Histology-based health assessment and estrogenic effect on *Labeobarbus natalensis* in the uMgeni River system

By:

Mandlenkosi Nkala (215008198)

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University of KwaZulu-Natal Westville Campus,

Durban

Supervisor: Dr. J Lebepe (SMU and UKZN)

Co-supervisors: Dr. D Vosloo (UKZN)

Prof. WJ Luus-Powell (UL)

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I **Dr. J. Lebepe** as the main supervisor approved the submission of this thesis titled as **Histology-based health assessment and estrogenic effect on *Labeobarbus natalensis* in the uMgeni River system** by Mandlenkosi Nkala (215008198).

Signed: [redacted] Date: 22/11/2024

I **Dr. D. Vosloo** as the co-supervisor approved the submission of this thesis titled as **Histology-based health assessment and estrogenic effect on *Labeobarbus natalensis* in the uMgeni River system** by Mandlenkosi Nkala (215008198).

Signed: [redacted] Date: 25/11/2025

I **Prof. W. Luus-Powell** as the co-supervisor approved the submission of this thesis titled as **Histology-based health assessment and estrogenic effect on *Labeobarbus natalensis* in the uMgeni River system** by Mandlenkosi Nkala (215008198).

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Signed: Mandlenkosi Nkala
Student No.: 215008198 (UKZN)
Date: 22 November 2024

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LIST OF ABBREVIATIONS

CF:	Condition Factor
DO:	Dissolved Oxygen
DWAF:	Department of Water Affairs
EC:	Electric Conductivity
EDCs:	Endocrine Disrupting Chemicals
ELISAs:	Enzyme-Linked Immunosorbent Assays
GSI:	Gonadosomatic Index
HAI:	Health Assessment Index
HSI:	Hepatosomatic Index
ICP:	Inductively Coupled Plasma
K:	Fulton's Condition Factors
KZN:	KwaZulu-Natal
NMP:	Natal Metamorphic Province
OCPs:	Organochlorinated Pesticides
PCA:	Principle Component Analyses
SA:	South Africa
SASS5:	The South African Scoring System Version 5
SL:	Standard Length
TL:	Total Length
TWQR:	Target Water Quality Range
USA:	United States of America
VTG:	Vitellogenin
WHO:	World Health Organization

ABSTRACT

The uMgeni River is home to more than 40 fish species and is highly polluted due to anthropogenic activities. The study aimed to assess the overall health and oestrogenic effect on *Labeobarbus natalensis* in the uMgeni River system and information will benefit the community located along uMgeni River catchment area. The physical parameters of water were measured at Nagle and Inanda dams, and the tributaries feeding the uMgeni River (i.e., uMsunduzi, uMngcweni, and eSikelekehleni rivers) before it empties into the Inanda Dam. Water and sediment samples were also collected for chemical analyses in conjunction with physical parameters. Fish were sampled at the Nagle and Inanda dams riverine using an electro-shocker, and indices such as condition factor, organosomatic, and health assessment index were used to assess the health of fish. Moreover, a histopathologic assessment was done on the liver and gills, and organ indices were calculated. The oestrogenic effect was assessed by measuring vitellogenin concentration in the liver of male *Labeobarbus natalensis*. A high alkaline pH level was recorded at Inanda Dam and uMsunduzi River with a neutral pH recorded at other sites. Nutrients showed significant differences between sites with Inanda Dam showing significantly higher concentrations for most nutrients. Higher sulphate concentration was recorded in the uMsunduzi River with higher phosphate concentration recorded in the uMngcweni River. Inanda Dam showed significant differences for most metals in the water and sediment with lower concentrations being recorded for Nagle Dam. Coinciding with water quality parameters, the health of fish was generally better at Nagle Dam compared to Inanda Dam. However, negative allometric growth was observed for both Inanda Dam and Nagle Dam populations. The health assessment index and gonadosomatic index (female population) were significantly different between the Inanda and Nagle dams, with the Inanda Dam population exhibiting higher indices than the Nagle Dam population. Histopathologic alterations showed a higher prevalence in the gill and liver for the Inanda Dam population compared to the Nagle Dam population. Gill histopathologies such as necrosis, deposits, oedema, architectural and structural alterations, nuclear alterations, hypertrophy, and hyperplasia were the most common pathologies at Inanda Dam. In the liver, architectural and structural alterations, necrosis, nuclear alterations, vacuolar degeneration, and hypertrophy were the most prevalent pathologies at Inanda Dam. Similarly, organ indices were higher for the Inanda Dam population. However, both populations exhibited gill and liver indices >40, denoting, severe alterations. Nevertheless, no significant difference was observed for vitellogenin (VTG) levels between the Inanda and Nagle dams ($p>0.05$). However, some individuals showed increased VTG level at Inanda Dam.

Generally, the poor water quality from tributaries seems to have impacted the water quality in the Inanda Dam. Although the overall fish health seemed to be in an acceptable state, it is evident that these fish populations are living in stressful conditions, with Inanda Dam showing to be rapidly deviating from

normal conditions. There is a need to urgently address the water pollution issue in this river system for the sustainability of *Labeobarbus natalensis* and possibly other sensitive species.

CHAPTER 1

General introduction

1.1 Background

About 97 % of the total water on earth is saline water, while 2.5 % is freshwater of which approximately 1.75 % is in ice, snow, and glaciers. Soil moisture and groundwater constitute approximately 0.05 % of total water, whereas less than 0.01% is the surface water that is accessible to humans (Kaur *et al.*, 2018a). Although surface water makes up a small portion of water, pollution from human activities, including urbanization, industry, wastewater treatment, power plants, and agriculture, poses a danger to its availability (Kumwimba *et al.*, 2018). In most river systems, water pollution has drawn more media attention due to its detrimental effects on the environment (Naidoo and Olaniran, 2014).

Several aquatic organisms are exposed to extremely contaminated water, particularly in locations where wastewater is dumped into water bodies prior to any treatment to lower the contamination levels. Aquatic organisms acquire pollutants immediately when they are exposed to contaminated water and diet (Afshan *et al.*, 2014). According to Allen and Moore (2004), fish health is severely impacted by pollution, especially when pollutants are non-biodegradable, have a large potential for accumulation, have additive or synergistic effects on one another, or have high biological effects. Water pollution can affect the ecological balance of both marine and freshwater systems (Qadri and Faiq, 2020). Moreover, fish that are exposed to water pollution may experience a variety of changes, from biochemical changes in a single cell to changes in the population as a whole (Bashir *et al.*, 2020).

Fish response to environmental stress may be reflected in their behavioural, histological, physiological, and molecular component (Phrompanya *et al.*, 2021; Raza *et al.*, 2022). A range of biological responses can be utilised to assess the effects of pollutants on aquatic organisms (Akhtar *et al.*, 2021). Fish health assessment has been viewed as one of the reliable tools for monitoring water pollution in aquatic environments. Moreover, fish have proven to be an effective tool for producing data on the general health of ecosystems as they are abundant, diversity in aquatic habitats, and sensitivity to pollution, fish have proven to be an effective tool for producing data on the general health of ecosystems (Gaber *et al.*, 2013).

The uMgeni River system is home to more than 40 fish species such as *Oreochromis mossambicus, Clarias gariepinus, Channidae mudfish, Oreochromis niloticus* and *Labeobarbus natalensis* (Mdluli *et al.*, 2023; Misra *et al.*, 2024). However, this study focuses on *L. natalensis* which is commonly known as scaly or KwaZulu-Natal (KZN) yellowfish (Karssing, 2008). *Labeobarbus natalensis* is native to KZN and widely distributed across the province since it is capable of withstanding a wide range of temperatures and moderately polluted water but is sensitive to a decrease in dissolved oxygen (DO) (Karssing, 2007). Since they are large and widely distributed in KZN, they are commonly targeted by

subsistence and recreational fishermen (Skelton and Bills, 2008). However, the uncertainty of the pollution status in the uMgeni River threatens the sustainability of the *L. natalensis* population.

There has been an indication of water quality decline in the uMgeni River's as a result of an increase in agricultural drainage, insufficient sanitation services, effluent return, leachate from landfills, urban wash-off, agricultural drainage, and industries over the past few years (Rimayi *et al.*, 2018). The uMgeni River is one of the biggest rivers in KZN, which provides water to eThekwini municipality and surrounding areas, but anthropogenic activities have affected the water quality of this water body which has even resulted in fish mortalities (Petterson, 2019). In response to water pollution, fish may adapt to the changing environment or gradually disappear and go extinct in that area. Therefore, it is essential to closely monitor the health of the fish populations inhabiting the uMgeni River as it is imperative to ensure that any deviation from the current health condition can be detected in time.

Aneck-Hahn *et al.* (2009) emphasised that estrogenic compounds have been identified in rivers receiving effluents from wastewater works, industrial activities, and agricultural areas. The uMgeni River has shown increased concentrations of pharmaceuticals and other oestrogenic organic compounds (Gakuba *et al.*, 2019). Oestrogenic compounds are biologically active even at low levels (Ciślak *et al.*, 2023) and they are described as the subset a subset of substances that together form the class of molecules known as endocrine disrupting compounds (EDCs), which are significant environmental pollutants (Fernandez *et al.*, 2007). The presence of estrogenic compounds in the aquatic environment poses a significant problem since the presence of estrogenic chemicals influences fish reproduction by altering the properties of sperm, decreasing fertilisation success, and decreasing the ability to generate viable offspring (Sumpter and Jobling, 2013). It can also lead to a rise in adverse effects on fish such as birth defects, development of physical abnormalities, and feminization (Nie *et al.*, 2009). Despite *L. natalensis* being endemic to KZN and the high concentrations of oestrogenic compounds in the uMgeni River, no studies have been conducted to monitor the general health and endocrine disruption of this species in the uMgeni River system.

1.2 Problem statement

Water pollution is a global challenge as it affects the physical status as well as the well-being of organisms found within the aquatic environment. Due to its limited water resources, South Africa (SA) has been unable to meet the rising water demands of its rapidly expanding population. About 50 % of people living in rural areas do not have access to clean drinking water, and the majority of them use untreated water from other sources including lakes, rivers, and dams (Bazaanah and Mothapo, 2023). However, it is a known fact that surface water from most freshwater systems is not fit for human consumption (Chollom *et al.*, 2023). The uMgeni River is one of the main rivers in KZN, and its water quality has been deteriorating over the past few decades. The lower uMgeni River receives water from

a heavily polluted tributary, the uMsunduzi River, which drains a catchment characterised by metallurgic industries, urbanisation, a wastewater plant, and a landfill site (Sosibo, 2022). This tributary has been associated with pollution load in the Inanda Dam as the dam is located after the uMgeni-uMsunduzi rivers confluence. Moreover, a major ecological disaster was also observed in August 2019 (Figure 1.1) due to the spillage of caustic soda and oil from a factory in Pietermaritzburg. Recent studies by Olaniran *et al.* (2014) and Mdluli *et al.* (2023) reported metal concentrations above the recommended limit for aquatic ecosystems in the surface water and sediment in the Inanda Dam.

It is clear that the uMsunduzi River plays a role in water quality deterioration in the lower uMgeni River system, particularly, Inanda Dam. The river is home to diverse fish species and other aquatic organisms (Karssing, 2007). The fish species include endemic species, *L. natalensis* which is sensitive to pollution. *Labeobarbus natalensis* is among the critical components of the province's biodiversity, and it thrives in polluted rivers (Misra *et al.*, 2024; Skelton and Bills, 2008). However, its response to increased pollution levels in the lower uMgeni River, particularly Inanda Dam has not been explored. Given the importance of *L. natalensis* on the biodiversity integrity of KZN province, it is imperative to keep a close eye on its well-being to ensure that any significant deviation from the current condition could be detected before any deleterious effect. Despite, *L. natalensis* being endemic to KZN and its role in the biodiversity integrity of the province, its response to pollution levels in the uMgeni River has not been explored and hence, not known. Therefore, the present study aims to assess the general health and oestrogenic effect on *L. natalensis* from the Nagle and Inanda dams of the uMgeni River.

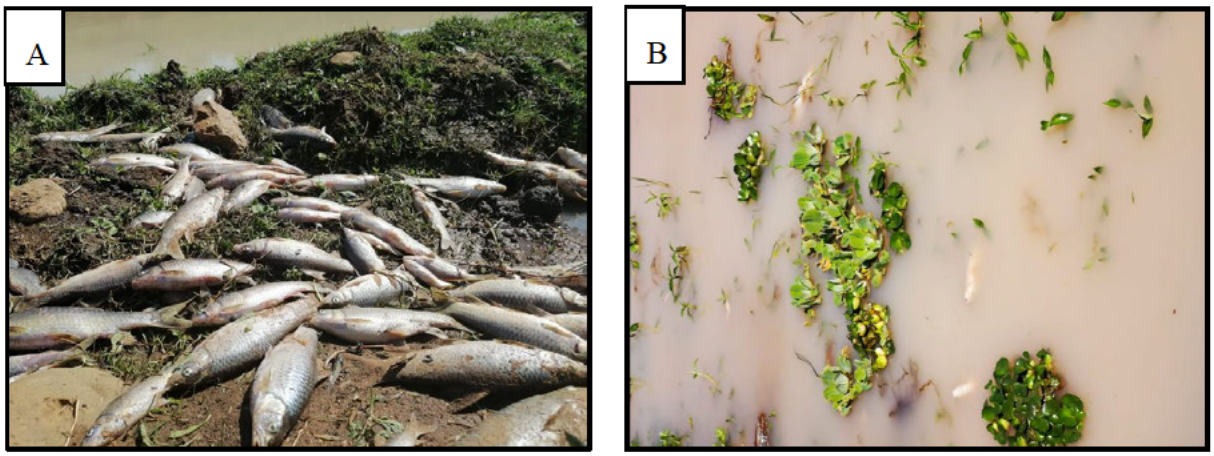


Figure 1.1: **A.** Fish found dead around the shore of Inanda Dam. **B.** Water at the uMgeni River turning grey as a result of the caustic soda-oil mixture spilled into the river (Kockott, 2019).

1.3 Aim

This study aimed to assess the general health and oestrogenic effect on *L. natalensis* at Inanda and Nagle dams in the uMgeni River system.

1.4 Objectives

The objectives of this study were:

- To determine water quality by measuring selected water parameters at the uMsunduzi, uMngcweni River and eSikelekehleni rivers, and Nagle and Inanda dams
- To compare the overall health of *L. natalensis* using the condition factor (CF), gonadosomatic index (GSI), and hepatosomatic index (HSI).
- To compare the health of *L. natalensis* from Nagle and Inanda dams using the histopathology-based health assessment index.
- To assess the possible oestrogenic effect on *L. natalensis* by measuring vitellogenin (VTG) concentration in male fish.

1.5 Research question

The main research questions for the study were:

- How do the uMsunduzi, uMngcweni and eSikelekehleni rivers impact the water quality of the Inanda Dam?
- Does the health of *L. natalensis* populations differ between the Nagle and Inanda dams in relation to water and sediment quality?
- Do *L. natalensis* populations at Nagle and Inanda dams exhibit any sign of oestrogenic effect?

1.6 Hypothesis

- The Inanda Dam receives water from highly polluted uMsunduzi, uMngcweni and eSikelekehleni rivers. It was, therefore, hypothesised that the water and sediment quality at Nagle Dam would be better compared to Inanda Dam and the health of the Inanda Dam fish population would be more compromised relative to the Nagle Dam population.

1.7 Thesis Outline

- This thesis consists of seven chapters. Chapter 1: Introduction. This includes background information, motivation, rationale, aim, objectives, research questions, and hypothesis; Chapter 2: Literature review; Chapter 3: Water and sediment quality; Chapter 4: General health condition of fish. Chapter 5: The histopathology of liver and gills; Chapter 6: Vitellogenin as a biomarker of oestrogenic effects; Chapter 7: Conclusion and recommendations.

CHAPTER 2

Literature review

2.1 The uMgeni River catchment area

The surface area of the uMgeni River catchment is approximately 4432 km^2 and is in the eastern part of SA (Figure 2.1 and 2.2). Its land use includes rural and semi-urbanized settlements, wastewater treatment plants, farmlands, industrial parks, recreational areas, and informal settlements (Abdel-Moneim *et al.*, 2012; Kaur *et al.*, 2018b). The uMgeni River catchment is classified as the most complex region in SA, due to having multiple water supply systems and diversified land usage (Strydom *et al.*, 2020). The basin is divided into twelve quaternary drainage areas, sometimes referred to as quaternary catchments. Two are in the lower part, three are in the middle part, and seven are in the uppermost part of the uMgeni basin (Namugize *et al.*, 2018). The catchment is drained by a 232 km mainstem which originates from the Drakensberg mountains and flows eastwards until it empties in the Indian Ocean (Banda and Kumarasamy, 2020). The uMgeni River is fed by six main cardinal tributaries which are uMsunduzi, iMpolweni, eSikelekehleni, uMngcweni, Karkloof, and Lions rivers (Rangeti, 2015).

2.1.1 Hydrology

The uMgeni River consists of four major dams: Albert Falls, Midmar, Nagle, and Inanda dams which are used to reserve and regulate the water flow (Misra *et al.*, 2024) (Figure 2.2). Inanda, Nagle, and Albert Falls dams provide water for the greater part of eThekwini Municipality whereas the Midmar Dam provides water mostly to Pietermaritzburg areas (Moodley *et al.*, 2023). The uMgeni basin has a seasonal rainfall pattern, with summer months receiving more rain than other seasons. The precipitation is highly variable, increasing from the western side of the river catchment towards the eastern side. The uMgeni basin normally receives rainfall ranging from 800 mm/y to 1000 mm/y, whereas the coastal areas receive the highest rainfall ranging from 1000 mm/y to 1500 mm/y (Rangeti, 2015). The uMgeni River has an average annual temperature that ranges between 12 ℃ to 22 ℃, this results in evaporation rates ranging between 1567 mm/y and 1737 mm/y (Namugize *et al.*, 2018).

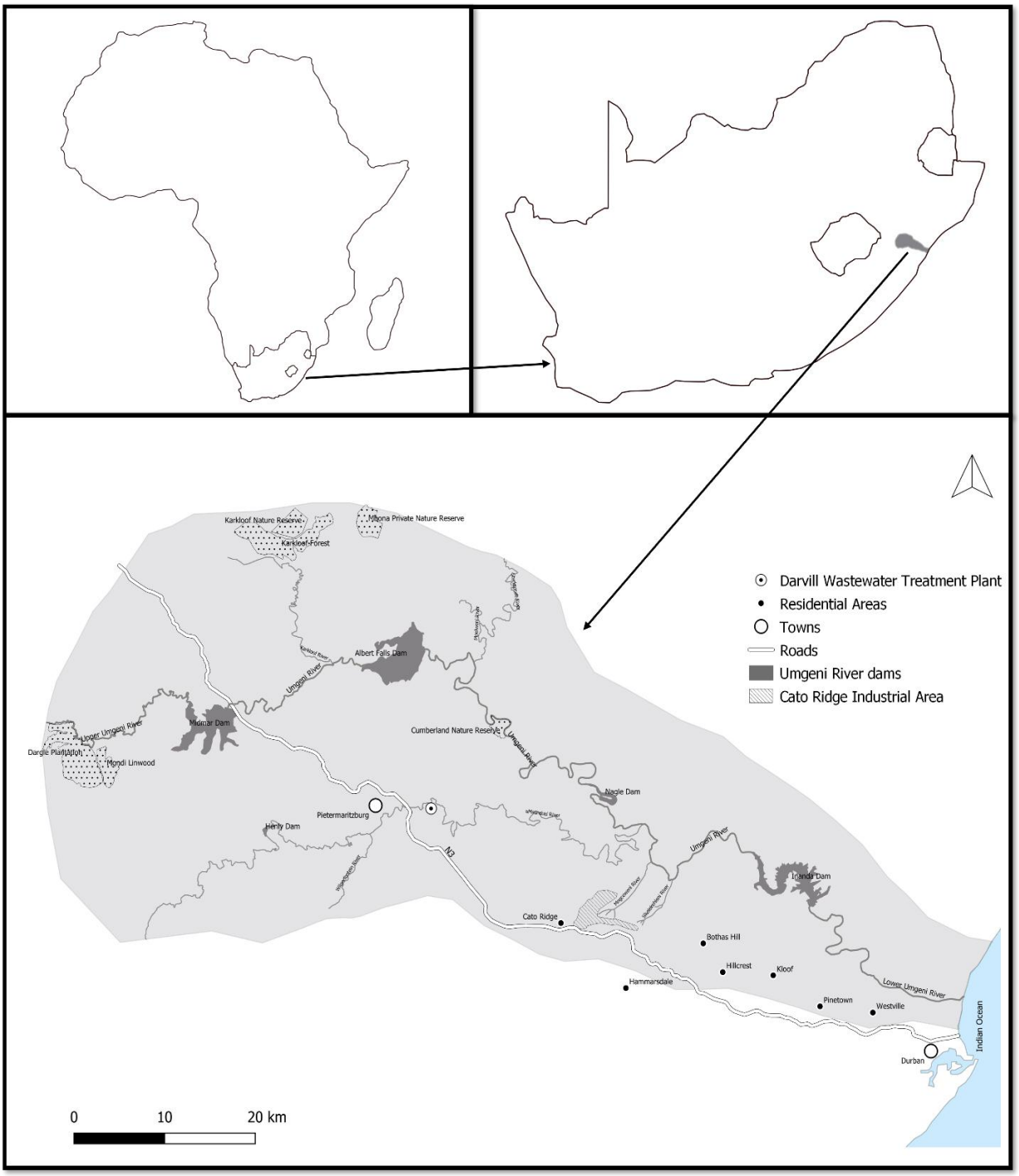


Figure 2.1: The uMgeni River catchment showing tributaries and impoundments in the system.

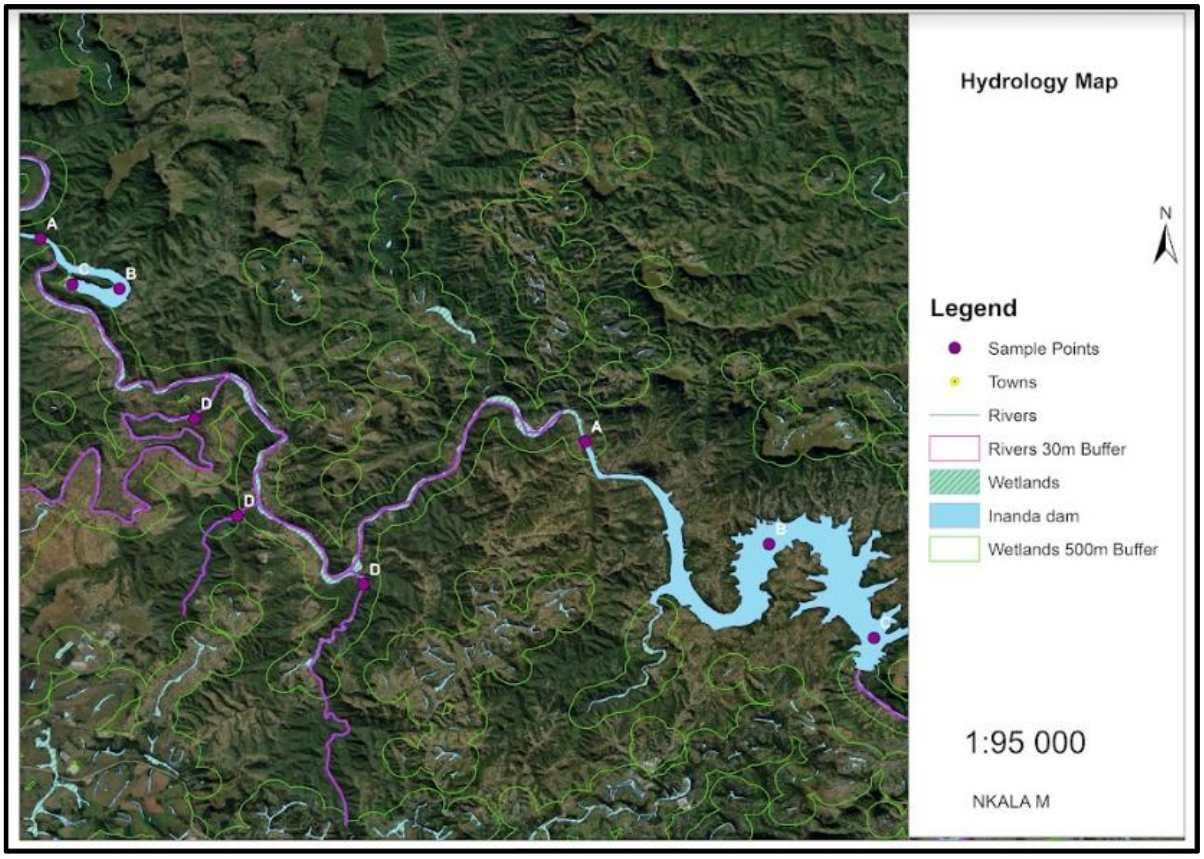


Figure 2.2: The hydrology map showing the water sampling point at Nagle Dam, uMsunduzi River, eSikelekehleni River, uMngcweni River, and Inanda Dam.

2.1.2 Geology

The upper catchment of the uMgeni River is dominated by the mudstone/shale of Ecca Group, and lithology around Howick and Pietermaritzburg. The tillite is also found along the uMgeni River catchment area. The Inanda and Nagle dams are concentrated with the gneisses/granite rocks of the Natal Metamorphic Province (NMP) (DWAF, 1999). As shown in Figure 2.3, the Inanda and Nagle dams are located within the Mapumulo and Maputaland Group geology type.

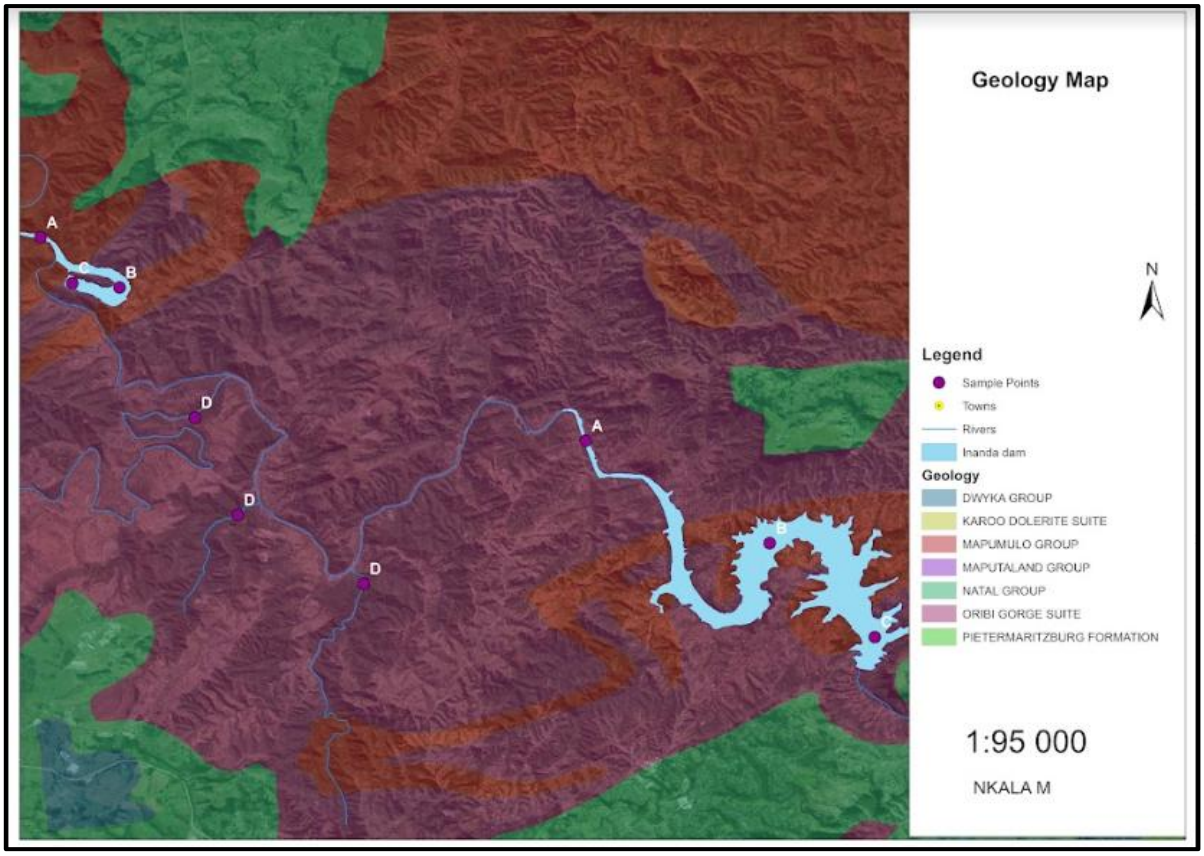


Figure 2.3: The geology map showing the underlying geology and water sampling point at Nagle Dam, uMsunduzi River, eSikelekehleni River, uMngcweni River, and Inanda Dam.

2.1.3 Land use

The uMgeni River catchment area is characterised as heterogeneous because it consisting natural forests, commercial agricultural farms, commercial sugarcane plantations, Port City of Durban, rural and urban areas (Moodley *et al.*, 2023). As shown in Figure 2.4, the river sustains the livelihoods of rural communities and informal settlements located within its catchment area that lack or have restricted access to municipal water (Hughes *et al.*, 2018; Namugize *et al.*, 2018). It has been noted that the community along the uMgeni River course relies on it for different household and domestic activities, such as livestock production and irrigation. There are approximately 300 farm dams located in the uMgeni River catchment area that are used for irrigation (Banda and Kumarasamy, 2020). The middle catchment of the uMgeni River is characterised by wastewater plants, waste disposal sites, and industrial activities.

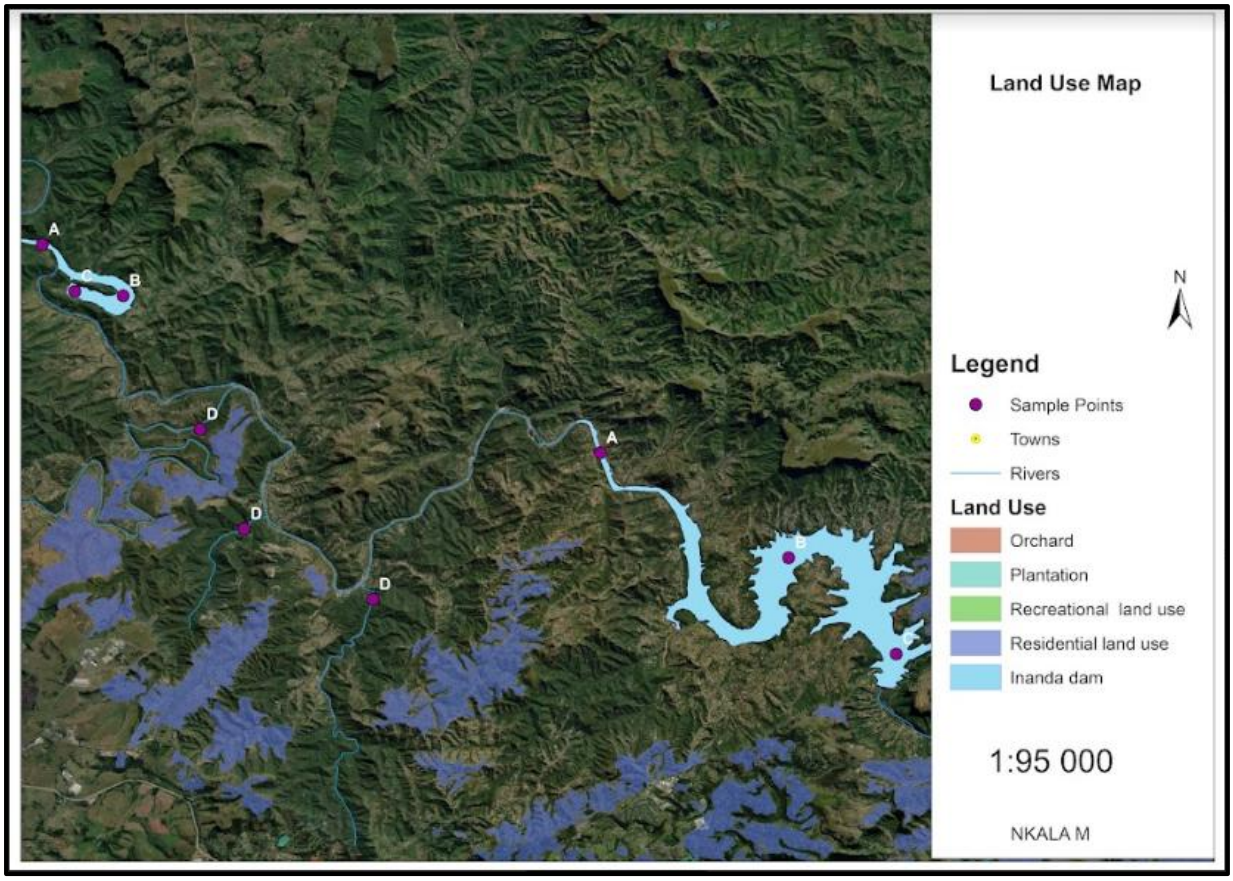


Figure 2.4: A land use map showing the different land use locations within the catchment of the study area and water sampling point at Nagle Dam, uMsunduzi River, eSikelekehleni River, uMngcweni River, and Inanda Dam.

2.2 Biomonitoring in the uMgeni River System

Abdelkarim (2020) defined freshwater biomonitoring as the integrated and comprehensive estimation of the environmental water quality. The concept incorporates the analysis of the condition of the environment by monitoring the communities, populations and species to understand changes due to various stressors over a long or short period. Adequate biomonitoring strategies are essential because the biological responses may emerge days or months after the chemical exposure has ceased or at the chemical concentration below the analytical detection limits (Zuykov *et al.*, 2013). Subsequently, ongoing biomonitoring programs currently include biomarkers, and other parameters at various levels of biological organisation, such as molecular, cellular, and physiological levels that indicate pollutant exposure, effect, or susceptibility (Santonen *et al.*, 2022).

In the uMgeni River, various biomonitoring tools have been used to explore the pollution problem and monitor its effect on the system. Van Deventer *et al.* (2022) employed macroinvertebrate assemblages (SASS5) as a biomonitoring tool for the ecological state of the river. Moreover, Mdluli *et al.* (2023)

employed general fish health, enzymatic, hormone, and histopathologic biomarkers of *Clarias gariepinus* whereas, Misra *et al.* (2024) employed bioaccumulation as a monitoring strategy by observing the selenium, arsenic and mercury concentration in different fish tissues.

2.3 Bioindicators

Bioindicators are defined as plants or animals that can reflect the ecological condition of the environment (Zaghloul *et al.*, 2020a). Hamza-Chaffai (2014) reported that bioindicators are considered useful when the indicative environmental factor is difficult to measure, when the indicative environmental factor cannot be measured, and when the indicative environmental factors can be easily measured but are difficult to be interpreted. Depending on how they respond to pollutant exposure, bioindicators may be categorised into compliance, accumulative, diagnostic, and early warning bioindicators (Kumari and Paul, 2020). The compliance indicators are used to evaluate the aquatic environmental health, and they use either the community or population species (Kumari and Paul, 2020). Accumulative indicators are organisms that are accumulating contaminants through feeding or from their surrounding environment, where concentrations in their tissue will reflect the contaminants level of the environment (Gerhardt, 2002). The early-warning and diagnostic indicators are sensitive to environmental changes and respond rapidly, and they can be measured at biochemical, cellular, or tissue levels (Gerhardt, 2002).

2.3.1 Fish as a bioindicator

According to Manzoor *et al.* (2021), fish are regarded as the ideal bioindicators for evaluating water pollution in the aquatic system. Fish are especially susceptible and exposed to water pollution due to living and feeding in the aquatic environment (Singh *et al.*, 2022). They are also not able to escape from this detrimental effect caused by pollutants (Saleh and Marie, 2015). Fish are suitable indicators of ecosystem health since they are more sensitive to several toxicants (Authman and Abbas, 2007), they are highly abundant, widely distributed and easy to sample (Khallaf *et al.*, 2003; Kime *et al.*, 1996) Moreover, fish accumulate pollutants in their fatty tissue such as the liver to concentrations exceeding their thresholds (Omar *et al.*, 2014; Sassi *et al.*, 2010). Fish have a long life span, and all its life stages are aquatic which enables comparing of responses between different stages of life (Ebrahimi and Taherianfard, 2011). Fish can take up pollutants either directly from the environment or indirectly by feeding on aquatic vegetation, invertebrates and small fish (Polat *et al.*, 2016). Van der Oost *et al.* (2003) emphasised that the response of fish to pollution can also be assessed at biochemical and tissue levels

2.4 Biomarkers

Biomarkers are defined as the quantifiable measurement of alterations in molecular or cellular components, functions, structures, and processes as a result of chemical exposure. Prabhu (2023) further defined biomarkers are early indicators of pollution and are usually unique to a certain class of pollutants. Biomarkers have been identified as a suitable and sensitive tool for detecting either the effect or exposure of pollutants since they can provide more comprehensive biologically relevant information on the effect of pollutants on the organism's health status (Depledge, 2020). Biomarkers are broadly categorized into markers of effect, exposure and susceptibility.

- Biomarkers of effect are best understood as indicators of early alterations that may eventually lead to clinical illnesses. They are defined as measurable changes in an organism's biochemistry, physiology, behaviour, or any other aspect that arises from exposure to or affected by environmental pollutants (Mutti, 1995).
- Biomarkers of exposure are the result of the xenobiotic's integration and a target cell or molecule that may be measured inside the organism's compartment.(Venier *et al.*, 2006).
- Biomarkers of susceptibility indicate an organism's acquired or inherent ability to respond to the pollutant's exposure (Amiard-Triquet *et al.*, 2013).

Effectively measuring the level of exposure in aquatic species to environmental pollutants requires the use of biomarkers for biomonitoring the natural aquatic system (Depledge, 2020). Biomarkers are increasingly recognized methods for evaluating the effects of pollution, despite the fact that their systematic and extensive use is uncommon. In other countries, some are already a part of environmental monitoring systems (Trapp *et al.*, 2014).

2.4.1 Histopathology as a biomarker

Most biomarkers used to study the effects of water pollution on aquatic organisms are narrow in their expression, but histopathology evaluates a wide range of factors since it focuses more on histopathological alteration in fish tissue in response to water pollution (Hinton, 2021). This is because exposure to environmental toxicants may lead to histological structural changes, which will result in changes in the significant function of organs and tissues (Zaghloul *et al.*, 2020b). These histological structural alterations can be reversible or irreversible when the stimulus is withdrawn, and they can even lead to the death of the aquatic organism (Elumalai *et al.*, 2023).

Histopathology is also the easiest method that can be used to assess both short and long-term effects of water pollution in organisms. Additionally, histopathology can provide early warning signs of diseases and injury in cells, tissues, or organs (Beegam *et al.*, 2020). It can be used to detect the acute and chronic changes in the organs and tissue of individual fish in response to environmental pollution (Salamat and Zarie, 2012). In both field and lab research, histopathology has been shown to be sensitive in identifying

the direct toxic effects of chemical substances in certain target fish tissues or organs (Guerrera *et al.*, 2021). Therefore, histopathology is considered a good biomarker when assessing the toxicological effects of water pollution in fish, but they are also invasive, expensive, and time-consuming (Reddy and Rawat, 2013).

2.4.1.1 Liver as a target organ for contaminants

The liver is highly associated with the biotransformation and detoxification processes (Topić Popović *et al.*, 2023). It is also the main organ for the accumulation, storage, and excretion of toxicants and is essential for basic metabolism (Zulkipli *et al.*, 2021). However, elevated concentration toxicant could result in structural damage. The histological changes in the liver structure are highly accurate and sensitive when assessing the effects of contaminants in fish both in the field and in experimental studies (Thophon *et al.*, 2003). Numerous studies have reported the accumulation of lesions and histological alterations in the liver following exposure to pollutants such as organic chemicals and heavy metals (Kitamura *et al.*, 2023; Lebepe *et al.*, 2020; Mdluli *et al.*, 2023). It has been reported that these contaminants also affect the pronounced metabolic capacity and function of the liver (Mohamed, 2009). Anderson and Lee (2006) have classified the histopathological changes in fish liver into different groups. These groups may be ranked based on their relative significance as an indicator of contaminants exposure:

- Degenerative lesions include the polymorphism and degeneration of hepatocytes and their nucleus.
- Foci of cellular alterations include the eosinophilic, basophilic, clear cell, and vacuolated foci.
- The neoplasm can occur in blood vessels, capillaries, bile ducts, cholangioma, and hepatocyte oedema.
- Malignant neoplasms include carcinoma, hepatocyte cholangiocarcinoma, and hemangiosarcoma.

2.4.1.2 Gills as a target organ for contaminants

Fish gills consist of different functional units and make up more than half of the entire surface of fish, which makes them more susceptible to contaminants. Moreover, fish gills are located in close contact with the outside water environment and are important for physiological functions such as maintaining ionic equilibrium (Evans *et al.*, 1999), excretion of nitrogenous waste, and maintenance of acid-base balance (Georgieva *et al.*, 2014). Wood (2017) reported that when exposed to toxicants, the gill experiences structural alterations as a response. Moreover, fish accumulate water-borne pollutants through gills which causes injuries to epithelium. The damage that occurs to the gill epithelium is influenced by the concentration of pollutants and the duration of exposure (Reddy and Rawat, 2013). Pathologies such as fusion of lamellae and the hypertrophy, hyperplasia, and epithelial lifting of the epithelial cells are considered to the defence mechanisms in response to contaminants (Kumar *et al.*,

2023). The notion is that these pathologies increase the distance between the blood and the external environment, acting as a barrier against pollutants (Fernandes, 2003). These changes in the gill structure are usually associated with chronic exposure rather than acute (Tavares-Dias, 2021). The changes decrease the interlamellar distance and increase the blood-to-water diffusion distance, and this results in the total reduction in the diffusive conductance of the gills to respiratory gases (Myers *et al.*, 1998).

2.4.2 Vitellogenin as a biomarker of oestrogenic effect

To evaluate exposure to or the impacts of environmental pollutants in aquatic ecosystems, several particular biomarkers have been employed (Zezza *et al.*, 2020). For example, VTG, also known as the glycolipophosphoprotein, is a reliable biomarker of estrogenic pollution exposure and is produced by oviparous species under the control of endogenous oestrogen (Thirumalaivasn *et al.*, 2023). In female fish, when oestrogen is released into the bloodstream from their ovaries, the oestrogen-receptor pathway is activated, which leads to the hepatic synthesis of egg proteins such as VTG. Vitellogenin levels in male and juvenile fish are very low and occasionally undetectable, but exposure to exogenous oestrogen can cause a substantial increase. (Thirumalaivasn *et al.*, 2023). Male VTG induction has been a commonly utilised biomarker to assess the presence of estrogenic EDCs (Hutchinson *et al.*, 2006). The presence of elevated VTG levels in fish, both male and juvenile, can therefore be a helpful biomarker for determining whether aquatic environments are contaminated with estrogenic EDCs (Souza *et al.*, 2023). Other studies did not find any clear relationship between the VTG biomarker response and estrogenic exposure (Brown *et al.*, 2023; Merola *et al.*, 2021) but few studies have reported a reasonable to good correlation relationship between the VTG induction in exposed fish to water pollution (Souza *et al.*, 2023; Thirumalaivasn *et al.*, 2023).

2.4.3 Indices for evaluating fish health

2.4.3.1 Health Assessment Index

Health assessment index (HAI) is a qualitative approach that was developed by Adams *et al.* (1993) and is used to analyse and evaluate the health condition of fish. This approach offers fish health profiles according to the extent of abnormalities found in the tissues and organs of fish taken from a population. The benefit of using HAI is that it is easy to use and inexpensive. Health assessment index has effectively been employed in several rivers in SA and Botswana to assess fish health (Crafford and Avenant-Oldewage, 2009; Heath *et al.*, 2004; Madanire-Moyo and Barson, 2010). Health assessment index has been further used by Crafford and Avenant-Oldewage (2009) using *C. gariepinus* to differentiate between the more polluted Vaal River Barrage and the less contaminated Vaal Dam. Even though the overall HAI score established could discriminate between localities, individual estimations differed significantly across the specimens examined (Crafford and Avenant-Oldewage, 2009). Fish health can therefore serve as a reliable indicator of the condition of a specific aquatic ecosystem. Poor

water quality is correlated with an increase in the index value, which in turn causes an increase in environmental stress (Adams *et al.*, 1993).

2.4.3.2 Conditional factor

The CF is an index that focuses on the weight-length relationship. It is also known as a somatic biomarker. It is defined as the fish health indicator that reflects the feeding conditions as well as the energy consumption and metabolism of fish (Famoofo and Abdul, 2020). Pollutants in the aquatic environment may affect fish growth in two ways: Directly, by altering metabolism and raising the energy needed for haemostasis, or indirectly, by decreasing food availability (Sadauskas-Henrique *et al.*, 2011). Conditional factor may also be used to assess the well-being of a fish (Konoyima *et al.*, 2020). It is assumed that fish in better conditions, such as pristine environments are heavier than a fish with the same length found in poor water quality, such as polluted environments (Gomiero and de Souza Braga, 2005). The CF may be affected by factors such as age and sex, environmental conditions, food source availability, and state of sexual maturity (Tessema *et al.*, 2020). The weight and length of a fish are used to calculate Fulton's condition factor (K), which is used to determine or describe the condition of a fish, specifically (Froese, 2006), and is thus used mainly in fish and fisheries biology studies.

2.4.4.3 Gonadosomatic index

The GSI is calculated as a percentage of gonad mass over the somatic or body mass (Cubillos and Claramunt, 2009). The GSI is a significant indicator of the overall health of a population of an organism under the effect of environmental pollutants or toxicity (Adams *et al.*, 1993). This is because the production of milk and eggs by fish is highly influenced by the environmental changes that they are exposed to (Kaur *et al.*, 2018b). Therefore, the GSI is a reliable indicator of changes in a fish's energy condition and nutrition. It is assumed that the larger gonads indicate that the fish is found within a better condition or pristine environment (Flores *et al.*, 2019).

2.4.4.4 Hepatosomatic index

The HSI is the ratio between the liver weight and the somatic or body weight expressed in percentage. It is widely used in fisheries research for the detection of water pollution and as an indicator of energy reserve in the liver (Kumari *et al.*, 2014). It is also defined as the general measurement of the growth status of the liver or the overall condition of fish, and it can be an excellent indicator of the adverse health of fish (El-Agri *et al.*, 2022). The HSI is measured to provide essential information regarding the health status of fish (Dane and Şişman, 2020). Moreover, the HSI serves as an initial screening tool that indicates exposure to and the impact of environmental pollutants even though they are not very sensitive and are still affected by non-pollutant factors (Van der Oost *et al.*, 2003).

Singh and Canario (2004) reported that the HSI was one of the most widely investigated biomarkers because of the liver's significant role in detoxifying pollutants. Darweesh *et al.* (2019) claimed that the

HSI is a useful indicator of chemical water pollution, while Dutta and Banerjee (2016) showed that the HSI was an inconsistent biomarker. It is also widely associated with seasonal changes, parasite infection, and the capacity to metabolize xenobiotics, this serves as an adaptative response of fish to environmental pollution (Heath, 1995).

2.5 *Labeobarbus natalensis*

Labeobarbus natalensis (Figure 2.5) is a useful bioindicator species in rivers situated in the province of KZN as it is distributed from Mkuze southward to the uMthavuna which forms a border between KZN and Eastern Cape (Skelton and Bills, 2008). This means that *L. natalensis* only occurs within the KZN province. Therefore, the conservation of *L. natalensis* should be a priority. However, according to the International Union for Conservation of Nature red data list, *L. natalensis* is of the least concern compared with other species (Cambray and Swartz, 2007). Therefore, to ensure the survival of *L. natalensis*, health monitoring programs should be undertaken. The *L. natalensis* population has been historically found in several rivers that are within the province, but the population has declined due to increases in the use of water sources (Karssing *et al.*, 2012). Factors that contribute to the decline in the population of *L. natalensis* include changes in water quantity and quality, the establishment of river barriers such as dams, and habitat loss (Olden *et al.*, 2006). Some factors include slow growth rates, late maturity, and long life span of *L. natalensis* species (Skelton, 2012). Karssing (2008) further reported that water pollution has been identified as a major factor that contributes to the decline of the *L. natalensis* population in KZN rivers.



Figure 2.5: The sentinel species, *Labeobarbus natalensis* (KwaZulu-Natal yellowfish)

CHAPTER 3

Water and sediment quality

3.1 Introduction

Pollution of aquatic ecosystems is one of the major and expanding issues in SA (Ngqwala and Muchesa, 2020). Moraru *et al.* (2020) reported that water quality in rivers may be affected by factors such as climate, biotic composition, soils, and geology of the region. Therefore, decision-makers and water quality specialists are faced with significant challenges in their efforts to manage surface water resources because of the complexity of the water environment (Elhatip *et al.*, 2008). Characterisation of source apportionment and spatial variation of water quality can improve the understanding of environmental conditions and assist scientists with establishing the priorities for sustainable water management (Chang, 2005).

River water quality degradation has emerged as one of the main risks to SA's ability to supply enough water of suitable quality to satisfy its needs and maintain environmental sustainability. Studies showed that the water quality of the uMgeni River has been deteriorating due to insufficient sanitation services, sand mining, human settlement, urban wash-off, effluent return, agricultural drainage, leachate from landfills, and industries (Olaniran *et al.*, 2014). The uMgeni River water quality has been significantly influenced by different polluting sources such as insufficient sanitation services, sand mining, human settlement, urban wash-off, effluent return, agricultural drainage, leachate from landfills, and industries (DEAT, 2006). Contaminants that were found to be problematic in the uMgeni River systems include organochlorinated pesticides (OCPs) (Gakuba *et al.*, 2019), pharmaceuticals (Matongo *et al.*, 2015), metals (Misra *et al.*, 2024; Olaniran *et al.*, 2014), and estrogenic compounds (Manickum and John, 2015). The levels of the pollutants have become a cause for concern as the river provides water for irrigation and livestock for communities along the river system.

The study aimed to assess the water and sediment quality of the uMgeni River system. Selected parameters were measured in the water and sediment at Nagle Dam, Inanda Dam and the uMgeni River tributaries namely, uMsunduzi, uMngcweni, and eSikelekehleni rivers. It was hypothesised that since the uMgeni River receives water from tributaries that drain industrialised catchment just before feeding Inanda Dam, the water and sediment quality would be better at Nagle Dam compared to Inanda Dam.

3.2 Methods

3.2.1 Study area

The surface area of the uMgeni River catchment is approximately 4432 km^2 with a river stretching for 232 km (Namugize *et al.*, 2018). The uMgeni River rises from the Drakensberg mountains and runs

eastwards to the Indian Ocean. It consists of six main cardinal tributaries which are uMsunduzi, Impolweni, eSikelekehleni, uMngcweni, Karkloof, and Lions rivers (Rangeti, 2015). The river is situated in the eastern part of SA and lies mostly within the province of KZN, SA (Hughes *et al.*, 2018; Rangeti, 2015). It also consists of four different dams, i.e., Midmar Dam, Albert Falls, Nagle Dam, and lastly the Inanda Dam (Figure 2.1). The uMgeni River passes through different rural and semi-urbanized settlements, wastewater treatment plants, farmlands, industrial parks, recreational areas, and informal settlements which are considered potential sources of anthropogenic pollutants.

3.2.1.1 Nagle Dam

The Nagle Dam is located within the Msinsi Nature Reserve property, and it was the first dam built in the uMgeni River system and it was officially opened in 1950. It is located in a place called Nonzila (KwaXimba) in Cato ridge. The Nagle Dam (29°35′40″S 30°38′30″E) is located 31 km east of the Pietermaritzburg. It is further located 20 km away upstream of Inanda Dam and 25 km away downstream of Albert Falls. The Nagle Dam has a total surface area of 1.56 km^2, capacity of 24.6 million m^3, total length of 393 m and a catchment area of 2545 km^2 (Namugize *et al.*, 2018). The Nagle Dam is under the jurisdiction of uMgungundlovu and Mkhambathini local municipalities. The sampling of *L. natalensis* was done on the Nagle Dam riverine (Figure 3.1).



Figure 3.1: The downstream of Nagle Dam.

3.2.1.2 Inanda Dam

Inanda Dam (29°42′1″S 30°52′1″E) is located on the uMgeni River, in Inanda, KZN, SA. It was built in 1989, and its major aim is to serve for home and industrial usage. Inanda Dam is located within eThekwini Municipality, and it is located 27 km away from Durban. The dam is in the lower catchment area of the uMgeni River and has a capacity of 241million m^3. Inanda Dam has a total length of 595 m and a surface area of 1463 ha. The Inanda Dam is located downstream of uMsunduzi and uMgeni rivers

confluence and it supplies water to part of Durban. The sampling of *L. natalensis* was done on Inanda Dam riverine (Figure 3.2).



Figure 3.2: The upstream of Inanda Dam.

3.2.2 Physical parameters

The physical water parameters such as DO, temperature, electric conductivity (EC), and pH were all measured *in* situ at the inflow, middle of the dam, and dam wall in the dams using the handheld multi parameters instrument (HANNA Instrument Model: HI98494) (Figure 3.3). These parameters were also measured in the uMsunduzi, eSikelekehleni, and uMngcweni rivers just before they joined the uMgeni River.



Figure 3.3: Handheld multi-parameter instrument (HANNA Instrument Model: HI98494) used to measure physical parameters.

3.2.3 Water and sediment sample collection

Water and bottom sediment samples were collected from the Nagle Dam inflow of the dam (A), middle of the dam (B), and dam wall (C); and Inanda Dam inflow (G), middle of the dam (H), and dam wall (I) during high flow (November to February) and low flow seasons (March to October) during the 2020 and 2021 surveys as shown in Figure 3.4. Water samples were further collected in three rivers that join the uMgeni River upstream of Inanda Dam but downstream of Nagle Dam. These rivers are the uMsunduzi River (D), uMngcweni River (E), and eSikelekehleni River (F) as shown in Figure 3.4. Water samples were collected at a depth of 0.5 m below the water surface using 1-liter acid pre-treated sampling bottles and they were then stored in laboratory ice pack containers immediately. The sediments were collected using the Van Veen sediment grabber (Figure 3.5) and immediately stored in ice-packed containers. The former Department of Water Affairs and Forestry (DWAF) target water quality range (TWQR) guideline was used as the main set of criteria for the evaluation process of water quality.

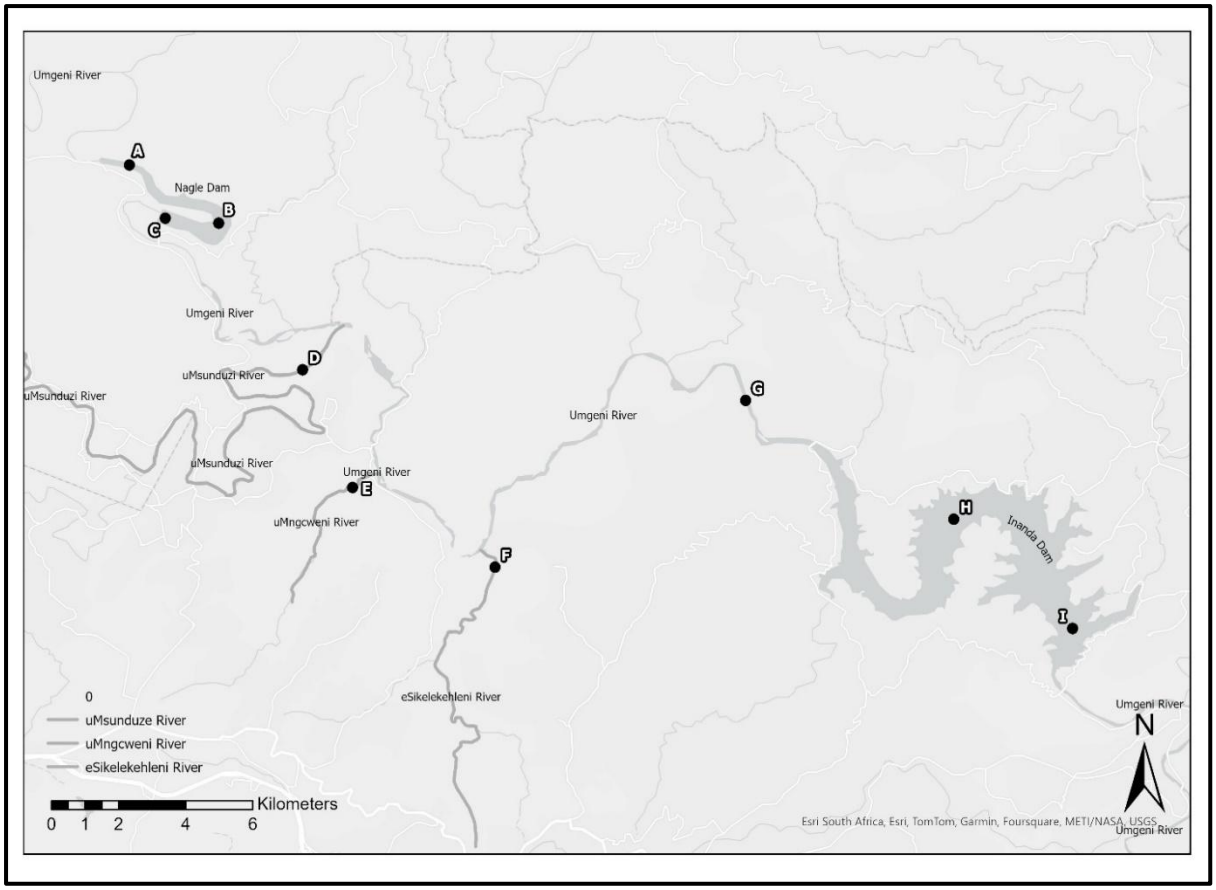


Figure 3.4: Water and sediment sample collection point in the Nagle Dam inflow (A), middle of the dam (B), and dam wall (C); uMsunduzi River (D); uMngcweni River (E); eSikelekehleni River (F); Inanda Dam inflow (G), middle of the dam (H), and dam wall (I).



Figure 3.5: The Van Veen grabber for sediments sample collection.

3.2.4 Water and sediment samples processing

3.2.4.1 Water sample processing

For metal analysis, approximately 150 ml of water sample was transferred to the beaker and acidified using hydrochloric acid. The 150 ml water samples were then filtered through the 0.45 µm Millipore filter paper into a filter flask. Thereafter, 50 ml of the solution was transferred to a 250 ml volumetric flask and topped up to 250 ml mark with deionized water. The solution of 15 ml was again filtered through a 0.45 µm pores syringe filter into 15 ml conical centrifuge tubes. The tubes were then stored in the fridge until analysis using inductively coupled plasma-optical emission spectrometry (ICP-OES). Metals i.e., aluminium, copper, iron, manganese, and zinc were analysed. Another 500 ml of water samples were sent to a commercial lab (uMgeni Water Analytical Lab) for nutrient and ion analyses. In the lab potassium, ammonia, nitrite, nitrate, sulphate, and phosphate were analysed. Duplicate chemical analyses were performed, and the results were reported in mg/l.

3.2.4.2 Sediment sample analysis

The sediment digestion was completed following the protocol done by Gaudino *et al.* (2007). In the laboratory, 2 g of sediments was digested as follows: The 2 g was placed in a beaker filled with 50 ml aqua regia solution (3HCL:1HNO_3). The beaker was then placed and heated on a hot plate for 2 hours. As the liquid levels decreased, an aqua regia mixture was added. In the last minutes, the mixture was allowed to boil until it reached an approximate volume of 10 ml in the beaker. The solution was allowed to cool down and then 50 ml of distilled water was added to the beaker. The solution was then filtered through 0.45 µm Millipore filter paper into a filter flask and then transferred to a volumetric flask. The 100 ml of distilled water was added to the volumetric flask. The 15 ml of the solution was again filtered

through a syringe filter and added to the ICP tubes. The ICP tubes were then stored in a fridge until analyses. Metal analyses were carried out using ICP-OES.

3.2.5 Data analysis

The R-3.1.1 (R Development Core Team 2014) was used to perform the statistical analysis with a level of significance set at $p<0.05$. The Shapiro-Wilk test was used to determine the normality of the data and the Levene's test was used to test the equality of variance between the data. Emphases were made on results that showed seasonal variation, whereas those that showed no significant seasonal variation were not stressed. Depending on whether assumptions were met a One-Way ANOVA or a Kruskal-Walli's test was used to test the differences in water parameters, nutrients in water, metals in water, and metals in sediment between the sampling sites. The PCA was used to assess spatial variations in study sites according to key water quality variations (R Core Team, 2024). All data were converted to Z-scores to standardize weighted variables.

3.3 Results

3.3.1 Physical parameters

Results for physical water parameters are presented in Table 3.1. A significant difference was observed for temperature with the mean value ranging from 22.46 to 28.76 °C ($p>0.05$). In contrast, a significantly higher DO was recorded in Inanda Dam, followed by uMsunduzi River, Nagle Dam, uMngcweni River and eSikelekehleni River, respectively ($p<0.05$). Similarly, Inanda Dam exhibited a significantly higher EC, followed by uMsunduzi River, Nagle Dam, eSikelekehleni River and uMngcweni River, respectively ($p<0.05$) (Table 3.1). The pH ranged from neutral to alkaline with a higher alkaline being recorded at the uMsunduzi River than other sampling sites.

Table 3.1: The Physical water parameters recorded in the uMgeni River system (Nagle Dam and Inanda Dam data shared with Mdluli *et al.* (2023))

	Nagle Dam	uMsunduzi River	uMngcweni River	eSikelekehleni River	Inanda Dam	TWQR
Temperature (°C)	24.09±2.12	28.76±2.04	22.46±1.18	23.24±1.65	25.16±3.74	n/a
DO (% of saturation)	90.37±1.28	124.5±1.94	86.25±1.10	83.95±1.63	152.70±111.7	80-120
DO (mg/L)	7.46±1.31	10.21±0.84	7.27±1.12	7.21±1.73	12.99±10.48	6-9
EC (µs/cm)	117.2±14.31	301±17.46	111.22±9.69	115.6±9.05	306.9±25.24	n/a
pH	7.93±0.46	9.01±0.53	7.87±0.27	7.93±0.43	8.30±0.10	6.5-9.0

Key: n/a= not available

3.3.2 Nutrients and metals in the water column

Potassium concentration in the water column was significantly higher at the uMngcweni River ($p<0.05$) compared to all other sites which showed no significant difference among themselves ($p>0.05$) (Table 3.2). No significant difference was observed for ammonia ($p>0.05$) (Table 3.2), whereas significant differences were observed for sulphate, nitrite and nitrate concentration between the sites ($p<0.05$). A higher sulphate concentration in the water was recorded in the uMsunduzi River, followed by Inanda Dam, uMngcweni, eSikelekehleni and Nagle Dam, respectively (Table 3.2). Nitrite concentration was higher eSikelekehleni River, followed by uMsunduzi, Inanda, uMngcweni and Nagle Dam respectively (Table 3.2). In contrast, nitrate was significantly higher at the uMngcweni River, followed by uMsunduzi, Inanda, Nagle and eSikelekehleni, respectively. Similarly, phosphate exhibited significantly higher concentrations at the uMngcweni River, followed by uMsunduzi River. However, Nagle Dam exhibited relatively lower phosphate concentration compared to other sites (Table 3.2).

Some metals have also showed significant difference between sites (Table 3.2). A significantly higher aluminium was recorded at eSikelekehleni River ($p<0.05$), followed by uMsunduzi River, Nagle Dam, Inanda Dam and uMngcweni River, respectively (Table 3.2). In contrast, no significant difference was observed for copper between the 5 sites whereas, iron showed significantly higher concentrations at eSikelekehleni ($p<0.05$), followed by uMsunduzi River, Nagle Dam, Inanda Dam and uMngcweni River (Table 3.2). Manganese and zinc showed no significant differences between the sampling sites ($p>0.05$) (Table 3.2).

Table 3.2: The nutrient and metal levels (mg/l) recorded in the water samples at the uMgeni River system. (Nagle Dam and Inanda Dam data shared with Mdluli *et al.* (2023)).

Parameters	**Nagle Dam**	**uMsunduzi River**	**uMngcweni River**	**eSikelekehleni River**	**Inanda Dam**	**TWQR**
Potassium (mg K/L)	5±0	5±0	17.75±1.20	5±0	5±0	n/a
Ammonia (mg NH_3/L)	0.23±0.21	0.41±0.2	0.12±0.02	0.1±0	0.13±0.04	0.5-2.5
Nitrite (mg NO_2/L)	0.1±0	1.03±0.12	0.14±0.05	1.67±2.20	0.62±0.65	n/a
Nitrate (mg NO_3/L)	0.23±0.11	2.27±0.35	7.57±1.32	0.15±0.06	1.07±0.96	0.5-2.5
Sulphate (mg SO_4/L)	3.45±1.67	24.5±6.05	19.25±2.05	13.4±0.85	19.83±2.58	n/a
Phosphate (mg PO_4^{3-}/L)	15.97±1.88	187.5±54.44	3485±395	84.7±34.36	16.97±1.36	n/a
Aluminium (mg Al/L)	0.31±0.49	0.63±0.41	0.108±0.01	1.17±0.60	0.28±0.12	n/a
Copper (mg Cu/L)	0.01±0	0.01±0	0.01±0	0.01±0	0.1±0	n/a
Iron (mg Fe/L)	0.53±0.70	1.19±0.89	0.129±0.05	2.35±0.09	0.38±0.49	n/a
Manganese (mg Mn/L)	0.04±0.02	0.09±0.04	0.025±0	0.1±0.04	0.16±0.04	0.18
Zinc (mg Zn/L)	0.025±0	0.025±0	0.025±0	0.025±0	0.025±0	n/a

Key: n/a= not available

3.3.3 Metals in sediments

Significant metal concentrations were observed in sediment (Table 3.3). Moreover, all metals showed significant difference between sites ($p<0.05$). The aluminium, copper, iron and zinc showed relatively higher concentration in the Inanda Dam and uMsunduzi River, respectively. All metals exhibited relatively higher concentrations at Inanda Dam compared to Nagle Dam (Table 3.3). Aluminium and iron concentrations were in a descending order Inanda > uMsunduzi > Nagle > eSikelekehleni > uMngcweni whereas Inanda > uMsunduzi > eSikelekehleni > uMngcweni > Nagle was observed for copper. For manganese, a higher concentration was recorded in the Inanda Dam followed by the Nagle Dam, eSikelekehleni River, uMsunduzi River, and uMngcweni River whereas zinc showed a relatively higher concentration at the Inanda Dam, followed by the uMsunduzi River, Nagle Dam, eSikelekehleni River and uMngcweni River (Table 3.3).

Table 3.3: The metal concentrations recorded in sediment samples in the uMgeni River system (Nagle Dam and Inanda Dam data shared with Mdluli et al. (2023)).

	Metals (mg/kg)					
	Nagle Dam	**uMsunduzi River**	**uMngcweni River**	**eSikelekehleni River**	**Inanda Dam**	**TWQR**
Aluminium	358.3±155.5	350±123.7	106.3±17.68	221.9±207.7	400±155.52	n/a
Copper	0.72±0.23	0.73±0.28	0.37±0.06	0.37±0.3	1.04±0.29	n/a
Iron	680.3±353.3	731.3±291.7	281.3±8.84	531.3±397.7	883.3±324.9	n/a
Manganese	17.59±11.60	11.99±9.02	6.78±0.22	10.53±8.18	18.23±5.22	0.18
Zinc	1.56±1.00	3.93±1.52	0.74±0.26	1.4±0.58	3.94±2.24	n/a

Key: n/a= not available

3.3.4 Correlation of the water and sediment quality variables

Manganese concentrations in the water and sediment showed a clear association with higher concentration being associated with Inanda Dam (Figure 3.6). The study sites are separated across principal component 1 (Dim1) accounting for 46% of the dataset's variation. Of the variables, Fe, Al, and Mn in sediment were significantly correlated with Dim1 (y-axis) and are the key drivers of the spatial variation among the study sites, clearly separating the two study sites, eSikelekehleni and uMngcweni rivers in the negative pole of x-axis from the remaining three in the positive pole of x-axis. The Inanda and Nagle dams as well as the uMsunduzi River in the positive pole of the x-axis indicating they are distinguishable due to their high sediment metal concentrations as opposed to the uMngcweni and eSikelekehleni rivers with lower metal sediment concentrations. The two river sites uMngcweni and eSikelekehleni rivers also have lower phosphorus, nitrate, and potassium compared to the remaining

river sites. The uMsunduzi River is also characterised by higher pH, ammonia and conductivity (Figure 3.6).

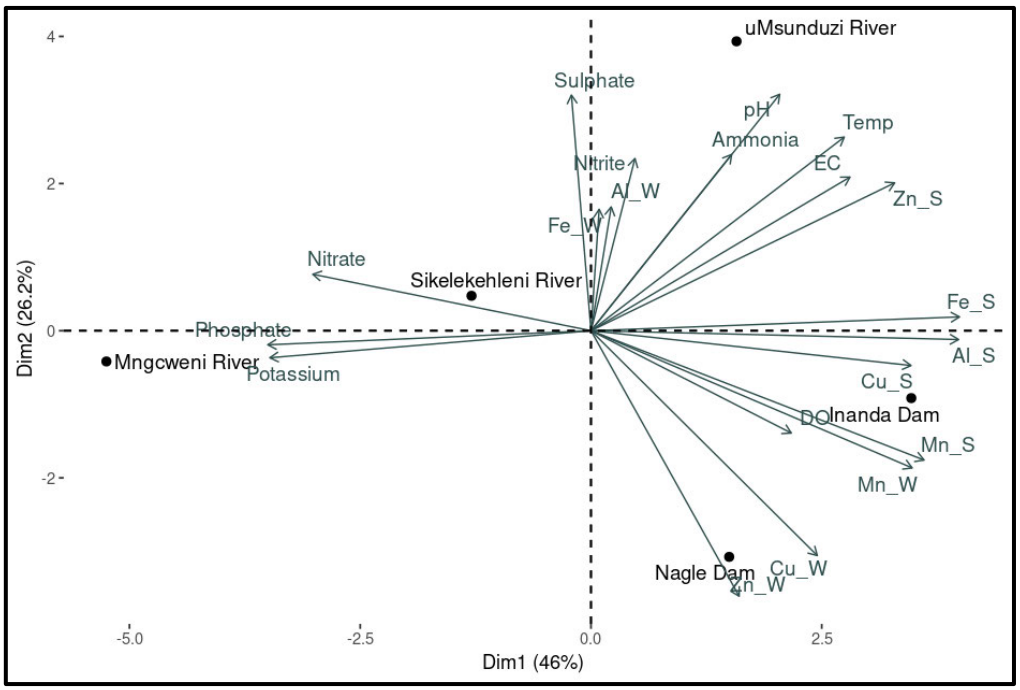


Figure 3.6: A principal component analysis biplot showing the spatial distribution and patterns of metals in sediment (S), metals in water (W), nutrients, and physical parameters across the four study sites.

3.4 Discussion

3.4.1 Physical parameters

3.4.1.1 Water temperature and dissolved oxygen

Water temperature is the most important parameter regulating biological, chemical, and physical processes in aquatic ecosystems (Banda and Kumarasamy, 2020). Zhu *et al.* (2019) mentioned that different aquatic species have different tolerance levels to water temperature. Moreover, water temperature affects the availability and solubility of DO, with solubility decreasing with increasing temperature (Halim *et al.*, 2018). In the present study, a significant difference was observed in water temperature and DO between the sampling sites. High water temperature was recorded in uMsunduzi River, followed by Inanda Dam, Nagle Dam, eSikelekehleni River, and uMngcweni River, whereas high DO (mg/l) was recorded in Inanda Dam, followed by uMsunduzi River, Nagle Dam, uMngcweni River, and eSikelekehleni River. Due to the natural changes in water temperature, there is no specific TWQR, but fish mortalities can occur when the water temperature becomes more than the thermal

tolerance of that fish species (Zhu *et al.*, 2019). The changes in water temperature could be the main factor for fish behavioural change. Crass (1964) mentioned that *L. natalensis* has been observed in low temperatures such as 5 °C during the austral winter and this temperature is considered a low threshold temperature. Burnett *et al.* (2021) found that there was a presence of *L. natalensis* in the uMgeni River when the water temperature decreased to 6 °C. This was close to the threshold or thermal tolerance limits for *L. natalensis* and resulted in *L. natalensis* seeking out a better habitat during these unfavourable conditions as it would decline if the temperature became lower than 5 °C. Despite being regulated, the uMgeni River catchment's water temperature indications have mainly stayed natural (Kusangaya *et al.*, 2018). Favouring higher water temperature, the uMgeni River water temperature is well suited for *L. natalensis* (Karssing, 2008).

Based on the TWQR as stipulated by DWAF (1996) for freshwater, the DO was within the TWQR of 80-120 % and 5-8 mg/l in the Nagle Dam, uMngcweni River, and eSikelekehleni River. The Inanda Dam and uMsunduzi River DO recorded were found to be outside the TWQR as stipulated by DWAF (1996). This shows that Nagle Dam, uMngcweni River, and eSikelekehleni River are well oxygenated in comparison to Inanda Dam and uMsunduzi River. Sharma *et al.* (2020) reported DO levels ranging from 8.6 to 9 mg/l at the Yamuna River which was impacted by wastewater effluents. In the present study, Inanda Dam and uMsunduzi River had higher DO (mg/l) compared to Sharma *et al.* (2020) which justifies that Inanda Dam and uMsunduzi River are heavily polluted by wastewater. Li *et al.* (2021) mentioned that the DO is an important water quality indicator as it affects the survival rate of the aquatic species and its reproduction rate. Moreover, Antonopoulos and Gianniou (2003) mentioned that DO is one of the water's quality parameters that is severely controlled by temperature changes. Dikole (2014) suggested that an increase in water temperature results in lower DO in water. Therefore, the high DO recorded in the uMsunduzi River and Inanda Dam could, at least in part, be attributed to lower water temperature than in the other sites because colder water retains oxygen better than warmer water.

3.4.1.2 Electrical conductivity

Electric conductivity is defined as the ability of an aqueous solution to transport an electric current and it is expressed in mS/cm (Dallas and Day, 2004). It is sensitive and dependent on the changes that occur in the mineral salts in water. Electric conductivity increases as the concentration of dissolved salts increases in the aquatic environment (DWAF (1996). There is no specific TWQR for EC. However, Chapman (2021) reported that the EC in most natural freshwater ranges from 1 to 100 µS/cm. In the present study, the EC recorded in all sampled sites was exceeding the suggested EC range by Chapman (2021). Therefore, Nagle Dam, uMngcweni, and eSikelekehleni rivers were found to be least safe since their EC is closer to 100 µS/cm whereas Inanda Dam and uMsunduzi River were found to be not safe as their EC levels were way above the stipulated range. Prabu *et al.* (2011) reported that high levels of ECs are often associated with untreated sewage from wastewater treatment plants and domestic waste

due to the enrichment of electrolytes and chlorine ions. Therefore, factors that may be contributing to the high levels of EC recorded in Inanda Dam and uMsunduzi River may be the sewage effluent from the wastewater treatment plants and domestic waste from the informal settlements located in Pietermaritzburg (Mdluli *et al.*, 2023). Moreover, the EC concentration reported in Inanda Dam and uMsunduzi River was significantly higher than the values recorded in a study done by Narayanan *et al.* (2021) in the polluted Thirumanimutharu River which receives wastewater from the waste treatment plant. The EC reported in the Thirumanimutharu River is slightly higher than the EC concentration that has been reported in the Nagle Dam, and the uMngcweni and eSikelekehleni rivers. Shabalala *et al.* (2013) mentioned that the agricultural activities can increase the level of EC in water if there is an enrichment of nutrients from these associated anthropogenic activities. Therefore, the EC concentration reported in Nagle Dam, uMngcweni River, and eSikelekehleni River could be related to the small-scale farming or agricultural activities in the catchment area.

3.4.1.3 pH

The pH is another important water quality parameter that has an impact on all aquatic animals' living conditions (DWAF, 1996). Any sudden changes in the pH level in water could significantly affect several aquatic animals. This is because certain aquatic species have preferable pH values, and some die whenever there is even a slight change in the pH level (Alavi *et al.*, 2019). In natural water, pH ranges from 5.0 to 9.0 and it is determined largely by the atmospheric and geological influences (Solovyev *et al.*, 2017). Therefore, pH may vary with regions without being influenced by anthropogenic activities. In the present study, the pH was within the DWAF (1996) TWQR of 6.5 to 9 at Nagle Dam, Inanda Dam, uMngcweni River, and eSikelekehleni River whereas the pH at uMsunduzi River was higher than the TWQR. The pH recorded in the uMsunduzi River and Inanda Dam were significantly higher than that reported by Narayanan *et al.* (2021) in a polluted Thirumanimutharu River. A high pH is associated with the nutrient input from agricultural practices stimulating local algae production (Banda and Kumarasamy, 2020). According to Singh and Serajuddin (2017), highly alkaline rivers may be partially associated with domestic and industrial wastewater nutrient input from agricultural practices stimulating local algae production. Moreover, the effects of anthropogenic eutrophication and industrial effluents on algae's photosynthetic and biological activities can lead to an increase in pH (Uzukwu, 2013). Therefore, the alkaline pH recorded in the Inanda Dam, uMngcweni River, and eSikelekehleni River may be associated with the wastewater discharge and agricultural run-off occurring around its catchment area, whereas the alkaline pH in Nagle Dam may be due to agricultural runoff. Moreover, the uMsunduzi River's alkaline pH may be due to the discharge of waste effluent from industrial activity and the municipality wastewater treatment plant whereas the alkaline pH in the uMgeni River may be associated with microbial processes (Singh *et al.*, 2022). Generally, the uMgeni River tributaries might have had a significant effect on the pH increase in the Inanda Dam, relative to that from Nagle Dam.

3.4.2 Nutrients

3.4.2.1 Potassium

In the present study, the potassium concentrations recorded in the water column ranged from 5 to 18 mg/l for all sampling points. There is no specific TWQR for potassium that has been stipulated for freshwater by DWAF (1996), however, the WHO (2009) emphasised that the safe limit of potassium is 12 mg/l. Potassium concentrations recorded in the uMngcweni River were higher than those reported by Narayanan *et al.* (2021) in the polluted Thirumanimutharu River. A high concentration of potassium in the uMngcweni River could be the result of the industrial activities and human infrastructure that is located within the river's catchment area.

High potassium levels in the water can promote photosynthetic algae reproduction, increasing the amount and quality of algal food sources available to consumers, which can also change the composition of communities, nutrient cycling, and water quality (Schindler, 2012). Bro Borvinskaya *et al.* (2017) further mention that excessively high amounts of potassium concentration in water can have detrimental impacts on fish health. High potassium concentration in water affects the ionic balance of recirculating aquaculture systems in water, and this disrupts the electrochemical gradient of potassium ions across the fish cell membrane, which affects all the physiological functions that are highly dependent on the intracellular and extracellular sodium and potassium concentration (Clement *et al.*, 2015). This includes physiological processes such as acid-base balance, nerve function, osmoregulation, and muscle contraction. It further explained that the sub-lethal effects associated with high potassium level include erratic swimming behaviour, stunted growth rate and gills irritation (Borvinskaya *et al.*, 2017).

3.4.2.2 Ammonia

Ammonia concentrations obtained from the water column ranged from 0.1 to 0.41 mg/l for all sampling points. All the ammonia concentrations recorded in the study were below the DWAF (1996) TWQR range of 0.5 to 2.5 mg/l. Nevertheless, Dallas and Day (2004) stated that 0.1 mg/l of ammonia is expected to be found in natural streams, while DWAF (1996) emphasised that ammonia concentrations between 0.3 and 0.8 mg/l are predicted to have potential sub-lethal effects on warm-water fish, which was the concentration recorded in the uMsunduzi River. Ammonia is a hazardous substance that can negatively affect fish health (Parvathy *et al.*, 2023). The nature and degree of toxicity of ammonia depend on many factors, such as the pH and temperature of the water, the life stage of the fish exposed to pollution, the chemical form of ammonia and the length of exposure (Soler *et al.*, 2021). In water, ammonia exists as NH_4^+ (ionized) and NH_3^0 (unionized). When the unionized form (NH_3^0) from the water enters the fish, some of them change into the ionized form (NH_4^+), which then results in cellular damage (EPA, 1999). At higher pH levels and temperatures, ammonia is considered to be more harmful to aquatic life. Therefore, higher ammonia concentration in the uMsunduzi River can be related to the higher pH and water temperature that was also recorded in the uMsunduzi River. As pH increases, so

does the fraction of unionized ammonia and the toxicity to fish (Parvathy *et al.*, 2023). It is further explained that organic pollution such as domestic sewage, agricultural wastes, and industrial effluents increases ammonia concentration in the aquatic ecosystem (Svoboda *et al.*, 1993). This could further explain the high ammonia concentration recorded in the uMsunduzi River as it receives wastewater from the municipality wastewater treatment plant located within its catchment area.

3.4.2.3 Nitrite

Nitrite can be harmful at 0.5 mg/l whereas a concentration of 1.6 mg/l could be lethal. Moreover, ideal concentrations in aquatic ecosystems range from 0-0.2 mg/l (Akhter et al. 2021). In the present study, a higher concentration was recorded at the eSikelekehleni River, followed by the uMsunduzi River, Inanda Dam and uMngcweni River. In Nagle Dam, the nitrite concentration was below the detectable limits. Generally, the three tributaries may have driven the increase of nitrate in Inanda Dam, whereas the high concentration recorded in uMsunduzi River may have been driven by the effluent from the municipality wastewater treatment plants (Raimonet *et al.*, 2015). In the eSikelekehleni River, the high nitrite concentration may have been driven by the extensive agricultural activities that are taking place within its catchment area. Due to the natural changes of nitrite, there is no specific TWQR. The nitrate concentration observed in eSikelekehleni River, uMsunduzi River and Inanda Dam was above the WHO recommended level of 0.2 mg/l, whereas Nagle Dam and uMngcweni River were below. The nitrite concentration recorded in the uMsunduzi River, uMngcweni River and Inanda Dam was below nitrite concentrations ranging from 1.54-1.58 mg/l that were recorded by Singh and Lin (2015) in the uMgeni River. In the eSikelekehleni River, the nitrite concentration recorded was above these ranges recorded by Singh and Lin (2015). Nitrite concentration recorded in the eSikelekehleni River was similar to the nitrite concentration recorded in the uMthinzima River in a study done by Van Deventer *et al.* (2022) on the upper catchment of Umgeni River. Therefore, the high nitrite concentration in the eSikelekehleni River is potentially due to the agricultural activities such as cattle farming and small scale farmers taking place within its catchment area.

3.4.2.4 Nitrate

Of all the inorganic nitrogen molecules, nitrate is the least harmful to fish with mortalities having been recorded when concentrations exceed 1000 mg/l (Svoboda *et al.*, 1993). When present in excess amounts, nitrate can disrupt the endocrine function in aquatic animals (Raimonet *et al.*, 2015). Halim *et al.* (2018) mentioned that the excessive amount of nitrate in water stimulates the production of algal bloom and then lowers the levels of DO. In the present study, a Weihe River higher concentration of nitrate was recorded in the uMngcweni River followed by uMsunduzi River, Inanda Dam, Nagle Dam, and eSikelekehleni River, respectively. Nitrate is one of the total nitrogen components influenced by domestic and industrial sewage, and agricultural activities (Zhang *et al.*, 2021). Nitrate levels greater than 10 mg/l could be an indication that agricultural and industrial pollutants are contaminating the

water (DWAF, 1996). Olaniran *et al.* (2014) recorded a relatively higher level of nitrate in the Inanda Dam than those recorded in the present study. Moreover, concentrations recorded in the Inanda Dam were comparable to those recorded by Mahabeer and Tekere (2021) in the uMdloti River system, KZN. Nitrate was generally higher at the Inanda Dam than Nagle Dam which could be explained by elevated concentrations in the three tributaries. However, the higher concentrations were still below 10 mg/l, which is the concentration expected for rivers impacted by industrial and agricultural wastes as per DWAF (1996). Nevertheless, the sewage pollution, industrial discharge, and agricultural practices in the uMngcweni and uMsunduzi rivers catchments seem to have played a role in increasing the level of nitrate in these rivers (Penerbit, 2020).

3.4.2.5 Total nitrogen

The total nitrogen was higher at the uMngcweni River, followed by the uMsunduzi River, Inanda, eSikelekehleni River and Nagle Dam. The Nagle Dam concentration was oligotrophic whereas a mesotrophic status was recorded at the Inanda Dam. Mesotrophic waters were also recorded at the uMsunduzi and eSikelekehleni rivers with the uMngcweni River exhibiting eutrophic condition. Nagle Dam exhibited an oligotrophic condition which suggests that the three tributaries had a significant influence on the trophic status of the Inanda Dam water. The total nitrogen recorded at the Nagle Dam was comparable to the concentration recorded by Singh and Lin (2015) in a water body impacted by negligible anthropogenic activities; the concentration recorded in the Inanda Dam was comparable to those recorded by Olaniran *et al.* (2014) in water bodies impacted by municipal and industrial wastewater effluents. It is evident that pollutants such as industrial and wastewater effluents are potential sources of nutrients in river systems. Therefore, the wastewater work in the uMsunduzi River catchment and the industrial activities in the Cato Ridge area could be associated with the nutrient enrichment in the Inanda Dam.

3.4.2.6 Sulphate

De Villiers and Mkwelo (2009) reported that elevated sulphate concentrations in surface water are a good indicator of pollution related to acid mine drainage and industrial wastewater. Mineral weathering, organic matter combustion, and decomposition are the natural sources of sulphate in rivers. (Zak *et al.*, 2021). The primary anthropogenic sources of sulphate concentration in water include solid waste from processing facilities, acid mine drainage, wetland drainage, and runoff from industrial and agricultural wastes (Oberholster *et al.*, 2010). In the present study, a high concentration of sulphate was recorded in the uMsunduzi River followed by the Inanda Dam, uMngcweni River, eSikelekehleni River, and Nagle Dam. Naturally, the concentration of sulphate in the surface water is around 5 mg/l (Banda and Kumarasamy, 2020) whereas, only Nagle Dam was below the natural sulphate concentration in surface water. The possible drivers of sulphate in Nagle Dam and eSikelekehleni River could be the mineral weathering and agricultural activities located within its catchment area. The municipality wastewater

treatment and industrial activities that are taking place within the uMsunduzi River are the main sources that contribute to the high sulphate concentration that was recorded in the river (Oberholster *et al.*, 2010).

In the uMngcweni River, the industrial activities that are taking place in Cato Ridge and the runoff from the agricultural activities could be the driving source of sulphate (De Villiers and Mkwelo, 2009). The presence of sulphate concentration in the Inanda Dam could be the result of the three tributaries that join the uMgeni River downstream of the Nagle Dam. It can also be the agricultural runoff and illegal sand mining that is taking place within the Inanda Dam catchment area. De Villiers and Mkwelo (2009) reported that elevated sulphate concentrations in surface water are a good indicator of pollution related to acid mine drainage and industrial wastewater. Nevertheless, the high concentration of sulphate may form sulphuric acid, which is regarded as a strong acid that reduces the pH level in the water and can also result in lethal effects of metals on aquatic life (Dallas and Day, 2004). The sulphate concentration recorded in the current study was lower than that recorded by Olaniran *et al.* (2014) in the uMgeni River. There is no guideline for sulphate concentration in surface water and aquaculture stipulated by DWAF (1996). Nevertheless, high concentrations of sulphate may form sulphuric acid, which is regarded as a strong acid that reduces the pH level in the water and can also result in lethal effects of metals on aquatic life (Dallas and Day, 2004).

3.4.2.7 Phosphate

Increasing agricultural and industrial activities have led to an excessive phosphate concentration in rivers. Although phosphate is the second most important nutrient for plants after nitrogen and is necessary for both plant and animal growth at ideal levels, it is typically regarded as a pollutant at certain concentrations (Badamasi *et al.*, 2019). There is no guideline on phosphate concentration in surface water stipulated for aquatic ecosystems and surface water, however, the concentration recorded in the present study was within the aquaculture guideline (DWAF, 1996). High phosphate concentration was recorded in the uMngcweni River followed by the uMsunduzi River, eSikelekehleni River, Inanda Dam and Nagle Dam. All three rivers are located within a very dense industrial area, and uMsunduzi River also receives wastewater from the municipality waste treatment plant, and this could be the driving source behind the elevated phosphate concentration in this river. Badamasi *et al.* (2019) mentioned that sewage and industrial waste are the main sources that significantly contribute to and result in elevated phosphate concentration in rivers. In Nagle Dam, the presence of phosphate concentration could be the result of natural processes such as the weathering of rocks and runoff from small-scale farming (Badamasi *et al.*, 2019). The Inanda Dam receives water from the uMsunduzi, uMngcweni, and eSikelekehleni rivers, which are located within a very dense industrial area, and there are having a high concentration of phosphate which they later deposit into the uMgeni River. Moreover, the presence of phosphate in the Nagle Dam could be driven by natural processes such as the weathering

of rocks and runoff from agricultural activities within its catchment area. The phosphate concentration recorded in the uMngcweni River was higher than the phosphate concentration recorded by Singh and Lin (2015) on the uMgeni River and similar to the phosphate concentration recorded in the uMsunduzi and eSikelekehleni rivers.

In reference to the DWAF (1996) guideline for the trophic status of phosphate within the freshwater aquatic environment, Nagle and Inanda dams were mesotrophic, whereas the uMsunduzi and eSikelekehleni rivers are in eutrophic condition. Lastly, the uMngcweni River showed a significantly higher average of phosphate measured in water. The concentration categorised the uMngcweni River as hypertrophic, which infers that this river might have contributed much in the phosphate concentration in the Inanda Dam.

3.4.3 Metals

3.4.3.1 Aluminium

Aluminium is one common chemical element that is discharged into the aquatic environment from the Earth's crust (Botté *et al.*, 2022) and it is classified as the 3rd most abundant element (Maya *et al.,* 2016). The natural source of aluminium is the erosion of Earth's crust whereas it can also be released by anthropogenic activities such as mineral extraction and industrial activities (Botté *et al.*, 2022). In the present study, a low concentration of aluminium was recorded in all water sampling points and there was no significant difference between all the sites. The aluminium concentration observed in the current study was higher than that observed by Mahabeer and Tekere (2021) in the uMdloti River, which ranged from 0.2 and 0.043 mg/l. However, there was a significant difference in aluminium for sediment samples between the sampling sites. A higher amount of aluminium concentration was recorded in the Inanda Dam followed by the uMsunduzi River, Nagle Dam, eSikelekehleni River, and uMngcweni River. The Inanda Dam receives wastewater from the tributaries such as the uMsunduzi, uMngcweni, and eSikelekehleni rivers, which are located in a highly dense industrial area and waste from the unregulated sand mining activities (Botté *et al.,* 2022). The high aluminium concentration recorded in Nagle Dam may have been driven by the weathering of rocks and erosion that is taking place within the river catchment area. Similar concentrations of aluminium that were recorded in the Inanda Dam and uMsunduzi River were recorded in the Weihe River affected by anthropogenic activities such as industrial activities in a study done by Wang *et al.* (2013). Therefore, high aluminium in Inanda Dam and uMsunduzi River could be the result of industrial activities located within the uMsunduzi River and eSikelekehleni River.

Aluminium solubility in aquatic systems depends on several chemical parameters, including temperature, salinity, pH, and the concentration of dissolved organic carbon (Rodríguez, 2023). It has been suggested that aluminium solubility increases when pH decreases. Therefore, aluminium can be easily absorbed by the bottom sediment in alkaline conditions and they are later released into the water

as the pH drops (Crafford and Avenant-Oldewage, 2010). The alkaline level of pH concentration that was recorded in the study may be the explanation for the low concentration of aluminium in water and high concentrations of aluminium in sediment. Based on DWAF (1996), there is no specific TWQR for aluminium in freshwater, however, the elevated level of aluminium in water environments may affect the success of aquatic species and communities in an acidic environment (Atkinson *et al.*, 2007).

3.4.3.2 Copper

Copper is an essential nutrient for fish, and therefore the intake of copper is essential to sustain basic metabolic processes (Wood *et al.*, 2012). Nevertheless, high levels of copper can potentially have detrimental consequences on various metabolic pathways (Delahaut *et al.*, 2020). Copper concentrations in water were below the detectable limit, however, notable copper concentrations were recorded in sediments. There is no specific TWQR for copper concentration in water and all copper levels in the sediments ranged from 0.37 to 1.04 mg/kg. Moreover, a significant difference in copper was observed in sediment samples between all sampling points. A high amount of copper concentration was recorded in Inanda Dam followed by the uMsunduzi River, Nagle Dam, uMngcweni River, and eSikelekehleni River. High levels of copper in the Inanda Dam and uMsunduzi River could be driven by the wastewater from municipality treatment works, industrial effluent, and agriculture run-off located in the uMsunduzi River catchment area (Poshtegal and Mirbagheri, 2019) whereas, concentration of copper in the Nagle Dam could be the result of fertilizer run-off and animal waste (Banda and Kumarasamy, 2020). The copper concentrations recorded in sediment in the Inanda Dam were similar to those obtained by Dikole (2014) in a study done at the uMgeni River. Furthermore, copper has a high solubility and mobility in acidic pH water, while it precipitates in alkaline water (Dallas and Day, 2004). This may account for the water column's low copper concentration. However, the high concentration in sediment remains a cause for concern as it could be problematic for the benthos and/or when the water pH changes.

3.4.3.3 Iron

In natural water, iron concentration varies in amounts depending on the various chemical properties and the geology of the water body. Iron is termed the fourth most abundant element on Earth's crust, and it is an essential micronutrient in living organisms (Wang *et al.*, 2020). There is no specific TWQR for iron concentration in both water and sediment whereas, based on DWAF (1996), iron concentration in unpolluted surface waters ranges from 0.001-0.5 mg/l. In the present study, iron concentration in water ranges from 0.38 to 2.35 mg/l with only uMsunduzi and eSikelekehleni rivers above the 0.001-0.5 mg/l range. This shows that the uMsunduzi and eSikelekehleni rivers receives polluted wastewater as they are both located in a very dense industrial area. A high concentration of iron in water was recorded in the eSikelekehleni River followed by the uMsunduzi River, Nagle Dam, Inanda Dam and uMngcweni River. In sediments, high iron concentration was recorded in the Inanda Dam followed by the

uMsunduzi River, Nagle Dam, eSikelekehleni River and uMngcweni River. The explanation for the high levels of iron concentration in Inanda Dam and uMsunduzi River can be that Inanda Dam receives water from the highly polluted uMsunduzi River which receives wastewater from industries and wastewater water treatment plants activities occurring within its catchment area. The presence of iron concentration in the Nagle Dam, uMngcweni River and eSikelekehleni River could result from the fertilizer run-off. Similar findings on iron concentration recorded in the Inanda Dam and uMsunduzi River were obtained from the Palmiet River by Moodley *et al.* (2014). It was further suggested that the anaerobic conditions and low pH in sediments can significantly influence the amount of iron released (Van Ha *et al.*, 2011). Higher iron concentration was observed in the study when compared to the iron concentrations that were observed by Shozi (2011) in a study done on the uMgeni River estuary. The high iron concentration recorded in sediment is because the iron precipitates and gets absorbed into sediment under alkaline conditions in water and it gets released back into the water body under acidic conditions (Fonseca *et al.*, 2011).

3.4.3.4 Manganese

As the eighth most common metal in the crust of the Earth, manganese is found naturally in rocks, soil, groundwater, surface water, and food but it may also be present in rivers due to pollution sources such as sewage, industrial effluents, and acid mine drainage (Banda and Kumarasamy, 2020). It is also a normal part of the diet of humans (Heal *et al.*, 2002). The concentration of dissolved manganese in water is highly affected by changes in DO, pH, organic matter and redox potential. Dallas and Day (2004) mentioned that elevated manganese concentration in water is toxic to vertebrates and can lead to disturbances in various metabolic pathways. The alkaline level of pH affects the solubility of manganese in the water body (Abesser and Robinson, 2010). Therefore, this can be the explanation for low concentration levels of manganese in the water sample than in the sediment sample (Dikole, 2014). The permissible limit of manganese is 0.05 mg/l in drinking water, as defined by WHO (2021). The manganese concentration for all sampling points in water was recorded to be below the manganese TWQR guideline for freshwater which is 0.18 mg/l according to DWAF (1996).

In sediment, high manganese concentration was recorded in the Inanda Dam followed by the Nagle Dam, eSikelekehleni River, uMsunduzi River and uMngcweni River. The low manganese concentration in the Nagle Dam, uMngcweni, and eSikelekehleni rivers could be the result of the agricultural runoff from the small-scale farmers, whereas in the uMsunduzi River and Inanda Dam could be highly driven by the wastewater from municipality treatment plants and industrial waste that is being discharged into the uMsunduzi River. Hasan *et al.* (2011) suggested that the municipality and industrial wastewater are major sources of manganese concentration in rivers. Varbanov *et al.* (2021) reported a high concentration of manganese in sediments compared to Inanda Dam on the Iskar River which receives wastewater from the wastewater treatment plant and three highly polluted rivers. A higher concentration

of manganese compared to the uMsunduzi River concentration was recorded in the Umdloti River in a study done by Mahabeer and Tekere (2021). Relativity lower manganese concentration was recorded in the Inanda Dam when compared to the manganese concentration provided in a study done by Banda and Kumarasamy (2020) on the uMgeni River system.

3.4.3.5 Zinc

For both plants and animals, zinc is a necessary dietary trace element. While fish are very susceptible to zinc poisoning, humans have a high tolerance capacity for excessive zinc concentrations (DWAF, 1996). When present in significant amounts, it can harm fish and other aquatic organisms. Excessive zinc concentrations can lead to system malfunctions that hinder fish growth and reproduction (Verma and Dwivedi, 2013). According to DWAF (1996) guideline, there is no specific TWQR for zinc in freshwater. However, Svoboda *et al.* (1993) mentioned that the zinc concentration may be lethal to fish at a concentration of around 0.5 to 1.0 mg/l for fish. In the present study, zinc in water samples was below detection level in all sampling points whereas a considerable level was recorded in sediment. Zinc concentration precipitates in the water column and becomes fixed in the sediments, resulting in a higher amount of zinc concentration based in sediment than in the water column.

A significant difference in zinc concentration in sediment samples between all sampling points was observed. A high zinc concentration was recorded in the uMsunduzi River followed by the Inanda Dam, Nagle Dam, eSikelekehleni River, and uMngcweni River. The presence of zinc concentration in Nagle Dam, eSikelekehleni River, and uMngcweni River could be the result of the agricultural runoff from the small-scale farming activities whereas, in the uMsunduzi River and Inanda Dam the possible drivers for high zinc concentration could be results of sewage waste from wastewater treatment and further by the industrial wastewater from industrial activities. Zinc concentrations recorded in Inanda Dam and uMsunduzi River are similar to those reported in the Olifants River system (Dabrowski *et al.*, 2014; Dabrowski *et al.*, 2013; Lebepe *et al.*, 2016). However, zinc concentrations observed in the uMsunduzi River were relatively higher than those recorded in the Vaal River which receives wastewater from the wastewater treatment work (Iloms *et al.*, 2020).

The physical parameters of the water were generally higher at the uMsunduzi River and Inanda Dam compared to other sites. Furthermore, nutrients were generally lower at the Nagle Dam with the uMngceweni River exhibiting higher concentrations for most nutrients. Metals were generally higher at Inanda Dam followed by Nagle Dam and uMsunduzi River, with lower concentrations observed in the other sites. It is evident that the tributaries are potential contributors of pollutants into the Inanda Dam. The agricultural activities, industrial wastewater and municipality wastewater treatment plants located in the uMsunduzi River catchment are potential drivers for water pollution in the uMsunduzi River and ultimately, the

Inanda Dam. Moreover, high metal concentrations observed in sediment at all sites become a cause for concern as these metals have the potential to get resuspended back into the water column and cause impact on aquatic biota. Therefore, measures to reduce pollutants influx into the uMgeni River systems should be taken to reduce the risks of continuous pollution of the river.

CHAPTER 4

General health condition of fish

4.1 Introduction

The rapid increase in the discharge of toxicants in the form of wastewater by domestic, agricultural, and industrial activities has resulted in the pollution of aquatic environments including freshwater systems (Malik *et al.*, 2020). In aquatic environments, fish have frequently been employed as biological indicators of pollution. Fish have often been used as biological indicators of pollution (Mdluli *et al.*, 2023). Aquatic pollution can result in morphological and physiological changes in fish which may, in some cases, result in sub-lethal conditions and/or death (Kavitha *et al.*, 2023). Therefore, these toxicants are a significant environmental hazard to aquatic environments and fish, in particular (Malik *et al.*, 2020).

Adams *et al.* (1993) mentioned that the means of monitoring and evaluating freshwater aquatic system health status is crucial. The assessment of the general condition of fish is used in combination with water quality, and it is done by assessing and comparing certain fish parameters such as growth, blood chemistry, age, weight, and liver structure among the affected and non-affected sites. The use of the health assessment index parameter in accessing freshwater pollution is easy and inexpensive, and has been successfully used by Madanire-Moyo *et al.* (2012), Mdluli *et al.* (2023), and Lebepe *et al.* (2020). Adams *et al.* (1993) developed a quantitative approach in the United States of America (USA) to evaluate the effect of pulp mill effluent on fish health status, and this index allows the statistical comparisons of fish health. Further modifications to the HAI were made by Heath *et al.* (2004) and Crafford and Avenant-Oldewage (2009), and are effective in discriminating water bodies with different water quality.

The HAI parameter evaluates the fish's health by assigning a score to stressor-induced abnormalities observed in different internal and external organs of the sampled specimens. This scoring system is based on the severity of damage that has been caused by parasite numbers and environmental stressors (Crafford and Avenant-Oldewage, 2009). A numerical value is given to each fish organ and tissue that is examined based on its condition/health. The total sum of the scored value for a certain fish is then referred to as the HAI for that particular fish, whereas, the average HAI of all fish sampled in a specific area is thought to be representative of that population (Madanire-Moyo *et al.*, 2012). A high HAI mean score is associated with poor water quality and an increase in physiological and behavioural stresses, while a low HAI mean score is associated with good water quality (Adams *et al.*, 1993; Crafford and Avenant-Oldewage, 2009). In SA the HAI has been used by Crafford and Avenant-Oldewage (2009) to evaluate the Vaal River system health status. It has been further used to evaluate the health status of the Limpopo and Olifants River systems (Madanire-Moyo and Barson, 2010; Madanire-Moyo *et al.*, 2012;

Watson, 2012), and the uMgeni River system (Hlatshwayo *et al.*, 2024; Mdluli *et al.*, 2023; Misra *et al.*, 2024).

Other indices that were shown to be reliable in discriminating water bodies characterised by dissimilar water quality are CF and HSI with the GSI indicating reproductive capacity and cycle of fish populations (Amina *et al.*, 2021). Moreover, the three indices are employed in fisheries to determine the well-being and the sustainability of stock (Banda and Kumarasamy, 2020). The study aimed to evaluate the overall health of *L. natalensis* in the Inanda and Nagle dams in the uMgeni River system. The objective was to compare the overall health of *L. natalensis* between Nagle and Inanda dams using the HAI, CF, GSI and HSI. The Inanda Dam receives water from the heavily polluted uMsunduzi River. Therefore, it was hypothesised that the health of the fish population from the Inanda Dam will be more compromised than those from Nagle Dam.

4.2 Methods and Materials

4.2.1 Fish sampling

Fish were caught using an electric shocker during low and high flow seasons in 2020 and 2021 at the riverine sites of the Inanda and Nagle dams (Protocol reference number: AREC/019/018) as seen in Figure 4.1. The fish were euthanised by severing the spinal cord just behind the head. The mass (g), standard length (cm) and total length (cm) of each fish specimen were recorded. The fish specimen was then cut open ventrally, and the gonads and liver were weighed. The liver and gill tissues were then stored on formalin for histopathology assessment, and another piece of male and juvenile liver tissue was stored on ethanol (99%) for VTG concentration analyses.



Figure 4.1: The electric shocker equipment for sampling in the riverine.

4.2.2 Health assessment index

The Adams *et al.* (1993) scoring system was used to grade the internal and exterior organs' appearance and condition of the fish in order to determine the HAI. The variables of the HAI were presented with a value ranging from 0-30, depending on the condition of the organs tested, with normal conditions indicated by 0 and abnormal conditions indicated by 30. The HAI value for each fish was calculated by adding the numerical values for all metrics and variables that were recorded (Adams *et al.*, 1993). The index value for the population was calculated by summing the indices of all fish examined and dividing by the number of fish that were examined.

4.2.3 Condition factor

The length and weight of the fish were measured, recorded and used to calculate the Fulton's condition factor (K) for each fish (Robinson *et al.*, 2008). The K was utilised to assess the overall health of fish and determine any potential effects of pollution on fish. The proportion of body mass to body length is calculated to determine it. The CF could be used as an indicator of fish conditions, and it was calculated using Equation 4.1.

$$K = \frac{W\ X\ 10^5}{L^3} \quad \text{..... Equation 4.1}$$

where L is length (mm) and W is mass (g).

4.2.4 Gonadosomatic index

The GSI was calculated as a ratio between the gonad's weight (g) and the body weight (g) of the fish. It is frequently employed to analyse and characterize the reproductive cycle of the fish (Ouréns *et al.*, 2012). The total weight (g) of each fish specimen was recorded using a handheld electronic balance whereas gonads were weighed using a bench-top balance. The calculation of the GSI follows the Equation 4.2:

$$GSI = \frac{Gonads\ Weight\ (g)}{Body\ Weight\ (g)} \text{X}100 \quad \text{..... Equation 4.2}$$

4.2.5 Hepatosomatic index

The HSI was calculated as a ratio between the liver weight (g) and the body weight (g) of the fish. Based on Van Dyk (2003), the fish tissue or organs can easily adapt to changes in the surrounding environment by undergoing either hyperplasia, hypertrophy, or atrophy. The HSI is used to indicate the liver size as a percentage of total body weight, hence, its value indicates the ratio of liver weight to body weight. Based on Marchand *et al.* (2012) the HSI was calculated using Equation 4.3.

$$HSI = \frac{liver\ weight\ (g)}{body\ weight\ (g)} \times 100 \quad \text{..... Equation 4.3}$$

4.2.6 Length-Weight relationship

The relationship between the length and weight was determined following Le Cren (1951) protocol using Equation 4.4.

$W = aL^b$ …...…….…….. **Equation 4.4**
Where: **W** is the weight of fish (g), **L** is the total length of fish (cm), **a** is the coefficient related to body form, and **b** is an exponentially expressed relationship between length and weight.

To estimate the parameters *a* and b, the logarithm transformation of the equation was expressed by Kahraman *et al*. (2014):

$LogW = Log\alpha + Logb$ …….. **Equation 4.5**

4.2.7 Data analysis

The R-3.1.1 (R Development Core Team 2014) was used to perform the statistical analysis with a level of significance set at $p<0.05$. The Shapiro-Wilk test was used to determine the normality of the data and the Levene's test was used to test the equality of variance between the data. Results demonstrating seasonal variations were emphasised, while those showing no seasonal variations were left out. Depending on the data distribution, the Wilcoxon-Mann-Whitney U-test or an independent sample t-test was used to evaluate the difference between the CF, HSI, HAI and GSI of the two dams.

4.3 Results

4.3.1 Health assessment index

The most prominent abnormalities that were observed in the liver and gills at the two dams (Figure 4.2). However, the magnitude of severity showed variation in each dam with fish size as well. Abnormalities in the skin, eyes, and fins were mild to negligible as some were associated with physical injuries during fish handling in both dams. As seen in Figure 4.2 A, fish from the Inanda Dam experienced liver discolouration (dark brown-black, as opposed to a lighter, red-brown), liver nodules and fatty liver as shown. In B, the discolouration of yellow fish gonads can be seen and showed asynchronous development. In C: The pale red colour was observed in fish gills located at the Inanda Dam.

The HAI results are presented in Figure 4.3. A significant difference was observed in the HAI between Inanda and Nagle dams ($p<0.05$) with the Inanda Dam exhibiting a higher index with a value of 45.09±27.34 compared to the Nagle Dam with a value of 19±22.22. Moreover, no seasonal variations were observed between the Inanda and Nagle dam populations ($p>0.05$).

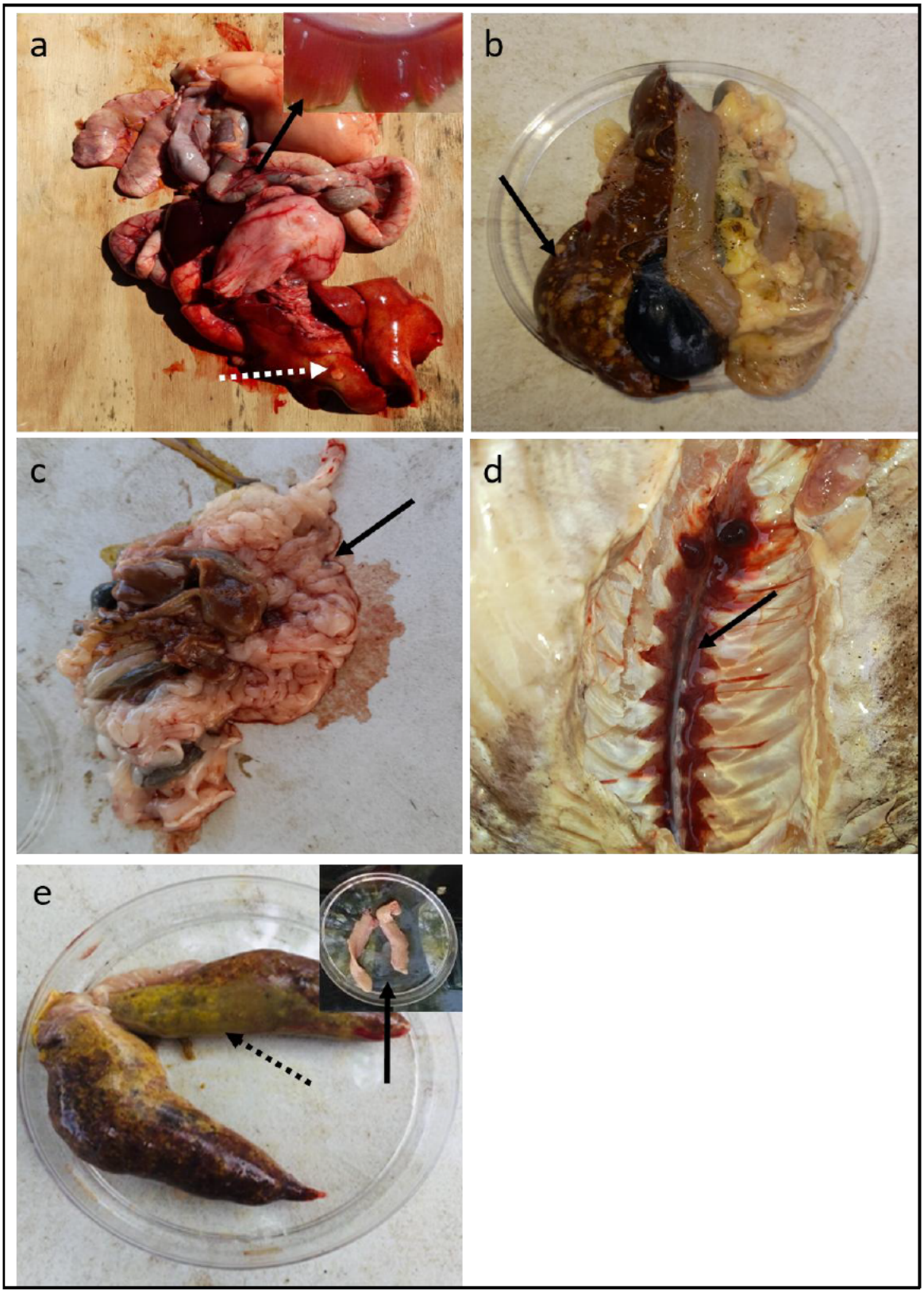


Figure 4.2: The observed abnormality in yellow fish at Nagle and Inanda dams: a. Cyst in the liver (dotted arrow), normal gill (solid arrow); b. Nodules, cysts and discolouration of the liver (arrow); c. High fat percentage covering the intestine and liver(arrow); d. Normal kidney (arrow); e. Fish gonads showing signs of discolouration and poor development (solid arrow), abnormal eggs (dotted arrow)

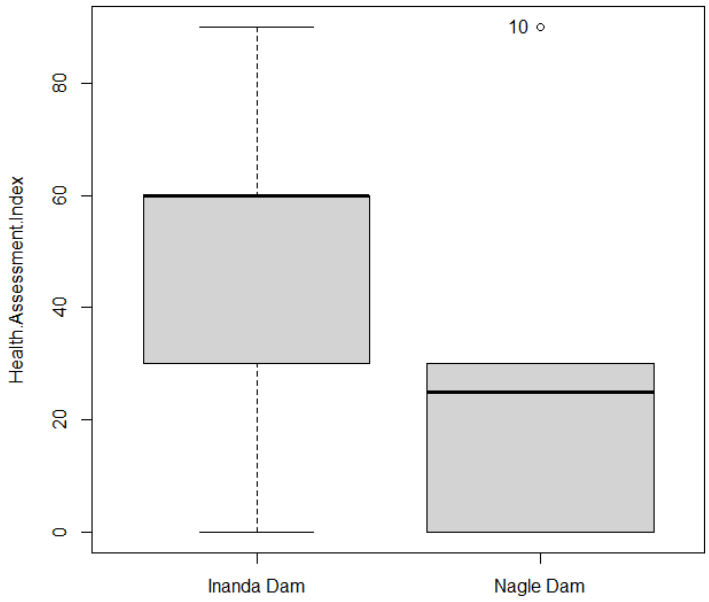


Figure 4.3: The HAI recorded for *Labeobarbus natalensis* in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

4.3.2 Overall fish health

4.3.2.1 Condition factor

No significant differences in K between the Inanda and Nagle dams' populations ($p>0.05$) as seen in Figure 4.4. The mean K recorded in Nagle Dam was 2.01±1.4 while Inanda Dam was 1.95±0.785. There was also no seasonal variation between the Inanda and Nagle dams ($p>0.05$).

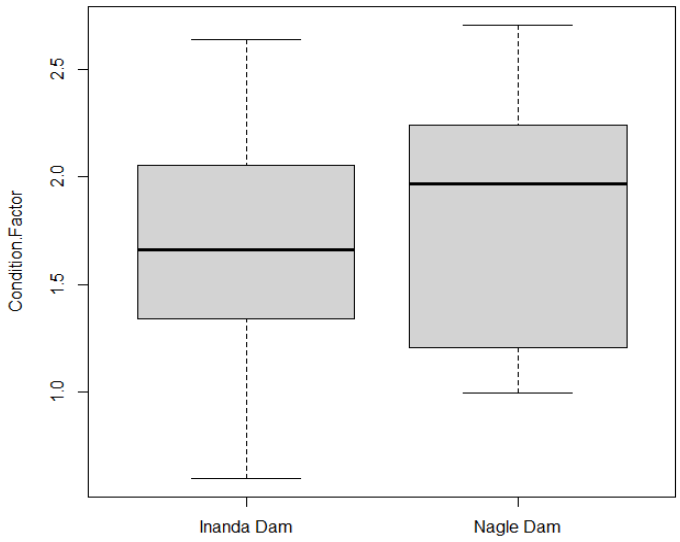


Figure 4.4: The K recorded for *Labeobarbus natalensis* in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

4.3.2.2 Hepatosomatic index

No significant difference for HSI between Inanda and Nagle dam populations ($p>0.05$) as presented in Figure 4.5. Inanda Dam had an average of 0.65±0.34 % and Nagle Dam had an average of 0.804±0.43 % for HSI. No seasonal variations between the Inanda and Nagle dams ($p>0.05$) were observed.

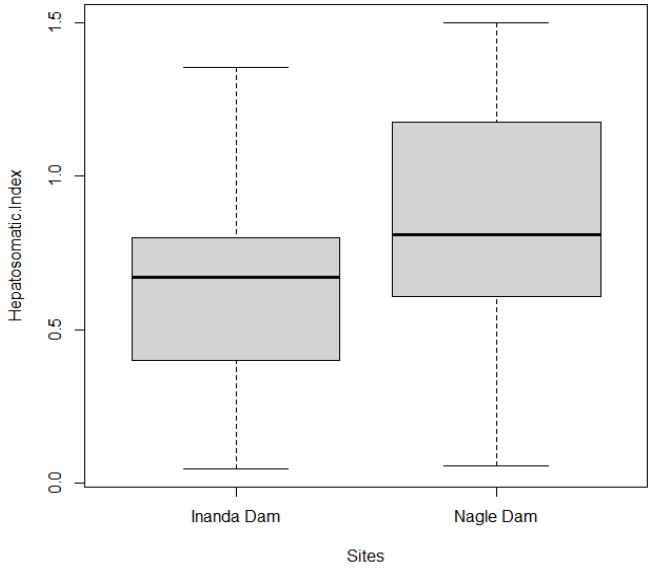


Figure 4.5: The HSI recorded for *Labeobarbus natalensis* in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

4.3.2.3 Gonadosomatic index

The GSI results are presented in Figure 4.6 and Figure 4.7. No significant difference in the male GSI between the Inanda and Nagle dams ($p>0.05$), whereas a significant difference was observed for female GSI between the Inanda and Nagle dams. There were no seasonal variations of the GSI for males ($p>0.05$). Inanda Dam males had a GSI average of 0.35±0.53 % and Nagle Dam males had a GSI average of 3.46±3.38 %, whereas females in Nagle Dam had 2.95±1.86 % had a GSI average of and Inanda Dam females had a GSI average of 1.79±0.24 %.

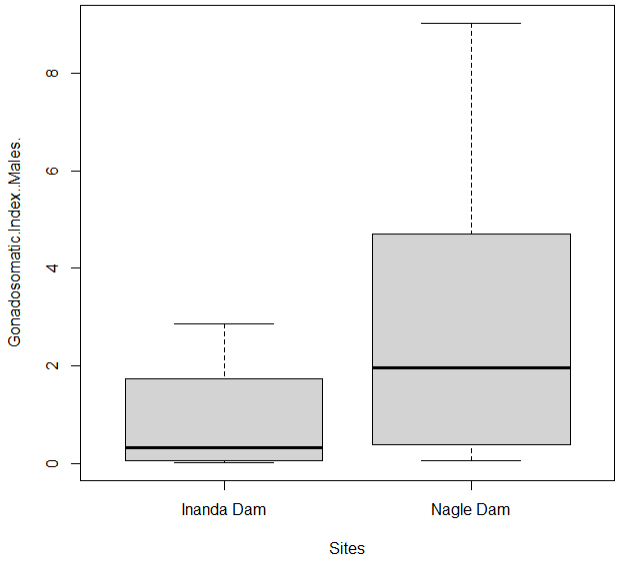


Figure 4.6: The GSI recorded for *Labeobarbus natalensis* in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

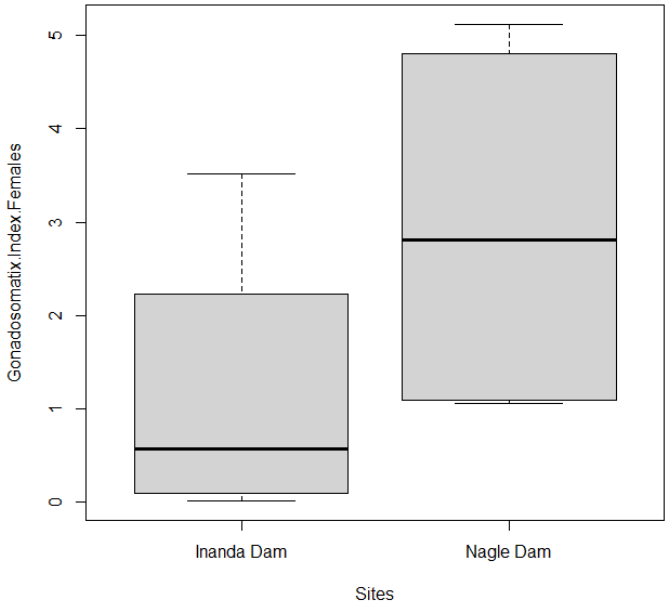


Figure 4.7: The GSI recorded for *Labeobarbus natalensis* (female) in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

4.3.3 Length-Weight Relationship

A negative allometric growth pattern in *L. natalensis* population was recorded, with a length-weight relationship of $W= 0.00149L^{2.132}$ for the male population and $W= 0.10762L^{1.272}$ for the female population (Table 4.1). The combined population of *L. natalensis* recorded in Inanda Dam had a negative allometric growth pattern with $W= 0.00842L^{0.651}$. The positive allometric growth pattern in *L. natalensis* male population was recorded in Nagle Dam with $W= 0.00000003L^{4.225}$ whereas, a negative allometric growth pattern ($W= 0.06653\ L^{1.508}$) in Nagle Dam female population was recorded. The combined population of *L. natalensis* recorded in Nagle Dam had a negative allometric growth pattern with $W= 0.0000000L^{0.889}$.

Table 4.1: Length-weight relationships of *Labeobarbus natalensis* recorded at Nagle and Inanda dams. (With n: Number of samples, a: The rate of change of weight with length (intercept), b: The weight at unit length (slope), R^2: The coefficient of determination, -: Negative allometric growth, +: Positive allometric growth)

Site	Sex	n	a	b	R^2	Growth pattern
Inanda Dam	Male	17	2.132	0.00149	0.672	-Allometric
	Female	4	1.272	0.10762	0.903	-Allometric
	Combined	21	1.789	0.00842	0.651	-Allometric
Nagle Dam	Male	16	4.225	0.00000	0.934	+Allometric
	Female	4	1.508	0.06653	0.916	-Allometric
	Combined	20	2.867	0.00000	0.889	-Allometric

4.4 Discussion

4.4.1 Health assessment index

Degradation of aquatic environments are mostly due to human impacts or human activity, and they may be caused by different pollutants such as toxic compounds, including heavy metals. A low HAI value is indicative of good water quality while a high HAI value indicates poor water quality (Yeom *et al.*, 2007). In the present study, the HAI values recorded in the Nagle Dam were comparable to those recorded by Yeom *et al.* (2007) in good quality Balule River and Okavango Delta (Van Dyk *et al.*, 2009). In contrast, the HAI for the Inanda Dam population was relatively higher than that of the Nagle Dam. The Inanda Dam HAI values were comparable to those recorded by Watson (2012) at a heavily polluted Loskop Dam.

4.4.1.1 Internal variables

Physiologically, the fish liver is responsible for essential metabolic processes including storage and processing of nutrients, bile formation and excretion, synthesis of enzymes and metabolism of xenobiotic compounds (Čižmek *et al.*, 2023). The liver is also referred to as the detoxification organ since it is primarily in charge of pollutants excretion (Topić Popović *et al.*, 2023). High amounts of pollutants can overwhelm its regulating mechanism, causing structural damage as a consequence (Van Dyk, 2003). In the present study, no liver abnormality was observed at the Nagle Dam during low flow and high flow. Several liver abnormalities were observed at the Inanda Dam during low flow and high flow. These abnormalities included fatty liver, liver nodules, focal discolouration, and general discolouration.

In fish, gonads are important organs that are responsible for the fish reproduction. Any changes in the gonad's activities can result in the reduction of the production ability of fish (Mohapatra *et al.*, 2021). In the present study, discolouration in one of the fish gonads caught in the Inanda Dam was observed, whereas no discolouration in fish gonads was observed in the Nagle Dam. Macroscopic assessment showed that the gonads were generally in good condition across all sites.

4.4.1.2 External features

Fins are unique anatomical features that are made of bony spines, and they are directly in contact with the aquatic environment. They are subjected to different kinds of pollutants that are available in the water body (Jooste *et al.*, 2005). Abnormally long fins and forked fins can be a result of genetic variation, or they may have originated from environmental influences (Ellison *et al.*, 2023). Furthermore, tail or fin rot observed in fish may specifically reflect the effect of pollution (Jan *et al.*, 2022). Various variables linked to anomalies in the fins include water quality, bacterial infection, feed type, exposure to excessive environmental contaminants, temperature, and overcrowding (Pelis and McCormick, 2003). The deformation of fin rays and fin/tail rot was not observed during low flow or high flow conditions in both dams.

The eyes are among the highly vascularised organs in fish that indicate the well-being of a fish in several ways (Ruchin, 2021). It is an important sensory organ in fish since it is responsible for gathering, focusing, and converting pictures into brain signals. Furthermore, the eye may be a significant pathway for the absorption of pollutants due to its large surface area and constant contact with the external environment (Pereira *et al.*, 2013). Lesions expected in fish eyes include the discolouration of the cornea and swelling of the orbits (Roberts, 2012). Moreover, sunken eyes as a result of severe internal illness, unfavourable conditions during winter, starvation, and chlorine poisoning may be observed (Stacy *et al.*, 2018). No eye abnormality was recorded in the present study in both Nagle and Inanda dams.

Fish skin is the primary defence against a wide range of chemical, physical, and biological stresses (Guardiola *et al.*, 2015). Due to its close proximity to the aquatic environment, the skin, despite being covered in scales, has been described as the initial organ to be attacked by a range of parasites and environmental pollutants. The fish skin is essential for fish to maintain appropriate osmoregulatory function and protection. It is composed of arrays of individual scales that form a textured surface (Esteban, 2012). The scales are embedded within the skin's epidermal layer. Fish skin functions as a semipermeable, natural, physical, and biological barrier that allows water, hormones, gases and gametes to be exchanged (Larsen *et al.*, 2013). Adams *et al.* (1993) reported that skin abnormalities may range from mild to moderate and severe aberrations. No skin abnormality was recorded in the present study in both Nagle and Inanda dams.

The fish gills are sensitive organs that are responsible for respiration and maintaining the optimal osmotic pressure. Additionally, they are responsible for regulating the exchange of water and salts (Strzyzewska *et al.*, 2016). The gills further play an important role in excreting harmful and unnecessary metabolic products (De Marco *et al.*, 2023). They assume a significant role in pH regulation, the discharge of nitrogenous waste products, and hormone production (Herrero *et al.*, 2018). Their vulnerability is subsequently extensive because they have a large surface area, and they are in contact with the external water environment, which makes them the most sensitive organs of the fish (Mohebbi *et al.*, 2020). This indicates that any irritating substance, whether dissolved or suspended in water, has the potential to harm them (Roberts, 2012). No gill anomalies were recorded in the Nagle Dam, whereas the Inanda Dam population showed a pale gill. Poleksić and Mitrović-Tutundžić (1994) mentioned that the presence of pollutants in water causes the alteration in the morphologic structure of gills and further interrupts the important functions that are carried out by the gills. Strzyzewska *et al.* (2016) mentioned that fish gills from wastewater polluted environments are pale pink as shown in Figure 4.2C, whereas fish gills from pristine environments are bright red. Similar results of pale pink gills were recorded in *O. niloticus* which was exposed to acute levels of Cu (Alkobaby and Abd El-Wahed, 2017). This justifies that the Inanda Dam has polluted water since it experienced structural changes in gills as compared to the Nagle Dam. The quantitative HAI does not give details to whether the anomalies observed were pollutants induced or not, therefore histological analysis was done and discussed in Chapter 5 to further investigate the anomalies observed.

4.4.2 Condition factor

The K is used as an indicator of water quality and the fish population's overall health that is inhabiting particular habitats or ecosystems (de Vries *et al.*, 2020). Komba *et al.* (2020) reported that the K is highly influenced by the biotic and abiotic factors of the environment, and it can be utilised to assess the condition of fish in aquatic systems. The K of fish varies with species since different species have certain different lengths and weights.

A K of 0.80, according to Barnham and Baxter (2003), indicate a very poor condition, while 1.60 indicates an excellent condition (Shobikhuliatul *et al.*, 2013). In the present study, mean K of 2.08 and 1.96 were recorded at Nagle Dam during low flow and high flow seasons, respectively while in Inanda Dam the mean K of 1.72 and 2.91 were recorded during low flow and high flow seasons, respectively. The K values recorded in Inanda Dam were higher than those reported by Gupta (2023) on *Eutropiichthys murius* species from the Narayani River in Nepal which is polluted by wastewater. In contrast, the K values recorded in the Inanda Dam were lower than those reported by Mdluli *et al.* (2023) for *C. gariepinus* in the Inanda Dam. It is believed that fish of the same length that are heavier are healthier than those that are lighter. The *L. natalensis* caught in Nagle Dam were bigger than the ones in the Inanda Dam with similar length this justifies that the *L. natalensis* located in the Nagle Dam are in better condition than the ones located in Inanda Dam. The K values of *L. natalensis* obtained in the Nagle and Inanda dams are higher than those reported by Marchand *et al.* (2012), and Crafford and Avenant-Oldewage (2009) in the Vaal River and the two dams in Gauteng, respectively. Although studies reported K as a useful indicator in differentiating fish populations from dissimilar water quality (Madanire-Moyo *et al.*, 2012; Watson *et al.*, 2012), it was not conclusive in distinguishing the overall fish health between the two dams.

4.4.3 Hepatosomatic index

The liver can degrade toxic compounds found in the body of an organism, but it may be overwhelmed by the elevated concentration of these pollutants (Van Dyk, 2003). Salamat and Zarie (2012) reported that hyperplasia or hypertrophy may act as an adaptive response to enhance the liver's ability for detoxification in response to elevated pollutants. The HSI values may increase or decrease due to hypertrophy, hyperplasia (increase in cell number), and cellular necrosis (Silveyra *et al.*, 2023). The HSI has been used as the indicator of the overall health of fish due to the energy storage and metabolic function of the liver (Prakash, 2022). The HSI is associated with contamination exposure (Savassi *et al.*, 2020).

Munshi and Dutta (1996) reported that the normal HSI values range from 1-2 %. Furthermore, Salamat and Zarie (2012) indicated that the HSI value below 1 % indicates possible atrophy of hepatocytes whereas the HSI score value of more than 2 % indicates a possible hypertrophy of hepatocytes. The HSI value of 0.62 % was recorded at the Inanda Dam with 0.52 % being recorded at the Nagle Dam. Both populations exhibited HSI below 1 % which suggests possible atrophy of hepatocytes. Moreover, the possible reasons for the HSI value in the Inanda Dam could be the result of the liver enlargement in fish that can be increased by enzyme activity for detoxification of the contaminants (xenobiotics) or high carbohydrate diets (Sindhe and Kulkarni, 2004). Therefore, increases in liver weight might have resulted in the increase in the HSI value at the Inanda Dam. Bekmezci and Nevin (2010) mentioned that wastewater decreases HSI in fish due to the depletion of the energy reserves in the liver. Similar to the

study, Prakash (2022) recorded a higher HSI of *Channa punctatus* collected from polluted and a lower HSI from fish collected from unpolluted sites. This is an indication that the populations of *L. natalensis* at Inanda Dam are exposed to elevated levels of wastewater contaminants in their aquatic environment than the Nagle Dam. However, HSI may be influenced by other factors such as food availability, parasites, and physiological development (Sanchez *et al.*, 2008). Darweesh *et al.* (2019) claimed that the HSI is a useful indicator of chemical water pollution, while Dutta and Banerjee (2016) showed that the HSI was an inconsistent biomarker. In the present study, the HSI was not conclusive in distinguishing the two populations.

4.4.4 Gonadosomatic index

The GSI is another significant parameter that shows changes during stressful conditions as well as the population's condition for future generations Prakash (2022) and it is further used to assess the level of ovarian ripening in fish (Banda and Kumarasamy, 2020). The GSI is a useful tool for figuring out an organism's reproductive cycle and for creating appropriate breeding plans for fish species that are endangered or that have an impact on any body of water (Kaur *et al.*, 2018b). Adams *et al.* (1993) claimed that the GSI is a significant indicator of the overall health of a population of an organism. On the contrary, Kaur *et al.* (2018b) claimed that the production of milt and egg by fish is highly influenced by the environmental contaminants.

In the present study, the female GSI recorded in the Nagle Dam was higher than the one recorded in the Inanda Dam. The high GSI is an indication that the fish in the Nagle Dam had larger gonads than those in the Inanda Dam. The industrial effluent and wastewater received from municipality wastewater treatment plants might have a toxic effect on fish gonads. This resulted in a decrease in GSI collected from Inanda Dam which is known to be highly polluted by tributaries joining the uMgeni River, especially uMsunduzi River which receives wastewater from industrial effluent and water treatment plants. West (1990) explained that the GSI assumes that proportionally larger gonads are an indication of greater development and better environmental conditions. Then the low GSI value could signify poor water quality as shown in Inanda Dam. Similar results of low GSI of *Channa punctatus* collected in polluted water were recorded in a study done by Prakash (2022) on the polluted site of Sawan Nallaha. In the present study, the GSI of *L. natalensis* collected in Inanda Dam decreased significantly as compared to the GSI of *L. natalensis* collected in Nagle Dam. It was further reported by Martínez-Gómez *et al.* (2012) that the GSI of *Mullus barbatus* decreased under the effect of heavy metals. The results collected in the study are further in accordance with those collected by Dewi and Prabowo (2017). On the other hand, Hassanin *et al.* (2002) found that a combination of the various pollutants in the river that receives sewage and industrial effluent caused a decrease in the GSI value.

4.4.5 Length-Weight Relationship

In the present study, *L. natalensis* observed in the Nagle Dam were larger than those from the Inanda Dam. Males in Nagle Dam were observed to display positive allometric growth, while males in Inanda Dam were observed to display negative allometric growth. Furthermore, a positive allometric growth was observed for the combined males and females in Nagle Dam whereas the Inanda Dam had the negative allometric growth for the combined males and females. Coinciding with the present findings, Stavrescu-Bedivan *et al.* (2015) reported positive allometric growth on *Carassius gibelio* from an unpolluted aquatic system and negative allometric growth from a polluted aquatic system. Similar findings were also recorded by Imam *et al.* (2021) and Dan-Kishiya (2013) reported negative allometric growth in all the Cichlid species located at the polluted Kano Dam and local reservoir in Nigeria. Other studies suggested that the positive allometric growth recorded in fish could be due to the high availability of food and oxygen content in the water (Amonodin *et al.*, 2018; Singh and Serajuddin, 2017). Nevertheless, pollution as a driver for negative allometric growth in the Inanda Dam population may not be dismissed.

The CF and HSI were not conclusive in the present study. The low HAI value recorded in the Inanda Dam was an indicative of poor water quality and the GSI assumes that the proportionally larger gonads is an indicative of better environmental conditions. A low GSI in females was recorded in Inanda Dam whereas a high GSI in females was recorded in the Nagle Dam. The results recorded in the present study show that the Inanda Dam population is heavily polluted due to the uMsunduzi River, uMngcweni and eSikelekehleni rivers which are potentially affected by wastewater pollution from industries and wastewater treatment plants located in its catchment area whereas the Nagle Dam population is still in good health.

CHAPTER 5

Liver and gill histopathology as biomarkers of *L. natalensis*

5.1 Introduction

Aquatic pollution has been an ever-increasing environmental problem globally due to anthropogenic activities such as soil erosion, agricultural, wastewater works, industrial and mining (Abdel-Moneim *et al.*, 2012; ORTAS, 2023). Aquatic biota has been shown to be affected negatively, with numerous mortalities of both lower and higher vertebrates (Malik *et al.*, 2020). According to Clements (2020), organisms respond differently to pollution with those occupying the higher level of the food chain reflecting the cumulative effect due to the increase of contaminants up the food chain.

Fish, being at the top of the aquatic food chain, can provide insight into the state of aquatic ecosystems, including possible pollution exposure (Saidon *et al.*, 2024). Thus, the establishment of links between water contamination, and the early detrimental effect on fish is of particular importance (Hogarh *et al.*, 2008). The evaluation and assessment of pollution effect and exposure using biomarkers such as histopathology have been successfully employed in various studies (Lebepe *et al.*, 2020; Mdluli *et al.*, 2023; Shahid *et al.*, 2022).

Histopathology has been defined as the easiest technique that can be utilised to assess both the short- and long-term effects of pollution on fish health. The histopathological changes in fish tissue are direct and reliable biomarkers of an environmental stressor in response to pollution (Muzaffar *et al.*, 2023). Both in laboratory and field investigations, histopathologic biomarkers have proven to be a sensitive tool for evaluating the direct toxic effects of chemical pollutants in fish organs including the liver and gills (Lebepe *et al.*, 2020; Mdluli *et al.*, 2023; Shahid *et al.*, 2022). Beegam *et al.* (2020) reported that histopathology can give early warning signs of injuries and diseases in cells, tissues, or organs. Therefore, the histopathology method is the gold standard when assessing the effect of pollutants and defining the toxicological effects in fish, although it is expensive, time-consuming, and invasive (Chris *et al.*, 2024).

The liver is the main organ responsible for the accumulation, metabolism, and elimination of toxicants from the body (Elumalai *et al.*, 2023). The liver frequently experiences lesions and other histological changes because of exposure to pollutants. Once more, pollutants have an impact on liver function and its metabolic capacity (Kim *et al.*, 2024). Several researchers have used liver histopathology to determine exposure to different contaminants (Lebepe *et al.*, 2020; Mdluli *et al.*, 2023). On the other hand, fish gills provide a large surface area relative to the total body surface directly in contact with water (Reddy, 2012). Gills are the main target of waterborne contaminants because of their complex structure and continuous contact with the surrounding water (Oliveira *et al.*, 2022). Mehana *et al.* (2020) reported that the contaminants enter through the gills and primarily affect the branchial epithelium. Fish

gill tissue alterations are thus one of the recognized responses to environmental stressors (Emam *et al.*, 2022).

In the uMgeni River, pollution destroys community structure by killing aquatic biota. Episodic fish kills have been occurring due to chemical spillage. As a result, fish health in this river system has consequently become a matter of concern. Therefore, the present study aims to use the histopathology of the liver and gills to determine the impact of water pollution on the health of *L. natalensis*. Given that the Nagle Dam is located before the uMgeni-uMsunduzi confluence with Inanda Dam found downstream, it was hypothesised that the condition of the Nagle Dam population would be better than that of Inanda Dam.

5.2 Methods and Materials

5.2.1 Tissue sampling and processing

In reference to Chapter 4.2, fish were caught during high and low flow seasons in 2020 and 2021 using an electric shocker at the riverine sites of Inanda and Nagle dams (Protocol reference number: AREC/019/018). After euthanising the fish, approximately a third of the fish's liver and gill arch were dissected out and immediately fixed in a 10 % neutral buffered formalin. The tissue processing was done based on the protocol by Humason (1962). The liver tissues were cut into 2-3 mm thick sections, and two liver tissues were inserted per cassette except for the gills. After that, the tissues were dehydrated using a series of alcohols to remove any excess water and unbound fixative. Therefore, the samples were passed through 50 % (30 min), 70 % (45 min), 80 % (45 min), 96 % (20 min), 99 % (20 min), and 100 % (45 min) of alcohol. This process was conducted slowly to minimise the tissue damage caused by shrinkage and dehydration. Specimens were then passed through xylene twice for 45 min each to make them clear and transparent for the light to pass through them when the specimen was viewed under the microscope. The specimens were then infiltrated with paraffin wax in a warm water bath with a temperature of 60 ˚C for 30 min, 30 min, and 45 min to support the tissue and allow thin sections to be cut. The paraffin wax was liquid at 60 ˚C and then solidified at 20 ˚C. The tissues were embedded in paraffin wax to allow the thin section to be cut. The 4-5 µm thick sections were cut using the microtome from a tissue block and immediately placed in a water bath to prevent damage and breakage. The slide was then used to mount the tissue ribbon from the water bath.

5.2.2 Tissue staining

The tissue staining was done using a protocol by Humason (1962). The slide was immersed in xylene in a closed container for 5 min to remove the wax. It was then hydrated through a series of graded alcohol concentrations of 100 % (2 min), 96 % (2 min), 70 % (2 min) and washed with distilled water for 2 min to remove xylene. The slides were immersed in haematoxylin for 10 min and thoroughly

rinsed with distilled water until no traces of haematoxylin came out. The slides were quickly dipped in acid alcohol (1 % HCl in 70 % alcohol) for 5 to 10 seconds. The slides were left in gently running tap water for 8 min or until turning blue and rinsed in 70 % alcohol for 2 min. They were stained in Eosin for 3 min and dehydrated in graded alcohol of 96 % (2 min), 96 % (2 min), and 100 % (3 min). The slides were then dipped in xylene for 1 to 2 min and mounted with entellan.

5.2.3 Histological analysis

Prepared histological sample slides were analysed using standard light microscopy. To determine the histological changes in the target organs, a qualitative histological evaluation was carried out, and the prevalence was recorded. A semi-quantitative histological protocol assessment protocol by Bernet *et al.* (1999) that was slightly modified by Van Dyk *et al.* (2009), was used to quantify the histological alterations observed in the selected target organs, which are based on the severity of the lesions (Abdel-Moneim *et al.*, 2012). Each reaction pattern contains various alterations that concern either an entire organ or functional units of the organ (Bernet *et al.*, 1999).

5.2.4 Data analysis

The lesion prevalence was calculated to determine the percentage of occurrence in each population at both dams. The frequency of histopathologic lesions was evaluated using the Chi-square test. All Chi-square assumptions were satisfied, and analyses were carried out using R-3.1.1 statistical software (R Development Core Team 2014). A significance level was set at $p<0.05$.

5.3 Results

5.3.1 Gills

The gill histopathology results are presented in Table 5.1. The gill histopathology alterations such as nuclear alterations, haemorrhage, oedema, deposits, architectural and structural alterations, necrosis, hypertrophy, and hyperplasia were observed in both dams with the Inanda Dam having the most prevalent alterations whereas in the Nagle Dam, the most prevalent alteration were deposits, plasma alterations, architectural and structural alterations. The plasma alterations percentage was higher at both dams with 80 % being recorded at Inanda Dam and 75 % at Nagle Dam. Alterations such as pillar cell rupture and nuclear alterations were among the regressive changes reported in both dams, with a higher alteration percentage being recorded for the Nagle Dam. Hypertrophy and hyperplasia were among the progressive changes reported in both dams, with a higher alteration percentage being recorded for the Inanda Dam. Exudate, activation of RES, and infiltration were among the inflammation changes recorded in both dams, with a higher alteration percentage being recorded for the Inanda Dam. In supporting tissue, the normal tissue structure with fewer histological alterations was observed. Atrophy

in the epithelium tissue for regressive changes showed a 60 % prevalence for Inanda Dam and a 53 % prevalence for Nagle Dam.

Table 5.1: Percentage of the histopathology prevalence of *Labeobarbus natalensis* gills recorded at Nagle and Inanda dams.

Reaction pattern	Functional unit of the tissue	Alteration	Nagle Dam (%)	Inanda Dam (%)
Circulatory		Haemorrhage/hyperaemia/aneurysm	51	77
		Intercellular oedema	52	88
Regressive	Epithelium	Architectural and structural alterations	76	92
		Plasma alterations	75	80
		Deposits	87	61
		Nuclear alterations	68	65
		Atrophy	53	60
		Necrosis	59	72
		Pillar cell rupture	65	63
	Supporting tissue	Architectural and structural alterations	49	70
		Plasma alterations	62	62
		Deposits	58	96
		Nuclear alterations	43	79
		Atrophy	13	13
		Necrosis	49	81
Progressive	Epithelium	Hypertrophy	44	74
		Hyperplasia	53	62
	Supporting tissue	Hypertrophy	37	34
		Hyperplasia	63	81
Inflammation		Exudate	27	50
		Activation of RES	37	55
		Infiltration	52	46

As shown in Figure 5.1, a higher histopathology prevalence for circulatory disturbance, regressive changes, progressive changes, and inflammation were observed in Inanda Dam than in the Nagle Dam. There was a significant difference in histopathology prevalence for gill alterations observed between Inanda Dam and Nagle Dam ($p<0.05$). A significant difference was observed in gills percentage prevalence between the Inanda and Nagle dams with Inanda Dam showing a high prevalence percentage as compared to Nagle Dam as seen in Figure 5.2.

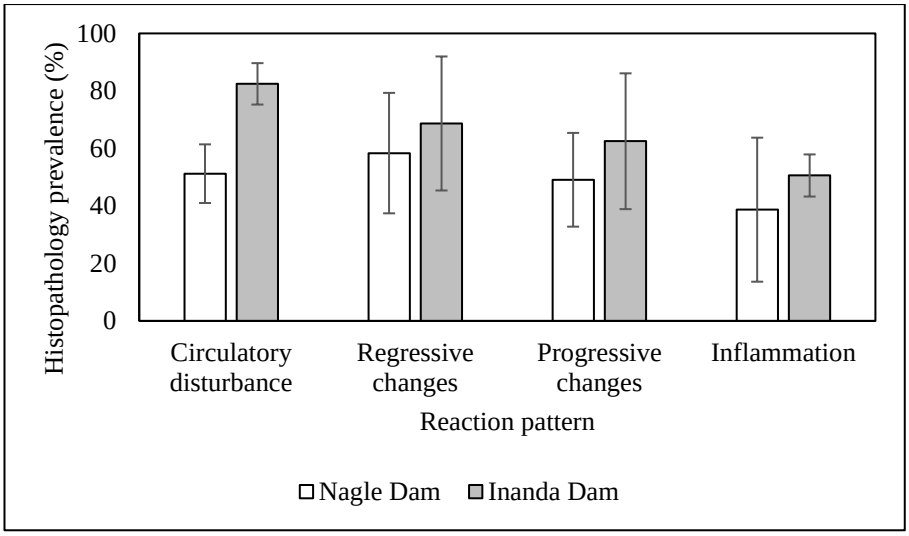


Figure 5.1: Histopathology prevalence of *Labeobarbus natalensis* gills recorded at Nagle and Inanda dams with bars (average) and error bars (standard deviation).

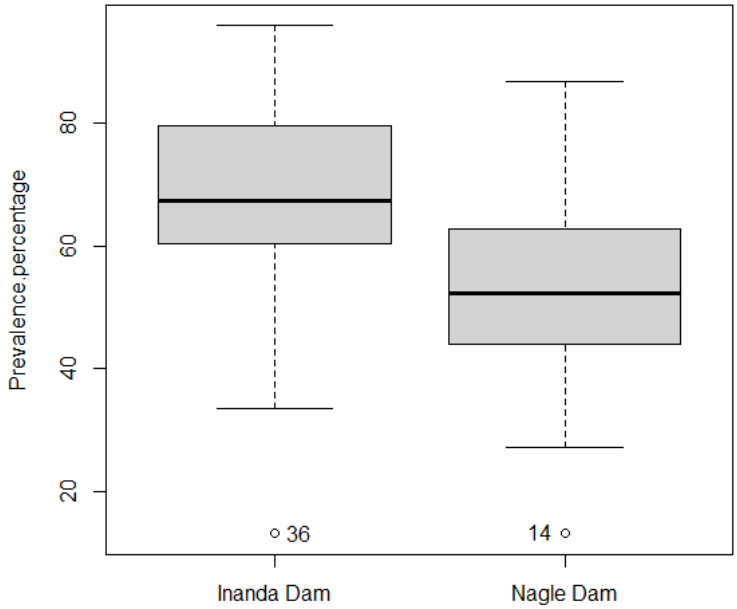


Figure 5.2: The prevalence percentage recorded for *Labeobarbus natalensis* gills in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

As seen in Figure 5.3, oedema resulted in epithelial lifting (solid arrow) and hyperplasia of epithelial cells resulted in the fusion of secondary lamella (dotted arrow) at both dams. The vacuolation, hypertrophied cells and nuclear dimorphism were observed in the liver of the two populations. Both populations showed similar pathologies, however, the magnitude of alterations were severe at the Inanda Dam.

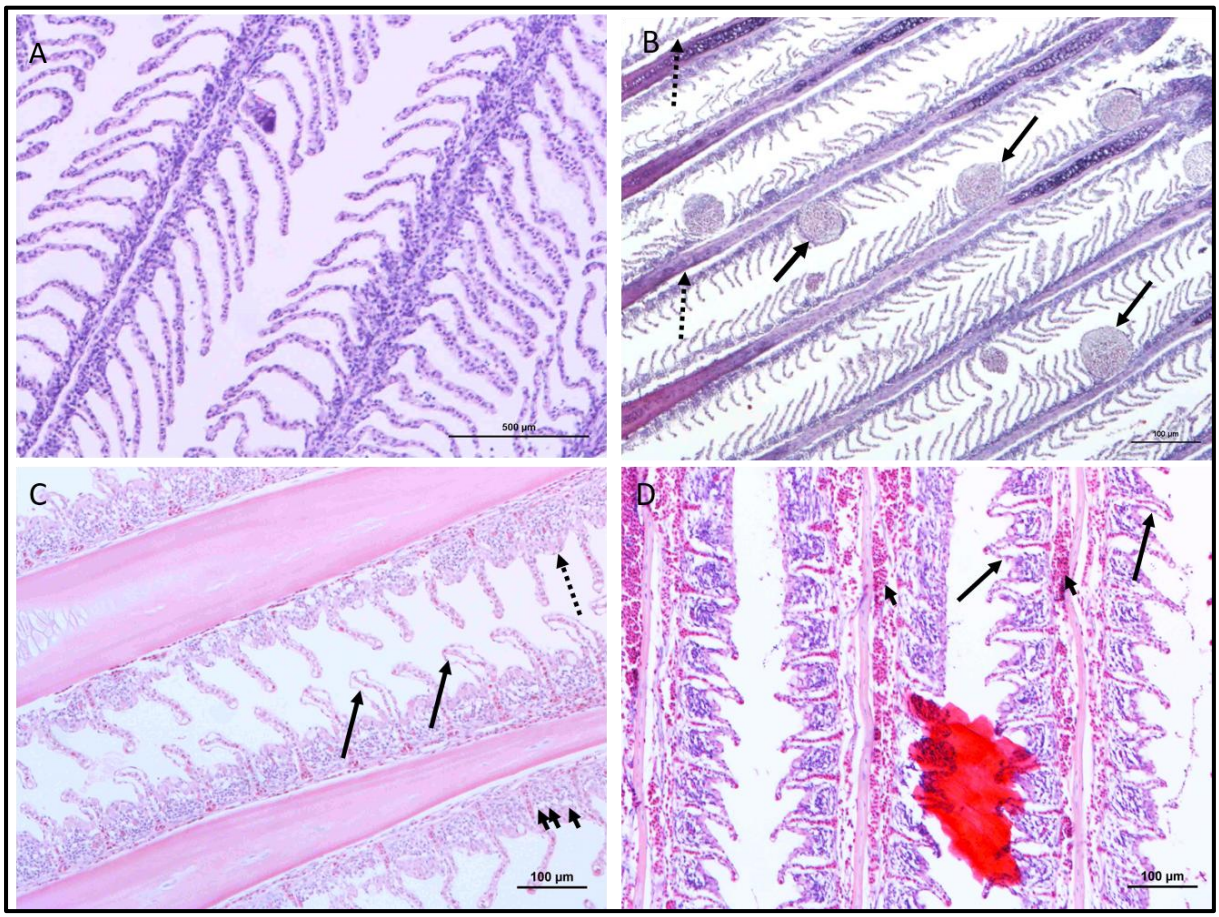


Figure 5.3: Some of the histopathological alterations observed in gills of *Labeobarbus natalensis* from the Inanda and Nagle dams. A. Normal gill; B. Aneurism (solid arrows), oedema accompanied by membrane lifting (dotted arrows); C. Epithelial lifting (solid arrows), hyperplasia of chloride cells (short arrows), hyperplasic of epithelial cells (dotted arrows); D. Epithelial cell lifting (arrows), haemorrhage (arrow head).

5.3.2 Liver

Architectural and structural alterations, necrosis, nuclear alterations, vacuolar degeneration, and hypertrophy were the most prevalent alterations recorded at the Inanda Dam whereas plasma alteration was the most prevalent alteration recorded in the Nagle Dam (Table 5.2). Infiltration for inflammation changes was prevalent at both dams with 85 % recorded at Nagle Dam and 91 % at Inanda Dam. The regressive changes such as plasma alterations, deposits, and atrophy were reported in both dams, with a higher prevalence alteration being recorded for the Nagle Dam. Hyperplasia was among the progressive changes reported in both dams, with a higher prevalence alteration being recorded at Nagle Dam. No pathological changes were observed in the activation of RES.

Table 5.2: Percentage of the histopathology prevalence *in Labeobarbus natalensis* liver recorded at Inanda and Nagle dams.

Reaction pattern	Functional unit of the tissue	Alteration	Nagle Dam (%)	Inanda Dam (%)
Circulatory		Haemorrhage/hyperaemia/aneurysm	46	54
		Intercellular oedema	57	41
Regressive	Liver tissue	Architectural and structural alterations	68	77
		Plasma alterations	56	27
		Deposits	60	52
		Nuclear alterations	58	75
		Atrophy	49	48
		Necrosis	58	70
		Vacuolar degeneration	66	81
Progressive	Liver tissue	Hypertrophy	72	88
		Hyperplasia	64	53
Inflammation		Exudate	33	39
		Activation of RES	0	0
		Infiltration	85	91

There was a significant difference in the circulatory disturbance prevalence percentage for liver alterations between Inanda Dam and Nagle Dam ($p<0.05$). High circulatory disturbance was observed in the Inanda Dam than in the Nagle Dam (Figure 5.4). No significant difference was observed for regressive changes, progressive changes and inflammation between the Inanda and Nagle dams. No significant difference was observed in liver percentage prevalence between Inanda, and Nagle dams as seen in Figure 5.5. Inanda Dam experienced a relativity high percentage prevalence of individuals as compared to Nagle Dam as shown in Table 5.2.

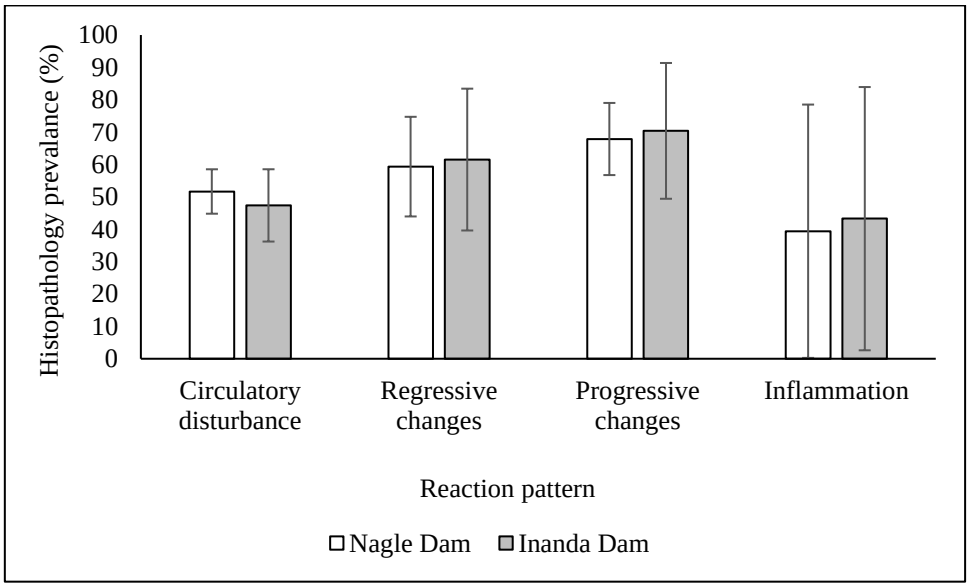


Figure 5.4: Histopathology prevalence of *Labeobarbus natalensis* liver recorded at Nagle and Inanda dams with bars (average) and error bars (standard deviation).

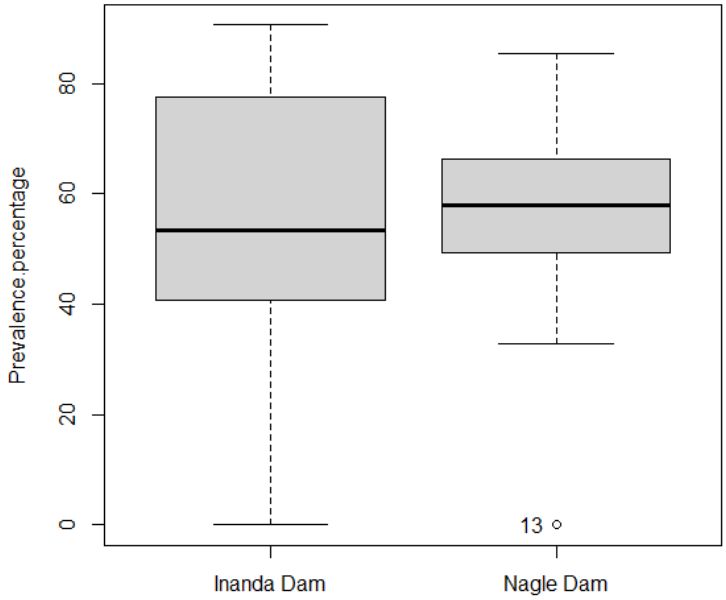


Figure 5.5: The prevalence percentage recorded for *Labeobarbus natalensis* liver in the Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

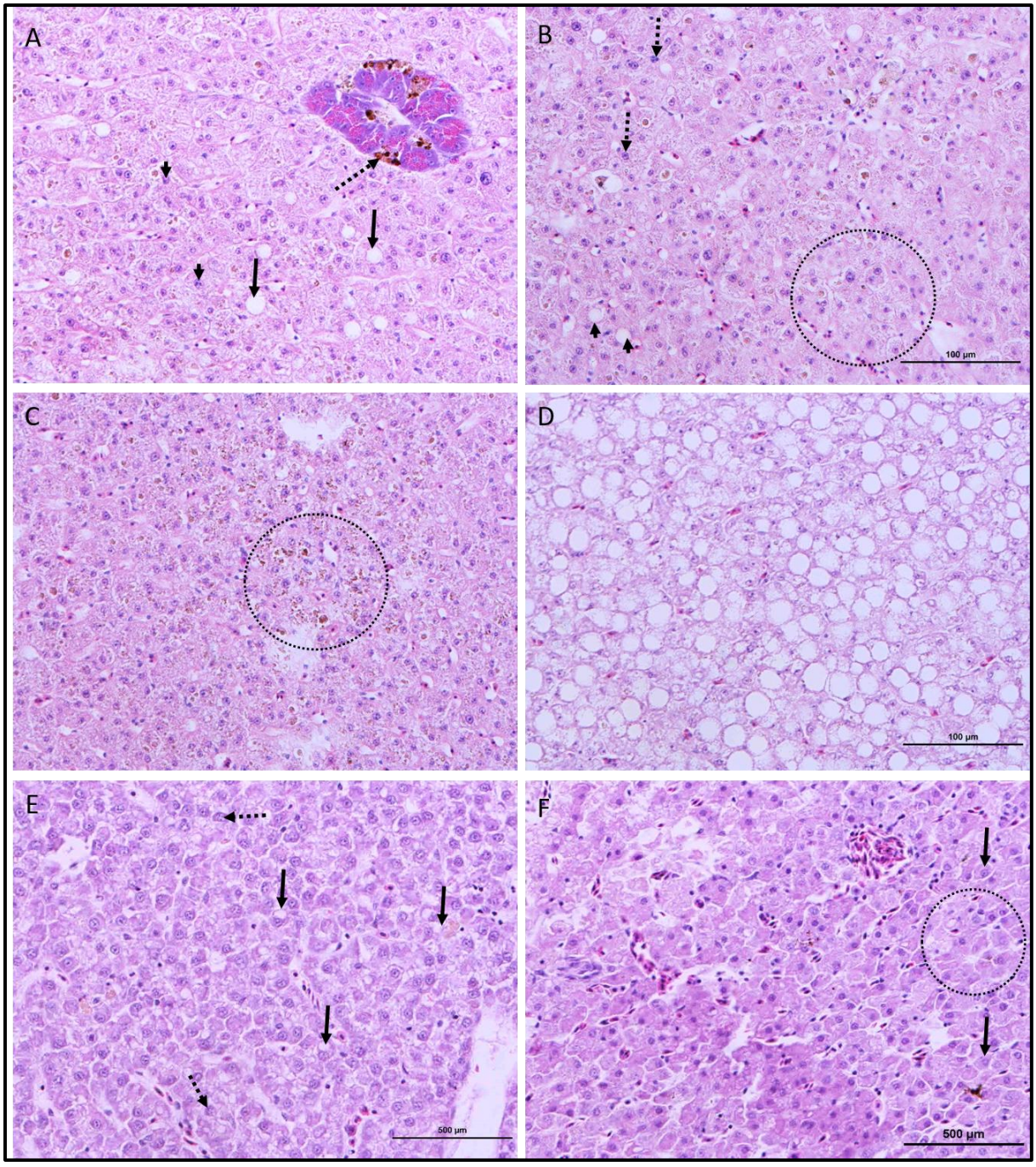


Figure 5.6: Some of the histopathologic alterations observed in the liver of fish from Inanda and Nagle Dam. A. Mild aggregation of MMCs (dotted arrow), nuclear alterations characterised by disintegration of the nucleolus (arrowhead); vacuolation (dotted arrows); B. Nuclear alterations characterised by disintegration of the nucleolus (dotted arrows); vacuolation (arrowhead), pleomorphism (encircled); infiltration of MMCs (encircled); D. Severe steatosis; E. Vacuolation (solid arrows), nuclear alterations characterised by disintegration of the nucleolus (dotted arrows); F. Pleomorphism (encircled), peripheral nucleus (arrows).

5.4 Discussion

5.4.1 Gills

Fish gills are said to be the most vital organs since they come into direct contact with the water. Pollution adversely affects the gills' ability to function by disrupting the gaseous exchange and ion regulation efficiency (Roberts, 2012). Macirella *et al.* (2020) found that gill structure is the best biomarker in fish to evaluate the impact of water-borne pollutants. Because of their exterior location, gills are extremely vulnerable to injury from any irritating element that is either suspended or dissolved in the water exchange (Roberts, 2012). Sharma *et al.* (2023) reported that the formation of hyperplasia, necrosis, lamellar oedema, and fusion of pillar cells leading to telangiectasia may occur if the irritating stimuli are more intense. Gill histological alterations such as necrosis, oedema, and hyperplasia reported in this study were comparable to those recorded in other studies conducted in polluted water bodies here in SA (Hasan *et al.*, 2022; Nephale *et al.*, 2021; Utete *et al.*, 2019).

A relatively high prevalence of hyperplasia and hypertrophy was observed in Inanda Dam. Das and Gupta (2012) mentioned that progressive alterations such as hypertrophy and hyperplasia of the epithelial cells are considered as being a general safety measure against pollutants by increasing the distance between the blood and the external environment and thus, acting as a barrier to prevent xenobiotics from entering (Mohamed, 2009). van Heerden *et al.* (2004) further mentioned that these alterations are also symptoms of inflammation, and this increases the diffusion distance for oxygen, which may cause hypoxia. Pereira *et al.* (2012) further suggested another deference mechanism is mucous cell hyperplasia, which secretes mucus, a coating of glycoproteins and glycolipids, to create a barrier of defence over the gill epithelium. Similar gill alterations observed in Inanda Dam, such as lamellar epithelial lifting, hypertrophy, and hyperplasia of the gill epithelium were observed in a study done by Dane and Şişman (2015) on *Capoeta capoeta* in polluted Karasu River, Erzurum. Similarly, Dragun *et al.* (2014) observed lamellar epithelial lifting, hypertrophy, and hyperplasia of the gill epithelium on *Squalius vardarensis* from the polluted Kriva River. Primary and secondary lamella fusion, aneurism, pillar cell rupture, haemorrhage, and epithelial lifting can affect gill function and hence make the body more vulnerable to various pathological conditions including opportunistic microbial infection (Katsumiti *et al.*, 2013). Furthermore, AL Taee *et al.* (2020) reported epithelial hypertrophy and necrosis after heavy metal exposure. Therefore, metals as one of the drivers for histopathologic alteration observed in *L. natalensis* should not be dismissed.

Haemorrhages, aneurysm, necrosis, and oedema had a high prevalence percentage in the Inanda Dam when compared to the Nagle Dam. Moreover, Marinović *et al.* (2021) mentioned that xenobiotic activity results in alterations such as necrosis, aneurism, or haemorrhage, which are typically thought to be irreversible. According to Cengiz and Unlu (2006), haemorrhages and aneurysms tend to be accompanied by pillar cell system collapses that release a significantly large amount of blood into the

lamella. Dane and Şişman (2015) found aneurysms in *Capoeta capoeta* that were subjected to polluted water such as wastewater effluent, which is consistent with the findings of the current study. Inanda and Nagle dams both showed signs of leukocyte infiltration. Koca *et al.* (2005) mentioned that leukocyte infiltration might indicate a fish's immune system fighting off toxicants in its surroundings. Moreover, Barišić *et al.* (2015) found the highest prevalence percentage of intracellular oedema in *Squalius vardarensis* from the extremely polluted Bregalcina River. It is evident that histopathology could be a reliable tissue for pollution exposure assessment, particularly, waterborne contaminants.

5.4.2 Liver

The fish liver is a reliable bioindicator of aquatic pollution since the liver's main function is to remove any poisons or pollutants from the body (El-Naggar *et al.*, 2009). It is one of the organs mostly impacted by pollutants since it is associated with detoxification and biotransformation progression (Mohamed, 2009). The histopathological alterations recorded in the present study include architectural and structural alterations, nuclear alterations, vacuolar degeneration, hypertrophy, necrosis, and infiltration.

Vacuolar degeneration was observed in both dams with Inanda Dam having high prevalence. Similar results were observed in freshwater fish *Channa punctatus* which was exposed to the herbicide paraquat dichloride (Badroo *et al.,* 2020). Santos *et al.* (2022) went on to explain that exposure to contaminated water leads to hepatocyte vacuolization, a sign of a degenerative process that suggests metabolic damage. The vacuolisation is a reversible lesion in which the cells can be able to recover to their normal functions when the pollutant (stressor) is removed. The cell recovery is further dependent on the duration and severity of exposure to stress (Iheanacho and Odo, 2020). The fish liver tissue in the Inanda Dam is unlikely to ever recover as the partly treated and untreated sewage continues to flow into the uMgeni River system.

Hypertrophy was more prevalent at Inanda Dam, whereas hyperplasia was more prevalent at Nagle Dam. Xia *et al.* (2000) mentioned that hyperplasia and hypertrophy are both regarded as compensatory actions to interstitial cell death. Hyperplasia and hypertrophy are further associated with hepatocellular pleomorphism. In the present study, hepatocellular pleomorphism was observed in both dams, with the Inanda Dam having a higher prevalence as compared to the Nagle Dam. Fernandes *et al.* (2008) reported that the hepatocellular pleomorphism is defined as the first toxic lesion resulting from exposure to environmental pollutants. Therefore, the high percentage prevalence observed in Inanda Dam may be related to an increase in metal toxicity observed in Inanda Dam as the uMgeni River system progressively receives polluted water from the uMsunduzi River and the other two tributaries. Moreover, the hepatocellular hypertrophy and hyperplasia observed in this study may be described as an adaptive response to increase detoxifying capability (Salamat and Zarie, 2012).

A high prevalence of necrosis was observed in Inanda Dam compared to Nagle Dam. Bernet *et al.* (1999) defined necrosis as an irreversible tissue or cell death. It was observed in fish species such as *O.*

niloticus and *Astyanax fasciatus* that were exposed to industrial and municipal sewage (Liebel *et al.*, 2013). Necrosis was further cited by Ayas *et al.* (2007) to justify it as the most significant histopathological reaction in the liver in response to pollutant exposure. Liebel *et al.* (2013) further explained that the presence of pollutants inside the hepatocytes, which disrupts the biochemical process, may result in necrosis in fish liver tissue. A high percentage prevalence of necrosis observed in Inanda Dam correlates with high metal concertation that was observed in sediment samples in Inanda Dam. Therefore, the high concentration of metals in Inanda Dam may be among the drivers of the high necrosis levels observed.

Infiltration alteration of cells in fish liver was prominent in both dams with Inanda Dam exhibiting a higher percentage prevalence of 91 % compared to Nagle Dam, with a percentage prevalence of 85 %. Similar results were observed by Valon *et al.* (2013) on *C. gariepinus* exposed to heavy metal pollution. Santos *et al.* (2022) observed leukocyte infiltration and hypermedia in *Cyprinus carpio* exposed to copper microparticles. Similar results of liver alteration such as nuclear degeneration, melano-macrophage centres, severe necrosis, cytoplasmic degeneration, and infiltration in freshwater cyprinid, *Labeo rohita* were induced by exposure to metal concentration (Kaur *et al.* (2018a). When fish are exposed to water pollution, different liver pathological abnormalities including deformed hepatocytes, vacuolization, necrosis, and leukocyte infiltration may be observed. Such observation has been reported in the liver of *Cyprinus carpio* exposed to lethal concentrations of chromium (Parvathi *et al.*, 2011), *C. gariepinus* to sewage/domestic wastewater containing copper, iron, lead, cadmium, manganese and zinc (Authman and Abbas, 2007) and *Tilapia zilli* to aluminium (Hadi and Alwan, 2012), and *Channa punctatus* exposed to sugar mill effluents (Javed and Usmani, 2019). In the present study, histopathological alterations in the liver showed to have been more prominent in the Inanda Dam population than the Nagle Dam population which was also associated with the water quality trend.

The Inanda Dam showed a higher prevalence of histopathologic alterations for both liver and gills. In fish, gills are directly exposed to metals in water and gills play a crucial role in metal accumulation (van Heerden *et al.*, 2004). Therefore, the high gills anomalies observed in Inanda Dam can be an indicative of metal exposure. In contrast to the high metals observed in Inanda Dam, the higher prevalence of histopathologic alterations observed in Inanda Dam is related to the high metals and nutrients observed in Inanda Dam. With Inanda Dam receiving polluted water from the three tributaries, the pollution impact as the driver for relatively higher prevalence may not be dismissed. This supported the hypothesis that the Inanda Dam population will exhibit compromised health compared to the Nagle Dam.

CHAPTER 6

Vitellogenin as a biomarker of estrogenic effects

6.1 Introduction

Instead of being exposed to particular pollutants in the environment, fish are continuously exposed to a mixture of chemicals (Heys *et al.*, 2016). This constant exposure makes fish a good sentinel species for pollution effect studies (Zezza et al., 2020). However, in aquatic environments, fish respond differently to various types and levels of pollution. Thorpe *et al.* (2003) showed that the presence of estrogenic compounds in the water can interact and disturb the fish's reproductive performance even at the individual level. Both laboratory and field studies done by Kidd *et al.* (2007) and Vajda *et al.* (2008) have demonstrated a variety of estrogenic effects including inhibition of gonad development, reduced fertilization rate, decreased egg production, remasculinization of males, the occurrence of gonadal intersex individuals, sex ratio bias in favour of females, the collapse of the entire fish population and the induction of VTG in males.

The VTG induction in fish is regarded as a crucial biomarker of oestrogenic exposure and its effect in aquatic environments. Vitellogenin is an egg yolk precursor protein that is produced by female fish in response to circulating oestrogen in the plasma, and its level can be utilised as a definitive indication of the reproductive stage of females (Gupta *et al.* (2021). Generally, only females can synthesise VTG, while in normal conditions the males contain exceptionally low or no measurable level of VTG. However, the exposure of males to oestrogenic compounds may result in the induction of VTG. Therefore, VTG induction in juvenile and male fish has been used as a biomarker of estrogenic pollutants in aquatic environments (Thirumalaivasn *et al.*, 2023). An increase in VTG in male fish can lead to decreased spermatogenesis, decreased testicular growth, increased susceptibility to illnesses, changes in sexual maturity, and kidney damage (Brown *et al.*, 2023). Most of these conditions affect the reproductive capacity of fish.

The lower uMgeni River receives water from a highly polluted uMsunduzi River and other tributaries draining industrialised catchments. This lower stretch has shown high concentrations of organic contaminants, including pharmaceuticals, which have the potential to cause an oestrogenic effect (Munzhelele *et al.*, 2024). Moreover, there is a high occurrence of native fish species in this river stretch including the endemic *L. natalensis* (Karssing, 2008). Despite signs of oestrogenic pollutants in the lower uMgeni River no study has been conducted to explore the oestrogenic response of this KZN endemic species, *L. natalensis*. Therefore, the present study investigates the oestrogenic effect on *L. natalensis* by measuring the VTG level in male individual fish from Nagle and Inanda dams. Nagle Dam is located upstream of the confluences where polluted tributaries join the mainstem whereas

Inanda Dam is located downstream. It was, therefore, hypothesised that male fish from Inanda Dam would exhibit elevated VTG levels relative to those from Nagle Dam.

6.2 Methods and Material

6.2.1 Fish Sampling

The fish were caught at the riverine sites of the Inanda and Nagle dams using an electric shocker as stated in Chapter 4. The collected fish were stored in aerated tanks and taken to the field lab for processing. Once the fish were euthanised and dissected (see Chapter 4), each liver was cut and immediately wrapped with aluminium foil and stored on dry ice in the field and later transferred to a –80 °C fridge in the lab for VTG analysis.

6.2.2 Whole-cell extraction from liver tissue

Before using the assay, all the standards, reagents, and samples were prepared. The liver samples were then crushed in dry ice and stored in 5 ml Eppendorf tubes. About 100 µg tissue was added to new Eppendorf tubes and homogenized in a 0.1 mol/L phosphate buffer (PBS). The solution was homogenized at a pH of 7.4 and centrifuged at 3000-4000 rmp for 20 min.

6.2.3 General Sandwich VTG ELISA

Two identical samples were used to test the VTG induction of male liver tissue, with an ELISA kit (Bioassay Technology Laboratory, catalogue number: E0020Fi). The 64-well plates were coated with 100 µl per well of purified anti-Vtg MAb (10 µg/mL) diluted in coating buffer (50 mM carbonate-bicarbonate, pH 9.6). The wells were washed once with the 200 µl wash buffer (PBS, pH 7.3, containing 0.05 % Tween-20). The plates were then inverted and tapped on the paper towel to absorb any excess liquid. The binding sites were then blocked with 200 µl of blocking buffer (1 % BSA in PBS) for 1 hr at room temperature. The plates were then aspirated, inverted, and tapped on a paper towel to absorb any excess liquid. Standards and samples (100 µl of each and in duplicate) were pipetted into a designated well. The well plate was then incubated at room temperature for 1 hr with a constant, gentle, continual shaking (~500 rpm). The wells were then aspirated and washed five times using the 200 µl Wash buffer per well. Further inverted and tapped on a dry paper towel to remove excess liquid.

The detection antibody solution of 100 µl was added in each well and incubated at room temperature for 2 hrs with gentle continual shaking (~500 rpm). The secondary antibody solution was prepared using the Streptavidin-HRP solution with blocking buffer by diluting 1:5,000. The 100 µl secondary antibody solution of streptavidin-HRP was added to each well and incubated at room temperature for 30 min with gentle continual shaking (~500 rpm). The 100 µl of TMB substrate solution was added to each well, and the well plate was then incubated for 30 min at room temperature. After 30 min, 100 µl of stop solution was added to each well to stop the reaction. The absorbance in the plate wells was then

measured using a multiwell plate reader at reader UV-VIS Spectrophotometer (UV-2600 Shimazdu) 450 nm within 30 min after adding the stop solution. The kit has a published intra-assay CV of <8 %, and an inter-assay CV of <10 %.

6.2.4 Data analysis

The statistical analysis was performed on R-3.1.1 (R Development Core Team 2014) with a level of significance set at $p<0.05$. To determine the normality of the data, the Shapiro-Wilk test was used, and Levene's test was used to test the equality of variance between the data. Results demonstrating seasonal variations were emphasised, whereas those showing no seasonal variations were overlooked. The VTG level was compared using an independent sample t-test or Mann-Whitney U test depending on the data distribution and homogeneity of variance. The relationship between VTG level and fish weight was evaluated using Pearson or Spearman's correlation test. The analyses were carried out using R with significance set at $p<0.05$.

6.3 Results

6.3.1 Vitellogenin concentration

The VTG concentration recorded in male *L. natalensis* from the Inanda and Nagle dams is shown in Figure 6.1. No signification difference was observed in VTG concentrations in male populations between the Inanda and Nagle dams ($p>0.05$). However, there were considerable variations within each population. In Inanda Dam, a VTG average concentration of 13.24±0.34 g/ml was observed, whereas the VTG average concentration of 11.98±0.52 g/ml was observed in the Nagle Dam population. No significant seasonal variation was observed at both dams.

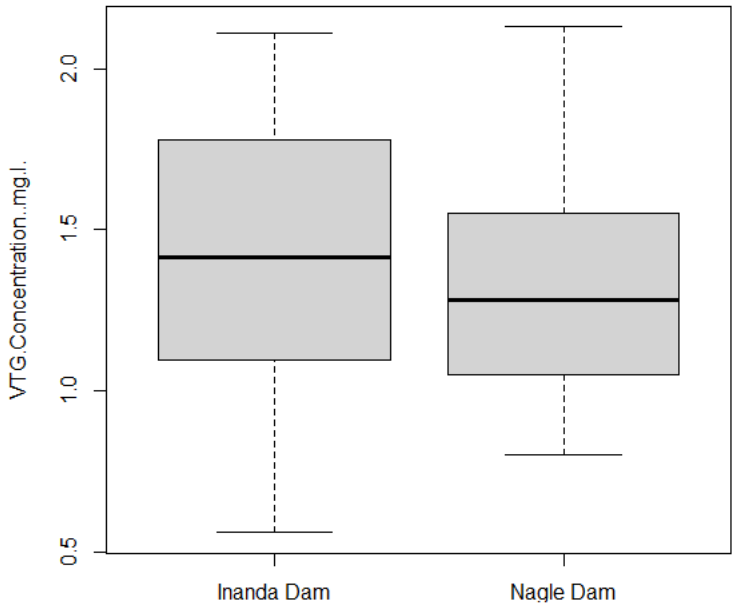


Figure 6.1: The VTG concentration (g/ml) in *Labeobarbus natalensis* recorded at Inanda and Nagle dams. The line inside shows the medium whereas the box shows the 25th percentile and the 75th percentile. The flat line at the bottom and the top shows the minimum and maximum concentration.

6.3.2 Relationship between fish size and VTG concentrations

As seen in Figure 6.2, the R^2 value is 0.6601 with r=0.81. This shows that there is a strong relationship between the weight and the VTG concentration of fish. As the fish weight increases, the VTG concentration of the fish also increases.

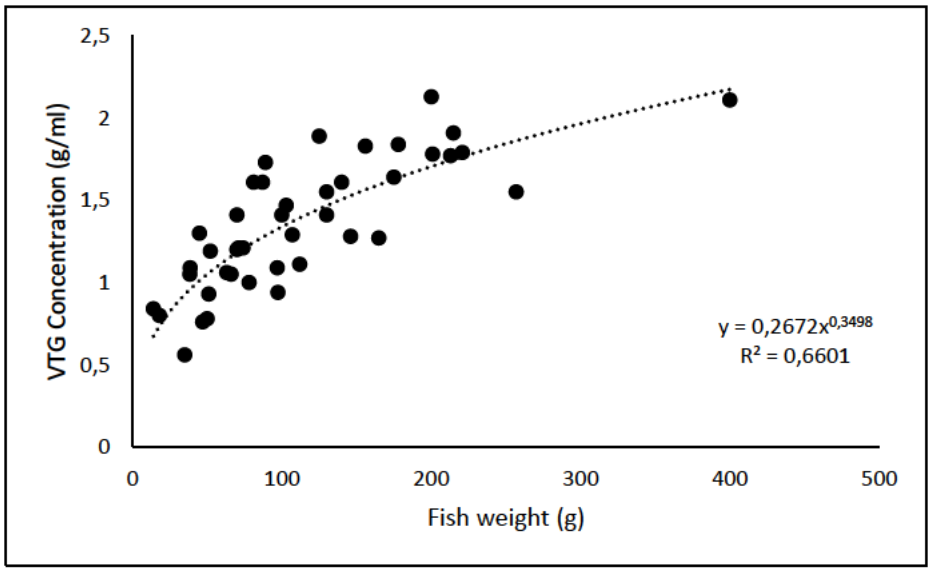


Figure 6.2: The relationship between the fish weight (g) and VTG concentration (g/ml) of *Labeobarbus natalensis* recorded at Inanda and Nagle dams. With R^2: The coefficient of determination.

6.4 Discussion

The VTG levels in male fish have been widely used as a biomarker for the assessment of the oestrogenic effect (Brown *et al.*, 2023) in response to xenoestrogens in water bodies (Badamasi *et al.*, 2020). Vitellogenin is a precursor yolk protein normally produced by females, however, it may be produced by males and juvenile fish when exposed to estrogenic compounds and is thus a sign of feminization (Thirumalaivasn *et al.*, 2023). Badamasi *et al.* (2020) reported that VTG can be a potential biomarker for the early detection of xenoestrogens in aquatic ecosystems due to its ecological relevance in biomonitoring of aquatic pollution.

In the present study, the male fish have shown a considerable amount of VTG level for both Inanda and Nagle dams. The Inanda Dam receives water from different polluted tributaries, including the uMsunduzi River, which is known to receive effluents from industrial activities and wastewater treatment plants (Olaniran *et al.*, 2014). However, the VTG concentration recorded between the Inanda and Nagle dams has shown no variation between the sampling sites. The presence of VTG concentration in Nagle Dam shows that it is exposed to oestrogenic pollutants upstream of the uMgeni River. The individuals showing the highest induction of VTG concentration were observed in Inanda Dam compared to Nagle Dam. Adeyinka et al. (2019), reported oestrogenic compounds organochlorine pesticides (OCPs) at the uMgeni-uMsunduzi confluence which could explain the presence of VTG concentration in Inanda Dam.

The VTG level obtained in the study coincides with those recorded in *Sarotherodon melanotheron* from organochlorine pesticides polluted aquatic environment in the Oueme River (Okoumassoun *et al.* (2002). Moreover, Brown *et al.* (2023) reported higher VTG concentrations than those recorded at Inanda and Nagle dams on *P. promelas* and *D. rerio* exposed to herbicides pendimethalin. *Clarias batrachus* has also exhibited induced VTG concentration in waterbody polluted by the herbicide pendimethalin Gupta and Verma (2020) whereas Yang *et al.* (2016) recorded a significant increase in VTG concentration of *D. rerio* exposed to industrial effluents. Furthermore, Holeton *et al.* (2011) mentioned that municipal wastewater treatment plants are point sources of a wide range of contaminants, such as metals, excess nutrients and pharmaceuticals. Therefore, the presence of VTG concentrations in the present study could be related to the higher OCP concentration observed in the uMgeni-uMsunduzi confluence, wastewater treatment plant located in uMsunduzi River, organochlorine pesticides, industrial effluents and agriculture activity.

Despite the absence of prominent anthropogenic activities around Nagle Dam, its population also exhibited VTG levels. Okoumassoun *et al.* (2002) reported induced VTG in fish from waterbodies receiving water from a catchment characterised by negligible anthropogenic activities such as small-scale agriculture. Moreover, Brown *et al.* (2023) questioned the credibility of VTG as oestrogenic biomarkers due to some inductions being observed without systemic toxicity. Despite Nagle Dam showing to be receiving water from the less industrialised catchment, some nutrients and metal concentrations were significantly higher than Inanda Dam, which might be the explanation for the presence of VTG levels in the Nagle Dam population. Furthermore, similar results of VTG concentration when compared to Nagle Dam were observed in less polluted Lake Mayor in male blackfin goodeid (Olivares-Rubio *et al.*, 2015).

Nitrate, one of the most common aquatic pollutants in the world, is currently included in the growing list of known and suspected EDCs (Fields, 2004). The level of VTG concentration in males is dependent on the amount of nitrate available, therefore the increase in nitrate concentration results in an increase in VTG concentration (Freitag *et al.*, 2015). In the present study, a relatively high amount of nitrate was recorded in the Inanda Dam with a relatively high VTG concentration as compared to the Nagle Dam. Therefore, the high level of nitrate concentration may be the possible reason for the relatively high VTG concentration that was observed in Inanda Dam. Martinović *et al.* (2007) reported an increase in VTG concentration in male fathead minnows when they were exposed to sewage treatment effluent with high nitrate concentration.

Despite the VTG level trends being inconclusive, Manickum *et al.* (2011) suggested the presence of estrogenic chemicals in the uMsunduzi River which feeds the uMgeni River before emptying into the Inanda Dam. Municipal sewage treatment plants are the most important sources of estrogenic compounds discharged into the aquatic environment. This could be one of the explanations for the VTG concentrations in male fish from the Inanda Dam whereas the VTG levels in the Nagle Dam may be explained by the natural oestrogen secretion in water by plants and aquaculture animals or agricultural activities (Lai *et al.* (2002).

CHAPTER 7

Conclusion and Recommendations

The physical water parameters recorded during the survey in the uMgeni River system were found to be within the TWQR range as stipulated by DWAF (1996) except for the DO and pH levels. The uMsunduzi River had the most alkaline pH followed by the eSikelekehleni River and uMngcweni River. A high DO was observed in the Inanda Dam, and a high water temperature and pH were observed in the uMsunduzi River. The water column exhibited low metal concentration, whereas elevated metal concentrations were observed in sediment at both dams. In metals in water, a significant difference was observed for aluminium, copper, iron and manganese between all sampling sites with higher metal concentrations of aluminium, copper and iron recorded in Inanda Dam, uMsunduzi River, Nagle Dam, eSikelekehleni, and uMngcweni rivers.

When water samples were analysed for nutrients, a significant difference was observed in phosphate and sulphate concentration between all the sampling sites, with high phosphate concentration recorded in the uMngcweni River and high sulphate concentrations in the uMsunduzi River. Concerning the DWAF (1996) guideline for trophic status, uMngcweni River was hypereutrophic for phosphate and eutrophic for nitrite, whereas uMsunduzi and eSikelekehleni rivers were mesotrophic for nitrite and eutrophic for phosphate. Inanda Dam exhibited a mesotrophic for nitrogen and phosphate status whereas the water within the Nagle Dam exhibited oligotrophic for nitrate and mesotrophic for phosphate. The uMsunduzi, eSikelekehleni, and uMngcweni rivers are in a highly industrialised area, they receive polluted water from industrial areas, and they are further polluted by runoff from agricultural activities and wastewater treatment plants. The uMngcweni and uMsunduzi rivers are two of the tributaries that deposit pollutants into the uMgeni River system.

Several liver abnormalities were observed at the Inanda Dam this included fatty liver, liver nodules, focal discolouration, and general discolouration. In gills, discolouration was observed in the Inanda Dam population with gills showing pale colour. A significant difference in HAI was observed between the Nagle and Inanda dams, with the Inanda Dam exhibiting a higher HAI than the Nagle Dam. This signifies that the fish population in Inanda Dam is experiencing poor water quality, whereas the fish population located in Nagle Dam is experiencing good water quality. Therefore, this shows that the Inanda Dam is polluted compared to Nagle Dam. No significant difference was observed in HSI, GSI, and CF. High GSI in females was observed in the Inanda Dam than in the Nagle Dam. The positive allometric growth was exhibited in the male population of *L. natalensis* recorded in Nagle Dam, whereas the Inanda Dam population of *L. natalensis* exhibited a negative allometric growth pattern. Therefore, in the present study, the HIS, GSI, and CF were not conclusive in distinguishing the two populations.

Inanda Dam exhibited a high prevalence of alterations for the liver and gills compared to the Nagle Dam. A significant difference was observed between the prevalence alterations of gills between Inanda and Nagle dams. Alterations such as oedema, nuclear alterations, necrosis, deposits, hypertrophy, and hyperplasia were prevalent pathologies in gills at Inanda Dam. The plasma alterations, deposits, architectural and structural alterations were more prevalent in Nagle Dam. Alterations such as necrosis, nuclear alterations, hypertrophy, vacuolar degeneration, and architectural and structural alterations were the most prevalent pathologies in liver alterations in Inanda Dam. Furthermore, a high liver and gills index was recorded in Inanda Dam, this justifies that fish populations located in Inanda Dam are under severe stress due to.

No significant difference in VTG concentration measure in the present study between the Inanda and Nagle dams. Despite the no significance, Inanda Dam had a relatively higher concentration of VTG for some individuals than Nagle Dam. This is evident that there are signs of estrogenic effect on the *L. natalensis* fish population in both Inanda and Nagle dams. The *L. natalensis* is currently regarded as the least concern, nonetheless, the species might be declining as a result of the ongoing pressure on KZN's aquatic environment.

The water and sediment quality at Nagle Dam was better when compared to water quality in Inanda Dam. Hence, the fish population in Nagle Dam are in better conditions as compared to fish population in Inanda Dam. Therefore, the hypothesis that the water and sediment quality at Nagle Dam would be better compared to Inanda Dam and the health of the Inanda Dam fish population would be more compromised relative to Nagle Dam population was accepted. It is recommended that further exploration of the effect of water pollution on fish health must be conducted on other native species that are known to be sensitive in response to water pollution. Furthermore, different multi-biomarker approaches should be utilized to get a better insight into the effect of anthropogenic oestrogen on fish species located within the uMgeni River.

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APPENDIX

Appendix 1: The raw data fish of *Labeobarbus natalensis* collected in Inanda and Nagle Dam during the low and high seasons.

Locality	Fish #	Season	Gender	Fish weight (g)	Total length (cm)	Std length (cm)	Liver weight (g)	Gonad weight (g)	Condition factor	HSI	GSI	HAI	Log10 (Weight)	Log10 (Standard length)
Inanda	1	Low	M	125	10.8	7.9	0.6	9.82	25.353	0.480	7.856	0	2.096910013	0.897627091
	2		M	100	20.6	15.6	0.5	1.7	2.634	0.500	1.700	30	2	1.193124598
	3		M	65	20.5	16.5	0.88	5.5	1.447	1.354	8.462	30	1.812913357	1.217483944
	4		M	65	23.5	15.8	0.05	3.1	1.648	0.077	4.769	60	1.812913357	1.198657087
	5		M	35	19.8	14.9	0.26	0.17	1.058	0.743	0.486	80	1.544068044	1.173186268
	6		M	50	19.5	15.5	0.2	0.16	1.343	0.400	0.320	90	1.698970004	1.190331698
	7		M	60	19.9	15.3	0.48	0.59	1.675	0.800	0.983	30	1.77815125	1.184691431
	8		M	85	20.5	16.5	0.68	0.26	1.892	0.800	0.306	60	1.929418926	1.217483944
	9		M	85	21.4	15.44	0.54	0.18	2.309	0.635	0.212	60	1.929418926	1.188647296
	10		M	50	20	15.5	0.43	0.19	1.343	0.860	0.380	60	1.698970004	1.190331698
	11		M	50	20.5	15	0.47	0.05	1.481	0.940	0.100	70	1.698970004	1.176091259
	12		F	145	32.9	28.9	0.4	1.38	0.601	0.276	0.952	0	2.161368002	1.460897843
	13		F	85	21.6	16.6	0.52	2.99	1.858	0.612	3.518	80	1.929418926	1.220108088
	14		F	65	20.2	16.1	0.36	0.12	1.558	0.554	0.185	60	1.812913357	1.206825876
Inanda	1	High	M	400	35	31.5	1.5	7.9	1.280	0.375	1.975	60	2.602059991	1.498310554
	2		M	85	21	17	0.6	0.05	1.730	0.706	0.059	0	1.929418926	1.230448921
	3		M	70	19	15.5	0.7	0.04	1.880	1.000	0.057	40	1.84509804	1.190331698
	4		M	80	18	14.5	0.6	0.01	2.624	0.750	0.013	30	1.903089987	1.161368002
	5		M	75	18.5	15	0.6	0.01	2.222	0.800	0.013	10	1.875061263	1.176091259
	6		M	60	15	14.3	0.6	0.01	2.052	1.000	0.017	70	1.77815125	1.155336037
	7		M	65	14.5	11.5	0.03	0.01	4.274	0.046	0.015	60	1.812913357	1.06069784

	8		F	65	19.5	16.5	0.1	0.01	1.447	0.154	0.015	30	1.812913357	1.217483944
Nagle	1	High	M	75	18.7	16.8	0.5	0.3	1.582	0.667	0.400	0	1.875061263	1.225309282
	2		M	49	24	17	0.4	0.3	0.997	0.816	0.612	0	1.69019608	1.230448921
	3		M	126	26	22	1.1	1.6	1.183	0.873	1.270	0	2.100370545	1.342422681
	4		M	130	18	12	0.1	0.1	7.523	0.077	0.077	30	2.113943352	1.079181246
	5		M	38.4	23	15.5	0.1	1.5	1.031	0.260	3.906	30	1.584331224	1.190331698
	6		M	18	17	12	0.01	0.01	1.042	0.056	0.056	30	1.255272505	1.079181246
	7		M	14	16	11	0.01	0.01	1.052	0.071	0.071	30	1.146128036	1.041392685
	8		F	300	33	29	3.5	3.4	1.230	1.167	1.133	30	2.477121255	1.462397998
Nagle	1	Low	M	105	22.3	18.9	0.83	0.39	1.555	0.790	0.371	0	2.021189299	1.276461804
	2		M	110	23	17.9	1.4	0.9	1.918	1.273	0.818	90	2.041392685	1.252853031
	3		M	170	22	19.5	1.62	13.3	2.293	0.953	7.824	20	2.230448921	1.290034611
	4		M	150	22.6	19.5	1.83	13.41	2.023	1.220	8.940	0	2.176091259	1.290034611
	5		M	140	21.6	19	2.1	12.65	2.041	1.500	9.036	30	2.146128036	1.278753601
	6		M	175	23.6	19.8	1.69	9.63	2.254	0.966	5.503	0	2.243038049	1.29666519
	7		M	215	24.3	20.7	1.18	16.45	2.424	0.549	7.651	0	2.33243846	1.315970345
	8		M	200	24.9	20.8	2.62	13.46	2.222	1.310	6.730	0	2.301029996	1.318063335
	9		M	165	23.5	19.8	1.32	5.32	2.126	0.800	3.224	30	2.217483944	1.29666519
	10		F	210	22.4	19.8	1.66	2.22	2.705	0.790	1.057	30	2.322219295	1.29666519
	11		F	470	40.6	33.3	3.54	24.07	1.273	0.753	5.121	0	2.672097858	1.522444234
	12		F	200	25	21	2.37	8.98	2.160	1.185	4.490	30	2.301029996	1.322219295