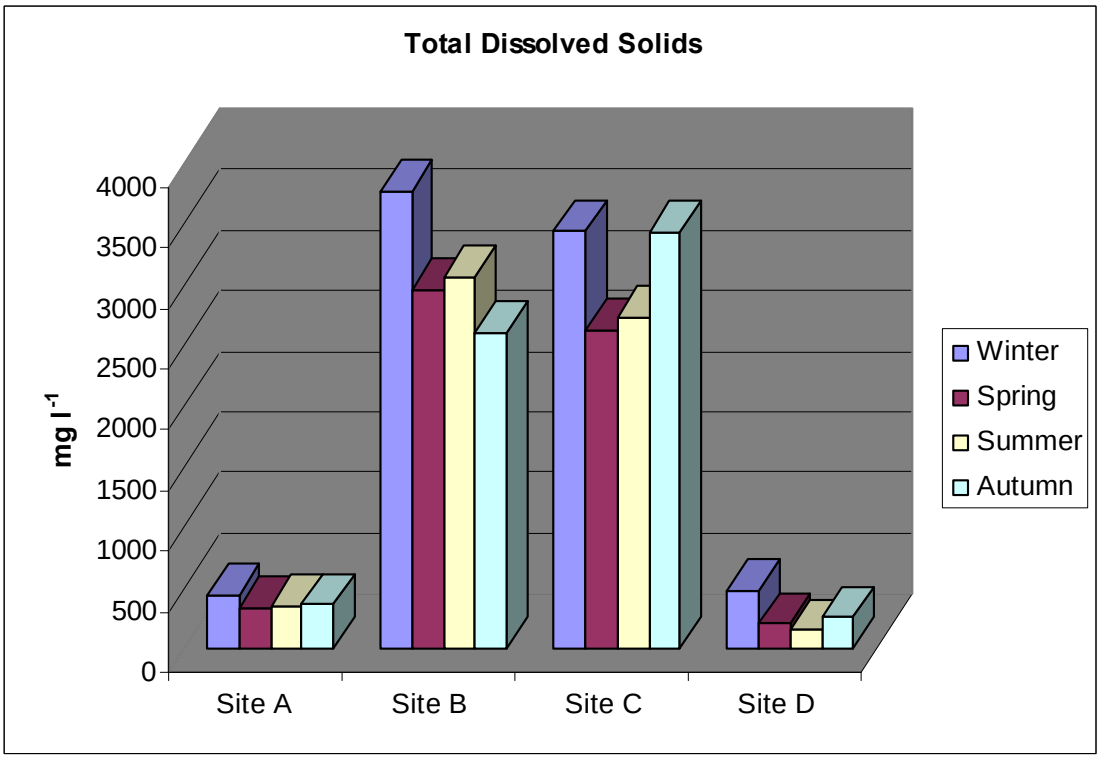

Figure 3.5: Seasonal TDS levels at the four sampling sites

The TDS levels at sites B and C were very high with a maximum concentration of 3764mg l^{-1} recorded at site B during Winter (Table 3.5 and Figure 3.5). The mean TDS value for site B was 3098.5mg l^{-1} with that of site C only slightly lower (3057.25mg l^{-1}) (Table 3.5). All the readings at the latter two sites were above the DWAF guidelines for domestic use of 450mg l^{-1} (DWAF 1996a). The TDS values of sites A and D are at more acceptable levels.

In natural aquatic ecosystems, the ions that form the bulk of the TDS are sodium, potassium, calcium and magnesium cations and chloride, sulphate, bicarbonate and carbonate ions, which are collectively known as the major ions (Davies and Day 1998). The high levels of these major ions (Tables 3.9 to 3.14) resulted in the very high TDS levels at sites B and C. These high TDS levels can furthermore be attributed to the mining activities (tailings water) as well as the geo-chemical contribution of the Phalaborwa Igneous Complex.

3.2.2 Salinity

Salinity refers to the saltiness of water (Davies and Day 1998). It was originally derived from the concentration of chloride ions in sea water. Sea water has a salinity of 35.5 or 35.5ă (parts per thousand) or 35 500 mg l^{-1} (Dallas and Day 2004). According to Dallas and Day (2004), salinity can adversely affect growth due to either a decrease of the osmotic potential (decreased water availability) caused by the high concentration of soluble ions or specific ion effects, which include toxicity of specific ions and/or unfavourable ratios of such ions. In addition, salinity disrupts nutrition by decreasing the activity of nutrient ions due to ionic strength, regardless of the substrate (Leske and Buckley 2003).

Every species of aquatic organisms is adapted to living in water of a certain quality, although some can tolerate wide differences in concentration of a wide variety of constituents, whereas others cannot (Davies and Day 1998). Changes in the dissolved salt concentration can have an effect on individual species, community structures and on microbial and ecological processes such as rates of metabolism and nutrient cycling (Dallas *et al.* 1998). Fish are generally tolerant to salinities in excess of 10 000mg l^{-1} TDS; however, larval fish are more sensitive than adults, while the eggs are more tolerant than larvae (Leske and Buckley 2003). Hart (1991) reported that fish are known to have coping mechanisms to deal with varying salinities in their surrounding environment, i.e. they use either hyper-osmotic (transport ions across gill surfaces) or hypo-osmotic regulation (loose water through gills). The Mozambique tilapia (*O. mossambicus*) is a euryhaline species and able to adapt to both freshwater and sea water (Skelton 2001). According to Uchida *et al.* (2000), the chloride cells in the gills of adult tilapia can respond to increasing environmental salinity.

Macroinvertebrate fauna are sensitive to salinity, with toxic effects likely to occur in most of the sensitive species at salinities in excess of 1000mg l^{-1} (Nielsen *et al.* 2003). Available data in Australia suggests that aquatic biota will be adversely affected when salinity exceeds 1000mg l^{-1} TDS (EC; 150mS m^{-1}) but there is limited information on how increased salinity will affect the various life stages of the biota (Nielsen *et al.* 2003).

Table 3.6: Seasonal salinity values in parts per thousand (ă) at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.24	1.64	1.6	0.25
Spring	0.26	1.66	1.58	0.14
Summer	0.24	1.57	2.07	0.1
Autumn	0.25	1.55	1.93	0.17
Mean	0.99	1.61	1.80	0.17

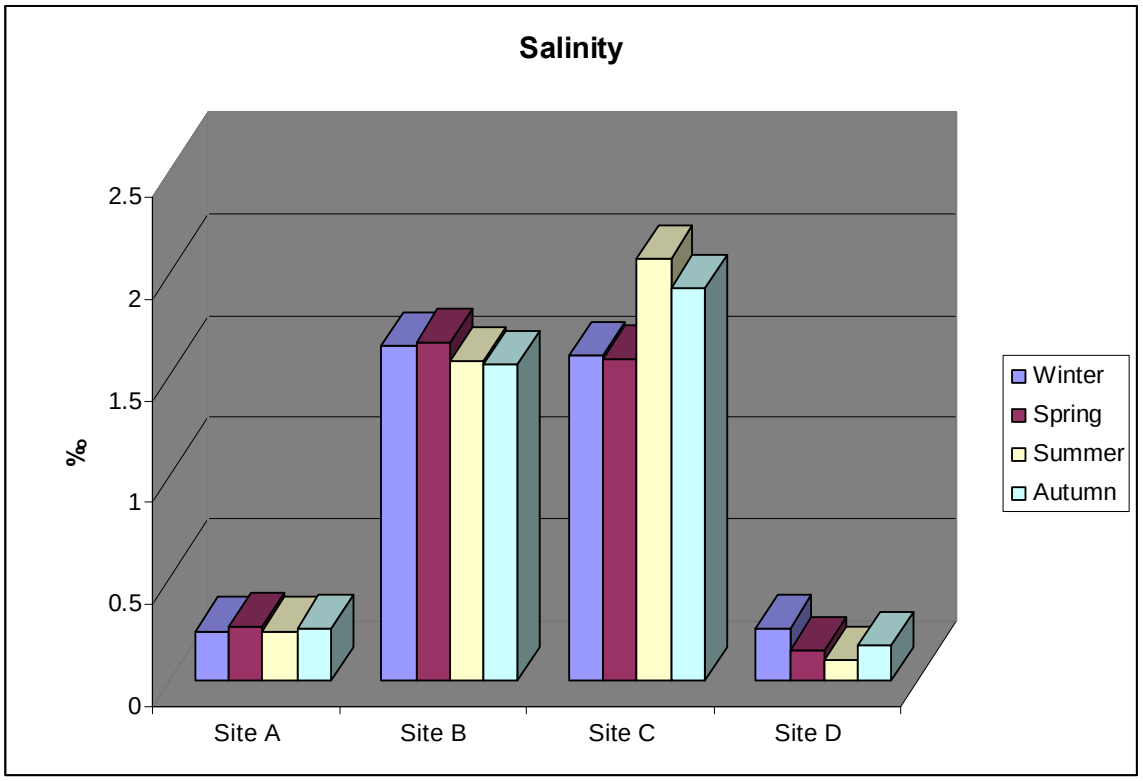

Figure 3.6: Seasonal salinity concentrations at the four sampling sites

Higher salinity levels were recorded during this study at sites B and C compared to the other two sites (Table 3.6 and Figure 3.6). The highest mean value was recorded at site C with the highest salinity level recorded during Summer (2.07ă). Little seasonal variation in salinity was recorded at sites A and D and a low mean value was recorded at both these sites. During Spring, salinity concentrations ranged between 0.14ă to 1.66ă with site D with the lowest value and site B with the highest value. Similar trends were measured during Summer and Autumn with the highest salinity value recorded at site C, and the lowest at site D (Table 3.6).

Evaporation might have contributed to the higher salinity levels recorded during Summer but the geological formations of the PIC might have further contributed to high levels at sites B and C (Figure 3.6). According to Palmer *et al.* (2004), high TDS concentrations correlated with high salinity originating from the composition of the tailings water, which was also the case at sites B and C during this study.

3.2.3 The Electrical Conductivity (EC)

Electrical conductivity (EC) is a measure of the ability of water to conduct an electrical current measured in mS/m^{-1}, where S is a "Siemen", which is the reciprocal of an ohm (the unit of electrical resistance). This ability is a result of the presence of certain ions in water such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, all of which carry an electrical charge (DWAF 1996c). The presence of these chemical constituents gives water the ability to conduct electricity. Thus, the EC of water is an indirect measure of its dissolved constituents and therefore another measure of dissolved material and is often used as a surrogate for TDS since the EC of water is a function of the number of charged ions in solution. The EC does not give specific information about the chemical species present in water, but it gives an indication of the TDS in the system, which is an acceptable indicator for water quality. The higher the conductivity, the greater the number will be of ions in solution (Dallas and Day 2004).

Table 3.7: Seasonal concentration values of the EC ($mS\ m^{-1}$) at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	50.4	313.6	304.8	51.6
Spring	53.2	318.4	303.4	30.7
Summer	49.4	303	393.9	24.5
Autumn	51.5	298	365.2	36.6
Mean	51.1	308.3	341.8	35.9

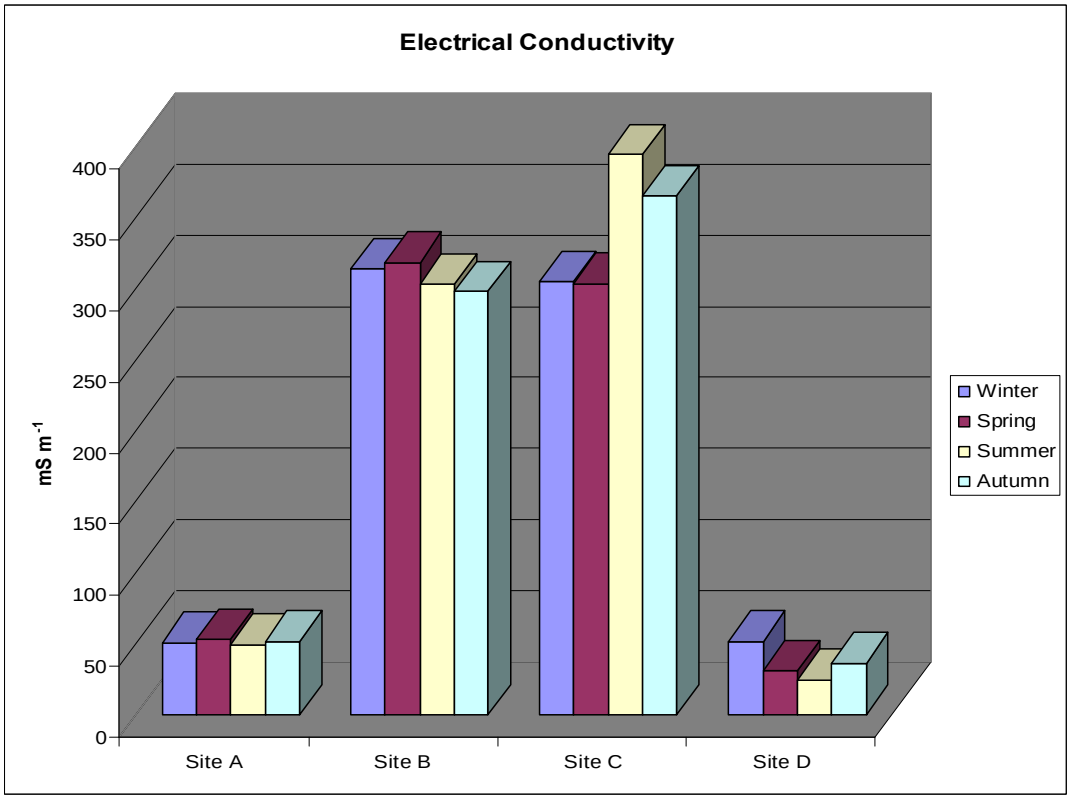

Figure 3.7: Seasonal Electrical Conductivity (EC) values of the four sampling sites

The EC readings varied between 24.5mS m^{-1} and 393.9mS m^{-1} during the four sampling periods (Table 3.7). The EC of sites B and C were much higher (about 10 times higher) than sites A and D throughout the sampling seasons (Figure 3.7). The EC reached 393.9mS m^{-1} at site C, with a mean of 341.8mS m^{-1} during Summer (Table 3.7). These high concentrations can be attributed to the high dissolved salt concentrations in the water, typical of effluent from mining activities. Both sites B and C recorded very high anion (chlorides, sulphates and nitrates) and cation (calcium, magnesium, sodium and potassium) concentrations in comparison with sites A and D.

3.2.4 Total Water Hardness

Total water hardness is the sum of the calcium and magnesium concentrations expressed as milligrams per litre (mg l^{-}) of calcium carbonate ($CaCO_3$). Other metals such as iron, aluminium, zinc and manganese will contribute to the hardness of water if

they are present, but the calcium and magnesium hardness usually predominates (DWAF 1996a).

Chapman and Kimstach (1996) stated that hardness may vary over a wide range; calcium hardness is usually prevalent (up to 70%), although in some cases magnesium hardness can reach 50 to 60%. The soft nature of the water can change the speciation of the metals in the water and thus potentially contribute to increased toxicity of metals (e.g. copper, zinc) found in an aquatic ecosystem (Dallas and Day 2004; Greenfield 2004). Seasonal variations of river water hardness often occur, reaching the highest values during low flow conditions and the lowest values during floods. Furthermore, the natural hardness of water is influenced by the geology of the catchment area and the presence of soluble calcium and magnesium minerals (DWAF 1996c).

The total hardness of raw water varies and may range from 0 - 1000mg $CaCO_3$ l^{-1} (DWAF 1996c). The total water hardness in surface waters rarely exceeds concentrations of 100mg $CaCO_3$ l^{-1} (DWAF 1996c). According to DWAF (1996a), water can be classified into four main classes according to its $CaCO_3$ mg l^{-1} concentrations: 0 to 50 = soft water, 50 to 100 = moderately soft water, 100 to 150 = slightly hard water, 150 to 200 = moderately hard water, 200 to 300 = hard water, and > 300 = very hard water.

Table 3.8: Total Water Hardness (mg $CaCO_3$ l^{-1}) values of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	220	1040	1180	216
Spring	236	1120	1248	230
Summer	83	289	957	49
Autumn	220	1040	1180	216
Mean	189.75	872.25	1141.25	177.75

The mean total water hardness values indicated that the water at sites A and D were moderately hard, while water at sites B and C was very hard (Table 3.8 and Figure 3.8). A maximum concentration of 1248mg $CaCO_3$ l^{-1} was recorded at site C during Spring and the minimum concentration of 49mg $CaCO_3$ l^{-1} was recorded at site D during

Summer (Table 3.8 and Figure 3.8). The high concentration of total water hardness can be related to the weathering of rocks, mainly that of calcium and magnesium minerals (Dallas and Day 2004).

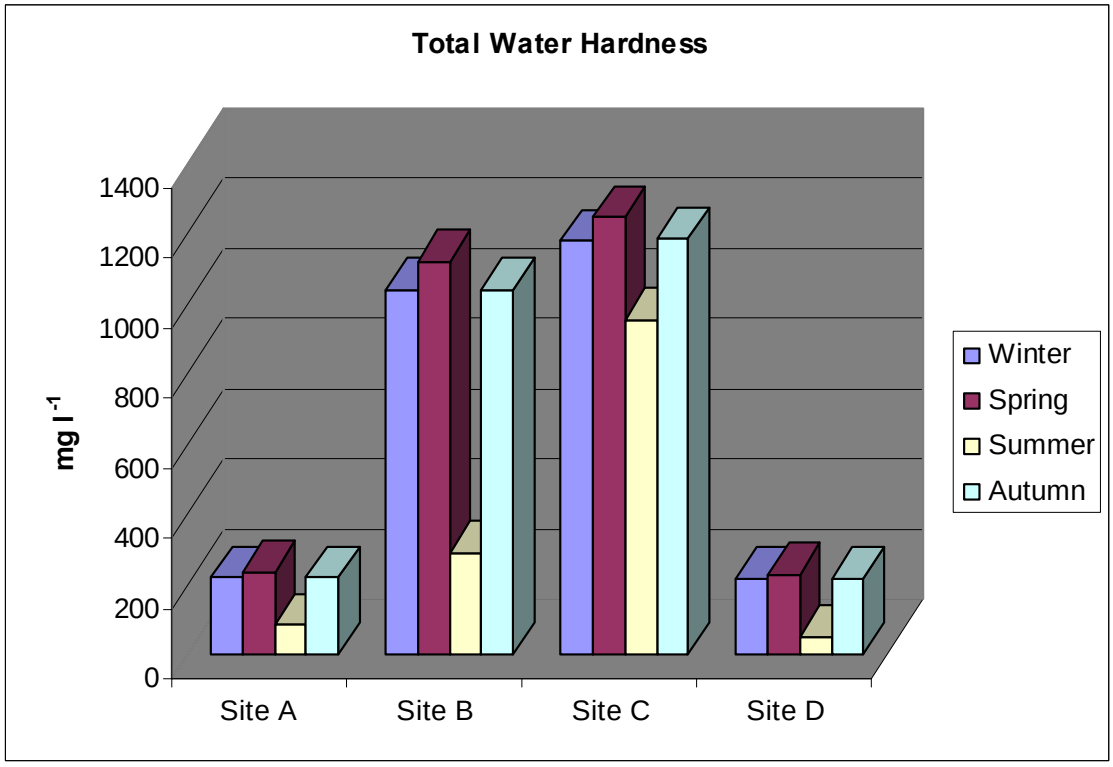

Figure 3.8: Seasonal Total Water Hardness concentrations at the four sampling sites

3.3 Major ions: Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Sodium (Na^{+}), Potassium (K^{+}), Chloride (Cl^{-}), Sulphate (SO_4^{2-}) and Bicarbonate (HCO_{3-}) are naturally very variable in surface and ground waters due to local geological, climatic and geographical conditions (Chapman and Kimstach 1996). The anions and cations contribute to the TDS, EC, alkalinity and salinity concentrations of aquatic ecosystems (Dallas and Day 2004).

3.3.1 Anions: chloride, fluoride, nitrate and sulphate.

3.3.1.1 Chloride

Most chlorine occurs as chloride (Cl^-) in solution. It enters surface waters with the atmospheric deposition of oceanic aerosols, with the weathering of some sedimentary rocks (mostly rock salt deposits) and from industrial and sewage effluents, and agricultural and road run-off (DWAF 1996c). Higher concentrations can occur near sewage and other waste outlets, irrigation drains, salt water intrusions, in arid areas and in wet coastal areas. As chloride is frequently associated with sewage, it is often incorporated into assessments as an indication of possible fecal contamination or as a measure of the extent of the dispersion of sewage discharges in water bodies (Van Vuren *et al.* 1999). High concentrations of chloride can make waters unpalatable and therefore, unfit for drinking. In pristine freshwaters, chloride concentrations are usually lower than 10mg l^{-1} and sometimes less than 2mg l^{-1} (Chapman and Kimstach 1996). Dallas and Day (2004) reported that chloride is the major anion in sea water and many inland waters in South Africa and it is an essential component of living systems. The chloride ions have no toxic effects on living systems, except if they increase the TDS levels. Therefore no TWQR values are available for chloride (DWAF 1996c).

Table 3.9: Seasonal chloride concentrations in mg Cl_2 l^{-1} at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	52	256	288	60
Spring	64	176	96	28
Summer	56	216	344	24
Autumn	60	244	340	36
Mean	58	223	267	37

The chloride concentrations at sites B and C were always higher than the other two sites during the four sampling periods (Table 3.9 and Figure 3.9). The highest concentration of 344mg l^{-1} was recorded at site C during Summer and the lowest value of 24mg l^{-1} was recorded at site D also during Summer (Figure 3.9). Chloride concentrations were always higher at site A compared to site D, except during the Winter survey. The highest mean value of 267mg Cl_2 l^{-1} was recorded at site C and the second highest mean value of 223mg Cl_2 l^{-1} was recorded at site B (compared to the

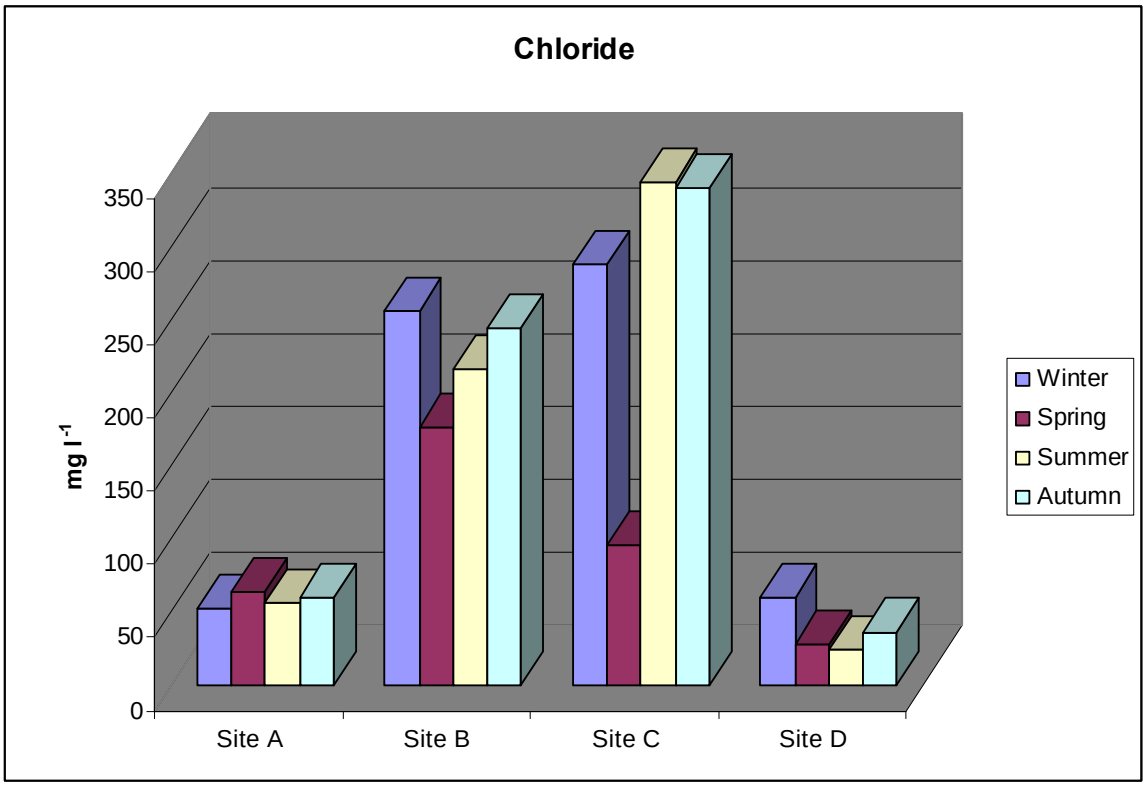

Figure 3.9: Seasonal chloride concentrations at the four sampling sites

lowest mean value of 37mg Cl_2 l^{-1} recorded at site D) (Table 3.9). High concentrations of chloride are usually present in tailings water (Yunxin and Sego 2001) which explain the high levels of chloride recorded at sites B and C. The geology of the region might have contributed further to the high levels of chloride.

According to DWAF (1996b) most fish can tolerate chloride levels up to 600mg Cl_2 l^{-1} under aquaculture conditions. Furthermore, according to Dallas and Day (2004), chloride ions exhibit no toxic effects on living organisms. The high chloride levels recorded during this study is thus of no concern as all measurements were under 600mg Cl_2 l^{-1}.

3.3.1.2 Fluoride

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 1996c). Fluoride occurs in the earth's crust at an average concentration of 0.3g/kg, most often as a constituent of fluorite (CaF_2), often known as fluorspar or calcium fluoride, in sedimentary rocks (DWAF 1996c). In natural waters, its concentration is dependent on geological variables such as the composition of the soils and rocks as well as climatic conditions, while anthropogenic activities such as industrial and agricultural pollution can lead to increased levels (Raubenheimer *et al.* 1991; Van Vuren *et al.* 1999).

Other important occurrences of fluoride are cryolite and fluorapatite in igneous rocks like the Phalaborwa Industrial Complex (Otto *et al*. 2007). Traces of fluoride (<1mg l^{-1}) occur in many aquatic ecosystems, whilst higher concentrations (often >10mg l^{-1}) can be found in ground waters derived from igneous rocks (Otto *et al*. 2007). Furthermore, fluoride is one of the common elements found in tailings (Wikipedia). Low concentrations of fluoride strengthen tooth enamel and bones in mammals. Toxicity includes skeletal fluorosis which may occur if exposure to intermediate fluoride concentrations occurs over long periods (DWAF 1996c). The TWQR for aquatic ecosystems for fluoride is 0.75ï 1.5mg l^{-1}, the CEV is 1.5mg $l^{-1}$and the AEV is 2.54mg l^{-1} (DWAF 1996c).

Table 3.10: Seasonal fluoride values (in mg F l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.5	3.8	2.1	0.4
Spring	0.68	2.6	2.4	0.24
Summer	0.6	4.5	3.1	0.2
Autumn	0.42	3.3	2.8	0.21
Mean	0.55	3.55	2.6	0.263

The concentrations of fluoride at sites B and C exceeded the TWQR as well as the CEV and AEV for aquatic ecosystems (DWAF 1996c) during most of the surveys (Table 3.10 and Figure 3.10). The lowest fluoride concentration recorded was 0.2mg l^{-1} at site D (with a mean of 0.26mg F l^{-1}). The high fluoride concentration of 4.5mg l^{-1} recorded at

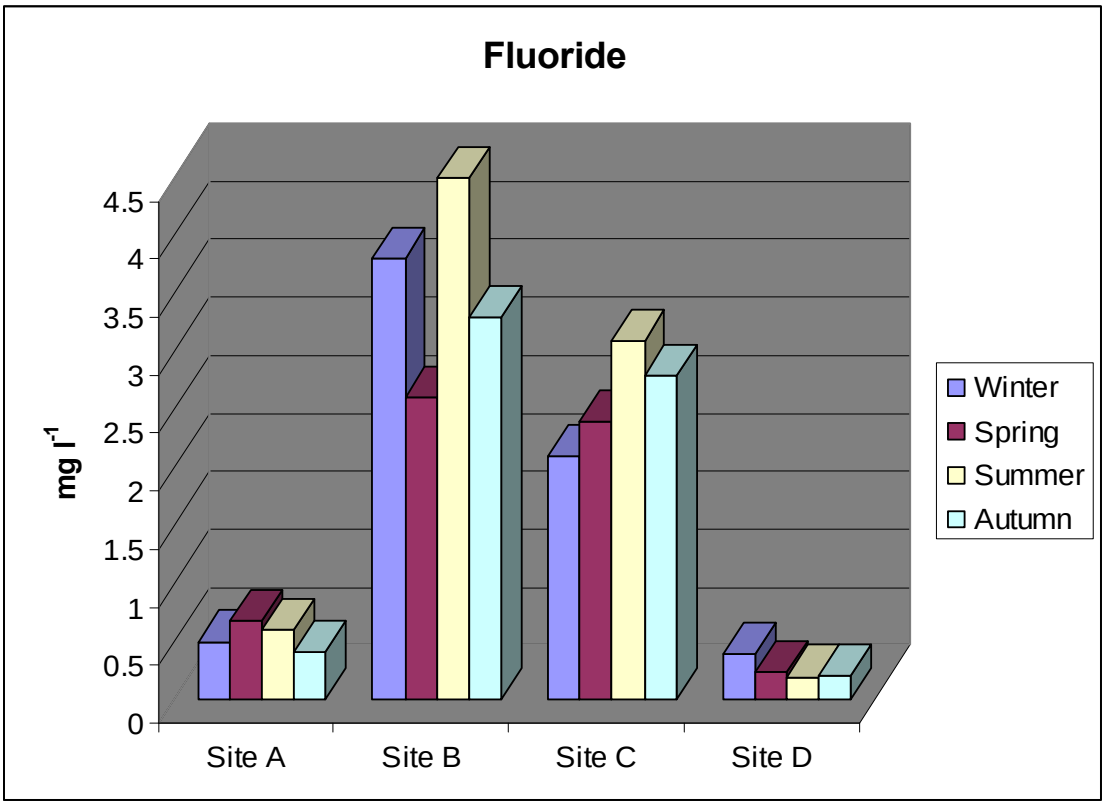

Figure 3.10: Seasonal fluoride concentrations of the four sampling sites

site B (which is a tailings dam) during Summer (with a mean of 3.55 F mg l^{-1}) can be attributed to the tailings water as tailings contain fluoride (Wikipedia). Fluoride is toxic at concentrations above 2.54mg F l^{-1} but reacts readily with magnesium and aluminium at alkaline pH values to form complexes which are not easily absorbed by aquatic biota (DWAF 1996c). Alkaline pH values were recorded at sites B and C throughout the study period (Table 3.3) reducing the possible toxic effect of high fluoride levels. Furthermore, increased water hardness level reduces the toxic effects of fluoride (DWAF 1996c) and very hard water was recorded at sites B and C (Table 3.8). The fluoride concentrations recorded at sites A and D were at acceptable levels throughout the study period (Table 3.10).

Furthermore, the geological formations of sites B and C include fluo-apatite rocks which host fluoride, therefore the geochemical origin of fluoride from igneous rocks at sites B and C, is also an important potential source of fluoride. Also, as mentioned above,

tailings contain fluoride which might also be the reason for very high and potentially toxic fluoride levels at site B.

3.3.2 CATIONS: calcium, magnesium, potassium and sodium

3.3.2.1 Calcium

Calcium is present in all waters as Ca^{2+} and is readily dissolved from rocks rich in calcium minerals, particularly as carbonates and sulphates, especially limestone and gypsum (Chapman and Kimstach 1996). It occurs naturally in varying concentrations in most waters and, together with magnesium, is one of the main components of water hardness. As mentioned earlier, soft waters contain low concentrations of calcium, while hard waters contain high concentrations of calcium (DWAF 1996c). Calcium is an essential element for all living organisms and is an important constituent of the bony skeleton of mammals, which consists of phosphates of calcium (Dallas and Day 2004). Occurrences of mineral deposits of calcium are common, usually as calcium carbonate, phosphate or sulphate (DWAF 1996a). There is no TWQR available for calcium for freshwater aquatic ecosystems but calcium levels up to 250mg l^{-1} are acceptable for all users according to DWAF (1996c). The TWQR for domestic use is 0 to 32mg Ca l^{-1} (DWAF 1996a).

Table 3.11: Seasonal calcium values (in mg Ca l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	28	51	173	26
Spring	23	50	166	25
Summer	10	18	155	11
Autumn	13	43	244	13
Mean	18.5	40.5	184.5	18.75

The calcium concentrations varied from 13mg l^{-1} to 244mg l^{-1} throughout the sampling period (Table 3.11 and Figure 3.11). Higher calcium concentrations were recorded at sites B and C during all the seasons, compared to the other two sites. Much higher calcium concentrations were recorded at site C (with a mean of 184.5mg Ca l^{-1}) with the maximum concentration (244mg Ca l^{-1}) recorded during the Summer survey (Table

3.11). These higher calcium concentrations at the latter sites are due to effluent from the mine waste water system. The lowest calcium concentration of 13mg l^{-1} was recorded during Autumn at both sites A and D with the lowest mean at site A (18.5mg Ca l^{-1}).

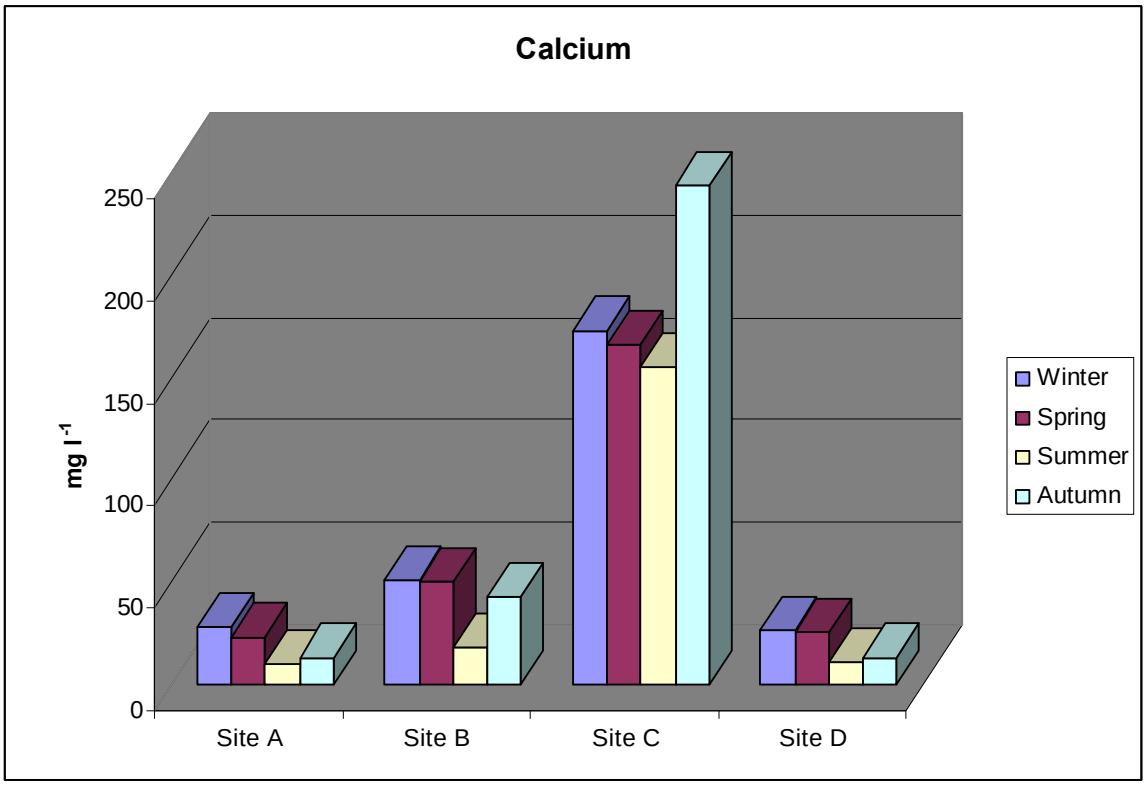

Figure 3.11: Seasonal calcium concentrations of the four sampling sites

Calcium is one of the cations associated with the weathering of rocks (Chapman and Kimstach 1996) and the high calcium concentrations can thus be associated with the volcanic rocks of the Phalaborwa Igneous complex which is underlain by pyroxenite rocks, hosting calcium. Moreover, calcium is present in high concentrations in tailings water (Wikipedia) explaining the higher concentrations recorded at sites B and C.

3.3.2.2 Magnesium

Magnesium is common in natural waters as Mg^{2+}, and along with calcium, is a main contributor to water hardness (DWAF 1996c). Magnesium arises principally from the

weathering of rocks containing ferro-magnesium minerals and from some carbonate rocks (Chapman and Kimstach 1996). According to Chapman and Kimstach (1996), it is an alkaline earth metal which reacts with oxygen and water to form magnesium oxide and magnesium hydroxide, respectively. The solubility of magnesium in water is governed by the carbonate/bicarbonate equilibrium and hence, the pH. Common minerals of magnesium are magnesium carbonate and various magnesium silicates (DWAF 1996a). Magnesium hydroxide is relatively soluble at a pH of 7, but gradually becomes less soluble as the pH increases. Magnesium bicarbonate, chloride, nitrate and sulphate are very soluble in water, whereas magnesium carbonate, silicate and phosphate are insoluble (DWAF 1996c).

According to Dallas and Day (2004), very little is known about the effects of magnesium on aquatic organisms although magnesium is also an essential nutritional element since it is found in relatively high concentrations, and as such it is unlikely to act as a limiting nutrient or toxin. According to DWAF (1996a) the concentration of magnesium in freshwater is between 4 - 10mg l^{-1} with the guidelines for domestic use 30mg $l^{-1}$and a TWQR between 50 ï 70mg l^{-1} will have no health effects on the consumers.

Table 3.12: Seasonal magnesium values (in mg Mg l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	25	221	173	27
Spring	31	232	183	25
Summer	11	56	142	6
Autumn	18	175	225	6

The magnesium concentrations ranged from 6mg l^{-1} to 232mg l^{-1} during the four sampling surveys (Figure 3.12 and Table 3.12). The concentrations were much higher at sites B and C during this study and higher than the TWQR as suggested by DWAF (1996a), but according to Dallas and Day (2004) not toxic. The magnesium levels at site A were always higher than those at site D, except during the Winter survey. During the

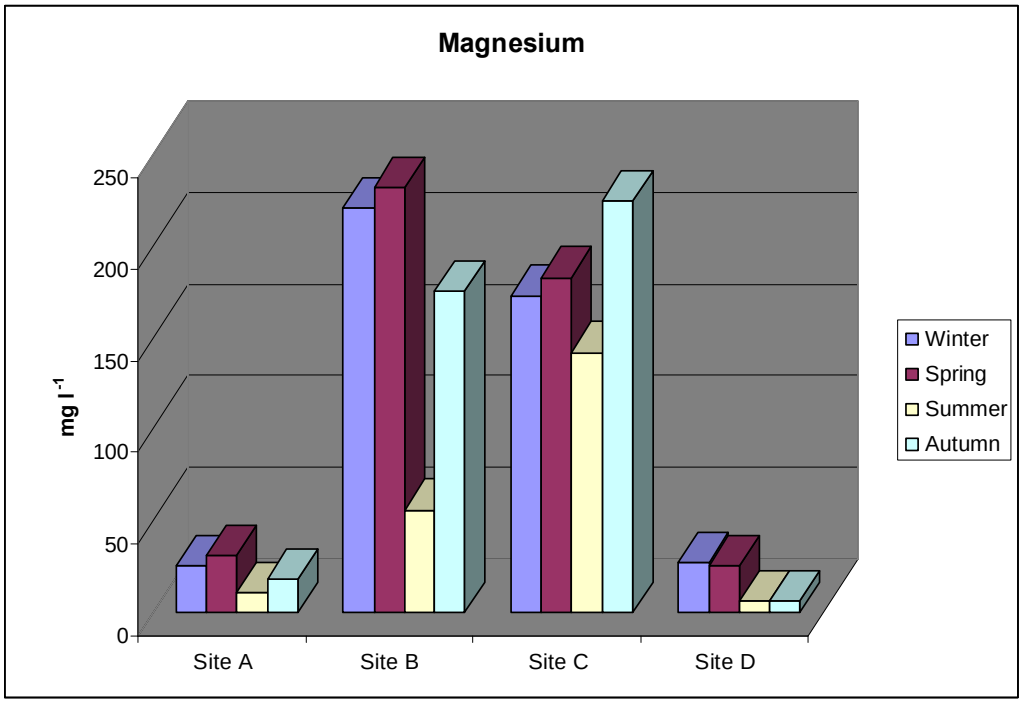

Figure 3.12: Seasonal magnesium concentrations of the four sampling sites

Summer survey the magnesium levels were the lowest at all sampling sites probably due to the dilution effect of the summer rains. The mean magnesium values of sites B and C were higher than that of sites A and D (Table 3.12). These high concentrations can also be associated with weathering of rocks at the PIC where the geological formations of sites B and C contain high levels of magnetite which host magnesium (Otto *et al*. 2007).

3.3.2.3 Potassium

Potassium usually occurs in natural waters in concentrations one-half to one-tenth than that of sodium (Trombley 2001). Even though potassium is fairly abundant in many of feldspars, and feldspars are among the most common silicate minerals, the potassium released by the weathering of such minerals appears to be rather quickly re-incorporated into clay minerals which largely resist further weathering (Chapman and Kimstach 1996). Potassium always occurs in water in association with anions, usually chloride, but can also occur with sulphate, bicarbonate or nitrate (DWAF 1996a). High concentrations of potassium may occur in runoff from irrigated lands and from fertilizer

production as well as domestic wastes. Potassium tends to remain scarce in most natural waters (Chapman and Kimstach 1996). There is no TWQR for potassium available for aquatic ecosystems. The TWQR for domestic use is 0ï 50mg K^+ l^{-1} (DWAF 1996a).

Table 3.13: Seasonal potassium values (in mg K l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.1	155	158	0.1
Spring	10	182	101	10
Summer	22	106	128	17
Autumn	0.1	165	137	0.1
Mean	8.05	152	131	6.8

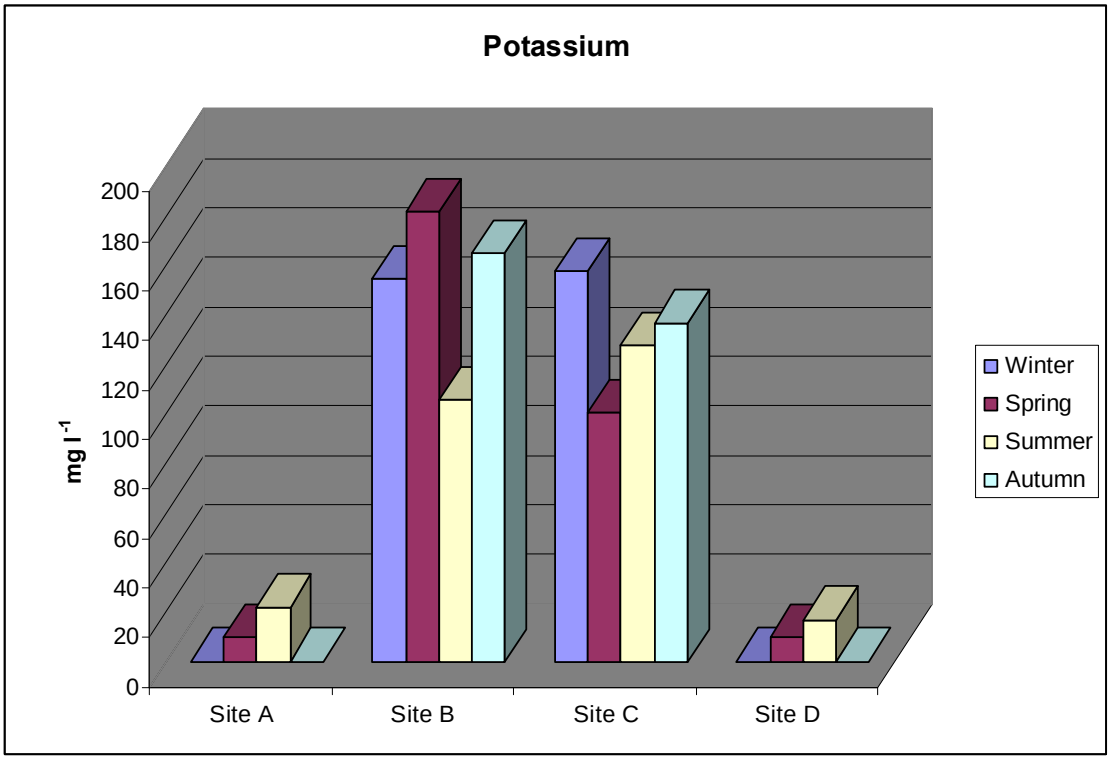

Figure 3.13: Seasonal potassium concentrations of the four sampling sites

Potassium concentrations were generally low (i.e. less than 1mg K l^{-1}) at sites A and D during the Winter and Autumn surveys (Table 3.13 and Figure 3.13). Very high concentrations of potassium were recorded at sites B and C throughout the four surveys. The highest value of potassium was recorded at site B (182mg K l^{-1}) during

Spring with a mean of 152mg K l^{-1} at this site (Table 3.13). The high potassium concentrations recorded at sites B and C can be attributed to mining activities (tailings), weathering of rocks (geological) and high concentrations of potassium may also occur in runoff from irrigated lands and from domestic wastes. Potassium always occurs in water in association with anions, usually chloride, but can also occur with sulphate, bicarbonate or nitrate (Dallas and Day 2004). During the present study the mean potassium concentrations were much lower than that of sodium which is in accordance with their natural occurrence in freshwater.

3.3.2.4 Sodium

Sodium is an alkali metal which reacts with water to form highly soluble, positively-charged sodium ions. It is found in the ionic form (Na^+) in plant and animal matter (DWAF 1996a). It is an essential dietary element important for the electrolyte balance and the maintenance of many essential physiological functions (DWAF 1996a). Sodium is ubiquitous in the environment and usually occurs as sodium chloride, but sometimes as sodium sulphate, bicarbonate or even nitrate (DWAF 1996c). Sodium is found as solid sodium chloride (rock salt) in areas where geological deposits occur (Otto *et al*. 2007). The levels of sodium in surface waters are generally low in areas of high rainfall but high in arid areas with low mean annual precipitation (Trombley 2001). Sodium is highly soluble in water and does not precipitate when water evaporates, unless saturation occurs. Hence, water in arid areas often contains elevated concentrations of sodium. Industrial wastes, especially processes that give rise to brines, contain elevated concentrations of sodium (DWAF 1996a). There is no TWQR for sodium available for aquatic ecosystems. The TWQR for domestic use of 100ï 200mg Na l^{-1} (DWAF 1996a) was used to discuss the results obtained during this study.

Table 3.14: Seasonal sodium values (in mg Na l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	39	213	158	47
Spring	59	275	221	30
Summer	56	216	344	24
Autumn	21	195	208	12

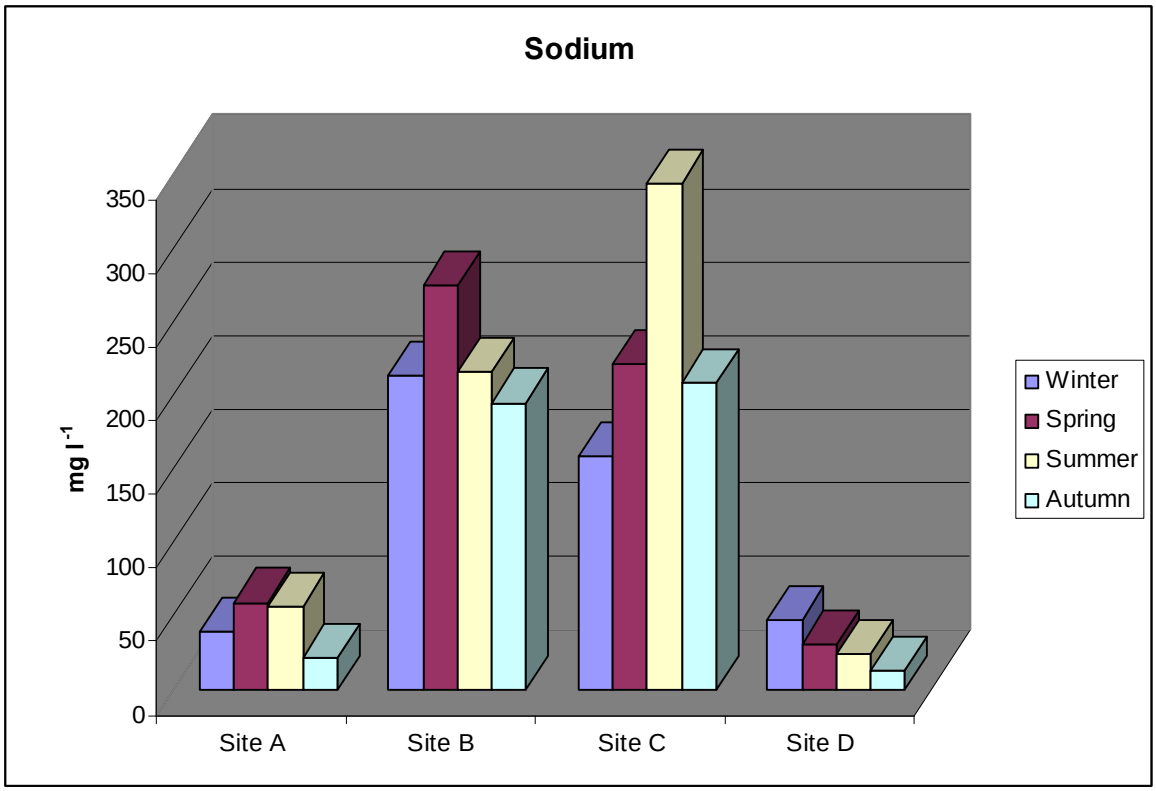

Figure 3.14: Seasonal sodium concentrations of the four sampling sites

Higher sodium concentrations were recorded at sites B and C throughout the sampling period compared to the other two sites (Table 3.14 and Figure 3.14). The highest concentration of sodium was 344mg Na l^{-1} recorded at site C in Summer which is much higher than the TWQR for domestic use of 100ï 200mg Na l^{-1} as suggested by DWAF (1996a). Very low sodium concentrations were recorded at sites A and D with the lowest concentration (12mg Na l^{-1}) recorded at site D during Autumn (Table 3.14). The sodium levels were always higher at site A than site D except during the Winter survey. Sodium levels recorded from the latter two sites are related to the geology and agricultural runoff. The high concentrations of sodium at sites B and C can be related to mining activities at these sites.

3.4 Nutrients

Nutrients (nitrogen and phosphate) are generally not toxic *per se*, but they can stimulate eutrophication if present in excess (Dallas and Day 2004). Nutrients are represented by

inorganic nitrogen (nitrate, nitrite, ammonia and ammonium) and inorganic phosphorus and sulphate (DWAF 1996c).

3.4.1 Total Nitrogen (Inorganic Nitrogen)

The term inorganic nitrogen includes all the major inorganic nitrogen components present in water (Davies and Day 1998). Both the dissolved forms of inorganic nitrogen and those adsorbed onto suspended inorganic and organic material are included, since they are all available for uptake by algae and higher plants (DWAF 1996c). Inter-conversions between the different forms of inorganic nitrogen are part of the nitrogen cycle in aquatic ecosystems. As various plant nutrients are required for normal plant growth and reproduction, it is nitrogen and phosphorus that are most commonly implicated in excessive plant growth resulting from nutrient enrichment (eutrophication) of aquatic systems (Dallas and Day 2004). Surface runoff from the surrounding catchment area, the discharge of effluent streams containing human and animal excrement, agricultural fertilizers and organic industrial wastes are the major sources of inorganic nitrogen which enters aquatic systems (Trombley 2001).

In highly impacted catchments, the inorganic nitrogen arising from human activities can greatly exceed "natural" sources (Dallas and Day 2004). In addition, many groups of bacteria are able to transform organic nitrogen to inorganic nitrogen during the decomposition of organic material. Browne (2002) reported that inorganic nitrogen is seldom present in high concentrations in unimpacted surface waters. 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. In South Africa, inorganic nitrogen concentrations in unimpacted, aerobic surface waters are usually below 0.5mg N l^{-1} but may increase to 5ï 10mg N l^{-1} in highly enriched waters (DWAF 1996c).

The average inorganic nitrogen concentrations levels (mg l^{-1}N) of a water body are used to classify it into a trophic status, thus Dallas and Day (2004) suggested the following guidelines for inorganic nitrogen:

- <0.5mg N l^{-1} - Oligotrophic conditions;

- 0.5 ï 2.5mg N l^{-1} - Mesotrophic conditions;
- 2.5 ï 10mg N l^{-1} - Eutrophic conditions;
- >10mg N l^{-1} - Hypertrophic conditions.

Table 3.15: Seasonal values of total nitrogen (in mg N l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	1.42	0.7	38.9	2.83
Spring	3.34	2.13	25.09	3.63
Summer	0.54	1.32	30.13	1.92
Autumn	4.31	4.1	58.13	8.21
Mean	2.40	2.06	38.06	4.15

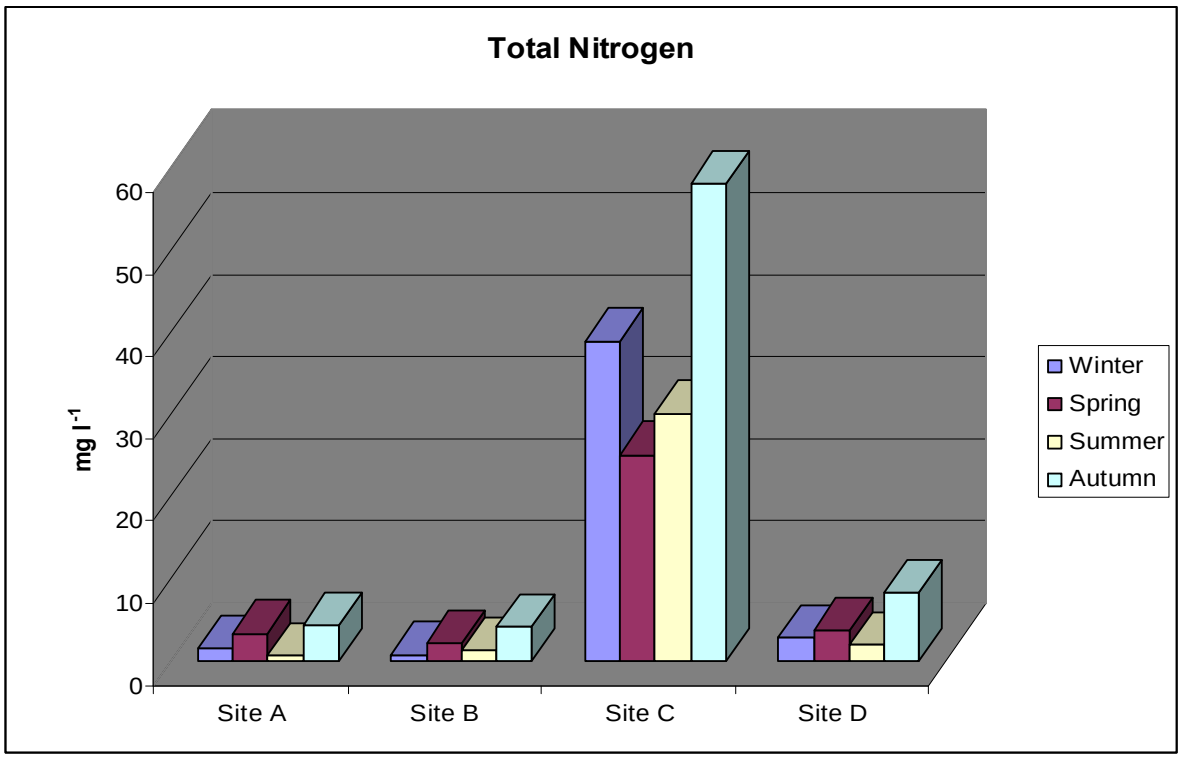

Figure 3.15: Seasonal total nitrogen concentrations of the four sampling sites

Very high total nitrogen levels were recorded at site C during all four surveys (thus hypertrophic conditions) according to Dallas and Day (2004), with the highest value (58.13 mg N l^{-1}) in Summer (Table 3.15 and Figure 3.15). Nitrogen levels, recorded at

sites A and B during Winter, suggest mesotrophic conditions, while an eutrophic condition was recorded at site D and hypertrophic conditions at site C during the same season. Eutrophic conditions were recorded at site D except during Summer when a lower nitrogen level (1.92mg N l^{-1}) was measured, indicating an oligotrophic condition. Throughout all surveys, mesotrophic conditions were recorded at site B, except during Autumn when a higher value was recorded (4.1mg N l^{-1}) indicating eutrophic conditions (Table 3.15). The high nitrogen levels at site C can be ascribed to industrial effluents and the nitroglycerine used in explosives. Furthermore, the hippopotami occurring at site C defecated in the water which added to the nitrogen load of the water. Under natural conditions, the dung is usually associated with providing much needed nutrients to aquatic ecosystems.

3.4.2 Nitrate

Nitrate (NO_3^-) is the end product of the oxidation of ammonia to nitrite and the latter to nitrate. According to Dallas and Day (2004), nitrates and nitrites occur together in the environment and interconversion readily occurs. Under oxidising conditions, nitrite is converted to nitrate by aerobic, autotrophic bacteria of the genus *Nitrobacter*, which is the most stable positive oxidation state of nitrogen and far more common in the aquatic environment than nitrite (DWAF 1996b). Nitrates are everywhere in soils and in the aquatic environment, particularly in association with the breakdown of organic matter and eutrophic conditions (Dallas and Day 2004). Nitrate levels tend to increase in shallow ground water sources in association with agricultural and urban runoff, especially in densely populated areas. Nitrate together with phosphate stimulate plant growth (Dallas and Day 2004).

In aquatic ecosystems, elevated concentrations of nitrate generally give rise to the accelerated growth of algae and the occurrence of algal blooms (DWAF 1996c). Algal blooms may subsequently cause problems associated with malodours and tastes in water and the possible occurrence of toxic conditions in aquaculture (DWAF 1996b). Dallas and Day (2004) reported that nitrate is not normally toxic, but high concentrations can be toxic to very young human infants because nitrate ion binds with foetal

haemoglobin to form a non-functional molecule, methaemoglobin. Upon absorption, in aquatic vertebrates, nitrate combines with the oxygen-carrying red blood pigment, haemoglobin, to form methaemoglobin, which is incapable of carrying oxygen (DWAF 1996b). There is no TWQR for nitrate in aquatic ecosystems, but for domestic use it is 0 ï 6mg l^{-1} (DWAF 1996a) and for aquaculture is < 300mg l^{-1} nitrate nitrogen (DWAF 1996b).

Table 3.16: Seasonal nitrate values (in mg NO_{3-} l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.9	0.7	38	2
Spring	3	2	23	3
Summer	0.1	0.1	28	0.1
Autumn	4	4	56	8
Mean	2	1.7	36.25	3.28

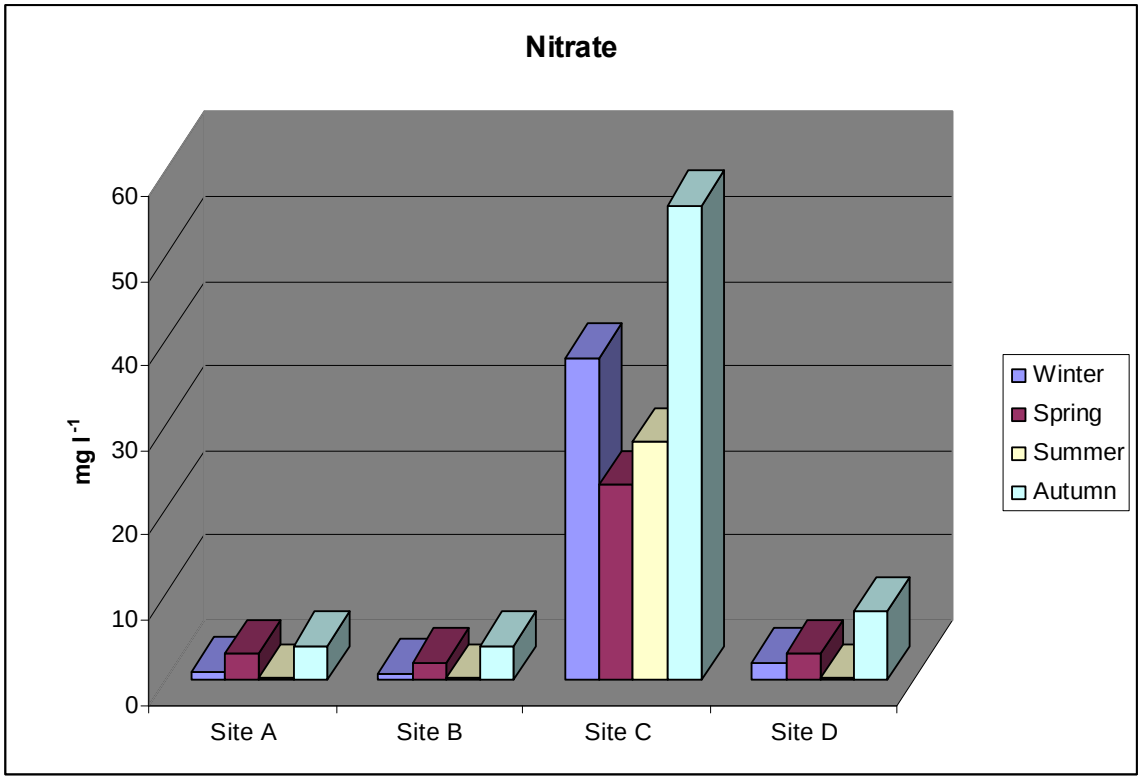

Figure 3.16: Seasonal nitrate concentrations of the four sampling sites

Site C had the highest nitrate concentrations throughout the study (Table 3.16) due to the contribution of nitroglycerine used in blasting rocks. Nitrates from nitroglycerine dissolve and accumulate in the open pit water. The highest nitrate concentration was 56mg l^{-1} recorded during Autumn (Table 3.16 and Figure 3.16). Sites A and D had the same concentration of 3mg NO_{3-} l^{-1} during Spring. The lowest nitrate concentration was 0.1mg l^{-1} recorded at sites A, B and D during Summer. Sites A and B had the same concentration of 4mg NO_{3-} l^{-1}, while twice that concentration was recorded at site C during Autumn. The highest mean value was 36.3mg NO_{3-} l^{-1} at site C and the lowest 2.0mg NO_{3-} l^{-1} at site A (Table 3.16).

The main source of nitrate in natural water is derived from the oxidation of plant- and animal debris and excrements (Dallas and Day 2004). Nitrate is a major contributor to the TDS of water. Nitrate levels in water are greatly enhanced through industrial and municipal discharges, leaching from waste disposal sites and landfills as well as from agricultural inorganic fertilizers (DWAF 1996c).

The elevated nitrate concentrations recorded at site C can be related to the inorganic (nitroglycerine) and organic matter (the excretory material of animals and decaying plants). According to Frempong and Clark (1996), nitroglycerine is an explosive compound formed by the combination of glycerol and nitric and sulfuric acids. The most important peaceful use of detonating explosives is to break rocks in mining. Groves and Vielreicher (2001a) reported that one important explosive used in mining, called ANFO, is a mixture of ammonium nitrate and fuel oil. Its use has revolutionized certain aspects of open-pit and underground mining because of its low cost and relative safety. The highest nitrate concentrations at site C can be attributed to these explosives used in the mining operations. None of the nitrate concentrations recorded during this sampling period was near the TWQR for aquaculture of < 300mg l^{-1} nitrate nitrogen (DWAF 1996b).

3.4.3 Nitrite

Nitrite is an intermediate product of inorganic oxidation and of the bacterially mediated

processes, nitrification and denitrification, which involve transformations of nitrogen in soil and water (Dallas and Day 2004). During nitrification, two groups of highly aerobic, autotrophic bacteria, mainly *Nitrosomonas* spp. and *Nitrobacter* sp., oxidise ammonia to nitrite, and nitrite to nitrate (DWAF 1996c). Nitrite is also an intermediate in the process of denitrification and usually involves a number of species of common facultative anaerobic bacteria which use nitrate as an exogenous terminal electron acceptor during the oxidation of organic compounds under anaerobic conditions (DWAF 1996c). As nitrite is an intermediate product of both nitrification and denitrification it does not usually accumulate, and concentrations of nitrite-nitrogen in inland waters are usually less than 0.1mg l^{-1} (DWAF 1996c). However, high nitrite concentrations are sometimes found in surface waters polluted with nitrogen-containing wastes, such as sewage or runoff from agricultural lands (DWAF 1996c).

Nitrite ions enter the fish through the chloride cells on the gills. The toxic effects of nitrite result from impairment of oxygen transport and cause anoxia in fish (Dallas and Day 2004). Exposure to low concentrations of nitrate manifests as stress responses, resulting in lower productivity, activity and growth, and poor health. Higher concentrations result in acute anoxia, loss of equilibrium and mortality (Dallas and Day 2004). Furthermore, nitrite toxicity is modified by water chemistry, especially by chloride concentration (toxicity increases as chloride levels decreases) as well as bicarbonate, calcium and nitrate levels and pH (Dallas and Day 2004). The TWQR for nitrite in aquaculture is between 0.06 and 0.25mg l^{-1}. This range is considered safe for warm water fish species (DWAF 1996b).

Table 3.17: Seasonal nitrite values (in mg NO_2 l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.02	0.001	0.7	0.03
Spring	0.24	0.03	2.09	0.43
Summer	0.34	1.12	1.93	0.92
Autumn	0.11	0	2.03	0.01
Mean	0.18	0.29	1.69	0.35

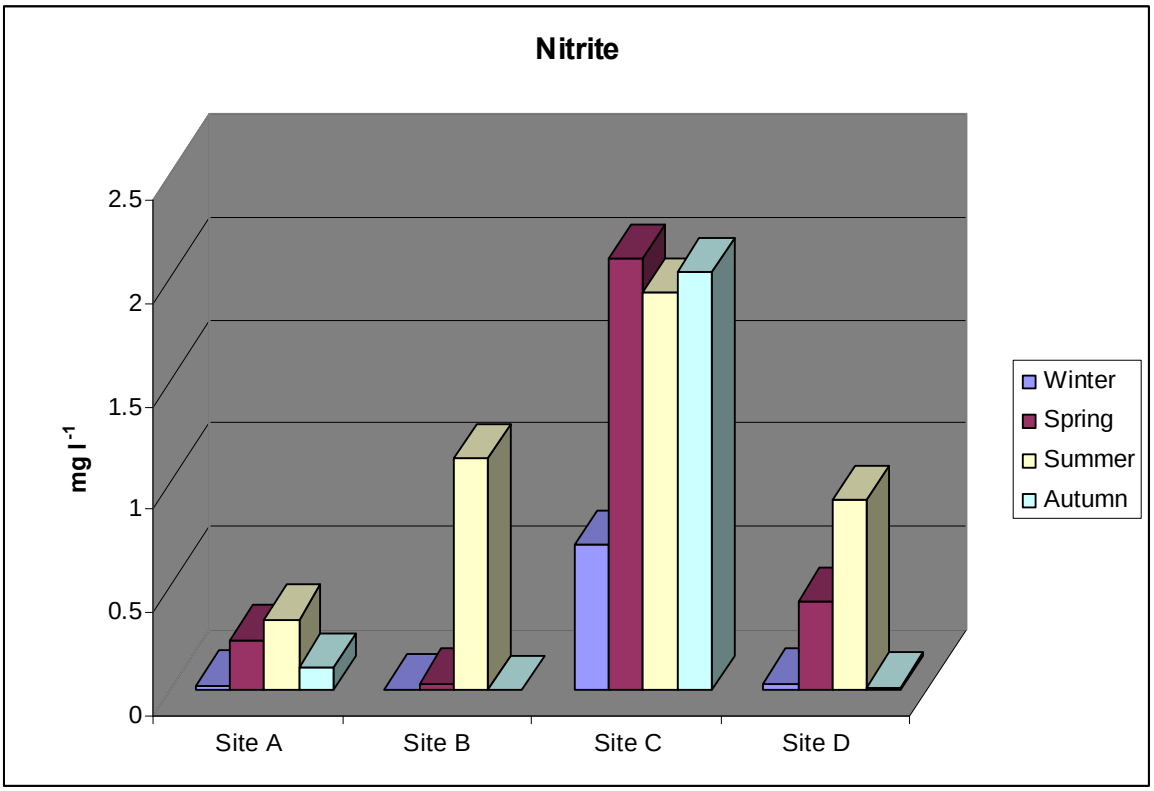

Figure 3.17: Seasonal nitrite concentrations of the four sampling sites

The nitrite concentrations at site C exceeded the TWQR for aquaculture (DWAF 1996b) during all four surveys with the highest value of 2.09mg NO_2 l^{-1} (Table 3.17 and Figure 3.17). These concentrations can be detrimental to fish and other aquatic organisms. Elevated levels of nitrite were also recorded at all the sites during Summer and all the concentrations exceeded the TWQR for this survey (Table 3.17). However, chloride concentrations recorded during this study were very high, especially at sites B and C (Table 3.9), which reduced the toxicity of nitrate. No concentration above 2.5mg NO_2 l^{-1} was recorded at any of the sites during the course of the study (Figure 3.17). This range is considered safe for the Mozambique tilapia as their 96 hr LC_{50} values are 10-15mg l^{-1} NO_2 (DWAF 1996b).

3.4.4 Ammonium

Ammonia may be present in the free, un-ionized form (NH_3) or in the ionized form as the ammonium ion (NH_4^+). Both are reduced forms of inorganic nitrogen derived mostly from aerobic and anaerobic decomposition of organic material and their relative

proportions are controlled by water temperature and pH (DWAF 1996c). They exist either as ions, or can be adsorbed onto suspended organic and inorganic material. The toxicity of ammonia is directly related to the concentration of the un-ionized form (NH_3), the ammonium ion having little or no toxicity to aquatic biota (Dallas and Day 2004). At low to medium pH values, the ammonium ion dominates, but as pH increases ammonia is formed, the latter being considerably more toxic to aquatic organisms (DWAF 1996c). The ammonium ion does, however, contribute to eutrophication in aquatic ecosystems. Prior exposure to ammonia increases the tolerance of fish to ammonia and enables them to withstand concentrations that would otherwise be acutely lethal (DWAF 1996c).

Ammonia is present in small amounts in air, soil and water, and in large amounts in decomposing organic matter. Ammonia, also associated with clay minerals, enters the aquatic environment through soil erosion (DWAF 1996c). Ammonia is also a major metabolic waste product from fish (Francis-Floyd and Watson 1990). The potential effect of ammonia on the aquatic environment is modified by the chemical species present, the relative proportions of each, and other factors such as pH, temperature and dissolved oxygen concentration (Dallas and Day 2004).

DWAF (1996c) suggested that ninety percent (90%) of all free ammonia estimates should be within the TWQR and that all free ammonia estimates should be below the Chronic Effect Value (CEV). In the case of accidental spills, chronic and acute toxicity effects will occur if ammonia estimates exceed the Acute Effect Value (AEV) (DWAF 1996c). The TWQR for aquatic ecosystems are calculated from the total ammonia concentration, that is, the sum of the un-ionized form (NH_3) and the ionized form (NH_4^+) (DWAF 1996c) and was used to discuss the ammonium results obtained during this study. The TWQR for un-ionized ammonia in ecosystems is < 0.007mg l^{-1}; the CEV is 0.015mg l^{-1} and the AEV is 0.1mg l^{-1} (DWAF 1996c).

Table 3.18: Seasonal ammonium values (in mg NH_4 l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.5	0.001	0.2	0.8
Spring	0.1	0.1	0.2	0.2
Summer	0.1	0.1	0.2	0.9
Autumn	0.2	0.1	0.1	0.2
Mean	0.23	0.08	0.18	0.53

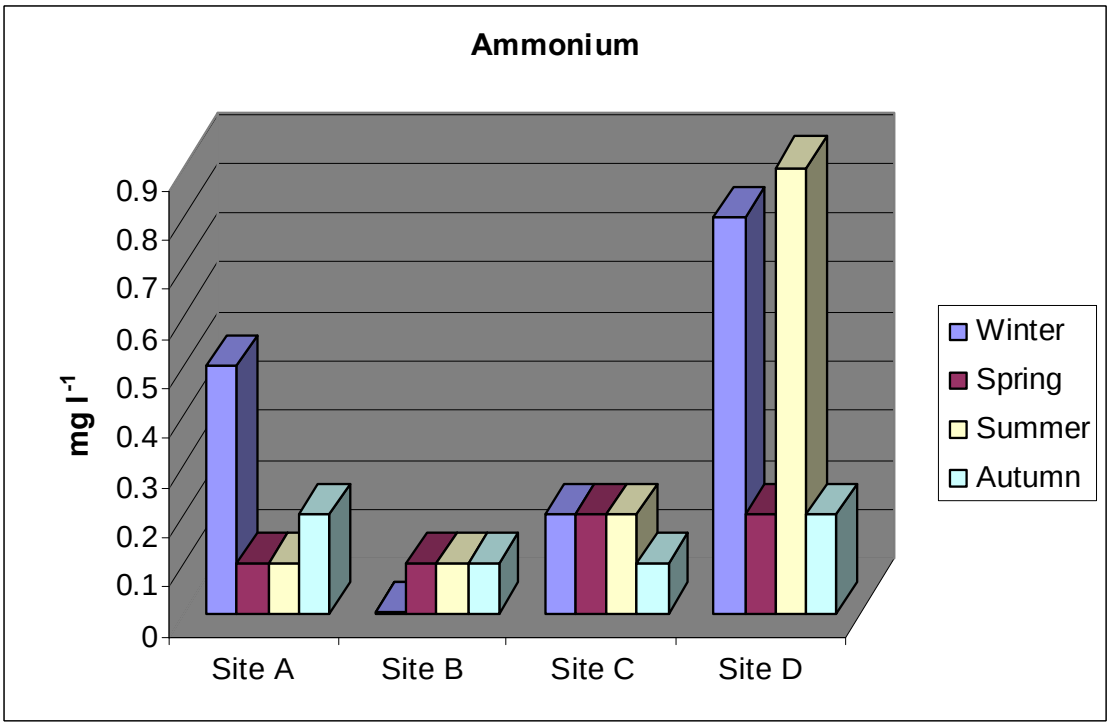

Figure 3.18: Seasonal ammonium concentrations of the four sampling sites

The highest mean ammonium concentrations were recorded from site D and the lowest at site B (Table 3.18 and Figure 3.18). The turbidity levels at site D were high (Table 3.4) caused by soil erosion, thus the ammonium can enter the water through clay minerals (DWAF 1996c). All the ammonium levels were above the un-ionized ammonia TWQR of 0.007mg l^{-1} of DWAF (1996c) for aquatic ecosystems. However, ammonia concentrations increase when pH increases, and the pH values of all the sites during this study were alkaline (Table 3.3), resulting in possible higher ammonia than ammonium concentrations which is the more toxic form. The mean concentration of ammonium at site D was over the TWQR of 0.3mg l^{-1} un-ionized ammonia for

aquaculture (DWAF 1996b), but within the possible sub-lethal range of 0.3 to 0.8mg l^{-1} un-ionized ammonia for warm water fish.

3.4.5 Phosphate

Phosphates may be formed by substituting some or all of the hydrogen of a phosphoric acid by metals (Chapman and Kimstach 1996). Depending on the number of hydrogen atoms that are replaced, the resulting compound is described as a primary, secondary or tertiary phosphate (DWAF 1996c). In nature, phosphorus occurs almost entirely as the inorganic phosphate ion (PO_4^{3-}). Elemental phosphorus (P) does not occur in the natural environment. Orthophosphates, polyphosphates, metaphosphates, pyrophosphates and organically bound phosphates are found in natural waters. Of these, orthophosphate species H_2PO_4 and HPO_4^{-2} are the only forms of soluble inorganic phosphorus directly utilizable by aquatic biota. Soluble Reactive Phosphate (SRP), or orthophosphate, is that phosphorus which is immediately available to aquatic biota which can be transformed into an available form by naturally occurring processes (DWAF 1996c). In unpolluted waters, orthophosphate is readily taken up by plants and converted into cellular structures by photosynthetic action. Inorganic phosphorus is the principal nutrient controlling the degree of eutrophication in the aquatic ecosystems (Dallas and Day 2004). Primary and secondary phosphates contain hydrogen and are acid salts. The primary phosphates tend to be more soluble. Secondary and tertiary phosphates, with the exception of those of sodium, potassium and ammonium are insoluble in water (Chapman and Kimstach 1996). Tertiary sodium phosphate is valuable as a detergent and water softener.

Phosphates, which are an important component of metabolism in both plants and animals, help in the first step in oxidation of glucose in the body. Primary calcium phosphate is an element of plant fertilizer (DWAF 1996c). The immediately available soluble reactive phosphorus, is seldom found in large quantities in non polluted water, as it is taken up by plants, or absorbed onto suspensoids or bonded to ions such as iron, aluminium, calcium and a variety of organic matter (Dallas and Day 2004).

The South African water quality guidelines (DWAF 1996c) recommended the following total inorganic phosphorus (TP) ranges to establish the trophic status of a water body:

- <0.005mg l^{-1} - Oligotrophic (nutrient-poor) conditions;
- 0.005 ï 0.025mg l^{-1} - Mesotrophic conditions;
- 0.025 - 0.25mg l^{-1} - Eutrophic conditions;
- >0.25mg l^{-1} - Hypertrophic conditions.

Table 3.19: Seasonal phosphate values (in mg PO_4^{3-} l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.2	0.2	0.2	0.2
Spring	0.16	0.08	0.06	0.01
Summer	0.1	0.1	0.1	0.1
Autumn	0.2	0.2	1.31	0.2
Mean	0.17	0.15	0.42	0.13

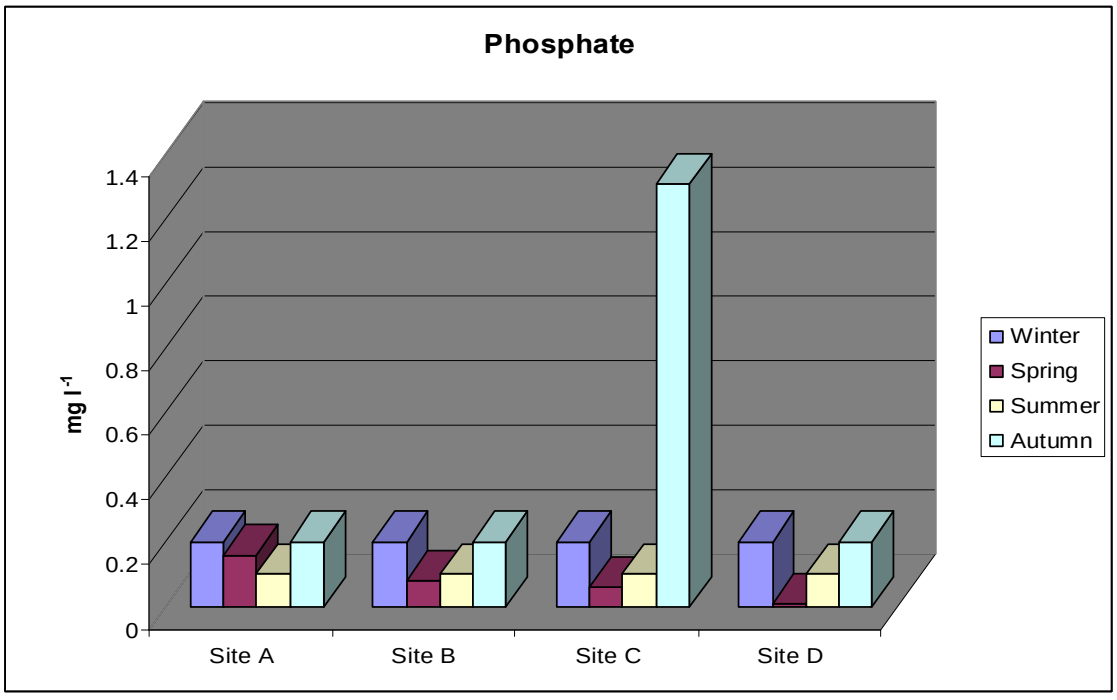

Figure 3.19: Seasonal phosphate concentrations of the four sampling sites

The highest phosphate value was 1.31mg l^{-1} and recorded at site C during the Autumn survey (Table 3.19). The lowest phosphate concentration was measured at site D during the Spring survey (Figure 3.19). The highest mean phosphate value was 0.42mg l^{-1} recorded at site C and the lowest mean was 0.13mg l^{-1} recorded at site D (Table 3.19).

Most of the sampling sites experienced eutrophic conditions during the sampling period. At site D during the Spring survey, a mesotrophic condition was measured due to the low phosphate level (0.01mg PO_4^{3-} l^{-1}). At site C, during the Autumn survey, a hypertrophic condition was measured due to an elevated phosphate level of 1.31mg l^{-1} (Table 3.19 and Figure 3.19).

Naturally, phosphate enters a water body by the weathering of rocks and the subsequent leaching of phosphate salts into surface waters, as well as the decomposition of organic matter (Correl 1998). Anthropogenic activities that release phosphates into the water include domestic and industrial effluents (point source pollution), atmospheric precipitation, urban runoff and drainage from agricultural activities (non-point source pollution) (Dallas and Day 2004). Site C receives amongst others, storm water and industrial effluents through the Loole creek which may be the reason for the higher phosphate levels recorded at this site.

According to DWAF (1996c) and Dallas and Day (2004), certain symptoms or effects are associated with selected ranges of inorganic phosphorus and nitrogen concentrations in aquatic ecosystems. In terms of the inorganic phosporus and nitrogen concentrations recorded during this study, it can be concluded that sites A and B were mesotrophic (according to the mean nitrogen concentrations recorded), indicating moderate or occasional water quality problems with occasional nuisance growth of aquatic plants and algal blooms (seldom toxic). But, the phosphate concentrations recorded at these two sites indicated eutrophic conditions at the latter two sites, meaning frequent water quality problems with frequent nuisance growth of aquatic plants and blooms of undesirable blue-green algae which may be toxic to man, livestock

and wildlife (Dallas and Day 2004). Hypertrophic conditions were recorded at site C, both for the nitrogen and inorganic phosphorus concentrations recorded, indicating almost continuous water quality problems with similar effects as mentioned above. At site D, eutrophic conditions prevailed, both in terms of the nitrogen and inorganic phosphorus concentrations at that site, indicating conditions with frequent water quality problems also with frequent nuisance growth of aquatic plants and blooms of undesirable blue-green algae.

3.4.6 Sulphate

Sulphate is the third most abundant anion. It is naturally present in surface waters as SO_4^{2-} ion (Dallas and Day 2004). It arises from the atmospheric deposition of oceanic aerosols and the leaching of sulphur compounds, either sulphate minerals such as gypsum or sulphide minerals such as pyrite, from sedimentary rocks (Chapman and Kimstach 1996). Since most sulphates are soluble in water, and calcium sulphate is relatively soluble, sulphates when added to water tend to accumulate to progressively increasing concentrations (DWAF 1996a). Sulphates are discharged from acid mine wastes and many other industrial processes that use sulphuric acid or sulphates (DWAF 1996a) and sulphur is thus also a common element in tailings (Wikipedia).

In living systems, sulphur is an essential component of proteins and is thus an essential element (Dallas and Day 2004). In most natural waters, sulphate ions tend to occur in lower concentrations than either bicarbonate or chlorides ions. According to Dallas and Day (2004), sulphate is not toxic *per se*, however in excess it forms sulphuric acid, which is a strong acid that reduces pH and can have devastating effects on aquatic ecosystems. Typical concentrations of sulphate in surface water is 5mg SO_4 l^{-1} although concentrations of several hundred mg SO_4 l^{-1} may occur where dissolution of sulphate minerals or discharge of sulphate-rich effluents from acid mine drainage takes place (DWAF 1996a). There is no TWQR available for sulphates in freshwater aquatic ecosystems, therefore TWQR for domestic use which is between 0 and 200mg SO_4 l^{-1} (DWAF 1996a) was used.

Table 3.20: Seasonal sulphate values (in mg SO_4 l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	40	903	1086	24
Spring	45	1127	1105	20
Summer	25	374	849	13
Autumn	72	899	1502	47
Mean	45.5	825.8	1135.5	26

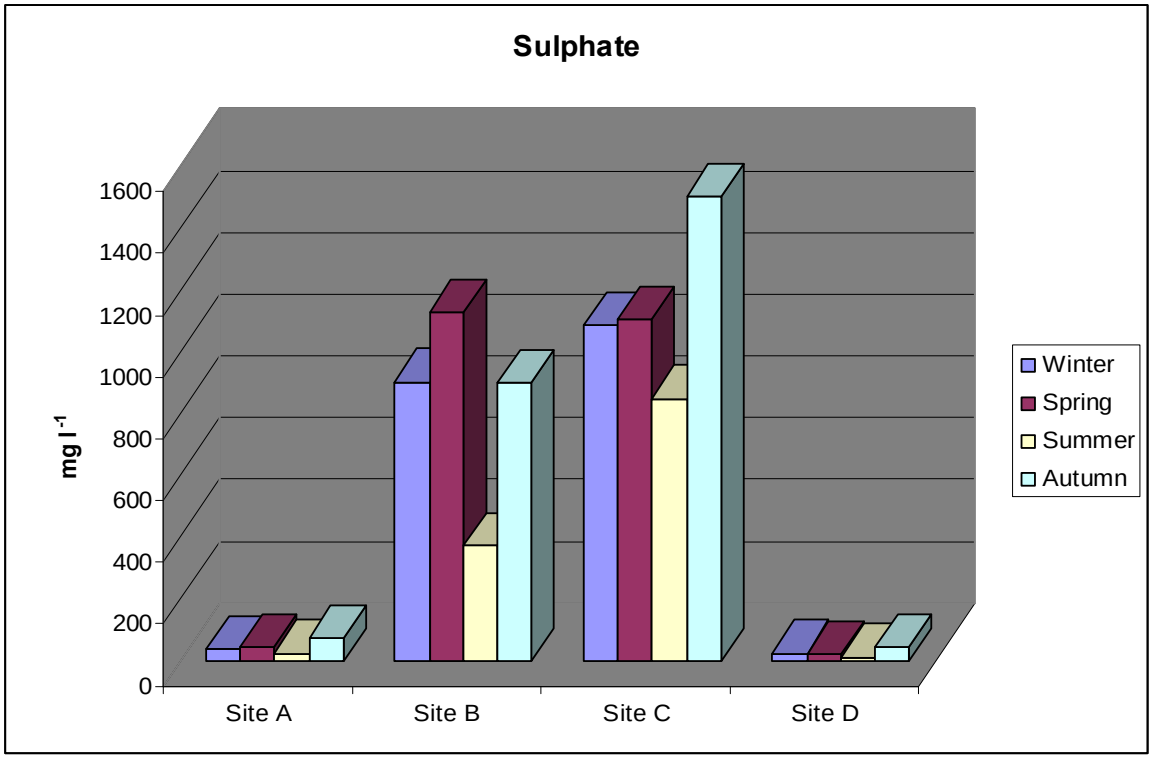

Figure 3.20: Seasonal sulphate concentrations of the four sampling sites

Sites B and C had the highest sulphate concentrations overall with a maximum of 1502mg l^{-1} recorded at site C during Autumn (Table 3.20 and Figure 3.20). These concentrations remained at unacceptable levels (for domestic use) throughout the sampling period. The lowest sulphate concentration of 13mg l^{-1} was recorded during Summer at site D (Table 3.20). The sulphate concentrations recorded at site A were always higher than those recorded at site D during the four sampling seasons. The highest mean concentration of sulphate was 1135.5mg l^{-1}, recorded at site C and the lowest level was 26mg SO_4 l^{-1} recorded at site D (Table 3.20). The elevated levels of

sulphate at sites B and C can be ascribed to the copper extraction process (with sulphur dioxide as a by-product) (Chapman and Kimstach 1996) and therefore the effluent (tailings) from the mining activities will increase the sulphate levels in tailing dams such as Site B. Sulphate also plays a vital role as one of the major ions contributing to the TDS concentrations in aquatic ecosystems (Dallas and Day 2004). Although high sulphate levels are not detrimental to fish, but when sulphate is reduced to hydrogen sulphide, it can be toxic to aquatic ecosystems (Dallas and Day 2004). The elevated sulphate concentrations at sites B and C can thus cause quick drops in pH resulting in acid water conditions according to Dallas and Day (2004).

3.5 Toxic constituents seldom occur in high concentrations in unimpacted systems (DWAF 1996c). Criteria are given as single numerical values associated with a specific level of risk or a lower value where no adverse effects are expected. Examples of typically toxic constituents are: inorganic constituents (e.g. aluminium, copper, fluoride, manganese and ammonium ions) and organic constituents (e.g. phenol and atrazine) (DWAF 1996c).

3.5.1 Aluminium

Aluminium is the most abundant metallic element in the lithosphere, but has little or no known biological function (Gensemer and Playle 1999). Aluminium can exist in a number of forms some of which may be soluble in water and some insoluble in water. It occurs as ionized aluminium (Al^{3+}), as ionised aluminium complexes, such as $Al^{2-}(OH)_6$ and insoluble compounds (Dallas and Day 2004). Aluminium is probably not an essential nutrient in any organism and is potentially one of the more toxic metals as soluble ionised aluminium is toxic to fish and aluminium complexes have been implicated in fish-kills (Dallas and Day 2004). The aluminium minerals, particularly the silicates of aluminium, are widespread. Some important minerals containing aluminium are hydrated aluminium oxide, magnesium aluminium oxide and the various aluminium silicates (DWAF 1996b).

Aluminium occurs in water in two main phases, as suspended aluminium minerals, and

as dissolved aluminium (DWAF 1996c). Aluminium is strongly influenced by pH levels and its solubility and toxicity is strongly pH-dependent. At alkaline pH levels it is present as soluble but biologically unavailable hydroxide complexes, while under acid conditions, aluminium occurs as the soluble, available and toxic species (Dallas and Day 2004).

The TWQR, CEV and AEV values of aluminium at different pH levels (DWAF 1996c) are indicated below:

Aluminum concentration (mg l^{-1})	**pH< 6.5**	**pH > 6.5**
TWQR (mg l^{-1})	0.005	0.01
Chronic Effect Value (CEV)	0.01	0.02
Acute Effect Value (AEV)	0.1	0.15

Aluminium is known to be toxic to various invertebrates and to plants and can interfere with the calcium ion metabolism (Dallas and Day 2004). Increases in acidity result in changes in the chemical structure of soils with the release of minerals such as aluminium into runoff water, which may enter lakes and fish farms. High concentrations of soluble aluminium may also be found in natural waters affected by acid rain and acid mine drainage (Dallas and Day 2004).

Table 3.21: Seasonal aluminium values (in mg Al l^{-1}) of the four sampling sites

Surveys	**Site A**	**Site B**	**Site C**	**Site D**
Winter	0.001	0.02	0.01	0.02
Spring	0.01	0.01	0.02	0.01
Summer	0.01	0.01	0.01	0.01
Autumn	0.01	0	0	0.01
Mean	0.008	0.01	0.01	0.0125

At sites A and C, the aluminium concentrations recorded during the Winter survey were within the TWQR of 0.01mg l^{-1} at pH >6.5 for aquatic ecosystems (DWAF 1996c), while the aluminium concentrations at sites B and D were equal to the CEV of 0.02mg l^{-1} (Table 3.21). During the Spring survey, only site C recorded a CEV concentration of 0.02 mg Al l^{-1} (Table 3.21 and Figure 3.21). The aluminium concentrations were constant (0.01mg Al l^{-1}) at the four sampling sites during the Summer and Autumn

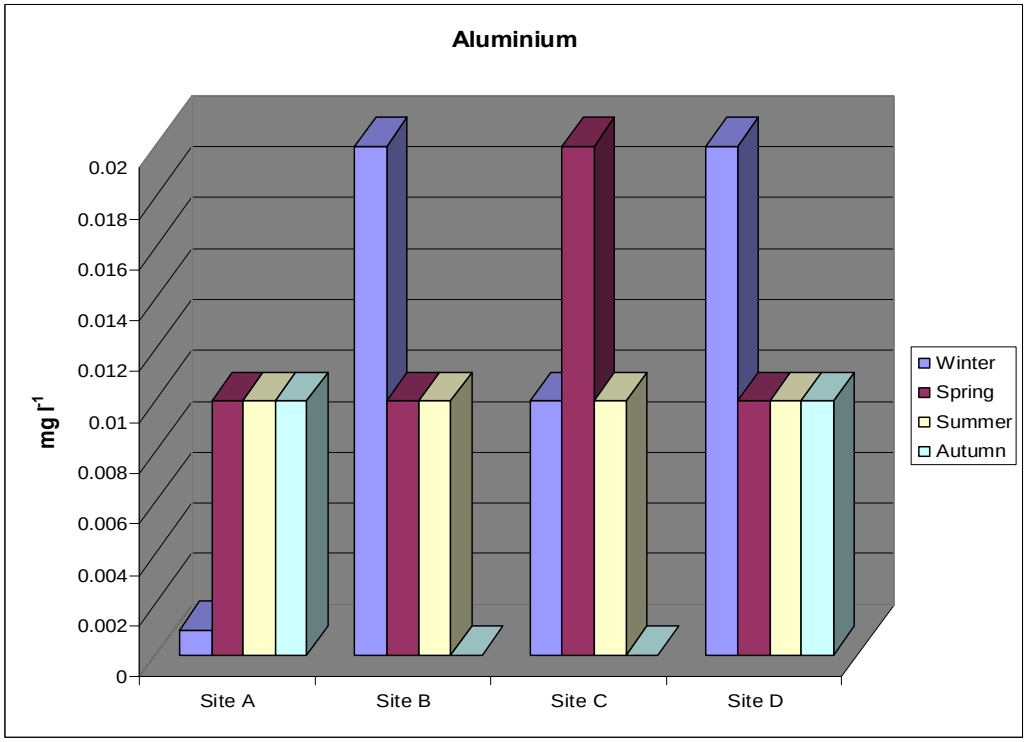

Figure 3.21: Seasonal aluminium concentrations of the four sampling sites

surveys except for sites B and C (Autumn) where the aluminium concentrations were too low to be determined by the spectrophotometer (Figure 3.21). The highest mean value was 0.0125mg Al l^{-1} recorded at site D (Table 3.21).

Dallas and Day (2004) stated that aluminium is pH dependent and at low pH values aluminium is largely in the aqua form which is soluble and very toxic. As the pH increases, hydrolysis of aluminum results in a series of increasing insoluble hydroxyl ions complexes ($Al(OH)^{2+}$ and $Al(OH)_2^{+}$). Taking this into consideration the aluminium concentrations recorded during this study were not toxic as the pH values ranged between 7.7 and 8.82 (Table 3.3) during this study. However, a CEV concentration of 0.02 mg Al l^{-1} (Table 3.21 and Figure 3.21) was recorded at three occasions which might be a matter of concern.

3.5.2 Copper

Copper is one of the world's most widely used metals and it occurs naturally in most waters (DWAF 1996c). Copper occurs in four oxidation states, the two most common forms are cuprous copper (I) and cupric copper (II). Cuprous copper is unstable in aerated aqueous solutions and will normally be oxidized to cupric copper (DWAF 1996c). Copper is a common metallic element in the rocks and minerals of the earth's crust, and is commonly found as an impurity in mineral ores and $CuFeS_2$ is the most abundant of the copper minerals (Villarroel 2000). Igneous rocks contain more copper than sedimentary rocks. According to DWAF (1996c) 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. Metallic copper is insoluble in water, but many copper salts are highly soluble as cupric or cuprous ions (DWAF 1996c).

Dallas and Day (2004) reported that copper is a micronutrient and an essential part of cytochrome oxidase and various other enzymes involved in redox reactions in cells. It is toxic at low concentrations in water and is known to cause brain damage in mammals (Olaifa *et al.* 2004). The toxicity of copper decreases when the total water hardness of an aquatic ecosystem increases (DWAF 1996c). Furthermore, the toxicity of copper increases as the pH and dissolved oxygen concentrations decrease (Benedetti *et al.* 1989; Greenfield 2004). According to DWAF (1996c), copper in aquatic ecosystems is correlated with water hardness as follows:

Water Hardness (mg $CaCO_3$ l^{-1})	< 60	60-119	120-180	> 180
TWQR (mg l^{-1}) Cu mg l^{-1}	0.0003	0.0008	0.0012	0.0014
Chronic Effect Value (CEV)	0.0005	0.0015	0.0024	0.0028
Acute Effect Value (AEV)	0.0016	0.0046	0.0075	0.012

During this study copper concentrations exceeded the TWQR of 0.0014mg l^{-1} at a total water hardness of >180mg $CaCO_3$ l^{-1} at all sites, as suggested by DWAF (1996c). Except for site D, the mean total water hardness was >180mg $CaCO_3$ l^{-1} at all sites (Table 3.8). The copper concentrations were high during the Winter and very high during the Autumn surveys at all sites (Table 3.22 and Figure 3.22). The highest

Table 3.22: Seasonal copper values (in mg Cu l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.02	0.01	0.022	0.021
Spring	0.01	0.02	0.005	0.009
Summer	0.01	0.018	0.004	0.002
Autumn	0.092	0.067	0.19	0.125
Mean	0.033	0.029	0.055	0.039

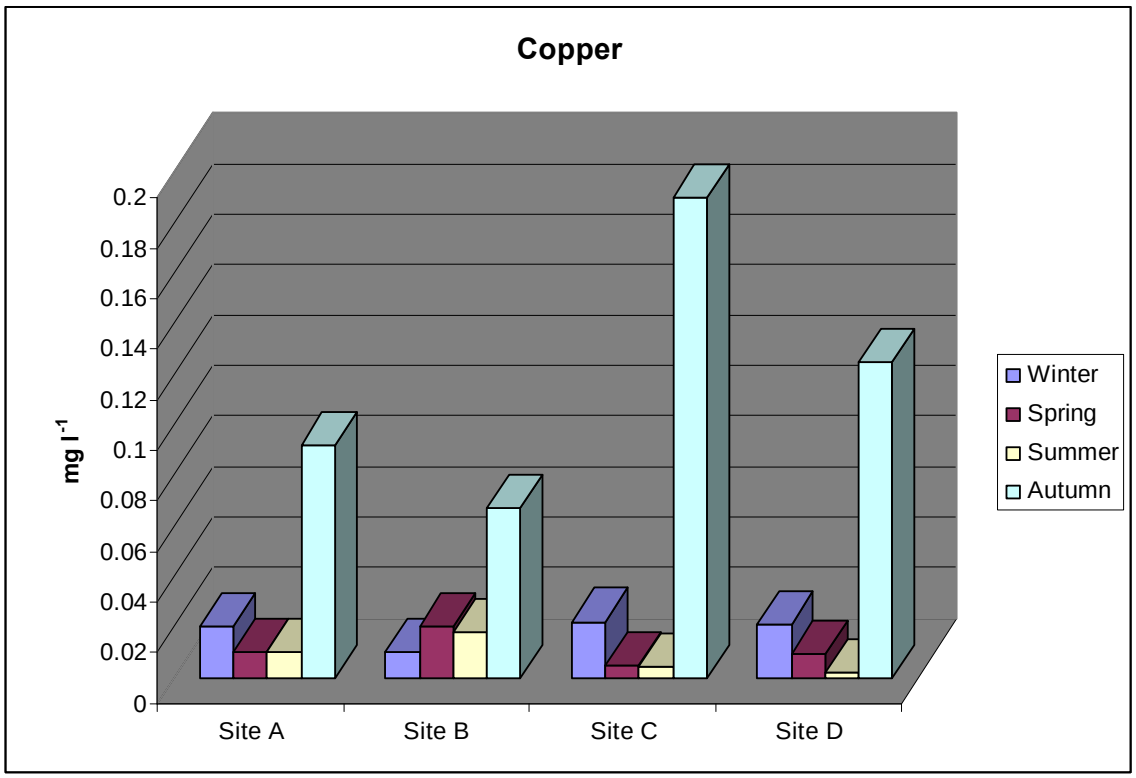

Figure 3.23: Seasonal copper concentrations of the four sampling sites

recorded concentration was 0.19mg Cu l^{-1} at site C with a mean value of 0.055mg Cu l^{-1} (Table 3.22). The mean concentrations exceeded the CEV and AEV (DWAF 1996c) for copper at a water hardness of >180mg $CaCO_3$ l^{-1} at all sites, but the toxicity of copper is reduced by the high water hardness levels (Table 3.8) as well as the alkaline pH vales recorded at all sites (Table 3.3). The high copper concentrations recorded during this study can mainly be attributed to industrial effluents and mine tailings (Von der Heyden

and New 2004) at sites B and C and geological weathering and atmospheric pollution (Moore and Ramamoorthy 1984) at sites A and D.

3.5.3 Iron

Iron is the second most abundant element in the earth's outer crust and may be present in natural waters in relation to the geology of the area and other chemical properties of the water body (DWAF 1996c). If iron is present in water in excessive amounts, it forms a red iron-oxide precipitate (Trombley 2001). Its redox pair, Fe(II) and Fe(III), has an oxidation potential that conveniently falls within the range of earth's surface conditions; Fe(II) is soluble and Fe(III) is highly insoluble. Thus, Fe(II)-containing minerals such as hornblende, pyrite, chlorite and biotite can weather, releasing dissolved Fe(II). Interaction of Fe(II) with dissolved oxygen in groundwater produces Fe(III)-oxides, -hydroxides and -oxyhydroxides (Dideriksen *et al.* 2007).

Iron is an essential element in the metabolism of animals and plants (Dallas and Day 2004). It is required in the enzymatic pathways of chlorophyll and protein synthesis, and in the respiratory enzymes of all organisms. It also forms a basic component of haeme-containing respiratory pigments (e.g. haemoglobin), catalyses, cytochromes and peroxidases (DWAF 1996c). According to DWAF (1996c) iron is naturally released into the environment from weathering of sulphide ores and igneous, sedimentary and metamorphic rocks. Leaching from sandstones releases iron oxides and iron hydroxides into the environment. DWAF (1996c) reported that iron is also released into the environment by human activities, mainly from the burning of coal, acid mine drainage, mineral processing, sewage and the corrosion of iron and steel. It has toxic properties at high concentrations, inhibiting various enzyme pathways (Dallas and Day 2004). The TWQR for iron in aquatic ecosystems should not be allowed to vary by more than 10% of the background value (BV) of dissolved iron concentration for a particular site or case, at a specific time (DWAF 1996a). Typical, in unpolluted surface water, iron concentrations range from 0.001 to 0.5mg l^{-1} (DWAF 1996b) but there is insufficient data to derive a CEV or an AEV for iron for aquatic ecosystems (DWAF 1996c).

Table 3.23: Seasonal iron values (in mg Fe l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.01	0.021	0.022	0.021
Spring	0.001	0.001	0.112	0.001
Summer	0.001	0.001	0.11	0.001
Autumn	0.031	0.035	0.043	0.01
Mean	0.011	0.015	0.072	0.008

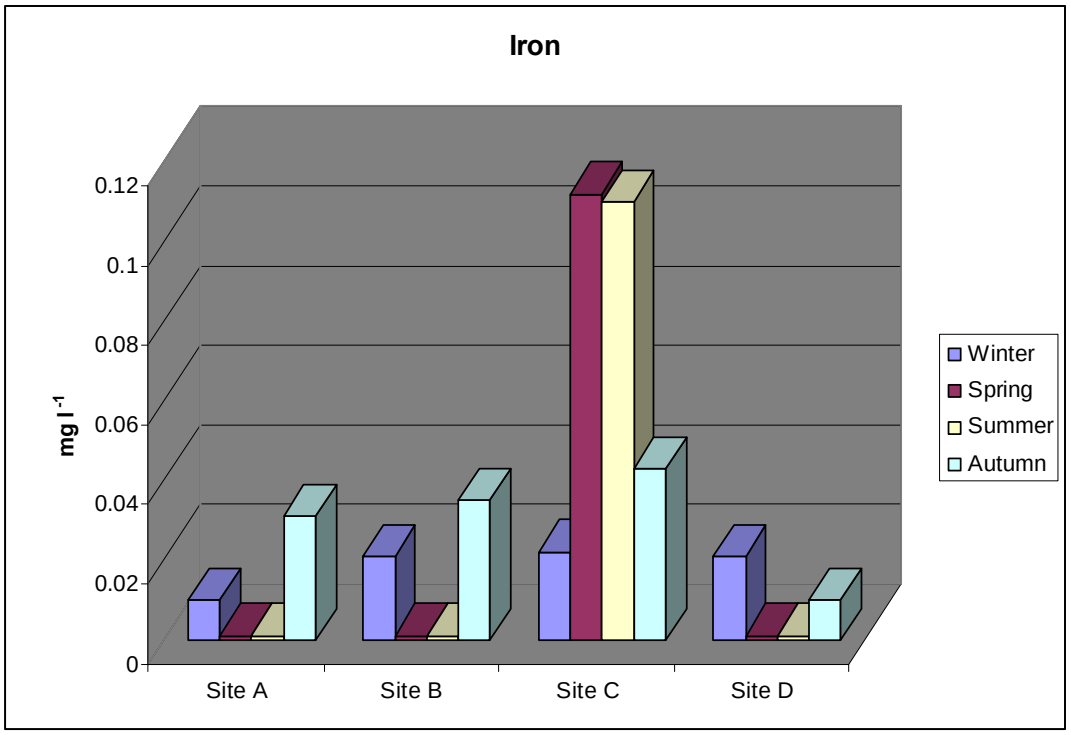

Figure 3.23: Seasonal iron concentrations of the four sampling sites

Kempster *et al.* (1980) suggested concentrations of iron between 0.2 and 1mg l^{-1} as optimal.

The concentrations of iron recorded during this study were within the range reported by DWAF (1996b) for unpolluted surface water as well as the optimum iron concentrations suggested by Kempster *et al*. (1980). The highest iron concentration was recorded at site C during Spring (0.112mg Fe l^{-1}) (Table 3.23 and Figure 3.23). The iron concentrations recorded at site C were still however, within the range suggested for

unpolluted surface water by DWAF (1996b). Iron levels increased during Autumn at all sites except at site C. The highest mean value was 0.072mg Fe l^{-1}**,** recorded at site C (Table 3.23) which can be attributed to mining activities as iron is one of the by-products (Chapman and Kimstach 1996) of the mining activities at site C.

3.5.4 Lead

Lead ranks about 36th in natural abundance among elements in the earth's crust. It is widely distributed all over the world in its sulfide form, the ore galena (DWAF 1996c). Anthropogenic outputs of lead in the environment outweigh all natural sources (e.g. weathering of sulfide ores, especially galena), and lead reaches the aquatic environment through precipitation, fall-out of lead dust, street runoff and industrial and municipal wastewater discharges (Gensemer and Playle 1999). Lead can be found in the air, water and soil and has no known beneficial effect on humans or animals.

Most of the lead entering aquatic ecosystems are associated with suspended sediments, while lead in the dissolved phase is usually complexed by organic ligands. It is a toxic metal that tends to bioaccumulate in living tissues and in the body of vertebrates where it is stored primarily in bones (DWAF 1996c). Low concentrations of lead affect fish by causing the formation of a film of coagulated mucous over the gills and subsequently over the entire body (DWAF 1996c).

Lead toxicity decreases with an increase in total water hardness and also increases with decreased dissolved oxygen in the water (Martinez *et al.* 2004). The water quality guidelines of DWAF (1996c) for lead in aquatic ecosystems are indicated below:

Water Hardness (mg l^{-1} $CaCO_3$)	**<60**	**60 - 119**	**120 – 180**	**>180**
TWQR (mg Pb l^{-1})	<0.0002	<0.0005	<0.001	<0.0012
Chronic Effect Value (CEV)	0.0005	0.001	0.002	0.0024
Acute Effect Value (AEV)	0.004	0.007	0.013	0.016

Table 3.24: Seasonal lead values (in mg Pb l^{-1}) of the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.002	0.007	0.009	0.003
Spring	0.001	0.009	0.008	0.001
Summer	0.001	0.001	0.024	0.001
Autumn	0.001	0.001	0.001	0.001
Mean	0.001	0.005	0.011	0.002

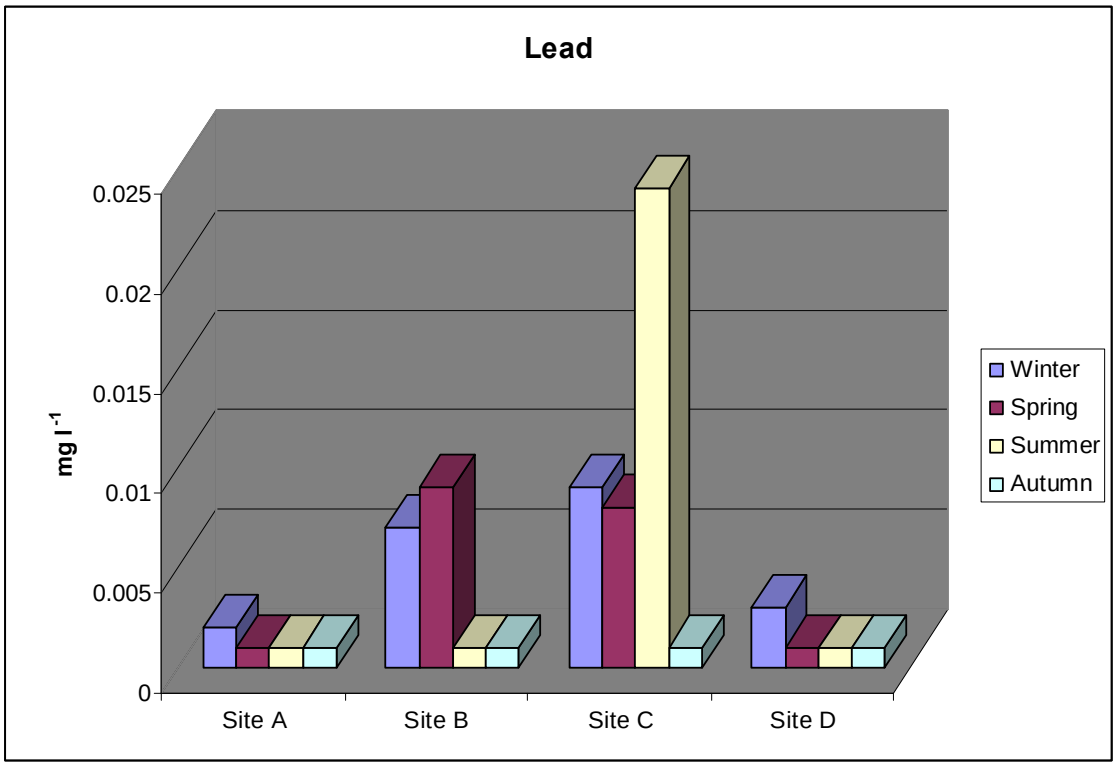

Figure 3.24: Seasonal lead concentrations of the four sampling sites

Lead concentrations recorded during this study exceeded the TWQR (DWAF 1996c) for lead in aquatic ecosystems (0.0012mg Pb l^{-1} in water hardness >180mg l^{-1} $CaCO_3$) during some of the surveys (Table 3.24 and Figure 3.24). Sites A and D had constant concentration values of 0.001mg Pb l^{-1} during the Spring, Summer and Autumn surveys as well as at all four sampling sites during Autumn (Table 3.24). The mean concentrations of lead at sites B and C were above the CEV of 0.0024 mg l^{-1}. Site C exceeded the AEV of 0.016 mg Pb l^{-1} in water hardness of >180mg $CaCO_3$ l^{-1} during the Summer survey only (Figure 3.24). The high values recorded at sites C and B can be

attributed to industrial effluents and mining activities and is toxic and harmful to aquatic life which is a matter of concern.

3.5.5 Manganese

According to Dallas and Day (2004) manganese is an essential micronutrient. Manganese is the eighth most abundant metal in nature, and occurs in a number of ores (DWAF 1996c). 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 (Galvin 1996). It may exist in the soluble manganous (Mn^{2+}) form, but is readily oxidised to the insoluble manganic (Mn^{4+}) form. The Mn^{2+} ion occurs at low redox potentials and low pH. Nitrate, sulphate and chloride salts of manganese are fairly soluble in water, whereas oxides, carbonates, phosphates, sulphides and hydroxides are less soluble. Soils, sediments, metamorphic and sedimentary rocks are significant natural sources of manganese (DWAF 1996c).

High concentrations of manganese are toxic to vertebrates leading to disturbances in various metabolic pathways (Dallas and Day 2004). Wang (1987) stated that the toxicity of manganese increases with a decrease in the pH levels of water. Industrial discharges also account for elevated concentrations of manganese in receiving waters (DWAF 1996c).

The TWQR for dissolved manganese in aquatic ecosystems is 0.18mg l^{-1}, the CEV is 0.37mg l^{-1} and the AEV is 1.3mg l^{-1} (DWAF 1996c).

Table 3.25: Seasonal manganese values in mg Mn l^{-1} at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.03	0.04	0.04	0.03
Spring	0.004	0.007	0.06	0.004
Summer	0.004	0.006	0.055	0.005
Autumn	0.02	0.009	0.14	0.003
Mean	0.015	0.016	0.074	0.011

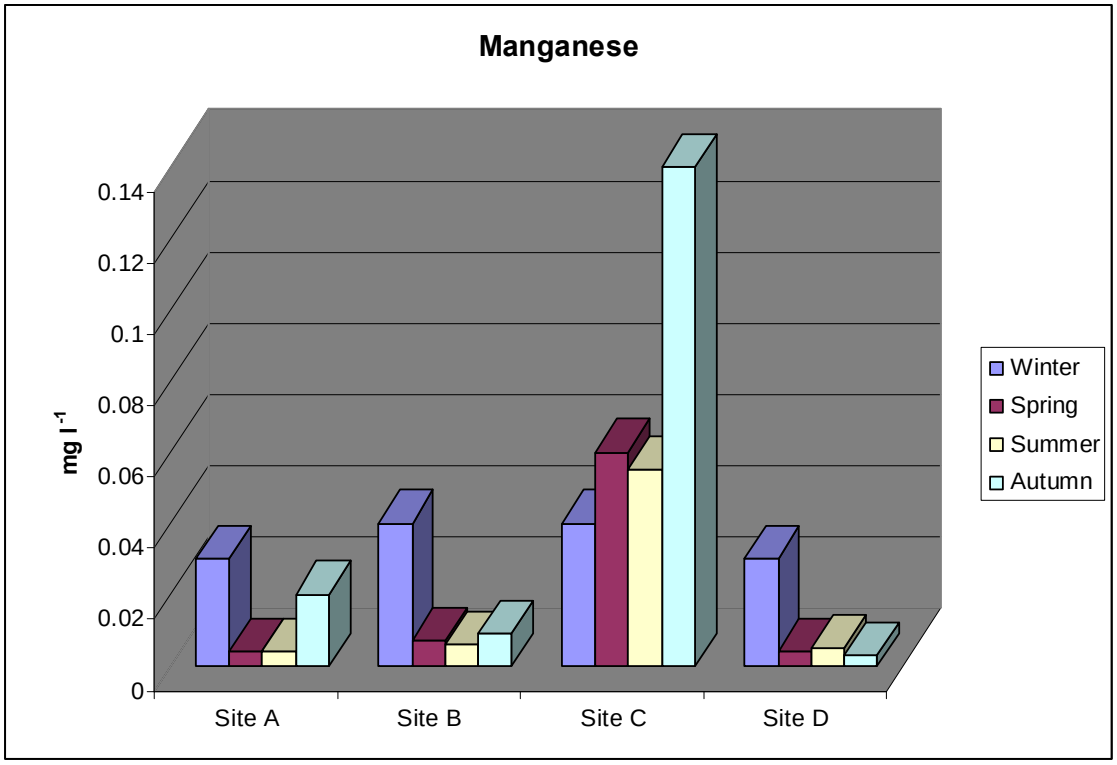

Figure 3.25: Seasonal manganese concentrations at the four sampling sites

All the manganese concentrations recorded during this study were below the TWQR of 0.18mg l^{-1} suggested for aquatic ecosystems by DWAF (1996c) at all sites (Table 3.25 and Figure 3.25). The manganese concentrations were very low throughout the sampling period except at site C. The highest manganese concentration of 0.14mg l^{-1} was recorded at sites C during Autumn and a mean of 0.074mg l^{-1}, but these values were still below the TWQR suggested by DWAF (1996c) (Table 3.25). Sites A and D had the same concentrations for manganese during Winter (0.03mg Mn l^{-1}) while slightly higher manganese concentrations were recorded at sites B and C during the same season (0.04mg Mn l^{-1}) (Figure 3.25). The results indicated that the mining activities did not contribute to manganese levels in the water and the low levels recorded during this study can probably be natural for the area.

3.5.6 Zinc

Zinc, like manganese, is a metallic element and an essential micronutrient for all organisms (Dallas and Day 2004). According to DWAF (1996a) zinc occurs in two oxidation states in aquatic ecosystems, namely as the metal, and as zinc (II) ions. In aquatic ecosystems the zinc (II) ion is toxic to fish and other aquatic organisms at relatively low concentrations (Robinson 1996). Zinc is also found as a carbonate, oxide or silicate and may occur in association with many other metal ores such as copper and arsenic (DWAF 1996a). It can enter aquatic ecosystems through both natural processes such as weathering and erosion, and through industrial activity. According to DWAF (1996c) zinc in aqueous solutions, is amphoteric, that is, it dissolves in acids to form the hydrated cations (Zn^{2+}) and in strong bases it forms zincate anions (probably of the form $Zn(OH^{2-})_4$). Organo-zinc complexes and compounds can also be formed (DWAF 1996c). In most natural waters, zinc exists mainly as the divalent cation, which is the potentially toxic form (DWAF 1996c).

Chemical speciation of zinc is affected primarily by pH and alkalinity. Greatest dissolved zinc concentrations will occur in water with low pH, low alkalinity and high ionic strength (DWAF 1996c). Soluble zinc salts (e.g. zinc chloride and zinc sulphate) or insoluble precipitates of zinc salts (e.g. zinc carbonate, zinc oxide and zinc sulphide) occur readily in industrial wastes (DWAF 1996c).

Zinc in its divalent form is toxic to aquatic organisms at relatively low concentrations (Dallas and Day 2004). Excessive absorption of zinc can also suppress copper and iron absorption. Toxic effects of zinc included the forming of insoluble compounds in the gill mucus and depressing white blood cell-thrombocyte counts of fish (Dallas and Day 2004). Furthermore, Kotzé *et al*. (1999) reported that the elevated bioaccumulation of copper and zinc in *Oreochromis mossambicus* from the Mamba weir in the Olifants River can be attributed to the mining activities in the Phalaborwa area.

The TWQR for zinc for aquatic ecosystems is <0.002mg Zn l^{-1}, the CEV is 0.0036mg Zn l^{-1}, and the AEV is 0.036mg Zn l^{-1} (DWAF 1996c).

Table 3.26: Seasonal zinc values in mg Zn l^{-1} at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0.030	0.041	0.051	0.022
Spring	0.001	0.001	0.020	0.001
Summer	0.001	0.001	0.024	0.001
Autumn	0.034	0.041	0.045	0.022
Mean	0.017	0.021	0.035	0.012

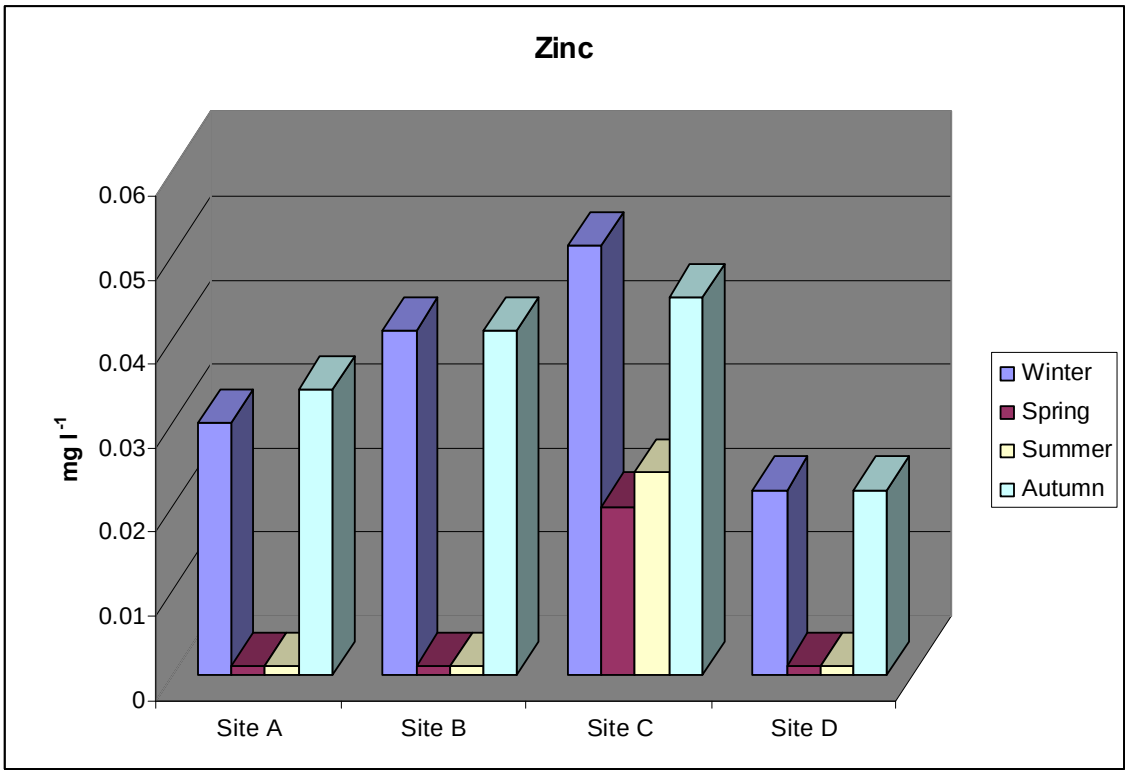

Figure 3.26: Seasonal zinc concentrations at the four sampling sites

Zinc concentrations were above the TWQR for aquatic ecosystems at all sites during the Winter and Autumn surveys but fell in the range during the Spring and Summer surveys, except at site C (Table 3.26 and Figure 3.26). The highest mean concentrations for zinc were recorded at sites B (0.041mg Zn l^{-1}) and C (0.051mg Zn l^{-1}). The elevated zinc levels can be attributed to the mining activities (Kotzé *et al*. 1999). The mean concentrations of zinc at all sites were above DWAFôs CEV of 0.0036mg l^{-1} (DWAF 1996c) during this study, but values recorded at site C exceeded the AEV of

0.036mg Zn l^{-1} during the Winter and Autumn surveys (Table 3.26). As mentioned previously, zinc is toxic to aquatic organisms in its divalent form and toxic effects include the forming of insoluble compounds in the gill mucus of fish (Dallas and Day 2004) and oedema and liver necrosis (DWAF 1996c). But, according to DWAF (1996c) the pH of the water influences the toxicity of metals. The alkaline pH recorded during this study (Table 3.3) decreased the toxicity of zinc in the water. Furthermore, the toxicity of zinc is reduced in hard water (DWAF 1996c) and the water was very hard (Table 3.8) at sites B and C, reducing the toxicity of zinc to the fish, but these levels were still unacceptable.

Conclusion

The Phalaborwa Barrage (site D) is the origin of the water used at the other sampling sites (sites A, B and C) of this study. The water quality of the Phalaborwa Barrage is influenced by agricultural activities as well as mining and industrial activities upstream. The Olifants River flows through many farms, and after heavy rainfalls, the agricultural pollutants are flushed into the river. The Foskor Limited mine (site B) and the Palabora Mining Company (site C) receive industrial water from the Barrage (Lepelle Water) which is used in the different mining processes. This results in different water quality at the different sites because of the dissimilar mining operations and processes. Water from the Barrage is also utilized in the industrial processes at the Sasol plant (site A), but as this site receives industrial water directly from the Barrage and no water from the Sasol Nitro industrial processes, water at this site is thus not impacted by mining or industrial activities. However, soil erosion, weathering and leaching (from the mine dumps) during rainy seasons, as well as evaporation may have an effect on the water quality at this site.

System variables: Results recorded for the system variables (pH, water temperature and dissolved oxygen) indicated that the pH levels and water temperature fell within the TWQR for aquatic ecosystems as suggested by DWAF (1996c) but the dissolved oxygen concentration was below 5mg O_2 l^{-1} during most of the surveys which may adversely affect the functioning and survival of biological communities (DWAF 1996c).

The pH of water ranged from 7.7 to 8.82 between the four sites, indicating an alkaline pH. When the pH of water is acidic, it can affect the chemical composition of various metals by rendering them more toxic, and this can be detrimental to aquatic biota (Dallas and Day 2004). The water temperatures varied during the four sampling periods with the lowest recorded in Winter as expected. The mean turbidity values were high at sites D, C, and A respectively which can be caused by particles such as silt, clay, fine organic matter and organisms that are suspended in the water. The elevated turbidity levels recorded during the rainy Summer survey at all four sites can be ascribed to runoff of organic material and sediments.

Non toxic constituents: The Total Dissolved Solids (TDS) and the electrical conductivity (EC) were very high at sites B and C throughout the four sampling surveys which may limit the growth, and may lead to death of aquatic organisms (Dallas and Day 2004). The total water hardness and salinity were very high at sites C and B which can be attributed to the mine tailings water as well as geochemical constituents of the region. High salinity levels can adversely affect growth in aquatic organisms due to a decrease of the osmotic potential (decreased water availability) as well as the toxicity of specific ions and/or unfavourable ratios of such ions. The cations and anions such as sodium, chloride, calcium, potassium and magnesium ions have contributed significantly to the elevated TDS and EC levels (DWAF 1996c).

Major ions: The cation (calcium, magnesium and potassium) and anion (chloride, fluoride and sulphates) concentrations were above the TWQR for aquatic ecosystems and domestic use (DWAF1996a and c) at all sites. The fluoride levels were high at two sites (i.e. sites B and C) but lower at sites A and D confirming the contribution of the tailings to fluoride levels (especially at site B). The fluoride concentrations were very high at site B which can pose a threat to animals and humans (DWAF 1996c). The mean levels recorded for sodium were low at sites A and D, but high at sites B and C. All the major ions contributed significantly to increased levels of TDS, salinity and EC (Dallas and Day 2004). The concentrations of dissolved salts varied significantly throughout the four sampling surveys. Sites B and C measured the highest

concentrations of major ions throughout the sampling period mainly because of mining activities, but also due to the catchment geology which is underlain by igneous rocks (Otto *et al.* 2007).

Nutrients: The nitrogen, sulphate and phosphate levels recorded during this study indicated that there was an influx of nutrients into the four sampling sites to varying degrees. The organic and inorganic matter can have an effect on eutrophication conditions at the sampling sites. The elevated levels of nitrogen caused eutrophication (nutrient enrichment) which increased the abundance of algae and aquatic macrophytes (like the pondweed, i.e. *Potamogeton* spp.) at sites B and C. Nitroglycerine used in mining operations contributed considerably to the elevated concentrations of nitrate nitrogen at site C throughout the sampling period. Elevated mean concentrations of total nitrogen, nitrate nitrogen, nitrite nitrogen, sulphate and phosphate were recorded at site C throughout the sampling period. The highest ammonium nitrogen concentrations were recorded at site D (Table 3.19). When considering the mean concentration values of nitrogen and phosphate at all sites, the water bodies of sites A and B experienced mesotrophic (according to the nitrogen concentrations) and eutrophic (according to the phosphate concentrations) conditions, site C was hypertrophic, while site D was eutrophic (DWAF 1996c). High sulphate levels are not toxic to fish but when it is reduced to hydrogen sulphide, it can have detrimental effect on aquatic life. The high sulphate levels at sites B and C can thus cause a quick drop in pH resulting in acid water conditions which is a matter of concern.

Toxic constituents: Trace metals and heavy metals differed considerably at all sites during the sampling period. The mean concentrations of aluminium recorded during this study were within the TWQR of 0.01mg l^{-1} at pH >6.5 for aquatic ecosystems (DWAF 1996c) at all sites. Furthermore, the aluminium concentrations were not toxic because of the alkaline pH recorded during this study. Copper levels were above the TWQR for aquatic ecosystems throughout the study. But, the toxicity of copper decreases in hard water and therefore the aquatic life can tolerate the higher copper levels recorded during this study. The mean concentrations of lead recorded during this study exceeded

the TWQR of aquatic ecosystems in water hardness >180mg l^{-1} $CaCO_3$ as suggested by DWAF (1996c) at all the sites except at site A. The mean concentrations of lead at sites B and C were above the CEV of 0.0024 mg Pb l^{-1}. Elevated concentrations of lead may be responsible for suffocation of fish due to the formation of a film of coagulated mucous over the gills (DWAF 1996c). Zinc concentrations varied significantly at all sites and exceeded the TWQR and CEV of aquatic ecosystems during some surveys (Table 3.27). The zinc concentration recorded at site C exceeded the AEV during the Winter and Autumn surveys. The toxic effects of zinc include the forming of insoluble compounds in the gill mucus of fish (Dallas and Day 2004) and oedema and liver necrosis (DWAF 1996c). But, the alkaline pH recorded during this study (Table 3.3) and the water hardness (Table 3.8) decreased the toxicity of zinc in the waters. Iron and manganese levels varied, however they were within the TWQR for aquatic ecosystems (DWAF 1996a).

The high concentrations of the trace and heavy metals recorded during this study at sites B and C can be attributed to mining activities (tailings) and the geological formations of the region. The overall water quality at sites A and D were much better compared to the other two sites impacted by mining activities. The results showed further that the water at site C was more impacted than that of site B. If the pH of the water at site C or B should change to be more acidic, it could affect the aquatic life in a serious manner (DWAF 1996c). During the rainy season, the water quality improved dramatically because of the dilution effect of the rain.

In conclusion, the mining activities adversely affect the water quality at sites B and C in terms of the dissolved salts, nutrients and trace and certain heavy metals. Some metals are however, dependant on the pH and water hardness, reducing the toxicity of metals in aquatic ecosystems with alkaline pH and hard water recorded at these two sites. Furthermore, certain metals, amongst others copper, iron, lead and zinc tend to bioaccumulate in aquatic organisms (Kotzé *et al.* 1999), and biomagnify in fish through the food chain, and this can be potentially hazardous to fish as well as quaternary consumers such as humans, birds and crocodiles (Carbonell *et al*. 2000).

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Chapter 4

Health Assessment Index and Parasite Index

Introduction

Chronic exposure of aquatic organisms to stressful polluted conditions have a cumulative impact on the three main physiological control systems, i.e. the immune system, the endocrine system and the nervous system as well as the metabolic systems, which provide energy for the proper functioning of an organism (Handy *et al.* 2003). Early toxic effects of pollution may, however, be evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance (Van Dyk 2003). Furthermore, fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect and give a good indication of the health status of a specific aquatic ecosystem.

Fish are good biological indicators because they are located at the top of the food chain, are a highly visible resource, and are known to accumulate toxicants (Streit 1998). In addition, they are in direct contact with pollutants in the water via their gills and their body surface. Fish are thus excellent indicators of aquatic health because they: live in the water all of their life, differ in their tolerance to amount and types of pollution, are easy to collect with the right equipment, live for several years, are easy to identify in the field, fish populations and individuals generally remain in the same area during summer seasons, they represent a broad spectrum of community tolerances from very sensitive to highly tolerant and respond to chemical, physical and biological degradation in characteristic response patterns (USEPA 2004). Harris (1995) indicated that fish possess three main attributes which make them useful for environmental monitoring programs: (a) fish are sensitive to most forms of human disturbances (this can be a disadvantage in

assessments of specific disturbances), (b) fish are useful for monitoring at all levels of biological organization and (c) fish monitoring programs have a favourable benefit-cost ratio.

Using fish for biological evaluation provides means to quantify ecological changes that result from the combination of physical, chemical and biological stressors (Oberdorff and Hughes 1992). Several approaches have been used over the past years to evaluate the stress on the health of fish populations but most of them is neither rapid nor inexpensive and cannot be applied to field studies. As an alternative to the more sophisticated methods, Goede and Barton (1990) and Goede (1992) developed and described an empirical necropsy-based system of organ and tissue indices to provide a fish health and condition assessment for fish populations. Adams *et al*. (1993) improved the method of Goede and Barton (1990) by developing a quantitative Health Assessment Index (HAI), intending to minimize the limitations of the necropsy-based system. The HAI comprises the evaluation of the external condition of fish (any aberrations of the skin, fins, opercules and eyes) as well as all internal organs and assigning values based on the degree of severity or damage observed, i.e. abnormal conditions can assume values of 10, 20 or 30 (depending on the severity of the conditions), while 0 represents normal conditions (Table 2.1). Statistical analysis of the assigned values is intended to show a correlation between fish health and pollution levels, as well as comparison of fish health between data sets of different populations.

According to Adams *et al*. (1993) the HAI has been successfully tested in the United States of America in the pulp-polluted Tennessee River basin (North Carolina, Tennessee, Alabama, Kentucky), the Hartwell Reservoir (Georgia, South Carolina) contaminated with polychlorinated biphenyls, and in the Pigeon River (Tennessee, North Carolina) that receives effluents from a bleached Kraft mill. Lohner *et al*. (2001) used the HAI to assess the possible effects of exposure to elevated selenium levels on sunfish populations. Kovacs *et al*. (2002) compared the HAI with other community-based approaches to assess the biological status of

fish in a river receiving pulp and paper mill effluents in Canada. Chaiyapechara *et al*. (2003) used the HAI to compare the health of rainbow trout that were fed diets containing adequate and high concentrations of lipid and vitamin E respectively. Schmitt *et al.* (2004; 2005) determined the effects of selected environmental contaminants on fish in the Rio Grande Basin using the following fish species, *Cyprinus carpio, Micropterus salmoides*, *Micropterus dolomieui,* and *Ictalurus furcatus.* Hinck *et al.* (2007) used three fish species to determine spatial trends in accumulative contaminants, health indicators (by applying the HAI) and reproductive biomarkers in fish from the Colorado River.

Avenant-Oldewage and Swanepoel (1993) suggested the use of fish health studies in South Africa. Subsequently the fish HAI was tested and adapted for local conditions through various studies on the Olifants River System (Avenant-Oldewage *et al*. 1995). The latter study made use of a variety of indicator fish species, including *Clarias gariepinus* (*cf* Marx 1996), *Oreochromis mossambicus* (*cf* Robinson 1996; Watson 2001), *Labeo* species (Luus-Powell 1997), and *Labeobarbus marequensis* (*cf* Watson 2001). After successful studies in the Olifants River, the HAI was also tested in the Vaal River System on *C. gariepinus* (*cf* Crafford 2000; Crafford and Avenant-Oldewage 2001), *Labeo* species (Groenewald 2000), *Labeo umbratus*, *L. capensis*, *Cyprinus carpio*, *Labeobarbus aneus* and *Lb. kimberleyensis* (*cf* Avenant-Oldewage 2001; Bertasso 2004). The HAI proved to be useful in bioassessment surveys and was incorporated in the ñField Biosurveys and Integrated Ecological Assessmentò by the Department of Water Affairs and Forestry (Killian 1996; Killian *et al*. 1997). Kotzé (2002) applied the HAI (as adjusted by Killian *et al*. (1997)) in the Klip River (Gauteng Province) and used *L. capensis*, *L. umbratus* and *Lb. aneus* as indicator species. Jooste *et al*. (2004) utilized the HAI to test the impact of mine effluents on the health of fish in the lower Ga-Selati River (Limpopo Province) with *C. gariepinus* and *O. mossambicus* as indicator species.

In the original HAI (Adams *et al*. 1993), parasites were only recorded as being absent or present. During testing and developing of the HAI in South Africa, a wide variety of fish parasites were encountered and a correlation between the abundance of endo- and ectoparasites and pollution levels was suggested (Avenant-Oldewage 1994; Luus-Powell 1997; Crafford and Avenant-Oldewage, 2001). The original denotation was thus expanded and endo- and ectoparasites were recorded as separate variables (Marx 1996, Robinson 1996; Luus-Powell 1997). These studies also showed that the presence of parasite *per se* were indicators of deteriorated health of the fish, and therefore also a deteriorated environment. This lead to the development of a separate index, i.e. the Parasite Index (PI) (Table 2.1) in the HAI (Avenant-Oldewage *et al*. 1995; Jooste *et al*. 2003, 2005; Luus-Powell *et al*. 2005).

The PI is a useful biomonitoring tool and gives a reliable indication of water quality (Avenant-Oldewage 1998; Jooste *et al*. 2004). According to Avenant-Oldewage (1998) a count of 10 to 20 ectoparasites can be expected in good quality water, but the count will drop to two, one or even zero if the water quality is poor. External parasites such as protozoa, monogeneans and copepods, usually attach to the skin, fins, mouth or gills of the host. Since these ectoparasites are constantly in contact with the external environment, they tend to reduce in diversity and abundance when water is heavily polluted (Barson 2004a). Conversely, endoparasites such as tapeworms, nematodes and digeneans, increase in abundance and diversity with increased pollution load. In polluted water, the number of endoparasites can rise to 2000 per fish. A possible explanation for this is the fact that the host protects the endoparasites from exposure to the surroundings (Luus-Powell 1997). Furthermore, because of the poor environment, the host might be stressed and its resistance to parasites might be lowered resulting in higher number of parasites.

Under normal or natural circumstances, a balance exists between the host and parasites controlled by the fishôs immune system. But if the fish is under stress

from pollution, the immune system may not be able to defend off large numbers of parasites and the host might die together with its parasites (Barson 2004b). When the HAI is supplemented by a parasite survey (PI), the value of the HAI can only be enhanced (Jooste *et al.* 2004).

Results and Discussion

In terms of indicator species, this study used a fish that is prevalent in most river systems of South Africa (Skelton 2001), namely the Mozambique tilapia (*Oreochomis mossambicus*). It is an important South African angling species and is also popular amongst subsistence fishermen, especially in rural areas in South Africa.

The health conditions of *O. mossambicus* were recorded during four surveys by making use of the fish Health Assessment Index (HAI) (Table 2.1). The data is presented as follows: the HAI with all external and internal variables (Addendum B; Tables 1 ï 4); the condition factor (based on the length-weight relationship); and the Parasite Index (PI). According to Schmitt *et al.* (2005) and Hinck *et al.* (2006) the HAI may vary depending on gender and gonadal stage of the fish. There were no sex-related differences for the HAI scores during this study, therefore the data for both sexes were combined for interpretation.

4.1 The Health Assessment Index

The HAI is a systematic method to identify external and internal lesions or abnormalities of each fish during a field necropsy (Schmitt *et al.* 2004). A higher HAI score indicates that a greater number of abnormalities were identified in/on the fish and a lower HAI score is indicative of better fish health and thus indirectly a better sampling site.

4.1.1 The population Health Assessment Index

Original field designations of all variables from the necropsy-based system were substituted with comparable numerical values into the HAI according to the methods used by Heath *et al.* (2004) and Jooste *et al*. (2004). All the variables of the HAI were represented by a value ranging from 0 - 30, depending on the condition of the organs, etc. tested, with normal conditions indicated by 0 (Table 2.1). To calculate an index value for each fish within a sample, numerical values for all variables are summed. By adding all individual fish health index values and dividing it by the total number of fish examined, the population HAI for a sampling site was calculated.

According to Goede and Barton (1990) when using an autopsy-based system such as the HAI, several assumptions are required:

i) when all organs and tissues appear normal according to the autopsy criteria, there is a good probability that the fish is normal;
ii) when fish are exposed to elevated levels of contaminants (fish under stress), tissue and organ function will change in order to maintain homeostasis;
iii) if a change in function persists in response to continuing stress, there will be a gross change in the structure of organs and tissues and
iv) if the appearance of an organ or tissue system departs from the normal or from a control condition, the fish is responding to changes brought about by the environmental stressor.

Table 4.1: The population Health Assessment Index values for *Oreochromis mossambicus* at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	65	83	99	57
Spring	94	71	92	95
Summer	66	73	127	109
Autumn	109	96	128	62

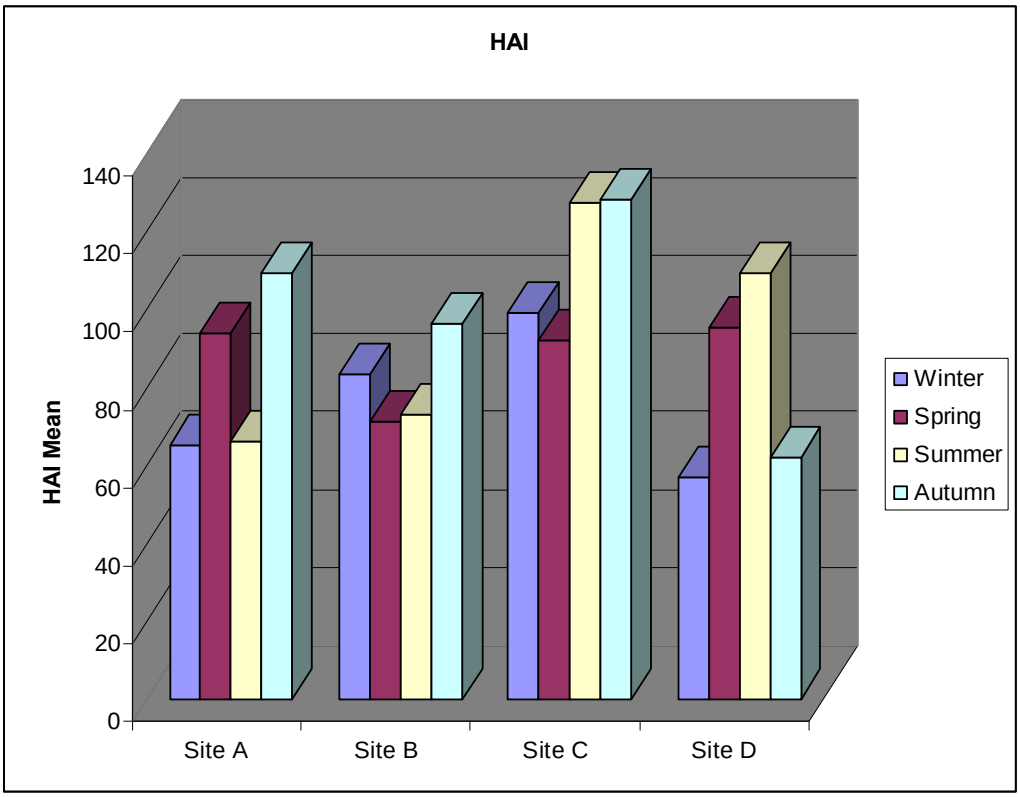

Figure 4.1: Seasonal population HAI recorded at the four sampling sites

The lowest population HAI values (indicating healthier fish populations) were not recorded constantly at a specific site but differed seasonally during this study (Table 4.1 and Figure 4.1). The lowest population HAI was recorded at site A amid Summer, at site B during Spring and at site D during Winter and Autumn (Table 4.1 and Figure 4.1). The population HAI score was the highest at site C for three surveys (Winter, Summer and Autumn). The high mean HAI values at sites A and C can mainly be attributed to liver discoloration and abnormal haematological parameters (Addendum B; Tables 1 ï 4). Comparing the population HAI between seasons at a specific site, the highest population HAI was scored during Autumn at three sites (sites A, B and C) while the highest population HAI was scored during Summer for site D. When comparing the HAI values with water quality results, one would expect lower HAI values at sites A and D (the sites with better water quality, see Chapter 3) and higher HAI values at sites B and C (the sites with poorer water quality). This was achieved for most of the surveys at site C where the highest

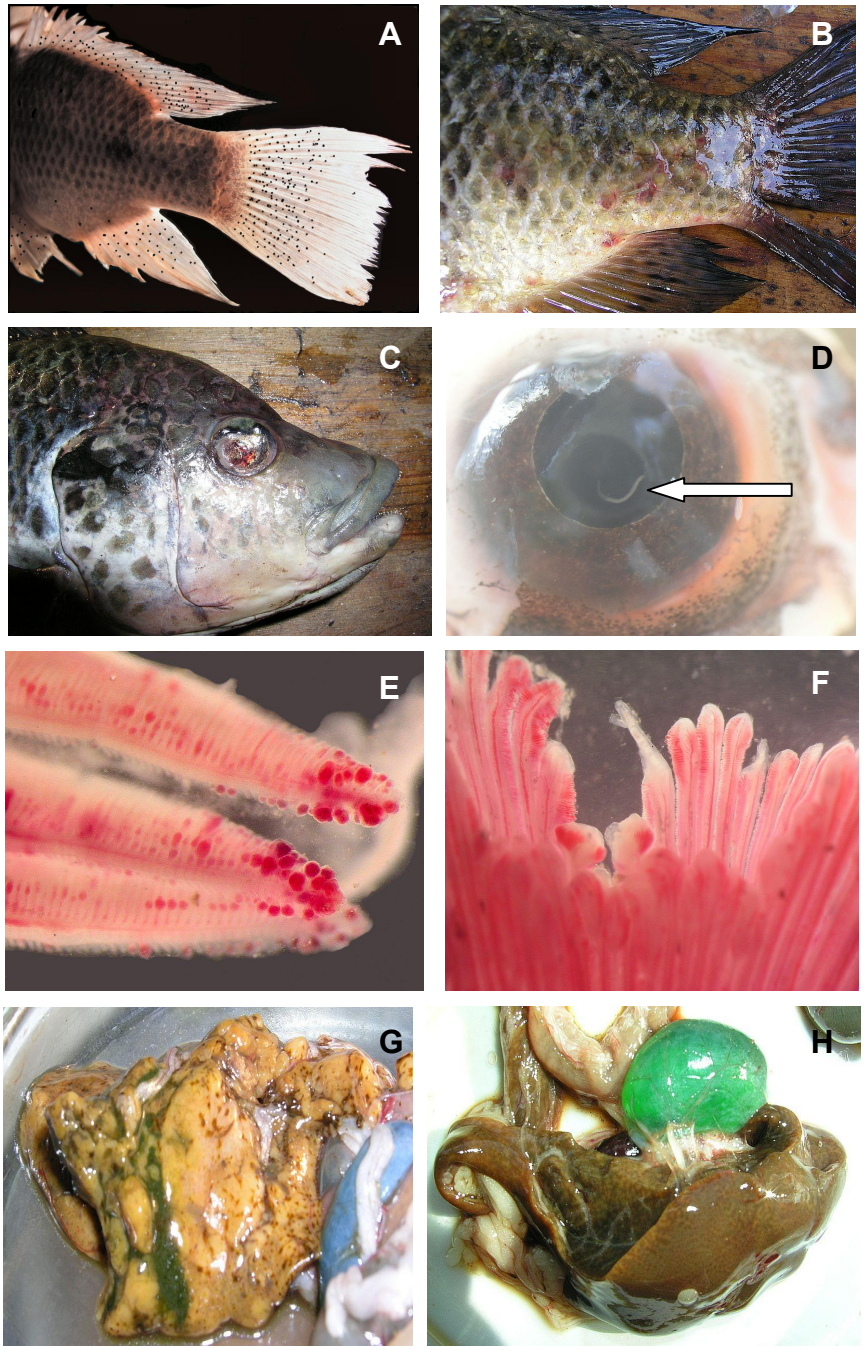

Figure 4.2: A-H. Abnormalities recorded from external surface and internal organs of *Oreochromis mossambicus*. **A**. Black cysts (caused by digenean larvae/trematode cysts) on body and fins. **B**. Lesions caused by *Lernaea cyprinacea.* **C**. Blind eye. **D**. Eye infected by digenean larva (arrowed). **E**. Bloody marks on gill filaments. **F**. Deformed gills. **G**. Focal discoloration of liver. **H**. Discolored liver with extended light green bile.

population HAI values were scored during three surveys. The lowest population HAI was scored at site D during two surveys. Site B had the lowest HAI score during Spring, but during the Spring survey the mean values of the different sites didn't differ considerably (Table 4.1).

4.1.2 External variables

4.1.2.1 Fins

Forked fins and abnormally long fins may be a result of genetic variation or they may originate from environmental influences. Inflamed and frayed fins might be due to fin rot, piscine tuberculosis, obstructed circulation (due to internal infection) or metabolic disturbances (Reichenbach-Klinke 1973). Water quality, parasites, bacterial infection and predation can also cause damaged fins (Ziskowski and Murchelano 1975; Austin and Austin 1999). Abbott and Dill (1985) reported no damage to the anal fins of steelhead trout (*Oncorhynchus mykiss*) and proposed that the anal fin was less susceptible to attack during aggressive encounters. Like the anal fin, the pelvic fins are located in an area that is less susceptible to attack by co-specifics. Many other factors have been implicated in causing fin erosion including overcrowding, water quality, temperature, feed type, malnutrition, bacterial infection, handling, and exposure to excessive sunlight and environmental contaminants (Pelis and McCormick 2003).

No forked or abnormally long or short fins were recorded at any sites for *O. mossambicus*, except once at site B when an eroded caudal fin was recorded from one specimen during Spring (Addendum B; Tables 1 – 4). During this study most of the fish exhibited black spots on the skin and fins (Figure 4.2A) caused by digenean larvae (metacercariae/trematode cysts). At site A, fin abnormalities due to trematode cysts were noted on five fish during survey one (Winter), one fish at site B, eight fish at site C and six fish at site D (Addendum B). During survey two (Spring) seven fish with abnormal fins due to trematode cysts were recorded from

sites A and B, five fish at site C and all the fish at site D were infested (although to a lesser degree, indicated by 10). During Summer (survey three) nine fish from site A, two fish from site B, all the fish at site C and five fish from site D exhibited trematode cysts on the fins. During Autumn (survey four) nine fish at site A, one fish at site B and seven fish at site C had trematode cysts on the fins. All the fish at site D had normal fins during the last survey. Thus, except from the trematode cysts recorded, the fins of fish were normal at most sites and surveys (Addendum B; Tables 1 ï 4).

4.1.2.2 Eyes

According to Fernald (1991) the eyes of fish are rounder than those of mammals because of the refractive index of water and focus is achieved by moving the lens in and out, not distorting it as in mammals. Teleost fish eyes grow throughout their life without compromising visual performance of the animal. This is made possible by a set of novel adaptations in the growth and development of the eye. Increased retinal area is achieved both by stretching the existing retina and by generation of new tissue at the retinal germinal zone at the margin of the eye (Fernald 2005).

According to Fernald (2005), rods are added in a fundamentally different fashion than are all other retinal cell types: they appear last as new retina is produced at the margin and they are inserted throughout the functional retina as it stretches. In this way, the animal maintains a constant rod density to preserve vision in low light level. Because the larger eye produces a larger image, visual acuity improves slightly as the animal grows. Blindness of long standings followed by atrophy of the optic lobes and those extensive lesions of the lobes are attended by either impairment or loss of vision (Fernald 2005). Seppälä *et al.* (2006) stated that cataracts may play a role in impairing the vision of fish and increasing their susceptibility to avian predation.

Eye flukes (metacercariae of larval digenean parasites) are known to induce cataracts due to metabolic excretions and mechanical destruction of the lens structures (Shariff *et al.* 1980) which can lead to much more acute effects (Southgate 2006). Several studies have reported white and opaque appearance of the eye lens associated with heavy infection of these parasites (Karvonen *et al.* 2004). According to Goede and Barton (1990) eyes are organs that indicate the well-being of fish in several ways.

Few eye abnormalities were noted during this study (Addendum B; Tables 1 ï 4). One fish with chronic inflammation of the lens capsule (Site A, Spring survey; Addendum B, Table 2) and three fish with blind eyes (Figure 4.2C) were recorded at site C during survey 3 (Summer survey, Addendum B, Table 3). The latter can most likely be attributed to parasitic infection but predation or mechanical injury cannot be excluded. Furthermore, parasites (digenean larvae) were recorded from the eyes during this study (Figure 4.2D) but when no obvious damage to the eye was observed, the eye was recorded as normal with a value of zero. The digenean larvae were however, recorded with the parasites and calculated with the Parasite Index (PI).

4.1.2.3 Gills

Fish gills are sensitive respiratory and ion regulatory membranes, which are directly and continuously exposed to environmental irritants (Playle 1998). The gills consist of horizontal flat filaments which are supported in the water stream by the bony gill arches. The secondary lamellae are found on the filaments which vary in frequency along a given filament from 10-60/mm with the higher numbers found in the more active species (Hughes 1995). Secondary lamellae are found on each primary lamella. The gills are multipurpose organs directly involved in a variety of functions including respiratory gas exchange, osmoregulation, acid-base balance and nitrogenous waste excretion (Heath and Heath 1995). A pseudobranch may also be found in the dorsal area immediately under the operculum of certain fish

species. It is made up of closely placed capillaries that resemble secondary lamellae. The function of this network of capillaries is thought to be supplying the retina and optic choroid with well oxygenated blood (Bowser 1999). A thin endothelial lined vascular channel lies between the pillar cells and function as the site of gas exchange, removal of nitrogenous waste and some electrolyte exchange (Roberts 2001).

The gills are very important due to their close direct contact with the external water and thus intimate ionic regulation and metal uptake occur primarily through the gills (Coetzee *et al.* 2002; Hubbard 2005). According to Mazon *et al.* (2002), the gills are the primary target organ for the toxic action of copper. The gills can act as a depot tissue, where the uptake of metals significantly exceeds the elimination thereof, leading to accumulation of certain metals (Hogstrand *et al.* 1994; Coetzee *et al.* 2002). Impacts of metals and acid on fish gill lamellae include a wide variety of changes including hyperplasia (increased number of cells) and hypertrophy (increased cell size measured as cell height and volume) of mucous cells, separation of the basilar membrane, and necrosis and fusion of secondary lamellae (Versteeg and Giesy 1986).

Most deformed gills (Figure 4.2F) were recorded from site C, i.e. two fish during survey one (Winter), four fish during survey two (Spring), none during survey three (Summer) and three fish during survey four (Autumn) (Addendum B; Tables 1 ï 4). Furthermore, one fish from site C had bloody marks on the gill lamellae (Figure 4.2E). Clubbed gills were recorded from one fish at site B during survey 2 (Spring) and one fish exhibited deformed gills during survey 3 (Summer). No gill abnormalities were observed at sites A and D throughout the sampling period. According to Versteeg and Giesy (1986) metals have an immense influence on the gills of fish and can cause a wide variety of changes in the gill lamellae leading to deformed gills. The deformed gills recorded from sites B and C can be ascribed to the higher metal concentrations recorded from these sites compared with sites A and D (see Chapter 3).

4.1.2.4 Opercula

The operculum of fish is the hard bony flap covering and protecting the gills. In most fish, the rear edge of the operculum roughly marks the division between the head and the body. The operculum is composed of four bones; the opercula, preopercula, interopercula and subopercula (Zapata *et al.* 1996). The morphology of this anatomical feature varies greatly between species. In some species, the operculum can push water from the buccal cavity through the gills and is vital in obtaining oxygen. It opens as the mouth closes, causing the pressure inside the fish to drop. Water then flows towards the lower pressure across the fish's gill lamellae allowing some oxygen to be absorbed from the water (Zapata *et al.* 1996).

According to Reichenbach-Klinke (1973) shortened and perforated opercula might be observed in fish as a result of calcium deficiency, environmental damage or predation or it might be of genetic origin. Furthermore, gill swelling may result in raised opercula (Reichenbach-Klinke 1973).

Of the 160 fish examined during this study none of the fish at any of the four sites exhibited abnormalities of the opercula and therefore a value of zero was recorded for all fish (Addendum B; Tables 1 ï 4).

4.1.2.5 Skin

The skin surface of fish differs from most of the higher vertebrates in that the epidermis is composed of non keratinized living cells (Roberts 2001). The outermost living cells of the skin are covered by a cuticle that is made up of mucus, mucopolysaccharides, immunoglobulin and free fatty acids. The epidermis is composed of stratified squamous cells and may be from 4 ï 20 cells thick. The dermis is immediately below the epidermis and the pigment cells (melanophores, xanthophores and iridophores). The scales are calcified plates that originate in the dermis and extend toward the exterior of the fish in an overlapping fashion and are

covered by the epidermis (Roberts 2001). The skin is extremely important for the ability of fish to maintain proper osmoregulatory function. The skin is directly exposed to contaminants and acts as the initial barrier to infections. It contains leukocytes and macrophages in addition to immunoglobulins (Bowser 1999). The skin tissue, together with the gill tissue, is characterized by a mucus layer on the outer surface. Bioaccumulation of metals in the edible muscle and skin tissue of fish is a major concern for fish consumers (Coetzee *et al.* 2002).

During this study, the skin of *O. mossambicus* displayed abnormalities in the form of trematode cysts and copepod lesions. No abnormalities for skin were recorded during surveys one (Winter), three (Summer) and four (Autumn) at all the sites (Addendum B; Tables 1 ï 4). Trematode cysts were recorded from the skin of one fish from site B and two from site C during survey two (Spring). Skin lesions were observed from fish (Figure 4.2B) infested with copepods (*Lernaea cyprinacea*) at sites A (Spring survey) and C (all surveys) (Addendum B; Tables 1 ï 4).

4.1.3 Internal variables

4.1.3.1 Bile

The bile is produced by the liver and stored in the gall bladder. The bile ducts pass through the hepatic lobules, collecting the gall or bile and passing it on to the gall bladder, which is a diverticulum of the common bile duct. When food enters the alimentary tract, bile is released through the bile duct, which opens into the gut just below the pyloric caeca (Udey and Shibagaki 2002). Bile serves a number of functions in that it (a) helps with emulsification and absorption of lipids/fats, (b) neutralizes hydrochloric acid from the stomach (and so prevents possible ulceration of the intestine) and (c) ensures that absorbed toxins are returned to the intestine for excretion. The size and fullness of the gall bladder is indicative of the feeding status of the fish. A large, distended bladder indicates that the fish has not eaten for some time whilst an empty flaccid bladder indicates that the fish has

recently eaten a meal (Love 1980 as quoted by Bertasso 2004). According to Wilson *et al.* (1998) yellow bile indicates the fish has fed recently whereas dark coloured bile indicates the fish last fed up to 5 days ago. Fish which have been caught in nets may not feed for whatever length of time they remain in the net which may affect the colour of the bile and fullness of the gall bladder.

Due to the colour variation of the bile as mentioned previously, it is complicated to distinguish between normal and abnormal conditions during the HAI. This variable was not included in HAI calculation proposed by Adams *et al.* (1993) and also not included in this study. The bile of the fish during this study was constantly almost full and dark green to light green in colour (Figure 4.2H).

4.1.3.2 Liver

The fish liver appears, as does the liver of other vertebrates, as a key organ which controls many life functions and plays a prominent role in fish physiology, both in anabolism and catabolism (including detoxification) (Gonzàlezi *et al.* 1993). The liver is a detoxification organ and essential for both the metabolism and excretion of toxic substances. Exposure to toxicants may cause histological changes in the liver, which in turn could be used as a biomarker to indicate prior exposure (Hinton and Laurén 1990). The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds, and could subsequently result in structural damage. Similar studies on various fish species, exposed to different toxicants, showed histopathological changes in the livers of the fish (Van Dyk 2003). The normal fish liver condition is generally considered to be solid red to light red in colour but is dependent upon both the species and the reproductive state of fish. Abnormal manifestations of the liver include: fatty and light tan in colour; liver nodules; focal discolouration and general discolouration (Goede 1992).

The liver of some fish from the four sites demonstrated discolouration during this study. Discolouration (Figures 4.2G and H) and black marks on the liver were frequently observed. The abnormal colour for liver observed in *O. mossambicus* was mostly a dark green liver (Figure 4.2H). During survey one (Winter), only one fish from site A had an abnormal liver while, at site B four fish had abnormal livers, at sites C and D, eight and one fish exhibited abnormal livers respectively (Addendum B; Tables 1 ï 4). During survey two (Spring) five fish with liver discolouration were recorded at site A, four fish from site B, and eight fish from site D while normal livers were recorded from site C. During survey three (Summer) three fish from sites A and B displayed abnormal livers and six fish at site C and nine fish at site D exhibited focal and general discolouration of livers. During survey four (Autumn) all fish from all four sites displayed abnormal livers, except for one fish from site A and one fish from site B (Addendum B; Tables 1 ï 4). Liver conditions contributed a great deal to the HAI score during this study.

The liver is the organ of detoxification and biotransformations, thus metabolic function of the body would be immediately disturbed under the influence of any toxic substance leading to abnormalities (Jafri and Shaikh 1998). Many cysts or nodules in/on the liver were also observed (which was not always parasitic however), as well as parasites, e.g. cestode larvae attached to the outer surface of the liver (see Figure 5.3A). These cestode larvae were recorded and calculated in the Parasite Index (PI).

4.1.3.3 Kidneys

The kidneys of fish are retroperitoneal (without a mesentery and associated with posterior body wall), as in mammals. The gross anatomy of the kidneys varies between different species from distinctly bilobed cranial and caudal kidneys, to kidneys that are fused and intimately embedded between the vertebrae (Ogawa 1962). The cranial or ñheadò kidney contains hematopoietic, lymphoid, and endocrine tissue. The caudal kidney is composed of nephrons surrounded by

hematopoietic and lymphoid tissue dispersed throughout the organ. Species variations in tubular segmentation also exist (Sakai 1985; Hentschel and Elger 1988). These variations are most evident when comparing freshwater and marine species, which is not surprising because their environments make different demands on their kidneys (Hickman and Trump 1969). In a freshwater fish the kidney conserves ions and excretes water. In a saltwater fish the kidney conserves water and excretes ions. According to Bowser (1999) abnormalities of kidney might include nodules, granular appearance, or the presence of parasites.

None of the mentioned abnormalities were observed during this study and the kidneys of all fish were normal at all sites (Addendum B; Tables 1 ï 4).

4.1.3.4 Hindgut

From the stomach the intestine comprises of the mid and hindgut which runs to the vent/anus (Sivadas 2005). The hindgut is the final site of digestion and absorption prior to defecation of waste products. In carnivorous fish the intestine is relatively short whilst that of herbivorous fish, which tend to lack a stomach, is long and much folded to increase the contact and absorption time. Several functions of the intestine include increasing the surface area for food absorption, specific site of carbohydrates and fat absorption and adding to the digestive functions of the stomach (Sivadas 2005). Redness and inflammation of the hindgut can be caused by parasites (Jooste *et al.* 2004), faulty feeding and poisoning.

During this study redness and moderate inflammation of the hindgut were noted from some fish during one survey at two sites (Addendum B; Tables 1 ï 4). One fish per site (sites B and C) exhibited slight inflammation of the hindgut during the Spring survey (Addendum B; Tables 1 ï 4). This redness of the hindgut could be ascribed to infection with dilepidid cestode larvae (Figure 5.2F). Many cestode larvae (encysted) were attached to the outer lining of the intestine. These cestode larvae were recorded and calculated in the Parasite Index (PI).

4.1.3.5 Haematological parameters

According to Jawad *et al*. (2004) hematological parameters have been recognized as valuable tools for the monitoring of fish health and in helping biologists interpret physiological responses to environmental stress. Blood parameters such as haematocrit, haemoglobin concentration and red blood cell (RBC) counts are related to environmental factors such as water temperature and salinity (Jawad *et al*. 2004). **Haematocrit values** reflect the percentage red blood cells to total blood volume (Schuett *et al.* 1997). It is assumed that elevated levels of haematocrit may represent a population under stress while low levels indicate the presence of disease (Goede and Barton 1990). According to Jawad *et al*. (2004) the haematocrit value will vary, depending on the health and physiological condition of the individual fish.

Ectoparasitic crustaceans can increase or reduce haematocrit values of freshwater and marine fish depending on the severity of infection (Bowers *et al.* 2000; Jones and Grutter 2005). If parasites simply act as a stressor, haematocrit can increase by splenic release of stored blood cells, by plasma loss or by erythrocytic swelling (Fange 1992). Sufficiently large or numerous ectoparasites can reduce haematocrit through blood feeding in marine fish (Horton and Okamura 2003; Wagner and McKinley 2004) or via osmoregulatory failure caused by exposed lesions (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997). Gallaugher *et al.* (1995) stated that in rainbow trout (*Oncorhynchus mykiss*) haematocrit values below 22% are deemed anaemic.

The haematocrit ranges and numerical values used for *O. mossambicus* are presented in Table 2.1 and adopted from Adams *et al*. (1993) and Jooste *et al*. (2004).

Table 4.2: Haematocrit values in % for *O. mossambicus* at the four sampling sites

Surveys		Site A	Site B	Site C	Site D
Winter	Mean	28.1	17.9	31.7	26.8
	Min - Max	24 - 38	14 - 30	21 - 44	19 ï 35
Spring	Mean	26.4	29.3	25.8	26.2
	Min - Max	13 - 39	19 - 39	21 - 42	19 ï 35
Summer	Mean	20.7	19.1	13.4	21.7
	Min - Max	12 - 41	11 - 46	11 ï 46	12 - 34
Autumn	Mean	32.7	27.6	20.3	36.1
	Min - Max	11 - 40	10 - 42	12 - 28	28 - 45

Normal haematocrit values range from 30 ï 45%, >45% is above normal, 19 ï 29% and <18% is below normal range (see Table 2.1). The lowest haematocrit value recorded for *O. mossambicus* was 10% at site B during survey four (Table 4.2) which is far below the normal range indicated anaemic fish. Higher haematocrit values were recorded during Autumn at site D with an average of 36.1% (Table 4.2). The haematocrit ranged from 10% to 46% throughout the four sampling periods. No blood could be drawn from some fish at sites A and B due to their small size or they were dead for some time before analysis. During this study, most haematocrit values were below the normal range (30 ï 45%) and received a numerical value of 20 (Addendum B; Tables 1 ï 4). According to Goede and Barton (1990) low haematocrit levels indicate the presence of disease in a population and can also be due to parasitic infestations.

The size, sex and state of maturity of fish may influence the **total plasma protein** levels recorded from fish (Douellou and Guillaume 1986). It may furthermore be influenced by environmental factors such as temperature or food availability (Douellou and Guillaume 1986). Low concentrations of plasma protein influence the colloid osmotic pressure in fish and are indicative of haemodilution (Wedemeyer and Yasutake 1977).

Most of the total blood plasma protein levels recorded during this study were below the normal range and received a numerical value of 30 (Addendum B; Tables 1 ï 4). Elevated levels were recorded during Spring at all sites (Table 4.3). Lower plasma protein levels were recorded at site A compared to the other sites during surveys one (Winter) and four (Atumn). According to Wedemeyer and Yasutake (1977) low plasma protein levels are indicative of haemodilution caused by infectious diseases, starvation, depletion of energy stores, or an impaired water balance.

Table 4.3: Plasma protein values (mg/100 ml) of *O. mossambicus* at the four sampling sites

Surveys		Site A	Site B	Site C	Site D
Winter	Mean	3.3	6.77	3.87	3.95
	Min - Max	1.91 - 4.5	1.75 - 14.7	2.16 ï 6.66	2.04 - 8.02
Spring	Mean	30.73	26.6	38.64	49.47
	Min - Max	1.4 ï 151.6	1.72 ï 129.2	2.9 ï 115.5	4.35 - 100
Summer	Mean	15.63	3.88	3.78	4.54
	Min - Max	2.56 - 44.5	1.57 - 7.4	2.38 - 5.93	1.9 - 6.47
Autumn	Mean	2.67	3.09	8.5	4.39
	Min - Max	1.04 ï 5.37	0.98 - 6.33	2.12 - 26.5	1.14 - 10.15

Erythrocytes contribute the largest percentage of blood cells in a circulatory smear of fish. Leucocytes or **white blood cells** (WBC) play a major role in the immune response and defense mechanisms of fish (Jurd 1985). An increase in the number of WBC is due to the fishôs reaction against foreign substances which can alter their normal homeostasis. WBC with a range of <4% were defined as normal (with a numerical value of 0) and >4% fell outside the normal range, indicated by a numerical value of 30 (Adams *et al*. 1993). White blood cell counts below the normal range were recorded for most fish from sites A, B and C, while most fish from site D had a normal WBC count (Addendum B; Tables 1 ï 4).

Many factors can influence the haematological results obtained from fish in such a way that it is difficult to interpret the results accurately (Van Vuren and Hattingh 1978) and therefore it is essential to obtain samples representing the true blood physiological status of the experimental animals. Therefore, when using haematological measurements in the HAI, the techniques must be standardized and stress factors must be minimized in order to reduce possible variation in the blood values.

4.1.3.6 Mesenteric fat

There is large variation among fish species in the way they store fat (Rutaisire and Booth 2005). In trout, the fish health profile rankings are based on the amount of fat deposited around the prominent pyloric caeca. Mesenteric fat is stored along the stomach and intestine. The categories for these species are based on the relative amounts of fat in the body cavity, and require some familiarity with what is usually encountered. Fish rarely store large amounts of mesenteric fat except as they approach sexual maturity and migration to spawning places (Rutaisire and Booth 2005).

Goede and Barton (1990) reported that mesenteric fat of fish reflects the intensity of feeding and energy deposition over the long term. This variable also gives an idea of the stress experienced by fish although it is not directly related to stress. Mesenteric fat can vary between seasons, sex of the specific species and aquatic systems (Goede and Barton 1990). According to Sinnhuber (1969) stored lipids are burned for fuel to enable body processes to continue during strenuous journeys (e.g. during fish spawning).

Due to the above-mentioned conditions Adams *et al.* (1993) did not assign values to this variable and therefore it was not included in HAI calculation during this study. More fat was noticed in the females during Spring followed by Winter,

Summer and Autumn during this study. This may be attributed to the seasonally preparation for reproduction.

4.1.3.7 Spleen

The spleen is the only lymph node-like organ to be found in the teleost fish (Fox 2000). It is situated near the greater curvature of the stomach or the flexure of the intestine (Roberts 2001). According to Goede (1992), black, red and granular spleens are all considered to be normal manifestations of spleen condition. Other manifestations of spleen condition such as enlarged tissue, nodules, and grey mottling can be due to constant contaminant exposure. Black *et al.* (1982) reported that the fish exposed to mining waste containing copper, lead and zinc are commonly affected with perivisceral masses resembling mesotheliomas that are usually associated with the mesenteric capsule of the spleen, but sometimes appear to be attached to the mesenteric fat of fish.

No abnormalities of the spleen were observed at any of the four sampled sites during this study (Addendum B; Tables 1 ï 4).

4.2 Condition Factor

The condition factor (CF) of fish, based on the analysis of length-weight data, indicates the health of fish in a habitat. Drops in condition factor values may indicate the reproductive period and/or changes in the foraging habits of certain species (Gomiero and Braga 2005). Barnham and Baxter (1998) stated that the value of the condition factor is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. With females, the condition factor value will decrease rapidly when the eggs are shed.

The condition factor of fish is classified as ideal when a value of one is recorded. This value is, however, dependent on the fish species, due to the variation in the length/weight ratio of different species. This is the reason why the condition factors of different species can not be compared. According to Barnham and Baxter (1998) a CF value of 0.80 indicates an extremely poor fish, resembling a barracuda (big head and narrow, thin body) while a value of 1.20 indicates a fair fish, acceptable to many anglers. Condition factors are believed to be good indicators of the general well-being or fitness of fish populations (Bolger and Connolly 1989) and are often used as indicators of pollutant exposure and the effect thereof (Kloepper-Sams *et al*. 1994).

Table 4.4: Condition factor of *O. mossambicus* at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	1.002	1.099	1.003	1.002
Spring	1.002	1.101	1.031	1.101
Summer	1.038	1.079	1.018	0.918
Autumn	1.022	1.055	1.018	1.210
Mean	1.017	1.083	1.017	1.058

The highest mean value was recorded at site B followed by site D (Table 4.4). The highest value (1.2) was recorded at site D during Autumn (survey four) and the lowest value (0.92) at site D in Summer (survey three) (Table 4.4 and Figure 4.3). A decline in the condition factor was observed during winter for *O. mossambicus*, probably indicating a change in feeding activity. The condition factor results were not very indicative in showing the difference between the four sampling localities during this study (Table 4.4 and Figure 4.3).

In highly polluted water the condition factor may show positive results, having an effect on the fishôs growth and reproduction. But in less severe polluted

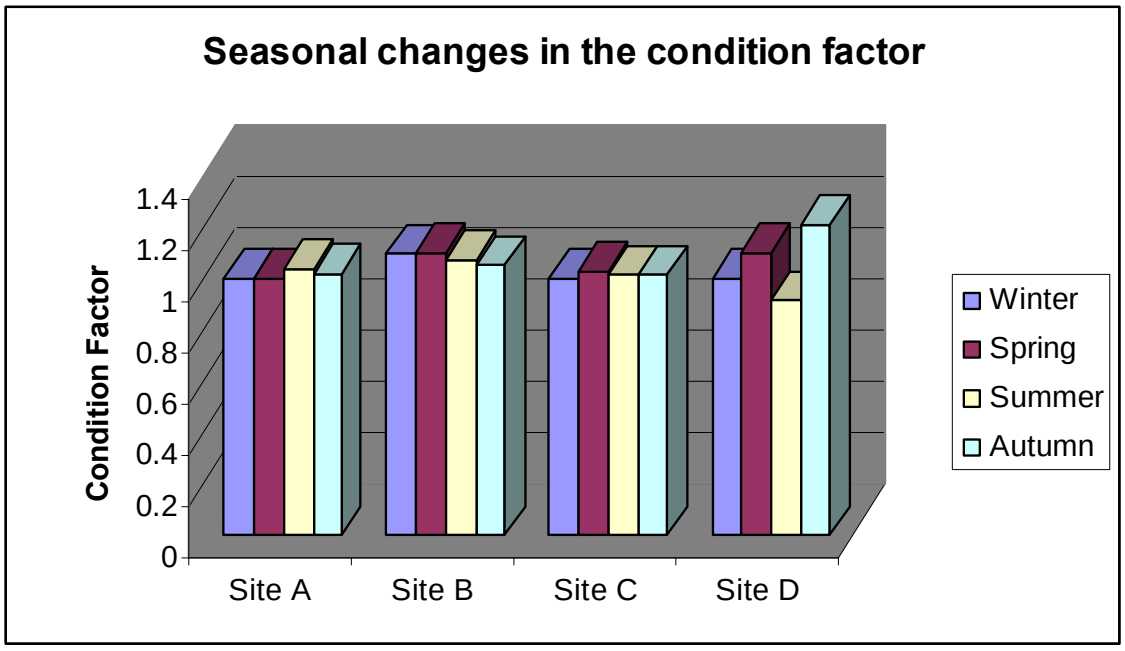

Figure 4.3: Seasonal changes in the condition factor of *O. mossambicus* at the four sampling sites

ecosystems, fish are not influenced to such an extent that the condition factor will change significantly. According to Lizama and Ambrósio (2002) higher condition factor results suggest that the species started their reproductive period. The condition factor was one or slightly above one at all sites during this study, indicating that fish was in a fairly good condition (Table 4.4 and Figure 4.3).

4.3 Parasite Index

According to Avenant-Oldewage (1998) the Parasite Index (PI) is a useful biomonitoring tool and provides a reliable indication of water quality. Since ectoparasites are constantly in contact with the external environment, they tend to reduce in diversity and abundance when water is heavily polluted and higher numbers of ectoparasites should be expected in good quality water (Avenant-Oldewage 1998; Luus-Powell *et al*. 2005). The number of endoparasites, on the

other hand, tends to increase in polluted water. This might be due to a lowered immune system and resistance of the fish due to continuous stress conditions.

Aquatic pollution may also have a direct effect on some stages in the development of the parasite or it may influence parasites indirectly, e.g. the composition of the ichthyo-fauna (definite host population), as well as the intermediate hosts (invertebrate fauna) (Möller 1987). Under certain conditions, pollution favours the survival of some groups of parasites by reducing the physiological and therefore immunological resistance of their hosts to stress (making the host more susceptible to toxic effects), or by providing favourable conditions for the enumeration of their intermediate hosts (Möller 1987; Khan and Thulin 1991).

In the original HAI, developed by Adams *et al*. (1993), parasites were recorded as ñmo observed parasitesò and ñfew observed parasitesò But during testing of the HAI in South Africa, parasites were incorporated as separate variables in the HAI because contaminants influence ecto- and endoparasites differently (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001). The number of parasites involved in the infection may also influence the condition of the fish and a further refinement of ecto- and endoparasites was done during the above mentioned studies. Furthermore, endoparasites are usually much higher in number than ectoparasites and up to 1000 trematode cysts or nematode larvae can be observed in a single host. Therefore it was decided to categorized endo- and ectoparasites as presented in Table 2.1 (Chapter 2).

The only ectoparasites recorded for *Oreochromis mossambicus* were monogeneans from the gills and branchiurans and copepods from the skin (see Chapter 5). A larger number of endoparasites were recorded, i.e. digenetic trematodes from the eyes, gills and swim-bladder and ñblack spotò (trematode cysts) from the skin and fins (as mentioned in the HAI section), cestode larvae from the liver and outer lining of the intestine, nematode larvae from the body cavity and heart and pentastomid larvae from the swim-bladder (infective stage).

Table 4.5: The Parasite Index (PI) of the four sampling sites

	Site A		Site B		Site C		Site D	
Surveys	**Ecto**	**Endo**	**Ecto**	**Endo**	**Ecto**	**Endo**	**Ecto**	**Endo**
Winter	8	11	5	10	14	17	3	11
Spring	8	13	4	11	21	13	7	10
Summer	0	12	0	11	2	17	0	10
Autumn	9	12	3	10	9	17	4	8

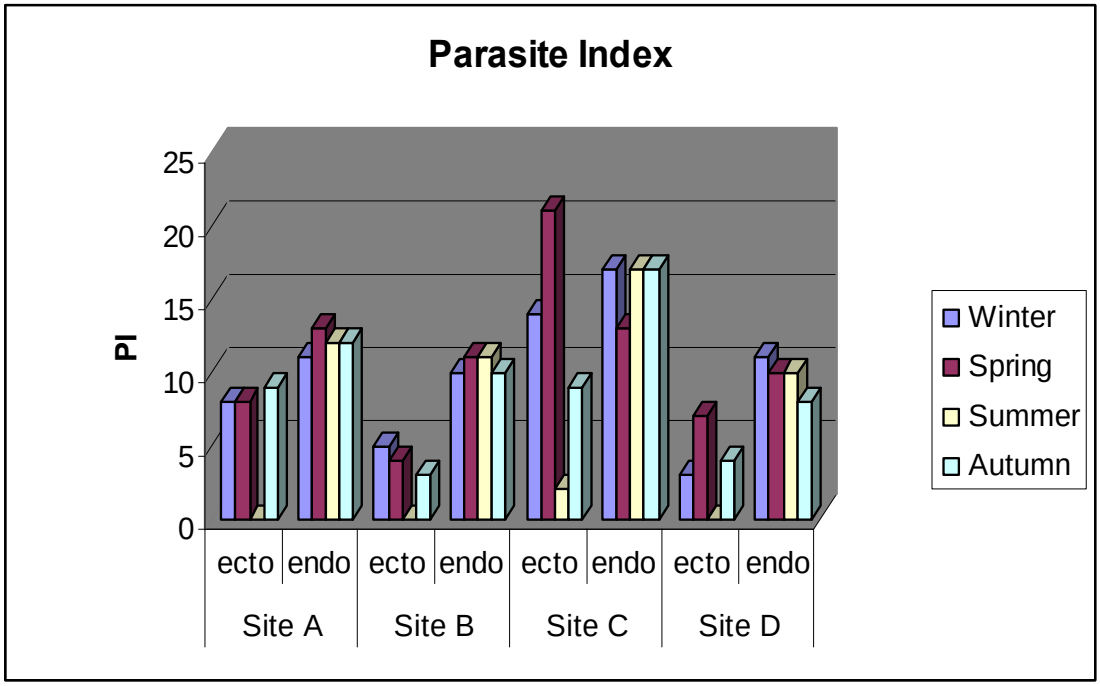

Figure 4.4: Seasonal Parasite Index (PI) of *Oreochromis mossambicus* at the four sampling sites

The minimum PI for endoparasites was eight recorded at site D in Autumn and the maximum was 17 recorded at site C during Winter, Spring and Autumn (Table 4.5 and Figure 4.4). Generally site C had the highest PI for endoparasites, followed by site A, B and D sequentially (Table 4.5 and Figure 4.4). The PI for endoparasites was always higher than the PI for ectoparasites at all sites except at site C during the Spring survey.

The lowest PI for ectoparasites was generally recorded from sites B and D while the highest PI for ectoparasites was at site C during Winter, Spring and Autumn. A

value of zero was recorded for the PI for ectoparasites at three sites during Summer (Table 4.5 and Figure 4.4). Generally, the PI for both ectoparasites and endoparasites at site C were higher than the other three sites (Table 4.5 and Figure 4.4). The PI for ectoparasites and endoparasites differed considerably at all sites during the four sampling surveys.

According to Hoffman (1958), the development of most trematodes is more rapid in warm than in cold water, and one would expect trematode infections to be higher in hard warm water (where snails are present). Many strigid trematode cysts (ñblack spotò) were recorded for *O. mossambicus* from all sites but with a very high abundance at site C and lower at site D (see Chapter 5, Table 5.1).

The presence of large numbers of ectoparasites in the contaminated sites is difficult to explain. The complexity of the pollution-parasite-host system makes the use of parasites as individual indicators of pollution effects difficult (Möller 1987). The ectoparasites recorded during this study included monogeneans, branchiurans and copepods. The branchiuran ectoparasite, *Argulus japonicus* is not endemic and this parasite is opportunistic and well established on a variety of fish species in our aquatic ecosystems. Only two *A. japonicus* specimens were encountered during this study and the number of these ectoparasites didnôt have a vast influence on the PI.

Larger numbers of monogeneans were generally recorded from the sites with better quality water (sites A and D, see chapter 5). Monogeneans are very sensitive to high salinity levels (Möller 1987) which may explain the lower numbers recorded at the mine sites. The number of monogeneans recorded during this study did not influence the PI to the same extent than the copepods.

The high abundance of copepods (*Lernaea cyprinacea*) at site C (see Chapter 5, Figure 5.5) contributed to the high ectoparasite PI recorded at this site. These findings were unpredicted as the number of ectoparasites is suspected to be lower

at sites with a poorer water quality. According to the water quality results (Chapter 3) site C had higher levels of certain water constituents and was overall in a poorer state compared to sites A and D. This variability may be attributed to the differential susceptibility of the parasites to the toxicity of different pollutants, their concentration, the exposure time and possible synergistic effects (Marcogliese 2005). According to Khan and Thulin (1991), contaminants have different influences on parasites and this might explain the difference in composition of ecto- and endoparasites at the different sampling locations.

Conclusion

Health Assessment Index

The HAI is a simple but systematic approach to assess the possible effects of stress on the health and condition of fish in a population. But, every aquatic ecosystem differs in terms of water quality, fish species, etc. Therefore the HAI will test differently in dissimilar aquatic ecosystem, depending primarily on the fish species used and types of stressors (Jooste *et al*. 2004). Nevertheless, this method can be used to provide biologists with useful information about their environment with minimal effort and expense.

The results obtained for the HAI during this study indicated that the fish population from site C was more affected (with a higher HAI) compared to the other fish populations. A higher population HAI was recorded at site C during three surveys (Winter, Summer and Autumn). When comparing the HAI values with water quality results, one would expect lower HAI values at sites A and D (the sites with better water quality) and higher HAI values at sites B and C (the sites with poorer water quality). This was achieved for most of the surveys at site C, as mentioned above, where the highest population HAI values were scored during three surveys. The lowest population HAI was scored at site D during two surveys (Winter and Autumn) and site A during Summer. Surprisingly, site B had the lowest HAI score

during Spring. However, during the Spring survey the mean values of the different sites didn't differ considerably (Table 4.1).

Schmitt *et al.* (2005) assumed that population HAI values ≤20 indicated un-impacted or minimally impacted sites, values >50 indicated intermediate sites and values >70 indicated heavily impacted sites. Therefore, taking the above values into consideration, all sites indicated to be intermediate to heavily impacted sites during this study.

Liver discolourations, eye abnormalities including whiteness of the cornea and lens (possibly indicating parasitic infestation) or missing and blind eyes, deformed gills, parasite infestation, hindgut infection due to parasite infestation and blood parameter values contributed to elevated HAI values in most of the cases. No abnormalities of kidneys, opercules and spleens were observed at any of the four sampling sites. Fin deformities, such as abnormal shapes or missing fins were not observed during this study, except once during Spring (eroded caudal fin) although scores were recorded for fins due to parasitic infections.

The abnormalities recorded from the fish at sites B and C may be related to stress due to poorer water quality because of mining activities and geological formations. With the exception of the liver condition and certain blood parameters of a number of fish, the health of fish was not significantly impaired at any of the sites during this study. All the fish appear to be in good physical form with no obvious health related problems although fish from site C showed more liver abnormalities than the other sites.

The HAI can not be used for the identification of compounds to which the fish response in the same way as physical-chemical techniques. The latter can also detect much lower concentrations of chemicals in freshwater aquatic ecosystems. Although the accuracy and reliability of the chemical analysis of water/sediment/tissue is very high, these methods are very expensive and require

extensive training and sophisticated equipment. Therefore, the more reliable and accurate the method, the more training is required with an accompanying increase in cost (expenses) and complexity of the method. But although the HAI does not require costly and sophisticated equipment, researchers must have a basic biological background and knowledge as well as some field experience (Heath *et al*. 2004).

Further limitations of the HAI are the subjectivity of this method. Individual researchers may interpret observations differently and this may influence the end results of the HAI. Numerical values given to variables are exact values, i.e. 0, 10, 20, and 30, and there is for instance a considerable difference between 10 and 20 while the physiological difference/state of disease might not be that significant. Huge leaps between values make a considerable difference to the end value of the HAI.

The HAI is not designed to be a substitute for the other monitoring methods, to be diagnostic or to solve specific problems related to fish health or environmental conditions. The primary objective of this method is not to understand the reasons for a change in population health, but to document in a relatively rapid and inexpensive manner the occurrence of a change in a fish population (Heath *et al*. 2004). More specific and intensive investigations may then be designed to determine if there are particular stressors in the environment that can be associated with the observed responses.

It is important to recognise that biological data do not replace chemical and physical data, any more than chemical data may adequately replace biological data. They provide converging lines of evidence that supplement each other but are not mutually exclusive. The ideal situation would, therefore, be one in which the HAI and physical-chemical techniques are applied in close association, using the former for general wide-spectrum screening purposes and the latter for the qualitative and quantitative analysis of potentially hazardous compounds detected

by methods such as the HAI. Continued biological monitoring and biological criteria provide a direct and effective way to determine if actions taken result in improvements in the water resource system.

Parasite Index

Ectoparasites are constantly in contact with the external environment and some tend to be more susceptible to a number of contaminants than certain fish species. Ectoparasites tend to reduce in diversity and abundance when water is heavily polluted and higher numbers of ectoparasites should be expected in good quality water (Avenant-Oldewage 1998). The number of endoparasites, on the other hand, tends to increase in polluted water. This might be due to a lowered immune system and resistance of the fish due to continuous stress conditions.

The hypothesis that the number of ectoparasites will be lower in more polluted water and the number of endoparasites will be higher was supported for O. *mossambicus* at all sites except at site C during Spring. The PI for endoparasites was higher at the mine sites but also at sites A and D (the less impacted sites). However, all sites used during this study were impacted to a lesser or higher degree and the number of endoparasites can thus be higher at all sites compared with the number of ectoparasites. It was however, expected that the number of endoparasites should have been higher at the mine sites (the more polluted sites) which was not the case during all surveys. Furthermore, some ectoparasites (especially the copepod, *Lernaea cyprinacea*) were present in high numbers at the more polluted site (site C), complicating the PI further. Thus it seems that the different groups of parasites, as well as certain specific parasites, differ in their response towards different water constituents. The latter can be best tested under controlled laboratory conditions and experimental work. But, the use of parasites in the HAI, or as indicators of pollution, has potential and requires much more attention and research.

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Chapter 5
Fish Parasites

Introduction

Biological communities are sensitive indicators of the relative health of their aquatic ecosystems and the surrounding catchments (Fausch *et al.* 1990; McCormick *et al*. 2000). The relationship between the biological, physical and chemical components of aquatic ecosystems is the basis of biological monitoring (Joy and Death 2002). Fish are potentially effective biological indicators of the condition of aquatic ecosystems because different species exhibit diverse ecological, morphological and behavioral adaptations to their natural habitat (Karr *et al.* 1986; Fausch *et al.* 1990; McCormick *et al.* 2000). Fish communities integrate the ecological processes of streams across both temporal and spatial scales (Hynes 1970; Harris 1995) and they can be useful indicators of aquatic degradation (Karr 1981; Berkman *et al.* 1986; Steedman 1988; Fausch *et al.* 1990; Karr 1991). Furthermore, because fish are a visible part of stream biological integrity they represent a measure of stream quality which is easily and intuitively understood by the public (McCormick *et al.* 2000). The parasites of fish form an integral part of their biology and reflect the life habits of the fish, including their interactions with the benthic, planktonic and other fish communities (Landsberg *et al.* 1998).

Ecosystems with a high degree of biotic integrity (healthy ecosystems) are composed of balanced populations of indigenous organisms with diverse structural and functional organizations. Healthy ecosystems have a complex trophic structure with many species forming the food web. In the aquatic ecosystem fish are at or near the top of that food web and their parasites are also an indigenous component of the food web (Landsberg *et al.* 1998).

Parasites of fish may rank among the most sensitive of bioindicators because parasitic infections of fish reflect the health of the entire aquatic community

(MacKenzie *et al.* 1995; Marcogliese and Price 1997). Most fish parasites have complex life cycles, e.g. a typical parasitic life cycle may include the fish as definitive host with one or more intermediate invertebrate hosts. For the parasite to survive, all hosts must co-occur in a stable community structure (Marcogliese and Cone 1997). Furthermore most parasites are specific to a host species or to a group of closely related hosts (Marcogliese and Price 1997). How specific a parasite is to its host can depend on the type of parasite and/or on the stage in its life cycle. Trematodes, for example, complete their development in at least two hosts, with the first intermediate host usually a mollusc (Roberts and Janovy 2000).

Each host is essential in the completion of the parasiteôs life cycle and if a parasite establishes itself in the wrong host, it often dies or can cause serious problems for that host. For this reason, parasites have been used for almost a century as biological indicators, tags, or markers to provide information on various aspects of host biology including fish stock separation, fish recruitment migrations, fish diet and feeding behaviour and host phylogenetics and systematics (Williams *et al.* 1992). Good biological indicators are sensitive to environmental alterations so that changes in their numbers can be used as a warning of deteriorating conditions before the majority of less sensitive organisms are seriously affected.

Parasitic infections are associated with a wide range of effects in fish, many of which manifest themselves as changes in behaviour. Frequently these behavioural changes in the host may be due to adaptations of the parasite, i.e. they appear to have evolved to facilitate transmission of the infection between hosts. Energy utilized by the parasites forces the host to intensify their search for food, resulting in decreased fitness and an increase risk of predation by entering the food chain of the definitive host (Bartoli and Boudouresque 2007). Furthermore, parasitic infections may influence the reproductive behaviour of the host by invading the gonads (Bartoli and Boudouresque 2007) or by altering attractiveness to potential mates (Barber *et al.* 2000).

According to Vidya and Sukumar (2002) potential factors determining the transmission of parasites include environmental conditions (affect the viability and behaviour of parasites) and feeding, movement, and defecation patterns of the host (determine the parasites encountered). The variation of parasitic loads may also be influenced by the hostôs lifespan, mobility, and physical/chemical stimuli. Site specificity, at least in some parasitic species, may be due wholly or partly to physiological factors. In some cases, morphological adaptations of parasites are at least partly responsible for site preference (Rohde 1993).

Parasites of fish could therefore be more sensitive indicators of environmental stressors than fish themselves. The adverse environmental conditions may decrease the ability of organisms to maintain an effective immunological response system. Poor water quality can cause a reduction in the level of unspecific immunity to disease (Svobodová *et al.* 1993; Sures 2006). As such, if the immune system of the fish is suppressed then the opportunistic diseases and parasitic infections may accumulate (Sures 2007). This is also supported by Möller (1987) who stated that fish in polluted waters tend to harbour more endoparasites than those from less polluted environments. In addition, high parasitic loads can be fatal to the host (Poulin 2000).

Different groups of parasites respond differently to various types of pollutants. Monoxenous parasites (direct life cycle) appear to be better adapted for survival in some polluted habitats (Dzikowski *et al.* 2003). Khan (1990) reported an increased rate of infection in Monogenea associated with oil pollution. It has been suggested that at chronic, sub-lethal levels of exposure, fish skin and gills secrete abundant mucus, which in turn may act to enhance monogenean and trichodinid rates of infection (MacKenzie *et al*. 1995). According to Marcogliese and Cone (1997) the apparent inconsistency of their findings with above mentioned reports may perhaps be explained by the variable susceptibility of parasite species, length of exposure time, and concentrations and the nature of the pollutant.

Oil, heavy metals and anaerobic conditions have been found to be toxic to adult trematodes inside their fish host (Overstreet 1988; Khan and Thulin 1991; Valtonen *et al*. 1997) and lethal to free-living stages (e.g. cercariae and miracidia) as well as to the mollusc intermediate hosts (Evans 1982; Siddall *et al.* 1993). Furthermore, parasite species that are directly or indirectly sensitive to pollution may disappear as the pollution levels increase, and may thus be considered good indicators for early detection of adverse environmental effects (Dzikowski *et al.* 2003).

Results and Discussion

The following parasites were recorded from *Oreochromis mossambicus* during the four sampling surveys: the **ectoparasites** included monogeneans (*Cichlidogyrus* sp. from the gills, Figure 5.1A); copepods (*Lernaea cyprinacea* from the skin, Figures 4.2B and 5.1B); and branchiurans (*Argulus japonicus* from the skin, Figure 5.1C). Each group will be discussed separately.

The **endoparasites** included several different digenean metacercariae: ñblack spotò from the skin and fins (Figures 4.2A, 5.2A), encysted larvae from the gills, *Diplostomum tregenna* (Figure 5.2B) from the brain, *Diplostomum* sp. from the swimbladder (Figure 5.2C) and eyes (Figure 4.2D) and *Neutraclinostomum* sp. (Figure 5.2E) from the branchial region; cestodes included dilepidid cestode larvae from the outer lining of the intestine (Figures 5.2F and 5.3B) and the liver (Figure 5.3A); nematodes (*Contracaecum* larvae) from the body cavity (Figure 5.3C) and sinus venosus of the heart (Figure 5.3D); adult acanthocephalans (Figure 5.3E) from the intestine; and infective stage larvae of pentastomids from two genera (*Subtriquetra rileyi*, Figure 5.3F and *Alofia* sp., Figure 5.3G) from the swimbladder.

Infestation statistics for all parasites are presented in Table 5.1. The total number of ecto- and endoparasites recorded during the four sampling surveys are available in Tables 1 ï 4 (Addendum C). Not all parasites were identified to genus and

species level as the study focused more on the specific groups, e.g. Monogenea, Digenea, Cestoda etc. to learn how the specific groups respond towards contamination levels. Therefore, when calculated the infestation statistics, the different species in a group were combined.

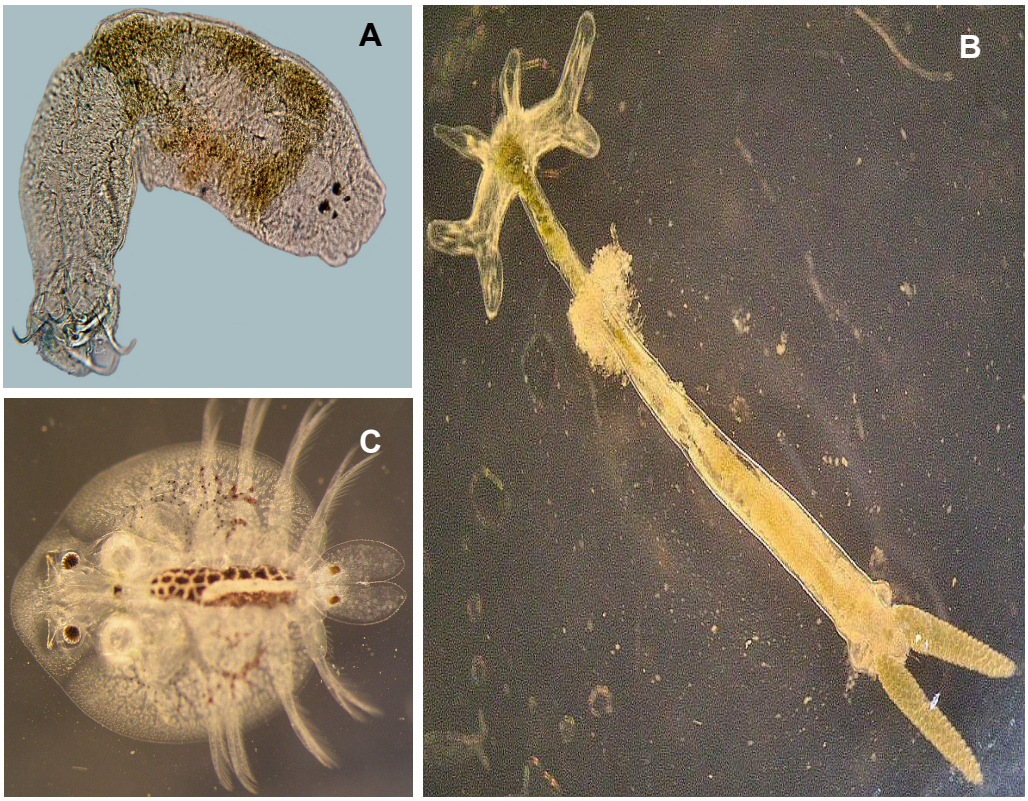

Figure 5.1: A-C. Ectoparasites recorded from *O. mossambicus.* **A**. *Cichlidogyrus* sp. from the gills, **B**. *Lernaea cyprinacea* from the skin, **C**. *Argulus japonicus* from the skin.

5.1 Infestation statistics

5.1.1 Ectoparasites

A prevalence of 70% was recorded for **monogeneans** at site A during all sampling periods with an exception during Summer when the prevalence decreased to 20%. The prevalence of monogeneans at site B was high in Winter (60%), decreased in

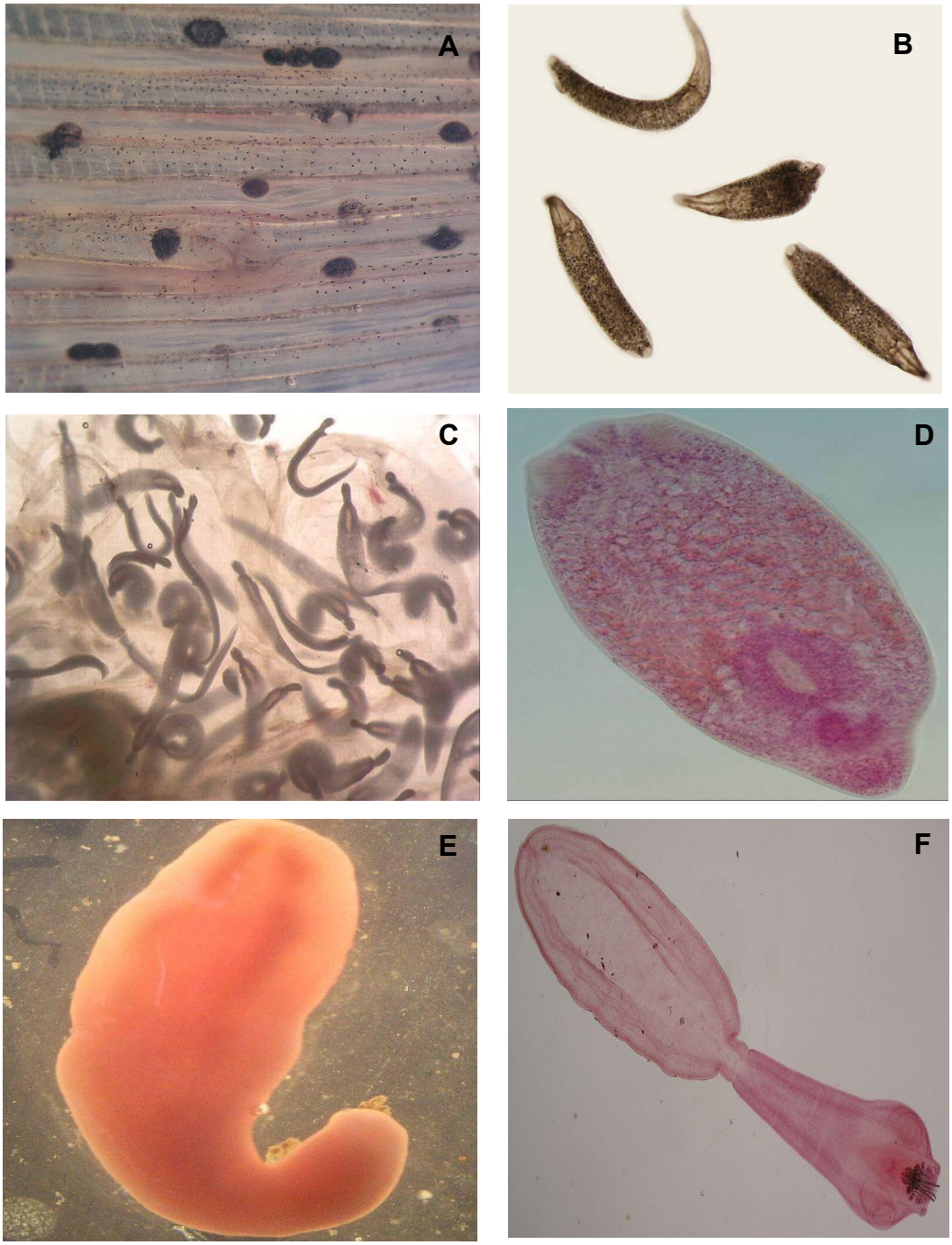

Figure 5.2: A-F. Endoparasites recorded from *O. mossambicus.* **A**. Digenea cysts (ëBlack spotí) from the fins, **B**. *Diplostomum tregenna* larvae from the brain, **C**. *Diplostomum* sp. metacercariae from the swimbladder, **D**. *Diplostomum* sp. metacercaria from the eye, **E**. *Neutraclinostomum* sp. larva from the branchial region, **F**. Dilepidid cestode larva (scolex everted) from the outer lining of the intestine.

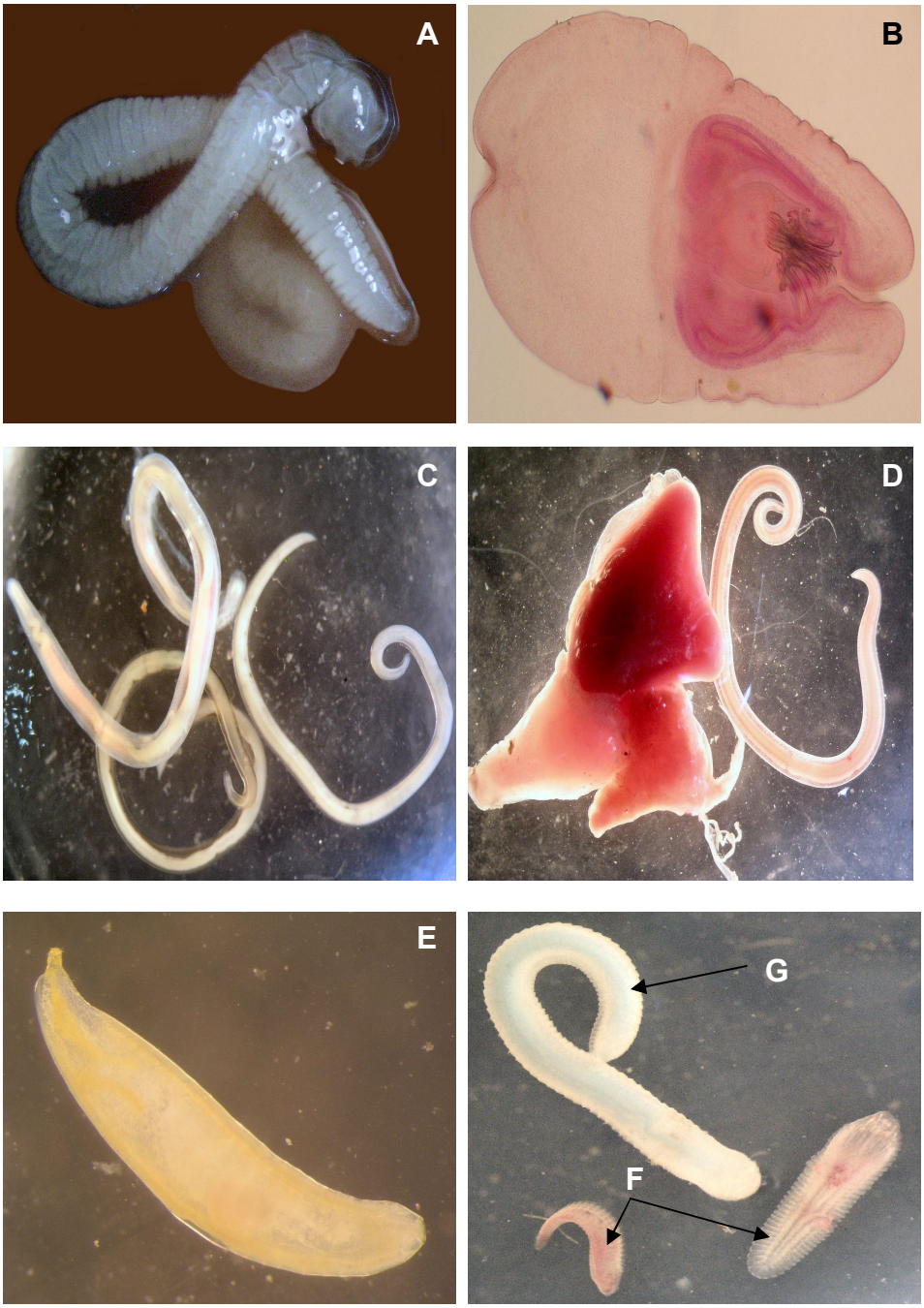

Figure 5.3: A-G. Endoparasites recorded from *O. mossambicus.* **A**. Dilepidid cestode larva from the liver, **B**. Dilepidid cestode larva from the intestine, **C**. *Contracaecum* sp. larvae from the body cavity, **D**. *Contracaecum* sp. larva from the sinus venosus of the heart, **E**. Adult acanthocephalan (proboscis everted) from the intestine, **F**. *Subtriquetra rileyi* (infective stage larvae) from the swimbladder, **G**. *Alofia* sp. (infective stage larva) from the swimbladder.

Spring (40%) and Summer (0%) and increased again in Autumn (30%). The same pattern was followed for the prevalence of monogeneans at sites C and D with no flukes recorded during Summer (Table 5.1). The highest mean intensity was 4.3 calculated at site D during Spring (Table 5.1).

A prevalence of 30% was recorded for **copepods** at site A during Spring while no copepods were recorded during the other seasons at this site. A 100% prevalence of copepods was calculated at site C during Winter, which was attributed to the occurrence of *Lernaea cyprinacea*. The abundance of copepods at site C was very high during Winter and Spring but decreased during Summer and increased again in Autumn (Table 5.1). The highest mean intensity for copepods was 26.7 recorded at site C during Spring (Table 5.1). No copepods were recorded at sites B and D throughout the sampling period (Table 5.1).

The only **branchiuran** recorded during this study was *Argulus japonicus* (Addendum C). A prevalence of 20% with a mean intensity of 1 was recorded for branchiurans at site A during Spring, while no branchiurans were noted at other sites during this study (Table 5.1).

5.1.2 Endoparasites

A prevalence of 100% was recorded for **digeneans** at sites A, C and D throughout the study (except during Autumn where a 60% prevalence was recorded at site D). These included cysts (ñBlack spotò) as well as *Neutraclinostomum* sp. and *Diplostomum* sp. larvae. The prevalence varied between 50% ï 90% at site B. The highest mean intensity (243.5) for digeneans was recorded during Summer at site C (Table 5.1).

A prevalence of 60% (survey 1) and 20% (survey 2) were recorded for **cestode** larvae at site A while 50% was recorded for the remaining surveys. Site B had the highest cestode mean intensity of 72 during Spring (Table 5.1). High cestode numbers were recorded during Summer at site C. The number of cestode larvae

was lower at site D with no tapeworm larvae recorded during Summer and Autumn at this site (Table 5.1). No adult cestodes were recorded during this study.

The prevalence recorded for **nematodes** was lower compared to digeneans and cestode larvae during this study. Low numbers of nematodes were documented at site A with no roundworms recorded during Winter and Autumn (Table 5.1). Higher numbers were recorded at site B and the prevalence varied between 40% ï 60% (Table 5.1). Low numbers of nematodes were also documented at sites C and D with no roundworms recorded during Spring and Summer at these sites (Table 5.1). The highest mean intensity (25) was recorded at site B during Spring. No adult nematodes were recorded during this study.

No **acanthocephalans** were recorded during Winter (at all sites) and at sites B and C during all seasons Table 5.1). Only one acanthocephalan was recorded at site D during Spring (Table 5.1). Acanthocephalans were recoded during all seasons (except Winter) at site A with the highest mean intensity of 5.67 amid Spring. The highest prevalence of 50% was recorded at site A during Summer (Table 5.1).

No **pentastomids** were recorded at site B during this study (Table 5.1). A prevalence of 20% was recorded at site A during Summer while no pentastomids were noted for the other seasons at this site. A prevalence of 50% and 10% were calculated at site C during Winter and Summer respectively, while no pentastomids were recorded during Spring and Autumn at this site (Table 5.1). A prevalence of 10% was recorded at site D during Winter and Spring, which increased to 60% during Summer and 30% during Autumn. The highest mean intensity (4.5) was calculated at site A during Summer. The highest pentastomid abundance (1.4) was recorded at site D during Summer (Table 5.1).

Table 5.1: Infestation statistics of parasites recorded at the four sites at the PIC and Barrage

	Site A				Site B				Site C				Site D			
PARASITES	**no**	**A**	**I**	**P**	**no**	**A**	**I**	**P**	**no**	**A**	**I**	**P**	**no**	**A**	**I**	**P**
Survey 1 (Winter)																
ENDO-PARASITES																
Digenea larvae	330	33	33	100	15	1.5	2.5	60	2136	213.6	213.6	100	469	46.9	46.9	100
Cestode larvae	172	17.2	28.7	60	24	2.4	3.43	70	29	2.9	14.5	20	14	1.4	7.0	20
Nematode larvae	4	0.4	0.1	40	14	1.4	2.3	60	1	0.1	1	10	1	0.1	1	10
Acanthocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pentastomid larvae	0	0	0	0	0	0	0	0	6	0.6	1.2	50	1	0.1	1	10
ECTO-PARASITES																
Monogenea	21	2.1	3.0	70	11	1.1	1.8	60	6	0.6	3	20	5	0.5	1.67	30
Copepoda	0	0	0	0	0	0	0	0	93	9.3	9.3	100	0	0	0	0
Branchiura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Survey 2 (Spring)																
ENDO-PARASITES																
Digenea larvae	741	74.1	74.1	100	109	10.9	13.6	90	881	88.1	88.1	100	133	13.3	13.3	100
Cestode larvae	27	2.7	13.5	20	173	17.3	21.6	80	8	0.8	1	10	29	2.9	14.5	20
Nematode larvae	6	0.6	3	20	125	12.5	25	50	0	0	0	0	0	0	0	0
Acanthocephala	17	1.7	5.67	30	0	0	0	0	0	0	0	0	1	0.1	1	10
Pentastomid larvae	0	0	0	0	0	0	0	0	0	0	0	0	3	0.3	1	10
ECTO-PARASITES																
Monogenea	28	2.8	4	70	8	0.8	2	40	11	1.1	2.2	50	26	2.6	4.3	60
Copepoda	3	0.3	1	30	0	0	0	0	240	24	26.7	90	0	0	0	0
Branchiura	2	0.2	1	20	0	0	0	0	0	0	0	0	0	0	0	0
Survey 3 (Summer)																
ENDO-PARASITES																
Digenea larvae	525	52.5	52.5	100	95	9.5	10.5	90	2797	279.7	279.7	100	195	19.5	19.5	100
Cestode larvae	33	3.3	6.6	50	320	32	35.6	90	55	5.5	7.9	70	0	0	0	0
Nematode larvae	3	0.3	1	30	11	1.1	2.2	40	0	0	0	0	0	0	0	0
Acanthocephala	10	1	2	50	0	0	0	0	0	0	0	0	0	0	0	0
Pentastomid larvae	9	0.9	4.5	20	0	0	0	0	1	0.1	1	10	17	1.7	2.8	60
ECTO-PARASITES																
Monogenea	2	0.2	2	20	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	3	0.3	1	30	0	0	0	0
Branchiura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Survey 4 (Autumn)																
ENDO-PARASITES																
Digenea larvae	641	64.1	64.1	100	53	5.3	10.8	50	2109	210.9	210.9	100	61	6.1	12.5	60
Cestode larvae	50	5.0	8.3	50	90	9.0	10	90	16	1.6	4	40	0	0	0	0
Nematode larvae	0	0	0	0	8	0.8	2.0	40	3	0.3	3	30	7	0.7	2.3	30
Acanthocephala	7	0.7	1.75	40	0	0	0	0	0	0	0	0	0	0	0	0
Pentastomid larvae	0	0	0	0	0	0	0	0	0	0	0	0	8	0.8	2.6	30
ECTO-PARASITES																
Monogenea	22	2.2	2.4	90	6	0.6	2	30	19	1.9	3.8	50	16	1.6	4	40
Copepoda	0	0	0	0	0	0	0	0	23	2.3	3.8	60	0	0	0	0
Branchiura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

No ⇒ Number of parasites

A ⇒ Abundance (relative density), total number of particular parasite species divided by the total number of hosts in a sample.

I ⇒ Intensity, total number of a particular parasite species divided by the number of infested hosts.

P ⇒ Prevalence, number of infested individuals of a host species divided by the number of hosts examined (expressed in percentage).

5.2 Ectoparasites

5.2.1 Monogenea

Monogeneans, commonly known as flukes, are ectoparasitic on the gill filaments of their fish hosts, but some are present on fins, body surfaces, in the nostrils, and buccal cavity of fish (Hendrix 1994). These flukes are classified in the Phylum Platyhelminthes and Class Monogenea (Brusca and Brusca 2003). They are small to medium sized flatworms and have direct life cycles (Hoffman 1999). The life cycle of most monogeneans include an egg, free swimming larval stage (oncomiracidium) and the adult. The exception to this pattern is the gyrodactylids which are viviparous (Bush *et al*. 2001). They hold onto their hosts via a combination of hooks, anchors, and suckers at their posterior end (opisthaptor), and use the anterior end (prohaptor) for feeding and assisting in moving for temporary attachment to other locations on the host (Hendrix 1994). Monogeneans generally display high levels of host specificity, and many species seem restricted to a single or several closely related host species (Cheung 1993; Benz and Bullard 2004). They are diverse both in terms of morphology and numbers and their phylogeny is well resolved, at least to the family level (Poulin 2002).

In the natural environment they usually do not cause many problems unless the host is stressed (Luus-Powell 2004). Monogeneans affect the host (1) through ingestion of mucus, skin, and blood so that much of the fish's protective coating is destroyed and then potentially dangerous secondary infections can set in, (2) by injury to gill tissue that leads to fusion and hyperplasia of gill tissues, and the subsequent decrease in gill surface area and thus a decrease in efficiency of respiration, and (3) by mechanical blockage of respiratory surfaces due to large numbers (Lawler 2003).

Table 5.2: Total number of monogeneans recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	21	11	6	5
Spring	28	8	11	26
Summer	2	0	0	0
Autumn	22	6	19	16
Mean	18.25	6.25	9	11.75

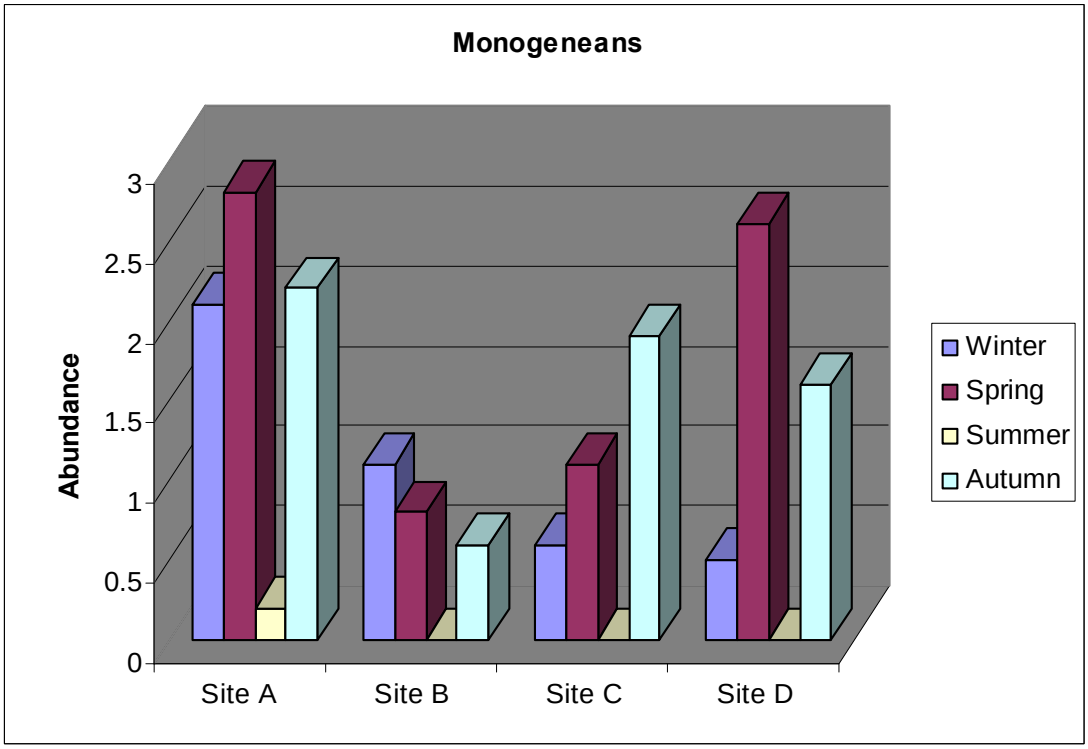

Figure 5.4: Seasonal variation in abundance of monogeneans at the four sampling sites

Cichlidogyrus sp. was recovered from the gill filaments of *O. mossambicus* during this study. The highest number of monogeneans was recorded at sites A and D during Spring (Table 5.2; Addendum C, Tables 1 ï 4). More monogeneans were recorded at site A compared to sites B and D during Autumn (Table 5.2; Addendum C, Tables 1 ï 4). The abundance of monogeneans declined towards Summer with only two monogeneans recorded at site A during Summer, and no monogeneans recorded at the other sites (Table 5.2). The numbers accrued again

during Autumn at all sites. The abundance of monogeneans were clearly lower at sites B and C compared to the other two sites (Figure 5.4) which can be ascribed to the high total dissolved salts and salinity levels at these two sites (see Chapter 3). Salt treatment is a well known method used in the treatment of monogenean infections in the aquaculture industry (Luus-Powell *et al*. 2006).

5.2.2 Copepoda

Copepods are found in fresh and sea water and are members from the phylum Arthropoda (subphphylum Crustacea, class Maxillopoda and subclass Copepoda) (Brusca and Brusca 2003). Most copepods are free living and are very important food items for a variety of aquatic life. Body shapes vary tremendously from generally cylindrical to flattened or saucer shaped. The body is divided into a head that is fused with the first or second thoracic segments (cephalothorax), thorax and abdomen (Williams and Bunkley-Williams 1996). One pair of thoracic appendages is modified into mouthparts, and five pairs are unmodified. The abdomen has no appendages, and usually terminates in a bifurcate tail (caudal rami). In parasitic forms, the life cycle is direct, but typically involves a series of planktonic stages off the host. Sexes are separate with males often much smaller than the females (Bunkley-Williams and Williams 2004).

Parasitic copepods generally occur on the gills or skin of fishes, but may burrow into the flesh or head sinuses, or crawl into the nose (nares) or eyes (orbits). They also associate with or parasitize a variety of invertebrates (Bush *et al*. 2001). Fish parasitic copepods are not known to infect humans. Those on fishes are permanent parasites, feeding on mucus, sloughed epithelial cells and tissue fluids (Bush *et al*. 2001). Copepods have not been found to directly transmit microbial diseases, but the wounds they cause may provide entry points for secondary diseases. Most of the copepods recorded from freshwater have been carried in from the saltwater environment by their euryhaline hosts (Bunkley-Williams and Williams 2004).

Table 5.3: Total number of copepods recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0	0	93	0
Spring	3	0	240	0
Summer	0	0	3	0
Autumn	0	0	23	0
Mean	0.75	0	35.8	0

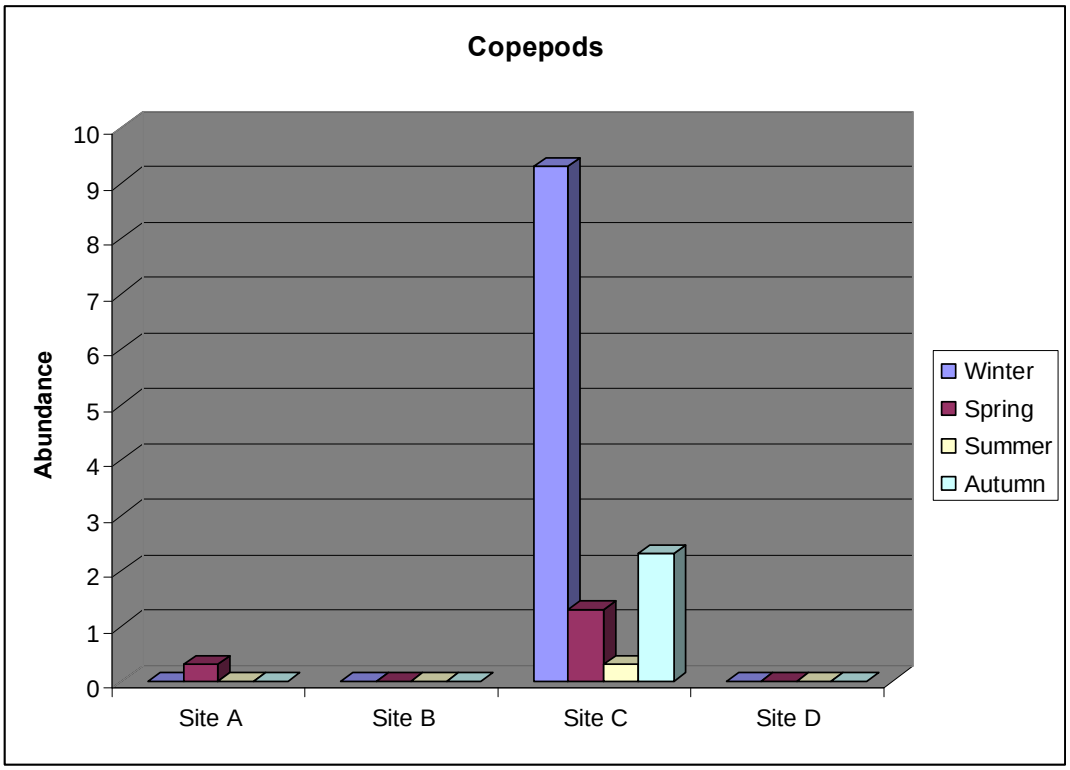

Figure 5.5: Seasonal variation in abundance of copepods at the four sampling sites

Lernaea cyprinacea was the only copepod recorded during this study. The anterior of the parasite was embedded in the hostôs flesh. They produced hemorrhagic lesions on the skin (Figure 4.2B). No copepods were recorded at sites A, B and D during the four sampling seasons except at site A during Spring (Table 5.3). The highest number of copepods (240) was recorded at site C during Spring (Addendum C, Tables 1 ï 4). The abundance of copepods was higher at site C compared to the other sites (Figure 5.5). These findings were unpredicted as the

number of ectoparasites is suspected to be lower at sites with a poorer water quality. According to the water quality results (Chapter 3), site C had higher levels of most water constituents and was overall in a poorer state compared to sites A and D. The unsuspected results for copepods may be attributed to the differential susceptibility of parasites to the toxicity of different pollutants, their concentration, the exposure time and possible synergistic effects (Dzikowski *et al.* 2003). Results from this study thus show that *Lernaea cyprinacea* has a high tolerance for certain water contaminants.

5.2.3 Branchiura

Argulus japonicus is an ectoparasite belonging to phylum Arthropoda (subphylum Crustacea, class Maxillopoda and subclass Branchiura), and are considered to be modified copepods (Brusca and Brusca 2003). They are parasites of both freshwater and marine fish (Heckmann 2003). The highly adapted maxillules are the primary organs for attachment to the host and provide the easiest means of identifying the genus since they are the most noticeable structures on the animalôs ventral surface (Brusca and Brusca 2003). After attaching to the fish, the argulids grow and metamorphose several times and become sexually mature in 30 to 35 days, depending primarily on the water temperature. They are typically found around the gills, operculum, mouth, pelvic, pectoral and dorsal fins of the host.

There are two structures used by these organisms for feeding, i.e. pre-oral spine (or stylet) and the mouth tube. The pre-oral spine punctures the hostôs integument and injects the fish with a cytolytic toxic secretion that can cause an inflammatory, hemorrhagic response in the host. Fish tissue is damaged by the repeated piercing of the skin by the pre-oral spine. Feeding sites often become hemorrhagic and ulcerated, and provide access to secondary infections by other parasites, fungi, bacteria, or viruses (Lester and Roubal 1995; Molnar and Szekely 1998; Heckmann 2003). One or two parasites, however, usually cause no clinical signs in large fish.

Table 5.4: Total number of branchiurans recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0	0	0	0
Spring	2	0	0	0
Summer	0	0	0	0
Autumn	0	0	0	0
Mean	0.5	0	0	0

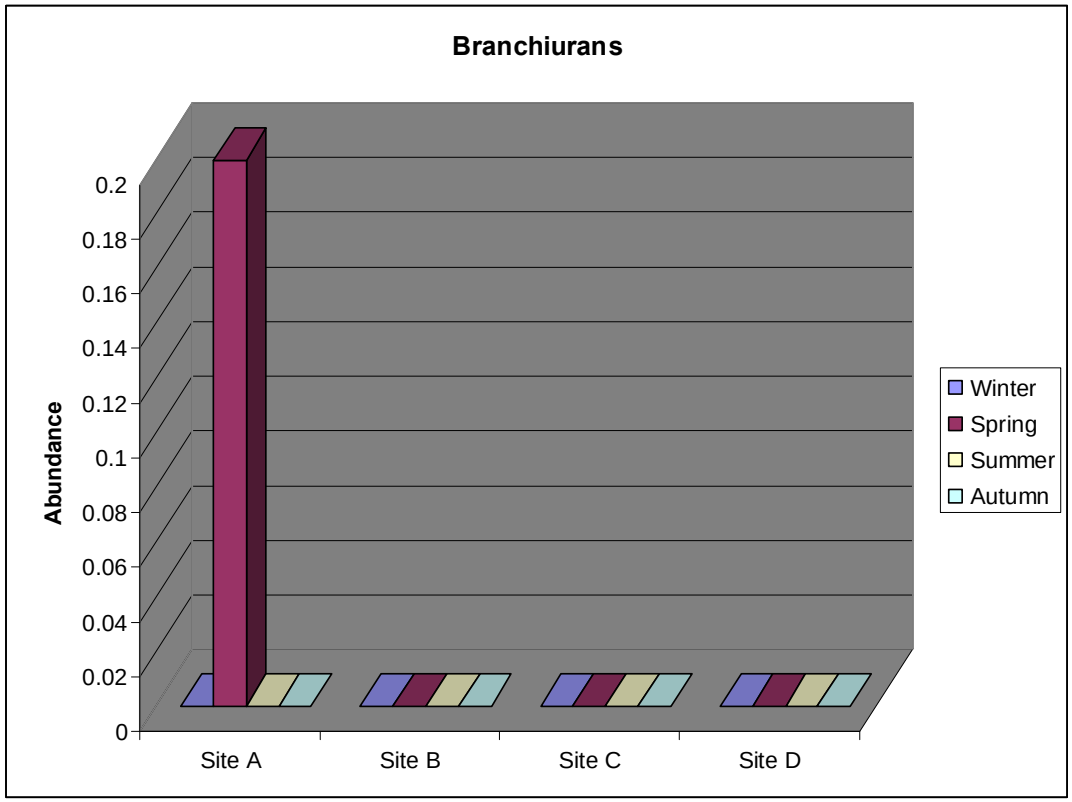

Figure 5.6: Seasonal variation in abundance of branchiurans at the four sampling sites

Argulus japonicus (Figure 5.1C) was the only branchiuran recovered from the skin of *O. mossambicus* during this study (Addendum C, Tables 1 ï 4). Only two specimens were recorded at site A during Spring, while no branchiurans were recorded at the other sites during the sampling period (Table 5.4 and Figure 5.6). No haemorrhages were noted on the skin of the fish which might be as a result of the low numbers of branchiurans recorded during this study from *O. mossambicus*.

5.3 Endoparasites

5.3.1 Digenea

Digeneans, commonly known as flukes, are members of the phylum Platyhelminthes (class Trematoda and subsclass Digenea) (Brusca and Brusca 2003). They are among the most common and abundant parasitic worms, second only to parasitic nematodes in their distribution (Roberts and Janovy 2000). Khalil and Polling (1997) listed 55 genera from 26 families of trematodes, occurring in freshwater fish in Africa. Digenetic trematodes (digeneans) are relatively host specific, heteroxenous flatworms which require typically a snail as the first intermediate host (Paperna 1996). Adult digeneans usually have oval, dorso-ventrally flattened bodies with a smooth, spiny or corrugated surface. A sucker is usually present at the antero-ventral mouth and a second sucker (acetabulum) is usually found mid-ventrally, both used for locomotion and attachment. The digestive system consists of a mouth opening, pharynx, short oesophagus and two blind caeca. Most adult trematodes contain both male and female reproductive organs.

Digeneans have a heteroxenic life cycle and can infect fish either in the adult form or as the metacercaria larvae (Bartoli and Boudouresque 2007). Metacercarial infections in fish have been recorded during various freshwater studies in Africa (Ortlepp 1935; Paperna 1964; Lombard 1968; Paperna and Thurston 1968; Khalil 1969; Prudhoe and Hussey 1977; Mashego 1982; Britz *et al*. 1984a, b; Van As and Basson 1984; Britz *et al*. 1985; Mashego and Saayman 1989; Paperna 1996; Luus-Powell 2004). Piscivorous birds, crocodiles, water monitor lizards and occasionally humans are the definitive hosts of the metacercariae found in fish. Most of the metacercariae form cysts in the flesh, on the gills and under the skin of fish, while others occur freely in the gall bladder, swimbladder, brain and the eyes. At low levels of infestation the host suffers no ill effects. However when infestation levels reach high levels, the fish may become morbid and loose condition.

Furthermore, according to Bartoli and Boudouresque (2007) the more the digenean load increases, the more the movement of the fish are disturbed which increases the probability of its capture by the final host. In addition, species of the genus *Diplostomum* can be significant pathogens causing a range of disease symptoms (i.e. exophthalmia, local hemorrhage, lens cataract and growth reduction) which may lead to fish mortality (Chappell *et al.* 1994; Dorucu and Yspir 2001).

Table 5.5: Total number of digeneans recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	330	15	2136	469
Spring	741	109	881	133
Summer	525	95	2797	195
Autumn	641	53	2109	61
Mean	559.25	68	1980.75	214.5

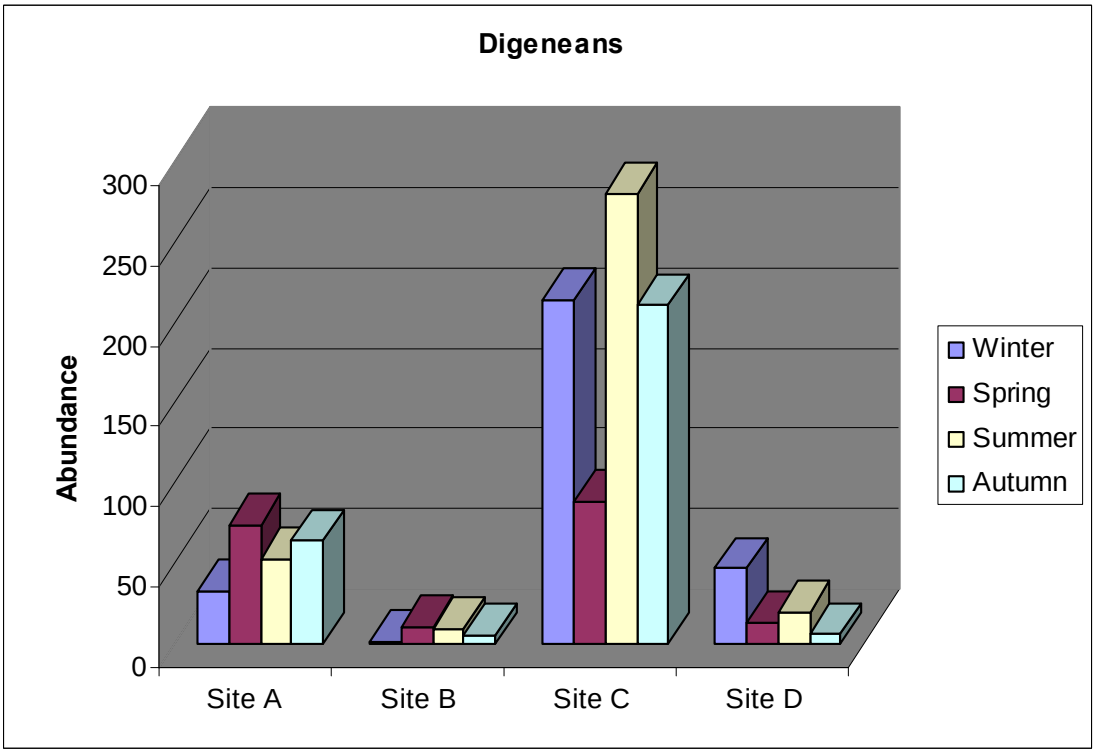

Figure 5.7: Seasonal variation in abundance of digeneans at the four sampling sites

Various types of diplostomid metacercariae were recorded from different organs such as eyes, gills, brain and swimbladder and one type of clinostomid metacercaria from the branchial region of *O. mossambicus* during this study (Addendum C, Tables 1 ï 4 and Figures 5.2B, C, D). All the digeneans were however grouped and is referred to as Digenea larvae as no adult digeneans were recorded during this study. The larval digenetic trematodes were identified to genus level only for most species, as no reproductive organs were noted in larval forms making it difficult to identify them to species level.

Neutraclinostomum sp. metacercariae (Figure 5.2E) were recorded from the branchial region of fish at sites A, C and D. No *Neutraclinostomum* sp. was recorded from site B indicating the possible absence of the specific snail intermediate host or that the water quality at this site might have an effect on the cercariae shed by the snails, but this aspect requires further investigation. Clinostomid infestations, commonly referred to as yellow grub, are not pathogenic to the host although mass mortality of fish at a fish station in South Africa was attributed to these parasites (Britz *et al*. 1985). Furthermore, the large conspicuous cysts will make the fish unattractive to human consumers, especially when large numbers are present in the muscle of fish. Clinostomids can utilize mammalians as definitive hosts and infections have been reported from domestic animals such as cats and also humans (although not from southern Africa) (Britz *et al*. 1985). Raw or undercooked fish as well as partially sun-dried fish may thus be a potential health hazard to human consumers.

As mentioned above, various types of diplostomid metacercariae were recorded from different organs. *Diplostomum tregenna* larvae were recorded from the brain (Figure 5.2B), sometimes in very large numbers (up to 125). The digenea metacercariae from the skin and fins were encysted between the integument and body musculature as well as on the fins of the fish (Figure 5.2A). The cyst is usually black in colour (formed by the parasites). According to Mashego and Saayman (1989) the black colour is due to dentritic pigmentation on the outer cyst

wall and is responsible for the clearly visible black spot on the fish and is thus commonly referred to as ôBlack spotô

Very high numbers of digenean larvae were recorded at site C, followed by site A and then site D, while lower numbers were recorded from site B (Table 5.5). The difference in abundance of larval digeneans between the two sites with poorer water quality (sites B and C) is difficult to explain. The lower numbers at site B may be due to possible lower numbers of the snail intermediate host at this site or the possible influence of certain water constituents on the cercariae shed by the snails. The abundance of digenea larvae was however much higher at site C compared to the other sites (Figure 5.7). It seems that the poorer water quality at the latter site had no effect on the snail intermediate host or the transmission of digenea cercariae to the fish intermediate host. The definitive hosts (piscivorous birds) were present in large numbers at all the sites.

5.3.2 Cestoda

The Cestoda or tapeworms form a large endoparasitic class of the flatworms belonging to phylum Platyhelminthes (Brusca and Brusca 2003). The common name derived from the long series of body segments which resemble a tape measure. They are always of concern because they can reduce growth and affect the reproductive success of fishes, and larval forms in fish can be harmful to humans. More than 5000 species of cestodes are known (Chub *et al.* 1987). Adults range from less than a millimeter to more than 30 meters in length.

Tapeworms usually consist of a chain of segments (proglottids) each with a set of reproductive organs. Most have both sexual organs in either the same individual or each proglottid with only a few species displaying separate sexes. The segments are continuously budded in the anterior portion of the body or neck, and enlarge and mature as they slowly move posteriorly. The scolex or "head" at the anterior end is armed with various attachments organs such as suckers, hooks and or

bothria (sucking grooves) to attach to the host's intestine (Brusca and Brusca 2003). Nutrients are absorbed through their body wall as their intestine has been lost. Due to their remarkable parasitic adaptations, larval forms occur in internal organs and adults in mesentery or free in the intestine of freshwater fishes (Yanong 2002).

Small aquatic crustaceans (copepods) or aquatic oligochaete worms (tubificids) serve as first intermediate host for fish parasitic cestodes (Bush *et al*. 2001). During feeding, the zooplankton makes it possible for the cestode larva to enter the fish. Fish may serve as the second intermediate host or as the definitive host. Cestodes that spend the larval stage inside a fish, reach the stage of sexual maturity in predacious fish, birds or mammals (including humans) (Bush *et al*. 2001).

Table 5.6: Total number of cestodes recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	172	24	29	14
Spring	27	173	8	29
Summer	33	320	55	0
Autumn	50	90	16	0
Mean	70.5	151.75	27	10.75

Heavy infections of encysted dilepidid cestode larvae were found in the liver (Figure 5.3A) and the outer wall of the intestine at all sites during all four surveys, except during Summer and Autumn at site D (Table 5.6). The dilepidid cestode larvae from the liver were much larger in size and morphological different from the ones recorded from the outer intestinal wall (Figures 5.2F, 5.3B). During this study, these larvae caused different degrees of damage to the liver and intestine, sometimes with red marks clearly visible on the intestine. No adult cestodes were recorded during this study.

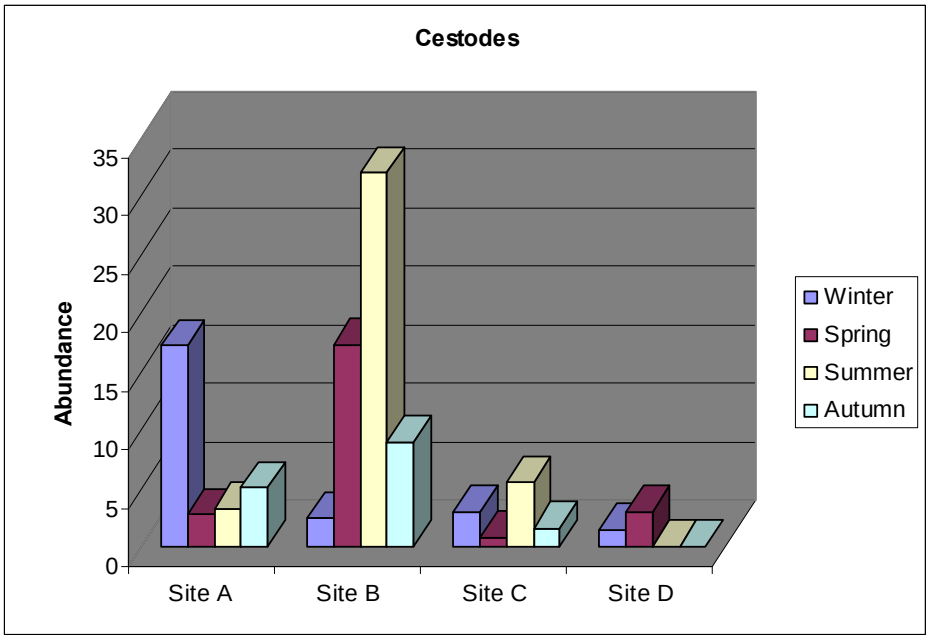

Figure 5.8: Seasonal variation in abundance of cestodes at the four sampling sites

The highest number of dilepidid cestode larvae was recorded at site B during Summer (Table 5.6). Lower numbers were recorded at site D with no dilepidid cestode larvae found during Summer and Autumn at this site (Table 5.6). A higher abundance of dilepidid cestode larvae was recorded at site B during most surveys (Figure 5.8) with the highest mean recorded at this site (Table 5.6).

5.3.3 Nematoda (roundworms)

Nematodes (Phylum Nematoda) are commonly known as roundworms. They are very distinctive in shape, with a solid tough cuticle which makes them last longer than flatworms in post-mortem conditions (Brusca and Brusca 2003). Most adult forms are large enough to be visible to the naked eye, but very small and hair fine forms are also present in fish hosts. Khalil and Polling (1997) reported 40 parasitic nematode species (representatives of 9 families) from freshwater fish in Africa. Adult nematodes are typically found in alimentary system of fish while larval forms are common in the body cavity. However, depending upon the species of

nematode and infected fish, adult and other life stages of nematodes can be found in almost any part of the fish, including the swimbladder, deeper layers of the skin or fins, and external muscle layers (Whitfield and Heeg 1977).

Larval nematodes either occur encysted in the tissue or free in the body cavity, but hardly if ever severely affect the fish (Paperna 1996). The occurrence of larval *Contracaecum* species have been widely reported in cichlids and catfish from several African countries such as Egypt (Amin 1978), East Africa (Aloo 2001; Yanong 2002), and South Africa (Mashego and Saayman 1981; Van As and Basson 1984; Boomker 1994; Barson 2003). Those that occur in the alimentary tract of the definitive host may in many cases cause lesions, ulcerations and tumors (Nagasawa *et al.* 1998). *Contracaecum* species that infect fish will be found as adults in the gut of the final host (piscivorous birds, notably pelicans, cormorants, herons and darters).

Table 5.7: Total number of nematodes recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	4	14	1	1
Spring	6	125	0	0
Summer	3	11	0	0
Autumn	0	8	3	7
Mean	3.25	39.5	1	2

Larval *Contracaecum* sp. (Figure 5.3C) belonging to the family Anisakidae was recorded from the body cavity of *O. mossambicus* at all sites during the study period (Table 5.7; Addendum C, Table 1 ï 4). The nematode larvae were found in the visceral membranes, embedded in the mesenteries and also recorded from the sinus venosus of the heart (Figure 5.3D). No adult nematodes were found during this study. It is very difficult to differentiate between nematode species at the larval stage, because the larva lacks genital systems and other features of adult stages which are used as taxonomic criteria. No nematodes were recorded at

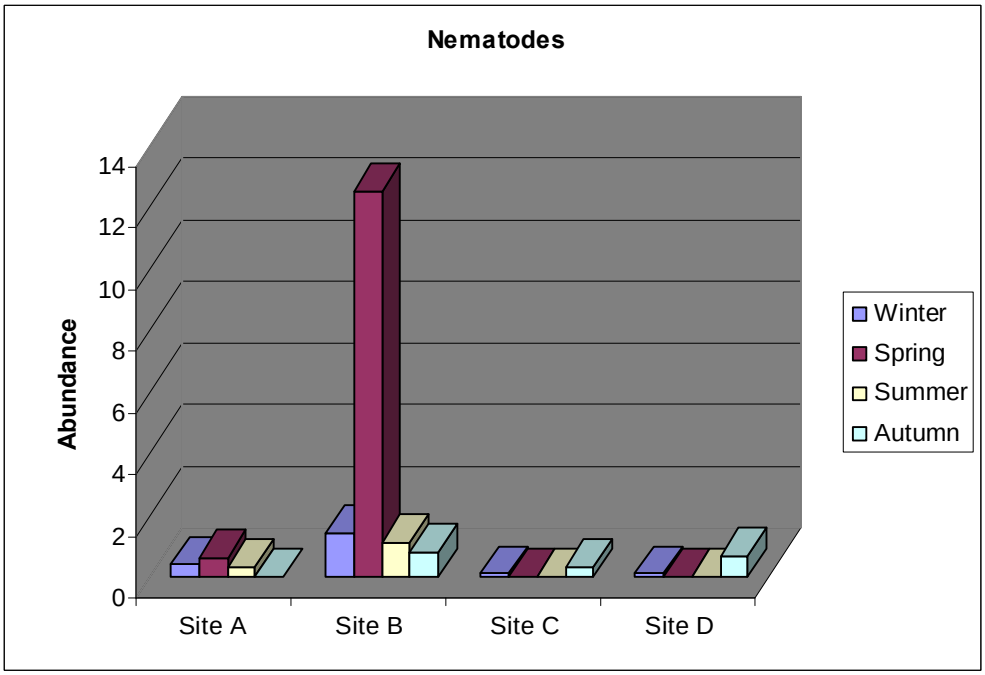

Figure 5.9: Seasonal variation in abundance of nematodes at the four sampling sites

sites C and D during Spring and Summer (Table 5.7 and Figure 5.9). Low numbers of larval nematodes were recorded from sites A, C and D (Table 5.7). Higher numbers were recorded at site B with the highest number (125) recorded during Spring (Table 5.7). *Contracaecum* species is one of the most frequent larval helminths occurring in freshwater fish. There is usually a change of host whereby the larval stages live in the visceral cavity or the mesentery and the sexually mature worms are found in the intestine of predacious fish or birds.

5.3.4 Acanthocephala

Acanthocephalans comprise a small phylum of parasites, commonly known as the spiny-headed worms. This phylum is divided into three classes based on the arrangement of the proboscis hooks, the nature of the epidermal nuclei, spination patterns on the trunk, and nature of the reproductive organs (Brusca and Brusca 2003). The classes include the Palaeacanthocephala, Archiacanthocephala and

Eoacanthocephala (Brusca and Brusca 2003). Comparing to parasitic platyhelminthes and nematodes, they are considered fairly rare (Roberts and Janovy 2000). Acanthocephalans are all permanent parasites in the intestine of mostly vertebrates, including humans. They are dioecious, oblong worms that lack a mouth and digestive tract and possess an eversible, spiny proboscis and a pseudocoel (Nickol 1995). They are bilaterally symmetrical, unsegmented, or only partially segmented externally and unsegmented internally. They attach in the gut of their host with a globular or cylindrical, protrusible, thorny proboscis. The proboscis protrudes and the spines fold out and lock like a compact umbrella. Muscles together with a hydraulic system (lemnisci) aid in the invert of the proboscis. Some species have thorny protrusions on the body. Sexes are separate, fertilization is internal, and embryos develop in the body of the female (Bunkley-Williams and Williams 2004).

The larva is enclosed within a shell (egg) and passes out with feaces of the vertebrate host (Bush *et al*. 2001). If the egg is eaten by certain insects (roaches or grubs) or by aquatic crustaceans (amphipods, isopods, ostracods), the larva bores through the gut wall of host and become lodged in the hemocoel (Brusca and Brusca 2003). The intermediate host is eaten by fishes, birds or mammals and the worm attaches to intestinal wall of vertebrate host. They can cause considerable damage to the intestinal wall when occurring in large numbers. No large numbers were recorded during this study and no damage was observed in the intestine.

Table 5.8: Total number of acanthocephalans recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0	0	0	0
Spring	17	0	0	1
Summer	10	0	0	0
Autumn	7	0	0	0
Mean	8.5	0	0	0.25

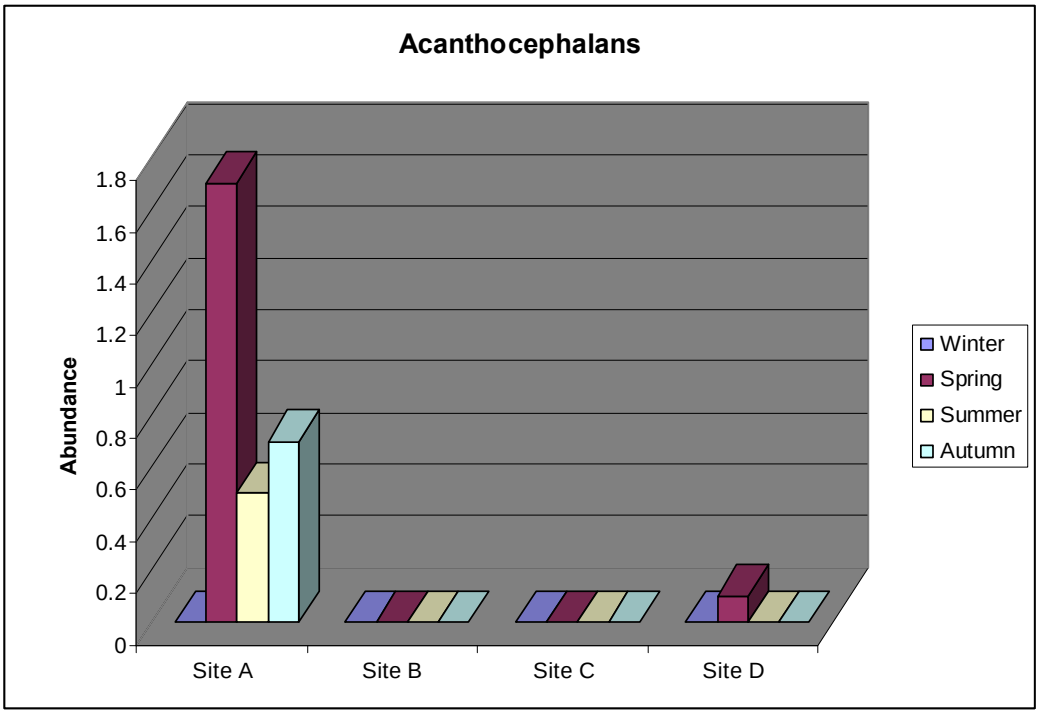

Figure 5.10: Seasonal variation in abundance of acanthocephalans at the four sampling sites

Adult acanthocephalans (Figure 5.3E) were recovered from the intestine of *O. mossambicus* from selected sites. No acanthocephalans were recorded in Winter during this study (Table 5.8). A maximum number of 17 were recorded during Spring at site A. A higher abundance was recorded at site A compared to the other sites (Figure 5.10). No acanthocephalans were recorded at sites B and C (Table 5.8 and Figure 5.10). The absence of acanthocephalans at these two sites might be ascribed to high levels of certain water constituents recorded from these sites that might affect the parasites, or due to the absence of the intermediate hosts.

5.3.5 Pentastomida

The Pentastomida is classified as a subclass of the subphylum Crustacea (phylum Arthropoda) because they are believed to be allied with the Onychophoroans but derived from the Branchiura (Brusca and Brusca 2003). On the other hand, many taxonomists classify them as a separate phylum. The pentastomids are a group of

worm-like parasites that infect many different species of fish. Infections have been found in several families of fish including the Cichlidae (tilapia), Cyprinidae (danios), Cyprinodontidae (flagfish), and Poecilidae (mosquitofish, swordtails, mollies, platies). Although pentastomids are small, they can be seen without the use of a microscope. Pentastomids have a complex, indirect life cycle. Infections cannot be transmitted directly from fish to fish (Yanong 2002). Pentastomids were first described in crocodiles more than a century ago and it was assumed from an early stage that fish were one of the intermediate hosts of these endoparasites (Junker *et al.* 1998). Several reptile species serve as final host including snakes, water monitor lizards, terrapins and crocodiles.

Pentastomatid larvae (infective stage larva) of two genera (*Subtriquetra rileyi*; *Alofia* sp.) were recorded from the swimbladder of *O. mossambicus* during this study (Table 5.9; Figures 5.3 F,G). The infective stage larvae of *S. rileyi* were freely mobile in the swimbladder of their hosts wherelse *Alofia* sp. were encapsulated in the swimbladder. Adults of the pentastomid species that infect fish are found in crocodiles and terrapins, which are considered to be their final hosts. Typically, adult stages of the parasite are found in the respiratory systems of reptiles, usually within the lungs and trachea.

Table 5.9: Total number of pentastomids recorded at the four sampling sites

Surveys	Site A	Site B	Site C	Site D
Winter	0	0	6	1
Spring	0	0	0	3
Summer	9	0	1	17
Autumn	0	0	0	8
Mean	2.25	0	1.75	6.5

Low numbers of pentastomids were recorded at all sites during this study (Table 5.9; Addendum C, Tables 1 ï 4). The highest number (17) was recorded at site D during Summer (Table 5.9). No pentastomids were recorded during Spring except at site D. The highest mean value and also the highest abundance were recorded

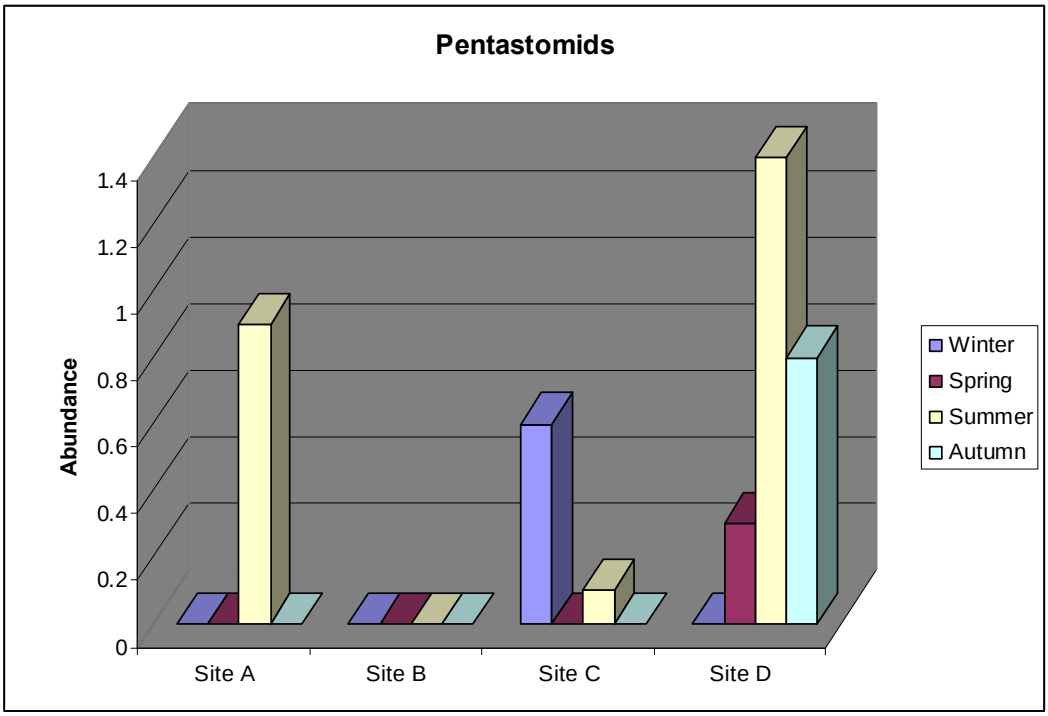

Figure 5.11: Seasonal variation in abundance of pentastomids at the four sampling sites

at site D (Table 5.9 and Figure 5.11). This can be ascribed to the higher presence of the definitive host (crocodiles) at this site. The life cycle usually includes an intermediate host for the larval stages (nymphs), usually vertebrates (fishes, amphibians, reptiles, or, rarely mammals) but the nymphs are also found in some insects. Pentastomiasis in humans is known from Africa, the Middle East and South-East Asia (Bush *et al*. 2001) and the infective stage larva encountered in *O. mossambicus* may thus pose a health hazard to human consumers. The occurrence of pentastomids in *O. mossambicus* recorded during this study is new distribution records for both pentastomid genera.

Conclusion

Monogenean parasites (*Cichlidogyrus* sp.) were recorded only from the gills (site specific) and were less abundant at the more polluted sites (sites B and C) throughout the sampling period. The highest monogenean abundance was recorded at sites A and D during Winter and Spring respectively. Thus more monogeneans were recorded during Winter and Spring with very low numbers encountered during the Summer survey indicating seasonal differences.

It seems that some ectoparasite species were not influenced by the water quality of the different sites and followed seasonal population changes. For example, high abundances of *Lernaea cyprinacea* (copepod) were found during Winter, Spring and Autumn at site C. Furthermore, the number of copepods at site C was very low during the Summer survey and absent from the other sites. More copepods were present at the impacted site (site C) than at site A (less impacted) with no copepods noted at sites B and D throughout the sampling period. Copepods were not affected by the poorer water quality of site C during this study.

A low branchiuran infestation was recorded during this study with only two specimens encountered at site A during Spring. The three recorded groups of ectoparasites (i.e. Monogenea, Copepoda and Branchuria) were all site specific on the host. *Cichlidogyrus* sp. and *Lernaea cyprinacea* were host specific while *Argulus japonicus* was also recorded from *Clarias gariepinus* at some of the sites (Jooste *et al*. 2004). The absence of certain ectoparasites at specified sites may suggest that some ectoparasites have been strongly influenced by the poorer water quality of sites B and C as some of the water constituents were above the target water quality range (TWQR) as suggested by DWAF (1996) (see Chapter 3). The specific water parameter/s that influenced the abundance of certain ectoparasites needs further investigation which would most probably include experimental work. *Lernaea cyprinacea* on the other hand, was not affected by these higher concentrations of certain water constituents.

Higher numbers of digenea larvae were generally recorded during Spring and Summer than the colder months. The abundance of digenea larvae was much higher at site C (the more polluted site) than the other sites throughout the sampling period. It seems that the poorer water quality at the latter site had no effect on the snail intermediate host or the transmission of digenea cercariae to the fish intermediate host. Furthermore, the immune system of the fish at this site might have been impaired due to constant pollutant stress leading to higher parasitic infestations.

Fish from sites A, B and C were heavily infected with dilepidid cestode larvae throughout the sampling period with lower numbers recorded at site D. A higher abundance of dilepidid cestode larvae was recorded at site B during most surveys. The water quality at this site thus had no effect on the first intermediate host (free-living crustaceans) and transmission to the fish (second intermediate host) was very successful. Furthermore, constant exposure to water pollution can lower the immune system of the host and cause higher susceptibility of fish to parasite infection and other diseases.

Nematode larvae (*Contracaecum* species) were recorded from all sites during this study but a higher abundance was recorded from site B throughout the study period. Piscivorous birds, notably cormorants, herons and darters serve as final host for *Contracaecum* species. Higher numbers of these birds were noted at site B which might be the reason for higher abundance of *Contracaecum* sp. larvae in the fish at this site. No definite seasonal variations were recorded for nematode larvae during this study.

Adult acanthocephalans were recovered from the intestine of *O. mossambicus* from site A only (with one specimen recorded at site D during Spring). No acanthocephalans were recorded from sites B and C (the more polluted sites). The absence of acanthocephalans at these two sites might be ascribed to high levels of certain water constituents recorded from these sites or due to the absence of the

specific intermediate hosts (aquatic crustaceans). Higher numbers were recorded during Spring at site A, while no acanthocephalans were recorded during Winter indicating seasonal variations.

Pentastomid larvae were recorded from all sites (except site B) during this study but in low numbers. The highest abundance was recorded from site D which might be due to the higher number of definitive hosts (water monitor lizards and crocodiles) at this site. No seasonal variation was observed for the pentastomids. The occurrence of the two pentastomid genera in *O. mossambicus* during this study is new distribution records and was presented at a national conference (Ramollo *et al*. 2006).

The number of endoparasites was generally higher at the more impacted sites (sites B and C) than at sites A and D. This can possibly be ascribed to increased physiological stress in the fish due to the constant exposure to poorer water quality. This may have lead to higher infection rates due to lowered immune systems of the fish. Although fish from sites B and C were more infected than fish from the other sites, the intensity of infection of different endoparasites varied considerably from site to site during certain surveys. The high numbers of digenean and cestode larvae could be attributed to the availability of intermediate hosts. The results from this study correspond with previous studies in South Africa which concluded that fish from more polluted water tend to harbour more endoparasites than those from less polluted waters (Robinson 1996, Luus-Powell 1997, Crafford 2000, Crafford and Avenant-Oldewage 2001, Jooste *et al*. 2004, Luus-Powell *et al*. 2005). In conclusion, results from the parasite composition of *O. mossambicus* recorded during this study may serve as an indicator of aquatic environmental changes but due to the variability and seasonal variation of some parasites, results should be interpreted with caution.

In general it is difficult to see the actual influence that parasitic infections have on fish as the presence of parasites does not necessarily imply manifestation of a

disease. Diseases caused by parasites are mostly manifested in fish that are stressed and suffer from stress factors that influence their ability to effectively protect themselves against parasitic infestations. No excessive high numbers of parasites were recorded during this study at any of the sites with the exception of trematode cysts (mainly ñBlack spotò on the skin and fins). However, large numbers of trematode cysts is not uncommon in freshwater fish. The prevalence and abundance of all parasites recorded during this study was relatively low and there is no reason for concern at this stage. However, it must be emphasized that the situation can change the moment fish are under constant stress and because of the tremendous reproductive potential of all parasites, numbers can increase rapidly resulting in fish mortalities.

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Chapter 6
General Summary and Conclusion

Water quality - Most of the system variables were within the target water quality range (TWQR) for aquatic ecosystems suggested by the DWAF guidelines (DWAF 1996a). There were, however, significant high levels of non-toxic constituents such as TDS (magnesium, sodium, calcium and potassium), salinity and electrical conductivity at sites B and C throughout the sampling period which can be attributed to the mining activities as well as the geological formations of the region. The high TDS and salinity levels are currently not impacting on the health of *O. mossambicus* as this is a euryhaline fish species and can tolerate high salinity levels. The total water hardness was very high at sites B and C which can be attributed to mining activities as well as the geochemical origin of these constituents at these sites. The cations such as sodium, calcium, potassium and magnesium, and anions (sulphate ions, chloride and fluoride) were above the TWQR for aquatic ecosystem (DWAF1996a) and domestic use (DWAF1996b) at all sites.

The nutrient levels (nitrate, nitrite, sulphate and phosphate) indicated that there is an influx of nutrients at all four sampling sites, with higher levels recorded at sites B and C. High levels of nutrient enrichment can increase the abundance of algae and aquatic plants (DWAF 1996a). The toxic water constituents (aluminium, copper, iron, lead, manganese and zinc) differed notably between the sites with higher levels generally recorded at sites B and C. The mean concentrations of aluminium, copper, lead and zinc were above the TWQR for aquatic ecosystems, while that of manganese and iron were within the TWQR for aquatic ecosystems (DWAF1996a).

The water quality results illustrated that the water at site C was more impacted than that of site B with less probability for sustainable aquatic life, because of the

specific mining activities as well as geochemical constituents at this site. The high concentrations of trace metals such as copper, lead and zinc at sites B and C can be attributed to the mining activities as well as geochemical constituents of the region. The water quality at sites A and D was much better compared to the other two sites impacted by mining activities. The contamination levels that were indeed recorded at site A can probably be related to the geological formations of the region as well as organic and inorganic matter entering the aquatic ecosystem from upstream activities or discharges and at site D it is probably due to agricultural return flows and other effluents from upstream activities.

In conclusion, the mining activities do affect the quality of water at sites B and C as far as dissolved salts, nutrients and certain trace metals are concerned, but their impact on the water quality is variable and dependent on the pH and water hardness at these sites.

Health Assessment Index (HAI) ï The HAI is a relatively simple and systematic approach to assess the possible effects of stress on the health and condition of fish in a population with minimal effort and expense. The results obtained for the HAI during this study indicated that the fish population from site C was more affected (with a higher population HAI) compared to the fish populations at the other sites. A higher population HAI was recorded at site C during three surveys (Winter, Summer and Autumn). When comparing the HAI values with water quality results, one would expect lower HAI values at sites A and D (the sites with better water quality) and higher HAI values at sites B and C (the sites with poorer water quality). This was achieved for most of the surveys at site C, as mentioned above, where the highest population HAI values were scored during three surveys. The lowest population HAI was scored at site D during two surveys (Winter and Autumn) and site A during Summer. Unexpectedly, site B had the lowest HAI score during Spring. However, during the Spring survey the mean values of the different sites didnôt differ considerably between the sites.

Schmitt *et al.* (2005) assumed that mean HAI values Ò20 indicated un-impacted or minimally impacted sites, values >50 indicated intermediate sites and values >70 indicated heavily impacted sites. Therefore taking this into consideration all sites indicated to be intermediate to heavily impacted sites during this study. But, Jooste *et al*. (2004) stated that the HAI will test differently in dissimilar aquatic ecosystems, depending primarily on the fish species used and types of stressors. Nevertheless, this method can be used to provide biologists with useful information about their environment with minimal effort and expense.

Liver discolourations, eye abnormalities including whiteness of the cornea and lens (possibly indicating parasitic infestation) or missing and blind eyes, deformed gills, parasite infestation, hindgut infection due to parasite infestation and blood parameter values contributed to elevated HAI values in most of the cases. No abnormalities of kidneys, opercules and spleens were observed at any of the four samplling sites. Fin deformities, such as abnormal shapes or missing fins were not found during this study (except once).

The fish abnormalities recorded at sites B and C may be related to continuous stress due to mining activities and the geochemistry of water but more detailed investigations are required. With the exception of the liver condition and certain blood parameters of a number of fish, the health of fish was not much impaired at any of the sites during this study. All the fish appear to be in good physical form with no obvious health related problems that could be observed while evaluating the fish using the HAI criteria. No detailed pathology tests were however done during this study. Furthermore, the condition factors (length-weight relationship) indicated that the fish of all sites were generally in good health.

The HAI can not be used for the specific identification of compounds to which the fish response in the water in the same way as physical-chemical techniques (Jooste *et al*. 2004). Although the accuracy and reliability of the chemical analysis of water is very high, these methods are very expensive and require extensive

training and sophisticated equipment. Therefore, the more reliable and accurate the method, the more training is required with an accompanying increase in cost (expenses) and complexity of the method. But although the HAI does not require costly and sophisticated equipment, researchers must have a basic biological background and knowledge as well as field experience (Jooste *et al*. 2004).

Further limitations of the HAI are the subjectivity of this method (Luus-Powell 1997). Individual researchers may interpret observations differently and this may influence the end results of the HAI. Numerical values given to variables are exact values, i.e. 0, 10, 20, and 30, and there is for instance a considerable difference between 10 and 20 while the physiological difference/state of disease might not be that significant. Huge leaps between values make a considerable difference to the end value of the HAI.

The HAI is not designed to be a substitute for other monitoring methods, to be diagnostic or to solve specific problems related to fish health or environmental conditions. The primary objective of this method is not to understand the reasons for a change in population health, but to document in a relatively rapid and inexpensive manner the occurrence of a change in a fish population. More specific and intensive investigations may then be designed to determine if there are particular stressors in the environment that can be associated with the observed responses.

It is important to recognise that biological data do not replace chemical and physical data, any more than chemical data can adequately replace biological data. These methods provide converging lines of evidence that supplement each other but are not mutually exclusive. The ideal situation would, therefore, be one in which the HAI and physical-chemical techniques are applied in association, using the former for general wide-spectrum screening purposes and the latter for the qualitative and quantitative analysis of potentially hazardous compounds detected by biomonitoring methods (Jooste *et al*. 2004). Continued biological monitoring

and biological criteria provide a direct and effective way to determine if actions taken result in improvements in the water resource system.

Parasites and the Parasite Index (PI) - Ectoparasites are constantly in contact with the external environment and they tend to be more susceptible to a number of contaminants than are some fish species. Ectoparasites tend to reduce in diversity and abundance when water is heavily polluted and higher numbers of ectoparasites should be expected in good quality water. The number of endoparasites, on the other hand, tends to increase in polluted water. This might be due to a lowered immune system and resistance of the fish due to continuous stress conditions (Sures 2006).

The hypothesis that the number of ectoparasites will be lower in more polluted water and the number of endoparasites will be higher was supported for O. *mossambicus* at all sites except at site C during Spring. The PI for endoparasites was higher at all the mine sites (except during Spring where the PI for ectoparasite was higher at site C) but similar results were obtained at sites A and D (the less impacted sites). However, all sites used during this study were impacted to a lesser or higher degree and the number of endoparasites can thus be higher at all sites. It was however, expected that the number of endoparasites should have been higher at the mine sites (the more polluted sites) and not at the better sites. Moreover, when a host is under chronic environmental stress it may result in impaired immunity. Some endoparasites of fish may thus take advantage from this and increase in number. This might have affected the PI results obtained during this study.

Constantly exposure to pollutants in aquatic ecosystems may lower the number of ectoparasites found on the host. *Lernaea cyprinacea* (an ectoparasitic copepod) was however recorded from water with higher dissolved salts and toxic constituents (compared to the other sites) during this study. More copepods were present at the more impacted site (site C) than at site A (less impacted) with no

copepods noted at sites B (more impacted) and D (less impacted) throughout the sampling period. It must be noted that fish from site C was overcrowded which might have contributed to higher number of copepods. Copepods were thus not affected by the poor water quality of site C during this study. But other ectoparasites, e.g. the monogenean parasites (*Cichlidogyrus* sp.) were less abundant at the more polluted sites (sites B and C) throughout the sampling period.

Higher numbers of digenea larvae (endoparasites) were recorded at site C (the more polluted site) and it seems that the poorer water quality had no effect on the snail intermediate host or the transmission of digenea cercariae to the fish intermediate host. A high abundance of dilepidid cestode larvae was recorded at site B during most surveys and the water quality at this site probably had no effect on the first intermediate host (free-living crustaceans), and transmission to the fish (second intermediate host) was very successful. Nematode larvae (*Contracaecum* species) were recorded from al sites during this study with a higher abundance at site B attributed to the higher presence of piscivorous birds which serve as final host for *Contracaecum* species. Adult acanthocephalans were recovered mostly from site A with no acanthocephalans recorded from sites B and C (the more polluted sites). The absence of acanthocephalans at these two sites might be ascribed to high levels of certain water constituents recorded from these sites or due to the absence of the intermediate hosts (aquatic crustaceans). Pentastomid larvae were recorded from all sites during this study but in low numbers. The highest abundance was recorded from site D which might be due to the higher number of definitive hosts (Nile monitor lizards and crocodiles) at this site.

The number of endoparasites was generally higher at the more impacted sites (sites B and C) than at sites A and D, thus supporting the hypothesis. This can possibly be ascribed to increased physiological stress in the fish due to the constant exposure to poorer water quality. This may have lead to higher infection rates due to lowered immune systems of the fish. Although fish from sites B and C

was more infected than fish from the other sites, the intensity of infection of different endoparasites varied considerably from site to site during certain surveys. The high numbers of digenean and cestode larvae could be attributed to the availability of intermediate hosts.

The abundance, prevalence and mean intensity of parasites from *O. mossambicus* differed between the four sites and between the different seasons during this study. Parasites recorded during this study showed to be site specific with monogeneans always recorded from the gills, copepods always from the skin, larval digeneans from the skin/fins, brain or swimbladder (depending on the species), acanthocephalans from the intestine, pentastomids from the swimbladder and cestodes always from the outer membrane of the intestine and the liver.

A high prevalence in parasites may affect the general health of fish by causing lesions (leading to secondary infections), stress and anemia in fish. It is however, difficult to estimate the actual harm to fish caused by the presence of parasites. As mentioned earlier, the presence of parasites does not necessarily imply manifestation of a disease and sometimes there is no sign of their existence. Diseases caused by parasites are manifested in fish that are stressed and suffer from stress factors that influence their ability to effectively protect themselves against parasitic infestations. No excessive high numbers of parasites were recorded during this study at any of the sites with the exception of trematode cysts (mainly ñBlack spotò). However, large numbers of trematode cysts is not uncommon in freshwater fish. The prevalence and abundance of all parasites recorded during this study was relatively low and there is no reason for concern at this stage. However, it must be emphasize that the situation can change the moment fish are under constant stress and because of the tremendous reproductive potential of all parasites, numbers can increase rapidly resulting in fish mortalities.

In conclusion, the health of the *Oreochromis mossambicus* populations did not manifest to be drastically negatively impacted by the pollutants (with limited abnormalities recorded from the skin, eyes, liver and fins, the latter mainly due to parasitic infections). Further studies of environmental factors that affect fish health need to be conducted before implications of abnormalities observed in freshwater fish populations can be properly addressed. The parasites of *O. mossambicus* may be used as indicators of impacted water but the fact that the different groups (eg. copepods and monogeneans, both ectoparasites) reacting differently towards different water constituents must be taken into consideration.

Metal bioaccumulation in fish, toxicity tests and human health risk assessment studies should be considered for future studies. The latter will be able to predict and evaluate the adverse effects that culminate when consuming contaminated fish and to verify the factor of metal biomagnification in all the trophic levels of aquatic ecosystems.

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Addendum A

Table 1: Water quality variables during the first survey (Winter)

Survey 1: June 2005	SASOL Site A	FOSKOR Site B	PMC Site C	Barrage Site D
Time	11:00	07:50	13:15	09:00
Water temperature ˚C	18.03	19.73	18.91	17.56
Dissolved oxygen (mg l^{-1} O_2)	4.75	2.73	5.02	4.65
pH	8.82	8.33	8.61	7.70
Conductivity (EC) mS/m^{-1}	50.4	313.6	304.8	51.6
Salinity ‰	0.24	1.64	1.6	0.25
TDS mg l^{-1}	444	3764	3445	473
Total Water Hardness (mg l^{-1})	220	1040	1180	216
Turbidity NTU	13	6	39	19
Nitrite (mg l^{-1} NO_2^-)	0.02	0.001	0.7	0.03
Nitrate (mg l^{-1} NO_3^-)	0.9	0.7	38.0	2.0
Ammonium (mg l^{-1} NH_4^+)	0.5	0.001	0.2	0.8
Total nitrogen (mg l^{-1} N_2)	1.42	0.701	38.9	2.83
Phosphate (mg l^{-1} PO_4^{3-})	0.2	0.2	0.2	0.2
Sulphate (mg l^{-1} SO_4^{2-})	40	903	1086	24
Chloride (mg l^{-1} Cl^-)	52	256	288	60
Fluoride (mg l^{-1} F^-)	0.5	3.8	2.1	0.4
Sodium (mg l^{-1} Na^+)	39	213	158	47
Potassium (mg l^{-1} K^+)	0.1	155	158	0.1
Calcium (mg l^{-1} Ca^{2+})	28	51	173	26
Magnesium (mg l^{-1} Mg^{2+})	25	221	173	27
Aluminium (mg l^{-1} Al)	0.001	0.02	0.01	0.02
Copper (mg l^{-1} Cu)	0.02	0.021	0.022	0.021
Iron (mg l^{-1} Fe)	0.01	0.021	0.022	0.021
Lead (mg l^{-1} Pd)	0.02	0.07	0.09	0.03
Manganese (mg l^{-1} Mn)	0.03	0.04	0.04	0.03
Zinc (mg l^{-1} Zn)	0.003	0.0041	0.0051	0.0022

Table 2: Water quality variables during the second survey (Spring)

Survey 2: October 2005	SASOL Site A	FOSKOR Site B	PMC Site C	Barrage Site D
Time	08:00	09:00	09:50	07:20
Water temperature ˚C	24.54	24.01	22.28	26.35
Dissolved oxygen (mg l^{-1} O_2)	6.15	4.55	4.73	3.51
pH	8.8	8.72	8.54	7.83
Conductivity (EC) mS/m^{-1}	53.2	318.4	303.4	30.7
Salinity ‰	0.26	1.66	1.58	0.14
TDS mg l^{-1}	337	2961	2620	204
Total Water Hardness (mg l^{-1})	236	1120	1248	230
Turbidity NTU	27	8	35	44
Nitrite (mg l^{-1} NO_2^-)	0.24	0.03	2.09	0.43
Nitrate (mg l^{-1} NO_3^-)	3.0	2.0	23.0	3.0
Ammonium (mg l^{-1} NH_4^+)	0.1	0.1	0.2	0.2
Total nitrogen (mg l^{-1} N_2)	3.34	2.13	25.09	3.63
Phosphate (mg l^{-1} PO_4^{3-})	0.16	0.08	0.06	0.01
Sulphate (mg l^{-1} SO_4^{2-})	45	1127	1105	20
Chloride (mg l^{-1} Cl^-)	64	176	96	28
Fluoride (mg l^{-1} F^-)	0.68	2.6	2.4	0.24
Sodium (mg l^{-1} Na^+)	59	275	221	30
Potassium (mg l^{-1} K^+)	10	182	101	10
Calcium (mg l^{-1} Ca^{2+})	23	50	166	25
Magnesium (mg l^{-1} Mg^{2+})	31	232	183	25
Aluminium (mg l^{-1} Al)	0.01	0.01	0.02	0.01
Copper (mg l^{-1} Cu)	0.010	0.020	0.005	0.009
Iron (mg l^{-1} Fe)	0.001	0.001	0.112	0.001
Lead (mg l^{-1} Pd)	0.001	0.009	0.008	0.001
Manganese (mg l^{-1} Mn)	0.004	0.007	0.06	0.004
Zinc (mg l^{-1} Zn)	0.001	0.001	0.002	0.001

Table 3: Water quality variables during the third survey (Summer)

Survey 3: January 2006	SASOL Site A	FOSKOR Site B	PMC Site C	Barrage Site D
Time	7:20	12:45	14:00	09:10
Water temperature ˚C	28.85	29.64	28.70	26.18
Dissolved oxygen (mg l^{-1} O_2)	2.68	2.87	3.78	3.27
pH	8.14	8.74	8.53	7.83
Conductivity (EC) mS/m^{-1}	49.4	303	393.9	24.5
Salinity ‰	0.24	1.57	2.07	0.10
TDS mg l^{-1}	355	3060	2730	150
Total Water Hardness (mg l^{-1})	83	289	957	49
Turbidity NTU	51	50	48	84
Nitrite (mg l^{-1} NO_2^-)	0.34	1.12	1.93	0.92
Nitrate (mg l^{-1} NO_3^-)	0.1	0.1	28.0	0.1
Ammonium (mg l^{-1} NH_4^+)	0.1	0.1	0.2	0.9
Total nitrogen (mg l^{-1} N_2)	0.54	1.32	30.13	1.92
Phosphate (mg l^{-1} PO_4^{3-})	0.1	0.1	0.1	0.1
Sulphate (mg l^{-1} SO_4^{2-})	25	374	849	13
Chloride (mg l^{-1} Cl^-)	56	216	344	24
Fluoride (mg l^{-1} F^-)	0.6	4.5	3.1	0.2
Sodium (mg l^{-1} Na^+)	30	90	145	11
Potassium (mg l^{-1} K^+)	22	106	128	17
Calcium (mg l^{-1} Ca^{2+})	10	18	155	11
Magnesium (mg l^{-1} Mg^{2+})	11	56	142	6
Aluminium (mg l^{-1} Al)	0.01	0.01	0.01	0.01
Copper (mg l^{-1} Cu)	0.010	0.018	0.004	0.002
Iron (mg l^{-1} Fe)	0.001	0.001	0.110	0.001
Lead (mg l^{-1} Pd)	0.001	0.001	0.024	0.001
Manganese (mg l^{-1} Mn)	0.004	0.006	0.055	0.005
Zinc (mg l^{-1} Zn)	0.001	0.001	0.024	0.001

Table 4: Water quality variables during the fourth survey (Autumn)

Survey 4: April 2006	SASOL Site A	FOSKOR Site B	PMC Site C	Barrage Site D
Time	8:40	9:15	9:30	15:40
Water temperature ˚C	22.26	23	22.78	22.37
Dissolved oxygen (mg l^{-1} O_2)	1.84	2.05	1.99	1.68
pH	7.72	8.47	8.15	8.52
Conductivity (EC) mS/m^{-1}	51.5	298	365.2	36.6
Salinity ‰	0.25	1.55	1.93	0.17
TDS mg l^{-1}	362	2609	3434	258
Total Water Hardness (mg l^{-1})	220	1040	1180	216
Turbidity NTU	35	6	25	16
Nitrite (mg l^{-1} NO_2^-)	0.11	0	2.03	0.01
Nitrate (mg l^{-1} NO_3^-)	4.0	4.0	56.0	8.0
Ammonium (mg l^{-1} NH_4^+)	0.2	0.1	0.1	0.2
Total nitrogen (mg l^{-1} N_2)	4.31	4.1	58.13	8.21
Phosphate (mg l^{-1} PO_4^{3-})	0.2	0.2	1.31	0.2
Sulphate (mg l^{-1} SO_4^{2-})	72	899	1502	47
Chloride (mg l^{-1} Cl^-)	60	244	340	36
Fluoride (mg l^{-1} F^-)	0.42	3.3	2.8	0.21
Sodium (mg l^{-1} Na^+)	21	195	208	12
Potassium (mg l^{-1} K^+)	0.1	165	137	0.1
Calcium (mg l^{-1} Ca^{2+})	13	43	244	13
Magnesium (mg l^{-1} Mg^{2+})	18	175	225	6
Aluminium (mg l^{-1} Al)	0.01	0	0	0.01
Copper (mg l^{-1} Cu)	0.092	0.067	0.19	0.125
Iron (mg l^{-1} Fe)	0.031	0.035	0.043	0.001
Lead (mg l^{-1} Pd)	0.001	0.001	0.001	0.001
Manganese (mg l^{-1} Mn)	0.02	0.009	0.14	0.003
Zinc (mg l^{-1} Zn)	0.0342	0.0412	0.0449	0.0221

Addendum B

Table 1: Health Assessment Index of *Oreochromis mossambicus* Survey 1 (Winter)

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hindgut	Kidneys	Blood values			Parasites		HAI values
	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site A (Sasol)																			
2S1	220	170	181.5	M	0	0	20	0	0	30	0	0	0	0	0	30	10	10	100
2S2	250	200	261.4	M	0	0	20	0	0	0	0	0	0	0	30	30	10	10	100
2S3	235	190	223.5	M	0	0	20	0	0	0	0	0	0	0	30	30	0	10	90
2S9	270	210	243.3	M	0	0	0	0	0	0	0	0	0	30	-	-	20	20	70
2S11	245	210	264.1	F	0	0	0	0	0	0	0	0	0	20	-	-	10	10	40
2S13	240	195	236.7	F	0	0	0	0	0	0	0	0	0	0	-	-	10	10	20
2S15	180	155	109.8	F	0	0	20	0	0	0	0	0	0	30	-	30	0	10	90
2S16	230	210	210.9	F	0	0	20	0	0	0	0	0	0	20	0	0	0	10	50
2S18	200	180	136.5	F	0	0	0	0	0	0	0	0	0	30	-	-	10	10	50
2S20	235	205	229.3	F	0	0	0	0	0	0	0	0	0	20	-	-	10	10	40
																		Final	**650**
																		Mean	**65**
Site B (Foskor)																			
2F1	181	140	96.0	F	0	0	0	0	0	30	0	0	0	30	-	-	10	10	80
2F2	250	200	286.8	F	0	0	0	0	0	30	0	0	0	30	-	-	10	10	80
2F3	240	185	217.8	F	0	0	0	0	0	0	0	0	0	20	30	30	0	10	90
2F4	272	210	367.9	F	0	0	0	0	0	0	0	0	0	20	30	30	0	10	90
2F8	198	155	121.4	F	0	0	0	0	0	0	0	0	0	30	-	-	0	10	40
2F10	273	211	367.1	F	0	0	0	0	0	0	0	0	0	20	30	30	10	10	100
2F12	192	150	106.8	M	0	0	0	0	0	0	0	0	0	30	0	0	0	10	40
2F17	185	149	100.0	M	0	0	0	0	0	0	0	0	0	30	-	-	0	0	30
2F19	194	159	118.9	M	0	0	0	0	0	30	0	0	0	0	30	30	10	20	120
2F20	393	302	1075	M	0	0	20	0	0	30	0	0	0	30	30	30	10	10	160
																		Final	**830**
																		Mean	**83**

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-	Gills	Liver	Spleen	Hind	Kidneys	Blood values			Parasites		HAI
												g							

	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site C (PMC)																			
2P24	330	280	656.2	F	0	0	0	0	0	30	0	0	0	20	0	30	20	20	120
2P25	342	267	674.0	F	0	0	0	0	0	0	0	0	0	20	30	0	10	10	70
2P26	313	255	638.3	F	0	0	20	0	0	30	0	0	0	20	0	30	20	20	140
2P27	330	260	706.9	F	0	0	20	0	30	30	0	0	0	0	0	30	10	20	140
2P28	329	264	619.0	F	0	0	10	0	0	30	0	0	0	0	30	30	20	20	140
2P29	330	260	677.6	F	0	0	20	0	0	30	0	0	0	20	-	-	10	20	100
2P30	295	240	514.5	M	0	0	20	0	0	30	0	0	0	0	0	0	10	10	70
2P31	315	260	635.1	F	0	0	10	0	30	0	0	0	0	0	-	0	10	20	70
2P32	312	250	563.3	F	0	0	10	0	0	30	0	0	0	0	0	0	20	20	70
2P33	336	270	735.8	F	0	0	20	0	0	30	0	0	0	0	-	-	10	10	70
																		Final	**990**
																		Mean	**99**
Site D (Barrage)																			
2B39	223	180	221.0	M	0	0	10	0	0	0	0	0	0	30	30	30	0	10	110
2B40	235	190	262.5	F	0	0	10	0	0	0	0	0	0	-	-	-	0	10	20
2B41	239	183	239.6	M	0	0	0	0	0	0	0	0	0	0	30	0	10	10	50
2B42	225	180	211.8	F	0	0	10	0	0	30	0	0	0	20	30	30	10	20	150
2B43	235	185	230.5	M	0	0	0	0	0	0	0	0	0	-	-	-	10	10	20
2B44	218	170	220.4	M	0	0	10	0	0	0	0	0	0	20	30	30	0	10	100
2B45	230	180	250.6	F	0	0	10	0	0	0	0	0	0	0	30	0	0	10	50
2B46	230	186	224.8	M	0	0	0	0	0	0	0	0	0	0	30	0	0	10	40
2B47	221	225	221.0	M	0	0	10	0	0	0	0	0	0	0	0	0	0	10	20
2B48	222	173	203.7	M	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10
																		Final	**570**
																		Mean	**57**

Table 1 Continued

Table 2: Health Assessment Index of *Oreochromis mossambicus* Survey 2 (Spring)

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-	Gills	Liver	Spleen	Hind	Kidneys	Blood values			Parasites		HAI
	TL	SL						cules				gut		Hct	Plasma	WBC	Ecto	Endo	values
Site A (Sasol)																			
BS1	242	190	240.5	M	0	0	10	0	0	0	0	0	0	0	30	0	10	10	60
BS2	264	219	311.8	M	0	0	10	0	0	0	0	0	0	30	30	30	10	10	120
BS3	250	200	257.0	M	0	0	10	0	0	30	0	0	0	30	30	30	10	10	150
BS4	265	220	277.0	M	0	0	10	0	0	30	0	0	0	0	0	30	0	20	90
BS5	258	203	289.8	M	0	0	0	0	0	0	0	0	0	20	0	0	10	10	40
BS6	262	205	285.7	M	30	0	20	0	0	30	0	0	0	20	30	30	10	20	190
BS7	250	200	248.1	F	0	0	10	0	0	0	0	0	0	20	30	0	0	20	80
BS9	266	200	283.4	M	0	0	0	0	0	30	0	0	0	20	0	0	10	10	70
BS10	234	184	224	M	0	0	10	0	0	30	0	0	0	0	0	0	10	10	60
BS11	245	203	253.4	F	0	0	0	0	0	0	0	0	0	0	30	30	10	10	80
																		Final	**940**
																		Mean	**94**
Site B (Foskor)																			
BF12	325	255	502.2	F	0	0	10	0	0	0	0	0	0	20	30	30	0	10	100
BF17	355	305	951.3	M	0	20	10	0	0	30	0	0	0	0	30	30	10	20	150
BF18	354	290	697.2	F	0	0	0	0	0	30	0	0	0	20	0	30	0	10	90
BF19	420	335	1388.8	F	0	0	0	0	0	30	0	0	0	0	0	30	0	10	70
BF20	290	259	448.0	F	0	0	10	0	0	0	0	10	0	20	30	30	10	10	120
BF24	261	214	334.0	F	0	0	10	0	0	0	0	0	0	20	-	0	0	10	40
BF25	245	195	230.1	F	0	0	10	0	0	0	0	0	0	0	0	0	0	10	20
BF26	289	238	417.2	M	0	0	0	0	0	0	0	0	0	20	0	0	10	10	40
BF27	287	232	391.2	F	0	0	10	0	0	0	0	0	0	0	0	0	0	10	20
BF28	330	240	421.5	F	0	0	10	0	30	0	0	0	0	0	0	0	10	10	60
																		Final	**710**
																		Mean	**71**

Fish	Length	Mass	Sex	Eyes	Skin	Fins	Oper-	Gills	Liver	Spleen	Hind	Kidneys	Blood values	Parasites	HAI

	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site C (PMC)																			
BP30	255	200	325.4	M	0	10	0	0	30	0	0	0	0	0	0	30	20	10	100
BP31	280	220	451.3	M	0	10	0	0	0	0	0	0	0	0	30	0	30	10	80
BP32	320	260	671.3	F	0	0	20	0	30	0	0	10	0	20	0	30	30	20	160
BP33	261	205	372.0	M	0	0	0	0	0	0	0	0	0	20	0	0	10	10	40
BP34	360	288	854.0	M	0	0	10	0	0	0	0	0	0	0	30	30	30	10	110
BP35	285	225	476.6	F	0	0	20	0	30	0	0	0	0	20	-	30	20	20	140
BP36	272	219	385.4	M	0	0	0	0	0	0	0	0	0	20	0	0	20	10	50
BP37	282	235	455.8	F	0	0	0	0	0	0	0	0	0	20	-	-	0	20	40
BP38	270	220	337.4	F	0	0	10	0	30	0	0	0	0	20	30	30	20	10	150
BP39	275	215	425.6	M	0	0	10	0	0	0	0	0	0	0	-	-	30	10	50
																		Final	**920**
																		Mean	**92**
Site D (Barrage)																			
BB1	215	165	196.2	M	0	0	10	0	0	30	0	0	0	0	30	0	10	10	90
BB2	235	185	250.1	M	0	0	10	0	0	30	0	0	0	-	-	-	0	10	50
BB3	190	150	124.0	F	0	0	10	0	0	30	0	0	0	0	30	0	10	10	80
BB4	217	175	201.2	M	0	0	10	0	0	30	0	0	0	30	30	30	0	10	140
BB5	234	181	204.5	M	0	0	10	0	0	30	0	0	0	30	-	-	0	10	80
BB6	217	171	159.2	F	0	0	10	0	0	30	0	0	0	20	30	30	10	10	110
BB7	230	188	154.7	F	0	0	10	0	0	0	0	0	0	20	30	30	20	10	130
BB8	222	177	163.3	M	0	0	10	0	0	30	0	0	0	30	30	30	10	10	140
BB9	220	176	146.2	M	0	0	10	0	0	30	0	0	0	20	0	30	0	10	100
BB10	225	185	175.0	M	0	0	10	0	0	0	0	0	0	0	0	0	10	10	30
																		Final	**950**
																		Mean	**95**

Table 2 Continued

Table 3: Health Assessment Index of *Oreochromis mossambicus* Survey 3 (Summer)

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Blood values			Parasites		HAI values
	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site A (Sasol)																			
3S1	185	140	114.9	F	0	0	0	0	0	30	0	0	0	0	0	30	0	10	70
3S2	190	150	128.4	F	0	0	10	0	0	0	0	0	0	30	30	0	0	10	80
3S3	175	142	99.0	F	0	0	10	0	0	0	0	0	0	-	-	-	0	10	20
3S4	230	180	227.6	M	0	0	10	0	0	0	0	0	0	30	30	0	0	10	80
3S5	195	155	137.8	M	0	0	10	0	0	0	0	0	0	20	0	0	0	10	40
3S6	180	140	102.2	F	0	0	10	0	0	0	0	0	0	30	0	0	0	10	50
3S8	250	195	225.5	M	0	0	10	0	0	30	0	0	0	20	-	-	0	20	80
3S9	242	190	224.1	M	0	0	10	0	0	0	0	0	0	30	0	0	0	10	50
3S10	240	193	233.3	M	0	0	10	0	0	0	0	0	0	20	0	0	0	10	40
3S11	260	201	251.1	M	0	0	20	0	0	30	0	0	0	20	30	30	0	20	150
																		Final	**660**
																		Mean	**66**
Site B (Foskor)																			
3F12	320	260	544.8	M	0	0	10	0	30	0	0	0	0	30	0	30	0	10	110
3F14	327	276	601.3	F	0	0	0	0	0	0	0	0	0	30	-	-	0	20	50
3F15	337	276	610.4	F	0	0	10	0	0	0	0	0	0	20	0	0	0	10	40
3F16	320	260	620.8	M	0	0	0	0	0	0	0	0	0	20	0	0	0	10	30
3F17	245	200	257.4	F	0	0	0	0	0	0	0	0	0	30	-	-	0	10	40
3F18	231	187	202.7	M	0	0	0	0	0	30	0	0	0	30	30	30	0	10	130
3F19	254	211	281.9	F	0	0	0	0	0	0	0	0	0	20	30	30	0	10	90
3F20	207	170	172.3	F	0	0	0	0	0	30	0	0	0	20	30	30	0	10	120
3F21	245	200	245.7	M	0	0	0	0	0	30	0	0	0	20	0	30	0	10	90
3F27	240	190	235.0	F	0	0	0	0	0	0	0	0	0	20	0	0	0	10	30
																		Final	**730**
																		Mean	**73**

Table 3 Continued

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper cules	Gills	Liver	Spleen	Hind gut	Kidneys	Blood values			Parasites		HAI values
	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site C (PMC)																			
3P25	370	295	800.5	M	0	0	20	0	0	30	0	0	0	20	30	30	10	20	160
3P27	314	265	623.6	F	30	0	20	0	0	30	0	0	0	20	30	30	0	20	180
3P29	365	290	777.6	M	30	0	20	0	0	30	0	0	0	0	30	30	0	20	160
3P30	365	290	692.8	M	0	0	20	0	0	30	0	0	0	0	30	30	0	20	130
3P31	345	285	656.8	M	30	0	10	0	0	30	0	0	0	0	30	30	0	10	140
3P32	342	275	639.9	M	30	0	20	0	0	0	0	0	0	0	30	30	0	20	130
3P34	248	200	297.8	F	0	0	10	0	0	0	0	0	0	0	30	0	0	10	50
3P35	370	300	745.7	M	0	0	20	0	0	0	0	0	0	0	30	30	0	20	100
3P36	309	255	566.8	M	0	0	10	0	0	0	0	0	0	10	30	0	0	10	60
3P40	315	270	682.5	M	0	0	10	0	0	30	0	0	0	30	30	30	10	20	160
																		Final	**1270**
																		Mean	**127**
Site D (Barrage)																			
3B43	260	200	285.0	M	0	0	10	0	0	30	0	0	0	30	30	30	0	10	140
3B44	205	165	151.9	F	0	0	0	0	0	30	0	0	0	30	0	30	0	10	100
3B45	224	183	202.5	M	0	0	0	0	0	30	0	0	0	20	30	30	0	10	120
3B46	230	182	212.3	M	0	0	10	0	0	0	0	0	0	20	30	0	0	10	70
3B47	220	175	197.9	F	0	0	0	0	0	30	0	0	0	20	30	30	0	10	120
3B48	230	180	206.2	M	0	0	0	0	0	30	0	0	0	30	30	30	0	10	130
3B49	240	190	255.8	M	0	0	10	0	0	30	0	0	0	20	30	30	0	10	130
3B50	210	165	177.2	F	0	0	10	0	0	30	0	0	0	20	0	30	0	10	100
3B51	213	172	172.1	M	0	0	0	0	0	30	0	0	0	0	0	30	0	10	70
3B52	230	190	174.7	M	0	0	10	0	0	30	0	0	0	0	30	30	0	10	110
																		Final	**1090**
																		Mean	**109**

Table 4: Health Assessment Index of *Oreochromis mossambicus* Survey 4 (Autumn)

Fish	Length		Mass	Sex	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Hind gut	Kidneys	Blood values			Parasites		HAI values
	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site A (Sasol)																			
4S37	240	190	260.5	F	0	0	10	0	0	30	0	0	0	0	0	30	10	10	90
4S38	230	180	212.8	F	0	0	20	0	0	30	0	0	0	30	-	-	10	20	110
4S39	230	180	219.6	F	0	0	20	0	0	30	0	0	0	20	0	30	10	20	130
4S40	210	160	169.1	F	0	0	10	0	0	30	0	0	0	20	30	30	0	10	130
4S41	210	170	143.4	F	0	0	10	0	0	30	0	0	0	0	30	30	10	10	120
4S42	210	180	189.0	F	0	0	10	0	0	0	0	0	0	0	30	30	10	10	90
4S43	210	170	165.5	F	0	0	0	0	0	30	0	0	0	20	30	30	10	10	130
4S44	200	150	127.0	M	0	0	10	0	0	30	0	0	0	20	-	-	10	10	80
4S45	200	150	127.1	F	0	0	10	0	0	30	0	0	0	20	30	30	10	10	140
4S46	195	155	132.0	F	0	0	10	0	0	30	0	0	0	20	-	-	10	10	80
																		Final	**1090**
																		Mean	**109**
Site B (Foskor)																			
4F25	270	210	364.0	F	0	0	0	0	0	30	0	0	0	20	30	30	0	10	120
4F26	290	230	433.8	F	0	0	0	0	0	30	0	0	0	20	-	-	0	10	60
4F27	290	260	460.0	F	0	0	0	0	0	30	0	0	0	0	30	30	0	10	100
4F28	280	220	396.1	F	0	0	10	0	0	0	0	0	0	0	-	-	0	10	20
4F29	285	210	383.0	F	0	0	0	0	0	30	0	0	0	0	0	0	0	10	40
4F30	255	210	315.2	F	0	0	0	0	0	30	0	0	0	20	30	30	0	10	120
4F31	245	205	254.8	F	0	0	0	0	0	30	0	0	0	20	30	30	10	10	130
4F34	260	210	155.7	F	0	0	0	0	0	30	0	0	0	20	0	30	10	10	100
4F35	235	190	209.0	F	0	0	0	0	0	30	0	0	0	30	30	30	0	10	130
4F36	210	170	154.0	F	0	0	0	0	0	30	0	0	0	30	30	30	10	10	140
																		Final	**960**
																		Mean	**96**

Fish	Length	Mass	Sex	Eyes	Skin	Fins	Oper-	Gills	Liver	Spleen	Hind	Kidneys	Blood values	Parasites	HAI

	TL	SL												Hct	Plasma	WBC	Ecto	Endo	
Site C (PMC)																			
4P6	295	255	564.5	F	0	0	10	0	0	30	0	0	0	30	0	30	10	20	130
4P7	245	270	640.8	F	0	0	10	0	30	30	0	0	0	30	0	30	10	20	160
4P8	280	220	414.6	F	0	0	10	0	0	30	0	0	0	30	30	30	10	20	160
4P9	310	250	600.3	F	0	0	10	0	0	30	0	0	0	20	-	-	0	20	80
4P10	270	210	367.5	F	0	0	0	0	0	30	0	0	0	20	0	30	10	10	100
4P11	360	290	665.8	F	0	0	20	0	0	30	0	0	0	20	0	30	10	20	130
4P12	350	180	583.0	F	0	0	10	0	0	30	0	0	0	20	30	30	10	20	150
4P13	370	210	339.5	F	0	0	0	0	0	30	0	0	0	30	30	30	10	10	140
4P14	360	290	702.2	M	0	0	20	0	30	30	0	0	0	20	-	-	10	20	130
4P15	250	190	402.2	F	0	0	0	0	30	30	0	0	0	20	-	-	10	10	100
																		Final	**1280**
																		Mean	**128**
Site D (Barrage)																			
4B1	190	150	88.4	M	0	0	0	0	0	30	0	0	0	0	-	-	10	10	50
4B2	140	110	42.7	F	0	0	0	0	0	30	0	0	0	0	30	30	0	10	100
4B3	130	110	33.7	M	0	0	0	0	0	30	0	0	0	0	-	-	0	0	30
4B4	120	100	25.4	M	0	0	0	0	0	30	0	0	0	0	-	-	0	10	40
4B5	100	80	17.5	F	0	0	0	0	0	30	0	0	0	20	-	-	0	10	60
4B6	95	75	12.5	F	0	0	0	0	0	30	0	0	0	0	-	-	0	0	30
4B51	215	170	142.0	M	0	0	0	0	0	30	0	0	0	0	30	30	10	10	110
4B52	200	160	128.0	F	0	0	0	0	0	30	0	0	0	0	30	30	10	10	110
4B53	200	160	132.0	M	0	0	0	0	0	30	0	0	0	0	-	-	0	10	40
4B54	175	130	81.6	F	0	0	0	0	0	30	0	0	0	0	-	-	10	10	50
																		Final	**620**
																		Mean	**62**

Table 4 Continued

Addendum C

Table 1: Total number of parasites recorded from *Oreochromis mossambicus* during survey 1 (Winter)

Sasosl (Site A)														
Species	**Fish #**	**Monog.**	**Copep.**	**Branch.**	**Ecto tot.**	**Digen. L**	**Digen. A**	**Cestode L**	**Cestode A**	**Nemat. L**	**Nemat. A.**	**Acantho.**	**Pentast.**	**Endo tot.**
O.mos.	AS1	3	0	0	**3**	15	0	0	0	1	0	0	0	**16**
O.mos.	AS2	1	0	0	**1**	48	0	0	0	0	0	0	0	**48**
O.mos.	AS3	0	0	0	**0**	45	0	7	0	1	0	0	0	**53**
O.mos.	2S9	11	0	0	**11**	105	0	100	0	1	0	0	0	**206**
O.mos.	2S11	1	0	0	**1**	7	0	50	0	0	0	0	0	**57**
O.mos.	2S13	1	0	0	**1**	4	0	0	0	0	0	0	0	**4**
O.mos.	2S15	0	0	0	**0**	46	0	8	0	0	0	0	0	**54**
O.mos.	2S16	0	0	0	**0**	47	0	0	0	1	0	0	0	**48**
O.mos.	2S18	2	0	0	**2**	2	0	2	0	0	0	0	0	**4**
O.mos.	2S20	2	0	0	**2**	11	0	5	0	0	0	0	0	**16**
Total	**10**	**21**	**0**	**0**	**21**	**330**	**0**	**172**	**0**	**4**	**0**	**0**	**0**	**506**
Foskor (Site B)														
O.mos.	2F1	1	0	0	**1**	0	0	11	0	3	0	0	0	**14**
O.mos.	2F2	1	0	0	**1**	2	0	1	0	2	0	0	0	**5**
O.mos.	2F3	0	0	0	**0**	0	0	1	0	1	0	0	0	**2**
O.mos.	2F4	0	0	0	**0**	1	0	0	0	3	0	0	0	**4**
O.mos.	2F8	0	0	0	**0**	1	0	0	0	0	0	0	0	**1**
O.mos.	2F10	6	0	0	**6**	3	0	0	0	4	0	0	0	**7**
O.mos.	2F12	1	0	0	**1**	1	0	1	0	0	0	0	0	**2**
O.mos.	2F17	0	0	0	**0**	0	0	2	0	0	0	0	0	**2**
O.mos.	2F19	1	0	0	**1**	0	0	7	0	0	0	0	0	**7**
O.mos.	AF12	1	0	0	**1**	7	0	1	0	1	0	0	0	**9**
Total	**10**	**11**	**0**	**0**	**11**	**15**	**0**	**24**	**0**	**14**	**0**	**0**	**0**	**53**

Table 1 Continued

Species	Fish #	Monog.	Copep.	Branch.	Ecto tot.	Digen. L	Digen. A	Cestode L	Cestode A	Nemat. L	Nemat. A.	Acantho.	Pentast.	Endo tot.
PMC (Site C)														
O.mos.	2P24	0	16	0	**16**	228	0	14	0	0	0	0	0	**242**
O.mos.	2P25	0	9	0	**9**	29	0	0	0	1	0	0	0	**30**
O.mos.	2P26	0	11	0	**11**	345	0	0	0	0	0	0	0	**345**
O.mos.	2P27	4	6	0	**10**	106	0	0	0	0	0	0	1	**107**
O.mos.	2P28	0	19	0	**19**	278	0	0	0	0	0	0	1	**279**
O.mos.	2P29	0	3	0	**3**	366	0	0	0	0	0	0	1	**367**
O.mos.	2P30	0	6	0	**6**	57	0	0	0	0	0	0	0	**57**
O.mos.	2P31	0	7	0	**7**	174	0	15	0	0	0	0	1	**190**
O.mos.	2P32	2	9	0	**11**	476	0	0	0	0	0	0	2	**478**
O.mos.	2P33	0	7	0	**7**	77	0	0	0	0	0	0	0	**77**
Total	**10**	**6**	**93**	**0**	**99**	**2136**	**0**	**29**	**0**	**1**	**0**	**0**	**6**	**2172**
Barrage (Site D)														
O.mos.	2B39	0	0	0	**0**	15	0	1	0	0	0	0	0	**16**
O.mos.	2B40	0	0	0	**0**	80	0	0	0	0	0	0	0	**80**
O.mos.	2B41	3	0	0	**3**	29	0	0	0	0	0	0	0	**29**
O.mos.	2B42	1	0	0	**1**	125	0	0	0	0	0	0	0	**125**
O.mos.	2B43	1	0	0	**1**	28	0	0	0	0	0	0	0	**28**
O.mos.	2B44	0	0	0	**0**	5	0	0	0	0	0	0	1	**6**
O.mos.	2B45	0	0	0	**0**	28	0	0	0	1	0	0	0	**29**
O.mos.	2B46	0	0	0	**0**	38	0	0	0	0	0	0	0	**38**
O.mos.	2B47	0	0	0	**0**	119	0	0	0	0	0	0	0	**119**
O.mos.	2B48	0	0	0	**0**	2	0	13	0	0	0	0	0	**15**
Total	**10**	**5**	**0**	**0**	**5**	**469**	**0**	**14**	**0**	**1**	**0**	**0**	**1**	**485**

O.mos. = *Oreochromis mossambicus*; L Ə Larva; A Ə Adult; Monog. Ə Monogenea; Copep. Ə Copepoda; Branch. Ə Branchiura; Digen. Ə Digenea; Nemat. Ə Nematoda; Acantho. Ə Acanthocephala; Pentast. Ə Pentastomida; Ecto tot. = total ectoparasites; Endo tot. = total endoparasites

Table 2: Total number of parasites recorded from *Oreochromis mossambicus* during survey 2 (Spring)

Species	Fish #	Monog.	Copep.	Branch.	Ecto tot.	Digen. L	Digen. A	Cestode L	Cestode A	Nemat. L	Nemat. A.	Acantho.	Pentast.	Endo tot.
Sasol (Site A)														
O.mos.	BS1	3	0	0	**3**	34	0	0	0	0	0	0	0	**34**
O.mos.	BS2	3	0	0	**3**	66	0	0	0	0	0	0	0	**66**
O.mos.	BS3	2	3	0	**5**	60	0	0	0	0	0	1	0	**61**
O.mos.	BS4	0	0	0	**0**	265	0	0	0	5	0	7	0	**277**
O.mos.	BS5	5	0	0	**5**	9	0	0	0	0	0	0	0	**9**
O.mos.	BS6	5	0	1	**6**	110	0	0	0	0	0	0	0	**110**
O.mos.	BS7	0	0	0	**0**	120	0	0	0	0	0	0	0	**120**
O.mos.	BS9	0	0	1	**1**	3	0	0	0	0	0	0	0	**3**
O.mos.	BS10	1	0	0	**1**	60	0	1	0	0	0	0	0	**61**
O.mos.	BS11	9	0	0	**9**	14	0	26	0	1	0	9	0	**60**
Total	**10**	**28**	**3**	**2**	**33**	**741**	**0**	**27**	**0**	**6**	**0**	**17**	**0**	**801**
Foskor (Site B)														
O.mos.	BF12	0	0	0	**0**	14	0	18	0	1	0	0	0	**33**
O.mos.	BF17	1	0	0	**1**	18	0	16	0	117	0	0	0	**151**
O.mos.	BF18	0	0	0	**0**	0	0	29	0	0	0	0	0	**29**
O.mos.	BF19	0	0	0	**0**	2	0	0	0	2	0	0	0	**4**
O.mos.	BF20	3	0	0	**3**	6	0	50	0	4	0	0	0	**60**
O.mos.	BF24	0	0	0	**0**	24	0	2	0	0	0	0	0	**26**
O.mos.	BF25	0	0	0	**0**	3	0	1	0	0	0	0	0	**4**
O.mos.	BF26	2	0	0	**2**	2	0	0	0	0	0	0	0	**2**
O.mos.	BF27	0	0	0	**0**	7	0	13	0	0	0	0	0	**20**
O.mos.	BF28	2	0	0	**2**	33	0	44	0	1	0	0	0	**78**
Total	**10**	**8**	**0**	**0**	**8**	**109**	**0**	**173**	**0**	**125**	**0**	**0**	**0**	**407**

Table 2 Continued

Species	Fish #	Monog.	Copep.	Branch.	Ecto tot.	Digen. L	Digen. A	Cestode L	Cestode A	Nemat. L	Nemat. A.	Acantho.	Pentast.	Endo tot.
PMC (Site C)														
O.mos.	BP30	3	9	0	**12**	52	0	0	0	0	0	0	0	**52**
O.mos.	BP31	1	36	0	**37**	11	0	0	0	0	0	0	0	**11**
O.mos.	BP32	0	37	0	**37**	103	0	0	0	0	0	0	0	**103**
O.mos.	BP33	0	9	0	**9**	1	0	0	0	0	0	0	0	**1**
O.mos.	BP34	0	94	0	**94**	29	0	0	0	0	0	0	0	**29**
O.mos.	BP35	0	19	0	**19**	185	0	8	0	0	0	0	0	**193**
O.mos.	BP36	2	11	0	**13**	66	0	0	0	0	0	0	0	**66**
O.mos.	BP37	0	0	0	**0**	325	0	0	0	0	0	0	0	**325**
O.mos.	BP38	3	12	0	**15**	14	0	0	0	0	0	0	0	**14**
O.mos.	BP39	2	13	0	**15**	95	0	0	0	0	0	0	0	**95**
Total	**10**	**11**	**240**	**0**	**251**	**881**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**889**
Barrage (Site D)														
O.mos.	BB1	3	0	0	**3**	11	0	0	0	0	0	0	0	**11**
O.mos.	BB2	0	0	0	**0**	13	0	0	0	0	0	0	0	**13**
O.mos.	BB3	1	0	0	**1**	6	0	0	0	0	0	0	0	**6**
O.mos.	BB4	0	0	0	**0**	8	0	0	0	0	0	0	0	**8**
O.mos.	BB5	0	0	0	**0**	23	0	0	0	0	0	1	3	**27**
O.mos.	BB6	4	0	0	**4**	23	0	13	0	0	0	0	0	**36**
O.mos.	BB7	11	0	0	**11**	10	0	16	0	0	0	0	0	**26**
O.mos.	BB8	5	0	0	**5**	7	0	0	0	0	0	0	0	**7**
O.mos.	BB9	0	0	0	**0**	13	0	0	0	0	0	0	0	**13**
O.mos.	BB10	2	0	0	**2**	19	0	0	0	0	0	0	0	**19**
Total	**10**	**26**	**0**	**0**	**26**	**133**	**0**	**29**	**0**	**0**	**0**	**1**	**3**	**166**

O.mos. = *Oreochromis mossambicus*; L Ə Larva; A Ə Adult; Monog. Ə Monogenea; Copep. Ə Copepoda; Branch. Ə Branchiura; Digen. Ə Digenea; Nemat. Ə Nematoda; Acantho. Ə Acanthocephala; Pentast. Ə Pentastomida; Ecto tot. = total ectoparasites; Endo tot. = total endoparasites

Table 3: Total number of parasites recorded from *Oreochromis mossambicus* during survey 3 (Summer)

Sasol (Site A)														
Species	**Fish #**	**Monog.**	**Copep.**	**Branch.**	**Ecto tot.**	**Digen. L**	**Digen. A**	**Cestode L**	**Cestode A**	**Nemat. L**	**Nemat. A.**	**Acantho.**	**Pentast.**	**Endo tot.**
O.mos.	3S1	0	0	0	**0**	7	0	0	0	0	0	1	0	**8**
O.mos.	3S2	1	0	0	**1**	41	0	2	0	0	0	0	0	**43**
O.mos.	3S3	0	0	0	**0**	27	0	0	0	0	0	0	6	**33**
O.mos.	3S4	0	0	0	**0**	43	0	0	0	0	0	6	0	**49**
O.mos.	3S5	0	0	0	**0**	11	0	0	0	0	0	0	0	**11**
O.mos.	3S6	0	0	0	**0**	37	0	12	0	0	0	3	0	**52**
O.mos.	3S8	1	0	0	**1**	90	0	8	0	1	0	0	3	**102**
O.mos.	3S9	0	0	0	**0**	25	0	10	0	2	0	0	0	**37**
O.mos.	3S10	0	0	0	**0**	83	0	1	0	0	0	0	0	**84**
O.mos.	3S11	0	0	0	**0**	161	0	0	0	0	0	0	0	**161**
Total	**10**	**2**	**0**	**0**	**2**	**525**	**0**	**33**	**0**	**3**	**0**	**10**	**9**	**580**
Foskor (Site B)														
O.mos.	3F12	0	0	0	**0**	29	0	53	0	0	0	0	0	**82**
O.mos.	3F14	0	0	0	**0**	5	0	107	0	0	0	0	0	**112**
O.mos.	3F15	0	0	0	**0**	45	0	14	0	0	0	0	0	**59**
O.mos.	3F16	0	0	0	**0**	3	0	0	0	0	0	0	0	**3**
O.mos.	3F17	0	0	0	**0**	7	0	27	0	1	0	0	0	**35**
O.mos.	3F18	0	0	0	**0**	2	0	14	0	1	0	0	0	**17**
O.mos.	3F19	0	0	0	**0**	0	0	46	0	6	0	0	0	**52**
O.mos.	3F20	0	0	0	**0**	1	0	24	0	1	0	0	0	**26**
O.mos.	3F21	0	0	0	**0**	2	0	21	0	0	0	0	0	**23**
O.mos.	3F37	0	0	0	**0**	1	0	14	0	2	0	0	0	**17**
Total	**10**	**0**	**0**	**0**	**0**	**95**	**0**	**320**	**0**	**11**	**0**	**0**	**0**	**421**

Table 3 Continued

PMC (Site C)														
Species	**Fish #**	**Monog.**	**Copep.**	**Branch.**	**Ecto tot.**	**Digen. L**	**Digen. A**	**Cestode L**	**Cestode A**	**Nemat. L**	**Nemat. A.**	**Acantho.**	**Pentast.**	**Endo tot.**
O.mos.	3P25	0	1	0	**1**	373	0	5	0	0	0	0	1	**369**
O.mos.	3P27	0	0	0	**0**	205	0	0	0	0	0	0	0	**205**
O.mos.	3P29	0	0	0	**0**	254	0	4	0	0	0	0	0	**258**
O.mos.	3P30	0	0	0	**0**	326	0	32	0	0	0	0	0	**358**
O.mos.	3P31	0	0	0	**0**	96	0	1	0	0	0	0	0	**97**
O.mos.	3P32	0	0	0	**0**	347	0	2	0	0	0	0	0	**349**
O.mos.	3P34	0	0	0	**0**	44	0	0	0	0	0	0	0	**44**
O.mos.	3P35	0	0	0	**0**	915	0	5	0	0	0	0	0	**920**
O.mos.	3P36	0	0	0	**0**	7	0	0	0	0	0	0	0	**69**
O.mos.	3P40	0	2	0	**2**	230	0	6	0	0	0	0	0	**236**
Total	**10**	**0**	**3**	**0**	**3**	**2859**	**0**	**55**	**0**	**0**	**0**	**0**	**1**	**2905**
Barrage (Site D)														
O.mos.	3B43	0	0	0	**0**	44	0	0	0	0	0	0	1	**45**
O.mos.	3B44	0	0	0	**0**	7	0	0	0	0	0	0	5	**12**
O.mos.	3B45	0	0	0	**0**	21	0	0	0	0	0	0	3	**24**
O.mos.	3B46	0	0	0	**0**	19	0	0	0	0	0	0	0	**19**
O.mos.	3B47	0	0	0	**0**	21	0	0	0	0	0	0	3	**24**
O.mos.	3B48	0	0	0	**0**	4	0	0	0	0	0	0	1	**4**
O.mos.	3B49	0	0	0	**0**	22	0	0	0	0	0	0	0	**23**
O.mos.	3B50	0	0	0	**0**	21	0	0	0	0	0	0	1	**22**
O.mos.	3B51	0	0	0	**0**	8	0	0	0	0	0	0	3	**11**
O.mos.	3B52	0	0	0	**0**	28	0	0	0	0	0	0	0	**28**
Total	**10**	**0**	**0**	**0**	**0**	**195**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**188**

O.mos. = *Oreochromis mossambicus*; L Ə Larva; A Ə Adult; Monog. Ə Monogenea; Copep. Ə Copepoda; Branch. Ə Branchiura; Digen. Ə Digenea; Nemat. Ə Nematoda; Acantho. Ə Acanthocephala; Pentast. Ə Pentastomida; Ecto tot. = total ectoparasites; Endo tot. = total endoparasites

Table 4: Total number of parasites recorded from *Oreochromis mossambicus* during survey 4 (Autumn)

Sasol (Site A)														
Species	**Fish #**	**Monog.**	**Copep.**	**Branch.**	**Ecto tot.**	**Digen. L**	**Digen. A**	**Cestode L**	**Cestode A**	**Nemat. L**	**Nemat. A.**	**Acantho.**	**Pentast.**	**Endo tot.**
O.mos.	4S37	1	0	0	**1**	70	0	12	0	0	0	1	0	**83**
O.mos.	4S38	3	0	0	**3**	122	0	23	0	0	0	0	0	**142**
O.mos.	4S39	2	0	0	**2**	153	0	0	0	0	0	1	0	**154**
O.mos.	4S40	6	0	0	**0**	35	0	0	0	0	0	4	0	**39**
O.mos.	4S41	1	0	0	**6**	41	0	1	0	0	0	0	0	**42**
O.mos.	4S42	0	0	0	**1**	47	0	0	0	0	0	0	0	**47**
O.mos.	4S43	2	0	0	**2**	13	0	14	0	0	0	1	0	**28**
O.mos.	4S44	3	0	0	**3**	45	0	0	0	0	0	0	0	**45**
O.mos.	4S45	2	0	0	**2**	24	0	0	0	0	0	0	0	**24**
O.mos.	4S46	2	0	0	**2**	91	0	0	0	0	0	0	0	**91**
Total	**10**	**22**	**0**	**0**	**22**	**638**	**0**	**50**	**0**	**0**	**0**	**7**	**0**	**695**
Foskor (Site B)														
O.mos.	4F25	0	0	0	**0**	0	0	23	0	0	0	0	0	**23**
O.mos.	4F26	0	0	0	**0**	7	0	6	0	0	0	0	0	**13**
O.mos.	4F27	0	0	0	**0**	35	0	0	0	0	0	0	0	**35**
O.mos.	4F28	0	0	0	**0**	8	0	7	0	0	0	0	0	**15**
O.mos.	4F29	0	0	0	**0**	1	0	0	0	0	0	0	0	**1**
O.mos.	4F30	0	0	0	**0**	0	0	4	0	1	0	0	0	**5**
O.mos.	4F31	2	0	0	**2**	0	0	16	0	0	0	0	0	**16**
O.mos.	4F34	1	0	0	**1**	0	0	8	0	1	0	0	0	**9**
O.mos.	4F35	0	0	0	**0**	0	0	6	0	2	0	0	0	**8**
O.mos.	4F36	3	0	0	**3**	2	0	20	0	4	0	0	0	**26**
Total	**10**	**6**	**0**	**0**	**6**	**53**	**0**	**90**	**0**	**8**	**0**	**0**	**0**	**151**

Table 4 Continued

PMC (Site C)														
Species	**Fish #**	**Monog.**	**Copep.**	**Branch.**	**Ecto tot.**	**Digen. L**	**Digen. A**	**Cestode L**	**Cestode A**	**Nemat. L**	**Nemat. A.**	**Acantho.**	**Pentast.**	**Endo tot.**
O.mos.	4P6	0	3	0	**3**	301	0	0	0	0	0	0	0	**301**
O.mos.	4P7	2	10	0	**12**	221	0	5	0	0	0	0	0	**126**
O.mos.	4P8	0	2	0	**2**	169	0	3	0	0	0	0	0	**172**
O.mos.	4P9	0	0	0	**0**	115	0	0	0	0	0	0	0	**115**
O.mos.	4P10	3	0	0	**3**	77	0	5	0	0	0	0	0	**82**
O.mos.	4P11	2	2	0	**4**	333	0	3	0	0	0	0	0	**336**
O.mos.	4P12	2	4	0	**6**	284	0	0	0	0	0	0	0	**284**
O.mos.	4P13	2	0	0	**2**	56	0	0	0	0	0	0	0	**56**
O.mos.	4P14	0	2	0	**2**	495	0	0	0	3	0	0	0	**498**
O.mos.	4P15	8	0	0	**8**	58	0	0	0	0	0	0	0	**58**
Total	**10**	**19**	**23**	**0**	**42**	**2109**	**0**	**16**	**0**	**3**	**0**	**0**	**0**	**2028**
Barrage (Site D)														
O.mos.	4B1	4	0	0	**4**	0	0	0	0	0	0	0	4	**4**
O.mos.	4B2	0	0	0	**0**	12	0	0	0	0	0	0	0	**12**
O.mos.	4B3	0	0	0	**0**	0	0	0	0	0	0	0	0	**0**
O.mos.	4B4	0	0	0	**0**	2	0	0	0	0	0	0	0	**2**
O.mos.	4B5	0	0	0	**0**	3	0	0	0	0	0	0	0	**3**
O.mos.	4B6	0	0	0	**0**	0	0	0	0	0	0	0	0	**0**
O.mos.	4B51	8	0	0	**8**	0	0	0	0	5	0	0	0	**0**
O.mos.	4B52	2	0	0	**2**	39	0	0	0	0	0	0	0	**39**
O.mos.	4B53	0	0	0	**0**	8	0	0	0	1	0	0	3	**12**
O.mos.	4B54	2	0	0	**2**	5	0	0	0	1	0	0	1	**7**
Total	**10**	**16**	**0**	**0**	**16**	**61**	**0**	**0**	**0**	**7**	**0**	**0**	**8**	**79**

O.mos. = *Oreochromis mossambicus*; L Ə Larva; A Ə Adult; Monog. Ə Monogenea; Copep. Ə Copepoda; Branch. Ə Branchiura; Digen. Ə Digenea; Nemat. Ə Nematoda; Acantho. Ə Acanthocephala; Pentast. Ə Pentastomida; Ecto tot. = total ectoparasites; Endo tot. = total endoparasites.