The evaluation of the health status of *Clarias gariepinus* from the Inanda and Nagle dams in KwaZulu-Natal, South Africa

by

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LIST OF ABBREVIATIONS

AChE	: Acetylcholinesterase
Al	: Aluminium
AMD	: Acid Mine Drainage
BCA	: Bicinchoninic acid
Cd	: Cadmium
CF	: Condition Factor
CNRC	: Canadian National Research Council
Cr	: Chromium
CRMs	: Certified Reference Materials
CV	: Coefficient of Variance
Cu	: Copper
DDT	: Dichloro-diphenyl-trichloroethane
DO	: Dissolved oxygen
DWWTP	: Darvill Wastewater Treatment Plant
DWAF	: Department of Water Affairs
DWS	: Department of Water and Sanitation
EC	: Electrical conductivity
ELISA	: Enzyme-linked immunosorbent assay
EDCs	: Endocrine disrupting chemicals
FAII	: Fish Assembly Integrity Index
Fe	: Iron

FRAI	: Fish Response Assessment Index
FWLRs	: Fish weight-length relationships
GSI	: Gonadosomatic Index
H_2O_2	: Hydrogen peroxide
H&E	: Haematoxylin & Eosin
Hg^{2+}	: Mercury
HSI	: Hepatosomatic Index
ICP	: Inductively Coupled Plasma-Optical Emission Spectrophotometer
I_{org}	: Organ index
K	: Potassium
KZN	: KwaZulu-Natal
LPO	: Lipid Peroxidation
MAP	: Mean annual precipitation
MMCs	: Melanomacrophages centres
Mn	: Manganese
N_2	: Nitrogen
NBF	: Neutral Buffered Formalin
NH_3	: Ammonia
NH_4^+	: Ammonium
NO_2	: Nitrate
NO_3	: Nitrite
NWWTP	: Northern Wastewater Treatment Plant

OCPs	: Organochlorinated pesticides
OPs	: Organophosphates
Pb^{2+}	: Lead
PCBs	: Polychlorinated biphenyls
PO_4	: Phosphate
RHP	: River Health Programme
ROS	: Reactive Oxygen Species
REMP	: River Eco-status Monitoring Programmes
SASS	: South African Scoring System
SA:V	: Surface area to volume ratio
sc	: Spermatocytes
SL	: Standard length
SO_4	: Sulphate
st	: Spermatids
sz	: spermatozoa
TNB	: 5-mercapto-nitrobenzoic acid
TL	: Total Length
VTG	: Vitellogenin
WMU	: Water Management Use
WHO	: World Health Organization
Zn	: Zinc

ABSTRACT

The uMgeni River is one of the most polluted freshwater ecosystems in KwaZulu-Natal. This river is home to about 48 fish species. The study aims to determine the effects of water quality on the health of *Clarias gariepinus* from the Nagle and Inanda dams. Water variables were measured *in situ* using a YSI meter. Water and sediment samples were collected at three sites in each dam. Fish were collected and euthanized by severing the spinal cord. Different fish biometrics were measured. Fish tissues were preserved based on the analysis to be carried out. Neutral to alkaline water pH was recorded at both dams. Although the Inanda Dam exhibited higher total nitrogen concentration, both dams were mesotrophic, whereas the phosphate concentration at the Inanda Dam was eutrophic. Generally, the Nagle Dam showed good quality water compared to the Inanda Dam. The Inanda Dam fish population showed a relatively higher prevalence of alterations in the gills and liver than fish from the Nagle Dam. The degree of alterations showed some variability within each population, however, there was no significant difference ($p>0.05$) between the two populations. Both populations exhibited organ indexes less than 20, hence, moderate alterations. Fish ovaries and testis showed slight alterations at both dams. Acetylcholinesterase (AChE) brain activity recorded no significant difference in between the dams ($p>0.05$). Male populations recorded no significant difference in vitellogenin (VTG) induction between the dams ($p>0.05$). Despite, AChE activity and VTG induction showing no significant difference between the two populations, there was a great variability within each population. The lowest AChE activity as well as the highest VTG level were observed in the Inanda Dam fish populations. Both histopathologic and biochemical biomarkers are signs of increased pollution effect at the Inanda Dam. Nevertheless, it is evident that the uMgeni River system which is supposed to be providing sanctuary to aquatic biota is becoming deteriorated. These findings provide baseline data or a point of reference for future studies as it is the first of its kind in this river system.

CHAPTER 1

1.1. GENERAL INTRODUCTION

Among our renewable natural resources, water is the most vulnerable. It makes up approximately 71% of the world's surface area but less than 1% constitutes of freshwater found in aquatic ecosystems such as rivers, lakes, ponds, wetlands and dams (Viman et al. 2010). Although comprising a small part of the aquatic ecosystems, it supports most life forms, it is responsible for ecological balance, enhance economic development and provide goods and services to people (Singh and Gupta 2017). Furthermore, freshwater ecosystems provide habitat to extraordinarily rich, endemic, and sensitive aquatic biota. Yet, they are mostly intensively altered ecosystems in the world through various anthropogenic activities such as dumping of waste into water bodies, industrial wastewater, effluent from river tributaries, agricultural run-off, sewage effluent from municipality treatment plants (Liu et al. 2007; Carpenter et al. 2011; McGrane 2016). Once polluted, it may be difficult to restore to its pristine state and its function cannot be substituted (Carpenter et al. 2011). Polluted water becomes very expensive to treat for human consumption. As a result, at least two-thirds of the global human population are still faced with a threat of safe and clean water shortage due to huge demand and extensive pollution rate to water bodies (Malik et al. 2014; Halder and Islam 2015).

Water pollution is a global problem affecting both developed and developing countries at varying degrees. In developing countries, an estimated 90% of waste is discharged incorrectly into rivers and streams without proper management and treatment (Tockner and Stanford 2002). Most African rivers flowing through cities, towns and agricultural landscapes are heavily polluted (Awoke et al. 2016). Moreover, limited access to good sanitation and poor regulatory policies have significantly contributed to the decline of water quality. In contrast, developed countries have good legislation, resources and governance by the state. The national and international authorities have successfully created platform to address the environmental implications of pollution to the public (McGrane 2016). Furthermore, most developed countries have well implemented monitoring and remediation programs than in most developing countries (Räsänen et al. 2012). In some parts of the world, rivers are heavily polluted such that water is declared unfit for industrial and domestic use (Morrison et al. 2001).

With the global evolution of industries and urbanization, improved agricultural practices and growing human populations, pollution into receiving water bodies have significantly increased (Liu et al. 2007). Land use activities range from one country to another, and the nature of environmental and socio-economic impacts are different. Developing countries undergo urbanization at a faster pace than developed countries: During 1990-1995, the annual average urbanization rate in developing nations were over 3.4% occurring at a large spatial scale as compared to only 0.7% in the developed nations occurring at local scales (Karn and Harada 2001; McGrane 2016). Most rivers in Asia are classified as eutrophic with extreme algal problems and huge pollution loading of arsenic (Abbaspour 2011). In Pakistan, most wastewater treatment plants are not properly working, discharging approximately 90% of untreated sewage. Furthermore, about 70% of the water provided by the government is deemed unsafe and result in waterborne diseases (Nabeela et al. 2014). In Nigeria, agricultural operations, oil refineries and daily market activities including abattoirs, street vendors and street mechanic operations are major contributors of different wastes into receiving water bodies (Galadima et al. 2011). Food security demand has resulted in intensive agricultural operations, resulting in about 38% of rivers in the United States of America classified as eutrophic (Evans et al. 2019).

For example, the effect of the diffuse and point source pollution arising from gold mines of the central and western basin is evident from the high salinity of the Vaal River in inland South Africa (McCarthy 2011) while the water quality of the Crocodile River in Mpumalanga, South Africa is affected by the 30-wastewater treatment works on the banks of the river. Also, this river is surrounded by intensive commercial and subsistence farming activities, mining and metal smelting activities, and various manufacturing operations (Che et al. 2021). Water quality of rivers such as the uMdloti and uMgeni in KwaZulu-Natal, South Africa are also affected by various pollution sources such as mining, agriculture and urban wash-off thus mostly affecting the river catchment lower reaches (Olaniran et al. 2014).

The uMgeni River system drains a highly industrialised catchment and is regarded as an important ecosystem as it provides goods and services to local people. However, it has succumbed to huge pollution load over the years. The river flows through industrial areas in Pietermaritzburg and rural areas before it opens into the Indian Ocean, Durban (Graham 2007). The uMgeni River receives water from the three polluted tributaries, the uMsunduzi, uMngcweni and Sikelekehleni rivers before feeding the Inanda Dam. Poor sanitation management, unprecedented population growth in surrounding rural areas, agricultural activities, the detergent run-off discharged from the household activities and unsewered human settlements which resulted in high level of nutrients and microbial contaminants have been evident along in the water quality of the uMgeni River system (Namugize et al. 2018).

1.2. RATIONALE & MOTIVATION

The Nagle and Inanda Dams are two of the four dams connected to the uMgeni River catchment in Durban, KwaZulu-Natal. The uMgeni River plays a fundamental role of providing water to about 3.5-5 million people of Durban and Pietermaritzburg metropolitan areas (Agunbiade and Moodley 2014). The river is very important to the informal settlers because they use it for household activities such as bathing, cleaning, washing and drinking and agricultural purposes such as irrigation and a water source for livestock (Gakuba et al. 2015). Recent studies have shown that the river's water quality is deteriorating because of various anthropogenic activities such as effluent from river tributaries, industrial waste, agricultural run-off, wastewater from municipality treatment plants (Namugize et al. 2018). These activities lead to the high concentrations of polychlorinated biphenyls (PCBs), *Escherichia coli*, pharmaceutical products, and heavy metals in the water column (Olaniran et al. 2009, 2014; Gakuba et al. 2015; Matongo et al. 2015). The uMgeni River is home to about 48 fish species both native and alien. Despite water quality deterioration in the uMgeni River system, no study has explored how the inhabitant fish are responding the level of water pollution.

Both dams are within nature reserves with the mandate of preserving freshwater biota and the ecosystems at large. Therefore, understanding the health of top predators in these water bodies will provide baseline data which the conservation authorities can use as a reference point for future studies. Hence, the present study employs *Clarias gariepinus* which can survive harsh conditions as a sentinel species.

1.3. AIM

To assess the health status of *C. gariepinus* from the Inanda and Nagle dams, in KwaZulu-Natal.

1.4. OBJECTIVES

a) To assess water and sediment quality by measuring selected variables such as pH, dissolved oxygen (DO) levels, temperature, and electric conductivity (EC) at the Inanda and Nagle dams.

b) Assess overall health of *C. gariepinus* by using the condition factor, hepatosomatic (HSI) and gonadosomatic indices (GSI), fish weight-length relationships, macroscopic health assessment and histopathology.

c) Assess possible oestrogenic effects and pesticides exposure by measuring vitellogenin (VTG) induction in the liver and acetylcholinesterase (AChE) activity in the brain, respectively.

1.5. RESEARCH QUESTION & HYPOTHESIS

The deterioration of water quality in the uMgeni River system has recently become a cause for concern as this river is home to about 48 freshwater fish species. The river is joined by three contaminated tributaries, the uMsunduzi, uMngcweni and Sikelekehleni rivers before feeding Inanda dam. The question of this study is whether the health of *C. gariepinus* population in the Inanda Dam differ from that of the Nagle Dam?

It is hypothesized that the water quality at the Inanda Dam will be poor as it is located downstream of the confluences with the three tributaries, and the health of *C. gariepinus* population in the Inanda Dam will be poorer compared to that in the Nagle Dam.

1.6. THESIS OUTLINE

The thesis comprises of six different chapters:

Chapter 1: General introduction, including aims, objectives, research questions and hypothesis,

Chapter 2: Literature review,

Chapter 3: Physico-chemical parameters of the water, and sediment quality,

Chapter 4: Overall fish health: condition factor, organ-somatic indices, macroscopic assessment, fish weight-length relationships (FWLRs) and histopathology-based health assessment,

Chapter 5: Biomarkers: AChE and VTG, and

Chapter 6: Summary and conclusion.

CHAPTER 2

Literature review

2.1. uMgeni River system

The uMgeni River is the largest freshwater system in KwaZulu-Natal (KZN) flowing for approximately 225km from the source to the mouth and drains a catchment area of 4416km^2. The river originates from the Drakensburg mountains passing through agricultural and industrial activities and urban and rural settlements. It rises in the uMgeni vlei in the highland plateau area of the KZN Midland, approximately 1900m above sea level (Graham 2007). Moreover, the geology of the catchment is made up of Cape and Karoo sedimentary rocks, complex granites and igneous rocks (Howard et al. 1995). The uMgeni River has four impoundments connected to its course namely: Albert Falls, Midmar, Nagle and Inanda dams (Moodley et al. 2014). It has four main cardinal tributaries namely: Lions, Karkloof, Impolweni and uMsunduzi rivers (Banda and Kumarasamy 2020) with other two smaller ones, uMngcweni and Sikelekehleni rivers. The uMgeni River is home to approximately 48 freshwater fish species consisting of 36 indigenous and 12 alien species (Agunbiade and Moodley 2014).

However, it is one of the most polluted rivers in KZN receiving treated and partially treated wastewater from the Northern wastewater treatment plant (NWWTP) situated along the catchment. Over the years, it has suffered huge pollution loading due to dumping of waste into water, agricultural run-off, poor infrastructure and surcharging sewers in areas such as Mpophomeni (low cost housing area around Midmar Dam) leading to high levels of nutrients, metals and *E. coli* (Olaniran et al. 2009).

The uMgeni River receives water from the uMsunduzi River which drains a highly industrialized catchment (Munyengabe et al. 2017). The uMsunduzi River also receives waste effluent from the Darvill wastewater treatment plant (DWWTP) and industrial activities that further contribute to the pollution load in the river (Howard et al. 1995). Such has resulted in the Henley Dam (connected to the uMsunduzi catchment course) no longer used for water supply to the surrounding communities. The uMsunduzi River has been reported to have increased nutrient and metal levels in the Inanda Dam (Munyengabe et al. 2017).

2.1.1. Nagle Dam

The Nagle Dam (29˚35’40’’S, 30˚38’30’’E) is located 25 km east of Pietermaritzburg, 46km downstream of Albert Falls and 48km upstream of the Inanda Dam. It was the first dam to be built along the uMgeni River to meet a huge water demand and was officially opened in 1950. It is within the jurisdiction of the uMgungundlovu and Mkhambathini local municipalities. It has a total capacity of 24.6 million m^3, surface area of 1.56km^2 and a spillway height of 24.1m. The dam is located in the Msinsi Nature Reserve property and is surrounded by traditional land and livestock watering (Namugize et al. 2018).

2.1.2. Inanda Dam

The Inanda Dam (29˚42’1’’S, 30˚52’1’’E) is located at the Valley of the Thousand Hills (KwaNgcolosi area) about 42km North of Durban, KZN. The Valley of the Thousand Hills is densely populated and local people rely on the Inanda Dam for drinking water, livestock watering and subsistence fishing (Oosthuizen and Ehrlich 2001). It was constructed in 1989 and supplies water to parts of Durban via Reservoir Hills. The dam is located at the lower catchment of the uMgeni River which, rises in the Natal Midlands and reaches the Indian Ocean. It has total capacity of 251.65 million m^3, a surface area of 14.63km^2 and spillway height of 31.2m. The structure of the dam exploits the local topography and geology with mass concrete thus forming the right bank, river bed and clay core on left bank (Tollow 1991). This dam is located just below the uMsunduzi-uMgeni River confluence. A recent study have reported that the water quality in the Inanda Dam is highly eutrophic with elevated metal concentrations (Munyengabe et al. 2017).

2.2. Land uses

The uMgeni River is located in KZN, the second most populated and highly ecologically disturbed province in South Africa (Chetty and Pillay 2019; Banda and Kumarasamy 2020). 85% of the contaminants found in the uMgeni River system comes from non-point sources such agricultural run-off, waste from household activities and illegal dumping of waste (Gakuba et al. 2015; Namugize and Jewitt 2018). The natural vegetation of uMgeni River is highly modified due to anthropogenic activities, population growth, food demand and socio-economic and policy drivers. (Namugize and Jewitt 2018; Namugize et al. 2018) (Figure 2.1). Upper reaches of the catchment are characterized by timber production, cultivation, and small-scale commercial and subsistence farming. Lower reaches are characterized by urban areas, unregulated sand mining activities around Inanda area, industries and municipality sewage treatment plant works (Banda and Kumarasamy 2020) (Figure 2.1).

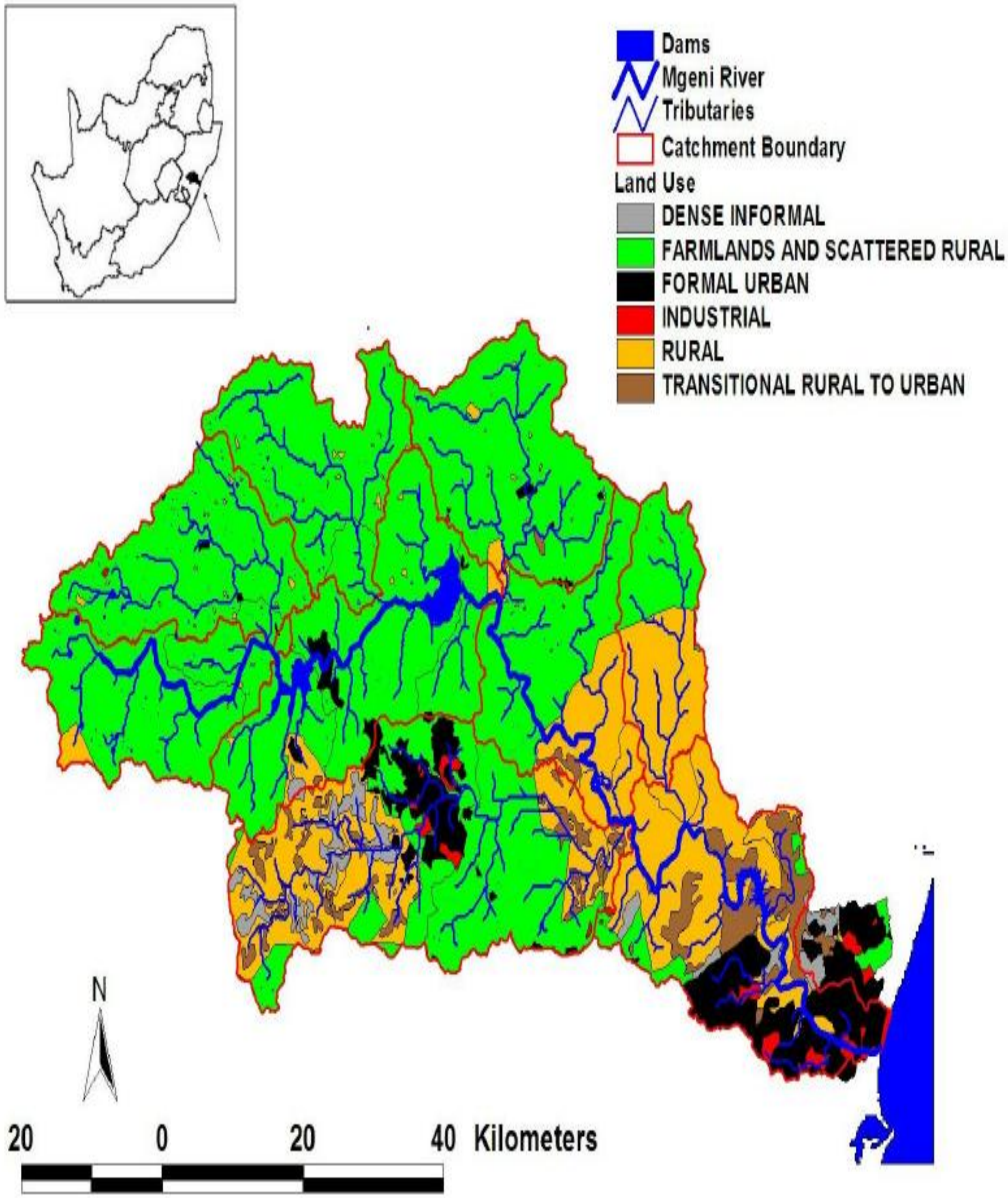

Figure 2.1: The locality map showing different land uses occurring along the uMgeni River catchment DWAF (1996).

2.3. Biomonitoring programs

Biomonitoring programmes aim to provide a direct measure of ecological integrity through the integration of various stressors. They provide a broad measure of the long term environmental synergistic impacts. In the early 1990s, the Department of Water and Sanitation (DWS) initiated an approach to monitor the health of rivers in South Africa under the South African River Health Programmes (RHP). The programmes were later incorporated into the River Eco-status Monitoring Programmes (REMP). The RHP/REMP was designed to do the following:

a) To measure, assess and report on ecological state and trends of aquatic ecosystems

b) Report on spatial and temporal changes in the aquatic ecosystem

c) Ecosystem health report reflect relevant scientific information

d) Create environmental awareness

Traditionally, South African rivers have been monitored by two methods: i) by chemical analyses to determine water quality and ii) use of bio-monitoring methods such the South African Scoring System (SASS) developed by (Chutter 1998) and Fish Assemblage Integrity Index (FAII) (Kleynhans 1999) that was later implemented into the Fish Response Assessment Index (FRAI). The bio-monitoring approaches are useful in integrating responses to combinations of contaminants and conclude on the overall quality of the aquatic ecosystem (Mangadze et al. 2019). Biomonitoring has received enormous attention in Southern African countries mainly because it is fast, reliable, integrative and cost effective to assess environmental stressors (Mangadze et al. 2019). However, water quality monitoring based on physico-chemical analyses have been used for a while in most African countries but has received criticism mainly because it is time consuming, costly and only provides water analyses of the time of sampling without taking into account temporal variations (Mangadze et al. 2019)

2.4. Fish as a bioindicator

Fish have been used as biological indicators of long-term effects and broad habitat conditions of rivers as they are sensitive to habitat alterations with regards to temperature, food availability, pollution and current velocity profiles (Schiemer 2000; Li et al. 2010). They are regarded as good bioindicators because they have long life-cycles, are mobile, are able to accumulate and concentrate contaminants in their body tissues, they are involved in energy flow through different trophic levels, they nearly inhabit every aquatic habitat and they reflect their environment's state of health from molecular to population levels (Gaber et al. 2013; Courtney et al. 2014; Sani and Idris 2016). Fish communities are frequently monitored to describe river conditions, used as sentinel species because of their important role in the trophic web, bioaccumulation of toxic substances and responses to low concentrations of mutagens (Schiemer 2000; De Andrade et al. 2004). Based on the level of biological organization, there are three types of bio-indicators namely: i) compliance indicators which are used to assess attainment and maintenance of the aquatic ecosystem, ii) diagnostic indicators which are used to provide insight into the cause of non-compliance indicators and iii) early warning indicators (biomarkers) used to manage environment before conditions have deteriorated (Weyl et al. 2016). Furthermore, the lower levels of biological organisations such as biochemicals and tissues provide more insight long before responses occur in both population and community levels in the ecosystem (Dennis and Dennis 2012).

2.4.1. Clarias gariepinus

Clarias gariepinus commonly known as the African sharptooth catfish is a native fish species in most African countries and have been successfully introduced in approximately 37 countries in Europe, Middle East and other parts of Asia (Weyl et al. 2016). It is widely distributed around the world and due to its commercial significance, it is successfully cultured in ponds and pens. It lives in a variety of freshwater environments such as lakes, rivers, swamps, areas of seasonal flooding and very adaptive to extreme environmental conditions. They are disease resistant with high fecundity and easy of reproduction in captivity. They are potamodromous fish migrating within streams and rivers (Olaleye 2005). The catfish can attain a weight of 60kg and a total length of 1.7m. During the breeding season, it lays the eggs in the vegetation and eggs hatch within 25-40 hours. The fish larva can swim and feed within 2 to 3 days from hatching, while photoperiod and water temperature influence the maturation process in juveniles (Weyl et al. 2016).

Clarias gariepinus has unique features of physiological, ecological, morphological, and behavioural traits that allows it to be a good indicator of environmental alteration. It has shown high trophic plasticity because it can feed on a variety of preys under diverse environmental conditions (Alimba et al. 2015). Furthermore, due to its wide distribution across many countries in the world, huge turnover rate, and high adaptability to laboratory conditions, *C. gariepinus* is considered as an ideal species for toxicology assessments (Alimba et al. 2015).

Figure 2.2 Clarias gariepinus

2.5. Target organs for contaminants

2.5.1. Gills

Fish gills play an important role in gaseous exchange, osmoregulation, maintain ionic homeostasis, acid-base balance and nitrogenous compounds excretion as they are in constant contact with the water (Machado and Fanta 2003). They make-up 50% of the total surface area of the fish and provide a large surface area for contaminant uptake. Organic pesticides, heavy metals, and industrial waste can change branchial epithelium and alter the activity of the Na-K-ATPase by altering the normal ion flow (Machado and Fanta 2003). When the water quality changes, epithelial detachment and wrinkling may be observed. Hyperplasia, characterized by cellular proliferation in the interlamellar region of the respiratory lamellae, can also occur thus decreasing the surface area making gaseous exchange difficult (Witeska et al. 2006). Additionally, other common gill changes include desquamation and necrosis, aneurism (related to the rupture of pillar cells) in secondary lamellae and lifting of the lamellar epithelium (Cengiz 2006; Camargo and Martinez 2007).

2.5.2. Liver

The liver is the largest and most important organ in the fish body. It is responsible for basic metabolism, protein synthesis, storing of energy sources detoxification and biochemical transformation of pollutants into less toxic water-soluble metabolites which are then excreted into the bile (Chovanec et al. 2003; Ruiz-Picos et al. 2015). Detoxification is carried out through Phase I & II biotransformation. Phase I biotransformation include oxidation, catalyzed by a variety of enzymes such as cytochrome P-450, monoxiganase, mixed function oxidase located on the endoplasmic reticulum of the cells (Chovanec et al. 2003). However, cytochrome P-450 induction varies with seasons, gender, hormonal status and temperature (Chovanec et al. 2003) and it is highly elevated in fish exposed to industrial and domestic waste. Phase II biotransformation metabolism involves the conjugation of xenobiotic compounds with endogenous substrate thus facilitating the excretion of chemicals by adding more polar groups to the molecule (Ferreira et al. 2006).

2.5.3. Gonads

Gonads (testis and ovaries) are important organs responsible for reproductive capacity are maintained by selected steroid hormones (Ebrahimi and Taherianfard 2011; Liebel et al. 2013). The reproductive system has been recognized as a major target for environmental pollutants in different ecosystems (Ebrahimi and Taherianfard 2011). Pollutants such as pesticides, agricultural and industrial wastes and different types of bacteria have histopathological effects on the reproductive tissue of fish gonads. Fish testes are the most dynamic organs in the body, with a high cell turnover during the reproductive period thus making them vulnerable to contaminant exposure (Sadekarpawar and Parikh 2013). Gonad alterations are amongst other, characterized by the presence of both inter and intra-tubular vacuoles (Gaber et al. 2013). Furthermore, disturbances in gonad activities include decreased sperm in gravid testis, condensation of spermatogenic cells through clump formations and disturbances in the development of germ cells thus resulting in reduced ability for reproduction. Furthermore, other disruptions include asynchronous development, interstitial changes, severe pathological alterations, enlargement of sperm ducts and the detachment on the basal membrane (Liebel et al. 2013).

In females, morphological signs of masculinization have been observed because of exposure to environmental contaminants (Ebrahimi and Taherianfard 2011). When exposed, female fishes develop a gonopodium, a modified anal fin that is normally used by male fishes for internal fertilization. Furthermore, other alterations include the alterations on the steroidogenesis in female fishes are commonly due to heavy metal exposure. Decreased oocyte diameter, disorientation of gonad shape, absence of yolk material characterized by the decrease in vitellogenin (VTG), yolk granular degeneration and chorion layer depletion are among other gonad alterations due to contamination exposure (Gaber et al. 2013; Liebel et al. 2013).

The gonadosomatic index (GSI) is an index of gonad size relative to fish weight. It has been used in different research to determine the spawning frequency (Al-Deghayem et al. 2017). The changes in GSI help determine the reproductive seasons in different fish species and provide useful information about the cyclic changes during the seasons (Jan and Jan 2017). However, reproductive system can also be affected by factors such as sex, age, seasons, reproductive stages and water temperature (Jan and Jan 2017). Fish species with a GSI value between 0 to 2% are not ready to spawn (Kaur and Dua 2014).

2.6. Biomarkers

2.6.1. Vitellogenin

Vitellogenin (VTG) is an egg yolk precursor of the two yolk proteins lipovitellin and phosvitin and synthesized in the liver of female oviparous vertebrates (Rankouhi et al. 2002). In the process of VTG synthesis, estrogen secreted from the ovarian follicles triggers the synthesis of VTG in the liver (Flammarion et al. 2000). Vitellogenin have been used as a biomarker of exposure with estrogenic characteristics in both laboratory and field studies (Hara et al. 2016). Exposure to environmental contaminants such as steroid estrogens, pesticides, phytoestrogens and alkyl-phenolic compounds can trigger vitellogenic response in male fish (Flammarion et al. 2000). These estrogenic compounds can mimic the estrogenic induced production of VTG in liver cells and therefore increasing blood levels of VTG in male and immature fish. Vitellogenin induction has been shown to occur concurrently with effects at the level of the gonad e.g. a reduced gonadal growth in maturing male trout and alterations in the development of specific cells in the testis (Archer et al. 2017; Horak et al. 2021).

2.6.2. Acetylcholinesterase

Acetylcholinesterase (AChE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing hydrolysis of the neurotransmitter acetylcholine into acetate and choline

(Pretto et al. 2010; Connell et al. 2020). Pollutants such metals, synthetic pyrethroid compounds, carbamate pesticides and organophosphates (OPs) are known to selectively inhibit AChE activity in fish (Mdegela et al. 2010). The fish brain has low levels of antioxidants and higher levels of peroxidizable unsaturated lipids. Moreover, lipid peroxidation (LPO) can interfere with membrane fluidity thus inhibit AChE activity (Pretto et al. 2010). During inhibition, the neurotransmitter acetylcholine is inactivated by the binding of OPs, AChE accumulates in the nerve synapses thus interfere with the normal functioning of the nervous system (Fulton and Key 2001). Acetylcholinesterase inhibition result in excessive stimulation of cholinergic nerves, behavioral alterations such as tremors, convulsions and erratic or lethargic swimming (Cattaneo et al. 2011). Moreover, when more than 70% of brain activity is lost fish die. However, brain activity is not uniformly distributed within the brain, sensitivity is species specific (Fulton and Key 2001; Chovanec et al. 2003).

2.7. Fish health biometric indices

2.7.1. Condition factor

Condition Factor (CF) is an important indicative tool of the fish health status, indicate nutritional status of organisms and is used to assess field contaminations (Chovanec et al. 2003). It represents a range of responses coming from different environmental changes such as the quality and quantity of nutrients, presence of pathogens and other pollutants that result in changes in individuals and organs. It is considered inexpensive, non-lethal and alternative to proximate analysis of fish tissues (Lenhardt et al. 2009). The alterations in growth rates are due to contaminants resulting in the reallocation of energy to detoxification processes thus depleting energy reserves that were designated for growth. Condition factor can be further affected by nutrition, sexual maturation, diseases and seasons (Jan and Jan 2017; Araújo et al. 2018). Condition factor greater than one indicate a good condition of fish and less than one indicate that the fish is not in a good condition (Shobikhuliatul et al. 2013).

2.7.2. Hepatosomatic index

The hepatosomatic index (HSI) is an important tool to indicate the metabolic condition of fish. It is expressed as liver weight relative to fish body weight, often used to estimate the reserve energy in fish (Hussey et al. 2009). The HSI is an indicator of the state of balance between food consumed and food required for metabolism. The HSI also plays an important role in gonadal development thus it can be correlated with GSI mainly because of the VTG (Araújo et al. 2018).

However, the link between HSI and GSI has been criticized for being inconsistent between seasons (Hussey et al. 2009).

2.7.4. Macroscopic fish health assessment

The Health Assessment index (HAI) is an approach developed by Adams et al. (1993) to analyze and evaluate fish health and condition. It provides health profiles of fish based on the degree of abnormalities on tissues and organs of fish sampled from a population. It has been successfully applied in fish health studies in South African rivers such as the Vaal, Olifants and Hout river systems (Crafford and Avenant-Oldewage 2009; Madanire-Moyo et al. 2012; Sara et al. 2014) and international rivers such as Tennessee, Pigeon and Hartwell river systems (Adams et al. 1993; Madanire-Moyo et al. 2012) in fish health research studies. One of the greatest advantages of using the HAI is that it is an easy-to-use and inexpensive method. However, it does not provide quantitative results for statistical comparison among sites, species and years (Adams et al. 1993).

CHAPTER 3

Physico-chemical parameters of the water, and sediment in the Inanda and Nagle dams

3.1. INTRODUCTION

Water quality can be defined as biological, chemical, and physical attributes that ensures the suitability of water to sustain all various uses and processes. The Department of Water Affairs and Forestry (DWAF) implemented target water quality range (TWQR) guidelines for all various water uses and processes (DWAF 1996; Cude 2001). Physico-chemical variations of river water may be influenced by climate, geology, soils and biotic regions in which they flow (Singh and Lin 2015). Water chemistry is further influenced by multiple natural and anthropogenic factors which are either spatially diffused or concentrated (Ahearn et al. 2005). Such factors include terrestrial factors (changes in soil structure and vegetation cover), resource-use factors (increased water demand and unsustainable water use) and hydrological factors (decreased dilution of point source emissions in the water bodies). Furthermore, land use and land cover (LULC) changes greatly influence soil properties, hydrological processes, geomorphology and stream water quality of most rivers all around the world (Namugize et al. 2018). In this regard, water quality is likely to decline where there is a high interspersion of various land use activities along the watershed (Uddin et al. 2014).

South Africa is a semi-arid and water stressed country experiencing rainfall below the global average and high evaporation rates. On average, the country experiences strong evaporation patterns and a mean annual precipitation (MAP) of 500mm per annum (Dennis and Dennis 2012). South Africa has different climate gradients, it receives annual rainfall of 497 mm, and the western side receives approximately 1000mm. As a result, aquatic biota adapts to different water qualities and flow patterns (DWAF 1996; Mantel et al. 2010). Moreover, with the anticipated population growth rates and trends in socio-economic developments, the South African water resources will not sustain the on-going patterns of water use and uncontrolled waste discharge (Dennis and Dennis 2012). Water from most South African rivers is not suitable for irrigation of produce due to the increased levels of pollution from land activities such as untreated sewage generated from incomplete sewer systems, agricultural and industrial activities (Dennis and Dennis 2012; Britz et al. 2013). Urban wash-off also carries detergents from households and deposit them into most South African rivers which has increased phosphate loadings (Griffin 2017).

The uMgeni River is one of the largest river systems in KwaZulu-Natal, the second-most populated province in South Africa. The river suffers from huge pollution load from different land uses (Chetty and Pillay 2019). High levels of nutrients, sediment and microbial contamination have been identified as major water quality problems in the uMgeni River system (Namugize et al. 2018). The river is showing signs of deteriorating water quality due to waste disposal from informal settlements, pollution from river tributaries, industries, agricultural run-off and treated and partially-treated sewage effluent. Gakuba et al. (2015) reported that 85% of the contaminants in the uMgeni River are from non-point sources. The lower reaches of the river is mostly affected by industrialization due to proximity to metropolitan areas whereas upper reaches are characterized and primarily affected by land cultivation, livestock farms and timber production (Singh and Lin 2015; Namugize and Jewitt 2018).

With the increasing pollution loads into the uMgeni River system from various land uses, water and sediment quality remains a cause for concern. People living in the vicinity of the uMgeni River are highly dependent on this river for various household water uses such as cleaning, washing, irrigation and livestock production (Namugize and Jewitt 2018; Banda and Kumarasamy 2020). Olaniran et al. (2014) reported that the uMgeni River have heavy metals such as Lead (Pb^{2+}), Cadmium (Cd^{2+}), mercury (Hg^{2+}), Aluminum (Al^{2+}) and Copper (Cu^{2+}) that have toxic effects on aquatic biota. However, there is limited information on the effect of anthropogenic activities on the physico-chemical properties of the water in the Umgeni River system. This chapter aims to evaluate the water and sediment quality at the Inanda and Nagle dams. Given that the Inanda Dam is located after the river has been joined by polluted tributaries, it was hypothesized that the Inanda Dam will exhibit declined water quality relative to the Nagle Dam.

3.2. MATERIALS AND METHODS

3.2.1. Study area

The uMgeni River (29º 48’36’’S, 31º 02’08’’) is the largest freshwater ecosystem in KwaZulu-Natal, drains a catchment area of 4000km^2, 225km long and has four impoundments connected to its course. It provides water to approximately 3.5-5 million people in both Pietermaritzburg and Durban metropolitan areas (Hart and Wragg 2009). It supports 60% of the province’s economy productivity through recreational, commercial and sport fishing activities (Namugize et al. 2018). Based on climate, land use and soil type the river is divided into 13 water management use (WMU) areas by the DWAF with six of these WMUs in the upper reaches, five in the middle and two in the lower reaches (DWAF 1996).

The catchment receives an average annual rainfall of 600-1500 mm per year with high rainfalls between October and March (Namugize et al. 2018). The present study focused on the two dams namely: the Inanda and Nagle dams. The Nagle Dam is located in the middle catchment of the uMgeni River, upstream from where the river is fed by the three polluted tributaries, the uMsunduzi, uMngcweni and Sikeleketeni rivers (Figure 3.1). The Nagle Dam has a catchment area of 2545 km^2, surface area of 156.13 ha and total capacity of 393 0050 m^3. The Inanda Dam is located after the river has been joined by the three polluted tributaries in the lower uMgeni River (Figure 3.1). The Inanda Dam has a surface area of 1463 ha and a total capacity of 241 700 000 m^3 (Moodley et al. 2014).

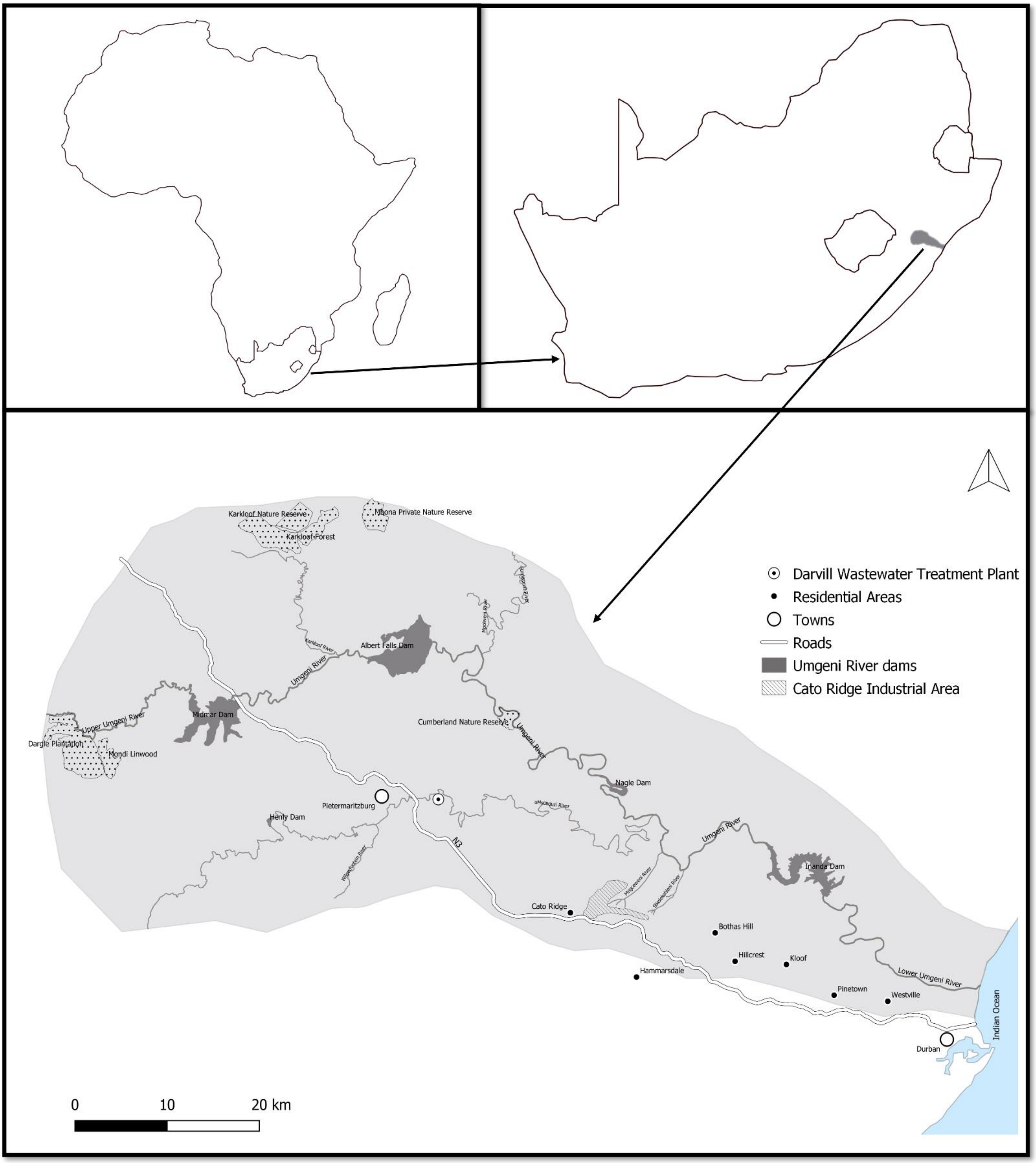

Figure 3.1: The locality map of uMgeni River system showing the four impoundments attached to its course, land uses, tributaries, and neighbouring communities along the catchment.

3.2.1. Sample collection

3.2.1.1. Water and sediment sampling

Water and sediment samples were collected at the Inanda and Nagle dams at the three sites (inflow, middle and dam wall) during the low and high flow seasons during the 2020 and 2021 surveys. The physical parameters such as temperature, salinity, DO levels, pH and electric conductivity were measured *in situ* using the YSI meter. Water samples were collected using acid pre-treated bottles and kept in a cooler box with ice. Sediments were sampled using Van Veen grab and put in 1L acid treated water sampling bottles and kept in cooler box with ice. The samples were transported to the laboratory and sediment were kept at -4℃ whereas water was kept in the fridge prior to chemical analysis. Water samples were collected with acid treated 1L water bottles. Heavy metals such as Al, Cu, Fe, Mn, and Zn were measured using the Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES).

3.2.2. Sample processing

3.2.2.1. Water samples

The nutrients analysis in the water was carried out at the Umgeni Water laboratory in Pietermaritzburg. Metal analyses were done using ICP-OES at the Chemistry laboratory, University of KwaZulu-Natal. Approximately 150ml water sample was transferred to a 250ml beaker. The sample was filtered, and 50ml was transferred into the 250mL volumetric flask. Thereafter, 1 ml of hydrochloric acid (HCl) was added to the sample and diluted to 250 ml with deionised water. Thereafter, 15ml of sample was transferred to the ICP vials and metal concentrations were determined with the ICP-OES. Metal concentrations were done in duplicates and results were expressed in mg/l.

3.2.2.2. Sediment samples

Sediment was digested following Gaudino et al. (2007) protocol where aqua regia was modified by adding hydrogen peroxide (H_2O_2) to enhance the destruction of organic matter. The sediment was placed into a 500 ml beaker in which 100 ml of modified aqua regia (3-HCl:1-HNO_3:1-H_2O_2) was added. The solution was then heated on a hotplate over medium-hot temperature, and as the acid level decreased, more acid was added. The process continued for about an hour. In the last 10 minutes, the solution was left to boil and reduce to 10 ml. The solution was left to cool down at room temperature and thereafter, made up to 250 ml in a volumetric flask, using deionised water. The metal analysis was carried out using ICP-OES.

For all digestions, the acid used was of ultra-pure analytical grade. Metal concentrations were done in duplicates and results were expressed in mg/kg. DORM-4 certified reference materials (CRMs) supplied by the Canadian National Research Council (CNRC) were used for validation, and the recovery range from 89.5 to 107%.

3.2.3. Statistical analysis

R-commander software was used for data analysis. Normality assumption was tested using the Shapiro-Wilk test and equality of variance was tested using the Levene's test. Depending on the assumptions test results, independent t-test or Mann-U Whitney test was used to test the differences in water parameters, nutrients in water, and sediment quality between the Inanda and Nagle dams. For inter-metal relationships in the water and sediment, results for each dam were pooled and evaluation was carried out using correlation analysis. Statistical significance was set at $p<0.05$.

3.3. RESULTS

3.3.1. Physical parameters and nutrients

Physical parameters and nutrient concentrations results are presented in Table 3.1. The Nagle Dam had a mean temperature of 22.2 ± 0.85 ℃ and 25.9 ± 0.73 ℃ during winter and summer seasons, respectively. The Inanda Dam had a mean temperature of 22.1 ± 2.46 ℃ and 28.2 ± 0.90 ℃ during the winter and summer seasons, respectively. There was no significant difference in water temperature between the two dams ($p>0.05$). Water temperature in relation with, DO showed no significant difference at both dams ($p>0.05$). Nagle dam had a mean DO of 96.3 ± 5.05% and 8.3 ± 0.45 mg/l in winter and 84.4 ± 16.6% and 6.54 ± 1.24 mg/l in summer seasons. The Inanda Dam had a mean DO of 200.37 ± 155.83% and 17.87 ± 14.26 mg/l during winter and 103.7 ± 6.24% and 8.12 ± 0.35 mg/l during summer seasons. Both dams had an alkaline pH during both winter and summer seasons ranging from 7.61 to 8.82 (Table 3.1). There was no significant difference in pH between the two dams ($p>0.05$). The Nagle Dam had a mean EC of 107.1 ± 1.3 µS/cm and 127.33 ± 20.6 µS/cm during winter and summer seasons, respectively. The Inanda Dam had a mean EC of 324.67 ± 32.42 µS/cm and 273.33 ± 24.01 µS/cm during the winter and summer seasons, respectively. There was a significant difference in EC between the two dams ($p<0.05$). The potassium concentrations were below the detectable limit at both dams (Table 3.1).

The Nagle Dam had a mean ammonia (NH_3) concentration of 0.30 ± 0.06 mg NH_3/L and 0.16 ± 0.33 mg NH_3/L during winter and summer seasons, respectively. The Inanda Dam had a mean NH_3 concentration of 0.14 ± 0.01 mg NH_3/L and 0.11 ± 0.07 mg NH_3/L during winter and summer seasons. Both dams showed no significant seasonal variation for NH_3 concentration. There was no significant difference in NH_3 concentrations between the dams ($p>0.05$). The Nagle and Inanda dams had mean NO_3 concentrations of 0.23 ± 0.12 mg NO_3/L and 1.08 ± 0.96 mg NO_3/L, respectively (Table 3.1). There was no significant difference in NO_3 concentrations between the two dams ($p>0.05$). Inanda Dam had a mean NO_2 concentration of 0.62 ± 0.65 mg NO_3/L. Nitrite was below the detectable limit at the Nagle Dam (Table 3.1). The Nagle and Inanda dams had a total inorganic nitrogen (N_2) of 0.56 and 1.82 mg N_2/L, respectively. There was a significant difference in total N_2 between the two dams ($p<0.05$).

The Nagle Dam had a mean sulphate (SO_4) concentration of 3.79 ± 0.82 mg SO_4/L and 4.05 ± 0.51 mg SO_4/L during winter and summer seasons, respectively. The Inanda Dam had a mean

SO_4 concentration of 20.77 ± 3.48 mg SO_4/L and 18.9 ± 1.39 mg SO_4/L during winter and summer seasons, respectively. There was a significant difference in SO_4 concentrations between the dams ($p<0.05$). The Nagle Dam had a mean phosphate (PO_4) concentration of 15.37 ± 2.71 mg PO_4/L and 16.57 ± 0.64 mg PO_4/L during winter and summer seasons, respectively. The Inanda Dam had a mean PO_4 concentration of 33.4 ± 79.43 mg PO_4/L and 62.3 ± 31.87 mg PO_4/L during winter and summer seasons, respectively. There was no significant difference in PO_4 concentrations between the two dams ($p>0.05$).

Table 3.1. Mean ± SD water variables recorded at the Nagle and Inanda dams during winter and summer surveys in 2020 and 2021. The DWAF (1996) target water quality range guidelines were used as a main set of criterion.

Water parameters	Nagle Dam	Inanda Dam	TWQR
Temperature (℃)	24.09 ± 2.24	25.16 ± 3.74	n/a
pH	7.95 ± 0.46	8.33 ± 0.12	6.5-9.0
DO (% of saturation)	90.2 ± 12.72	152.35 ± 111.67	80-120
DO (mg/L)	7.46 ± 1.31	12.99 ± 10.48	6-9
Electrical conductivity (µS/cm)	117 ± 17.12	299 ± 37.97	n/a
Potassium (mg K/L)	<5.00	<5.00	n/a
Ammonia (mg NH_3/L)	0.23 ± 0.21	0.13 ± 0.05	0.5-2.5
Nitrite (mg NO_2/L)	<0.10	0.62 ± 0.65	n/a
Nitrate (mg NO_3/L)	0.23 ± 0.12	1.08 ± 0.96	0.5-2.5
Sulphate (mg SO_4/L)	3.92 ± 0.75	19.83 ± 2.58	n/a
Phosphate (mg P/L)	15.97 ± 1.88	47.85 ± 56.39	n/a
Aluminium (mg Al/L)	0.30 ± 0.49	0.26 ± 0.31	n/a
Copper (mg Cu/L)	<0.010	<0.010	n/a
Iron (mg Fe/L)	0.53 ± 0.77	0.38 ± 0.49	n/a
Manganese (mg Mn/L)	0.04 ± 0.02	0.05 ± 0.02	0.18
Zinc (mg Zn/L)	<0.025	<0.025	n/a

Key: n/a= not available

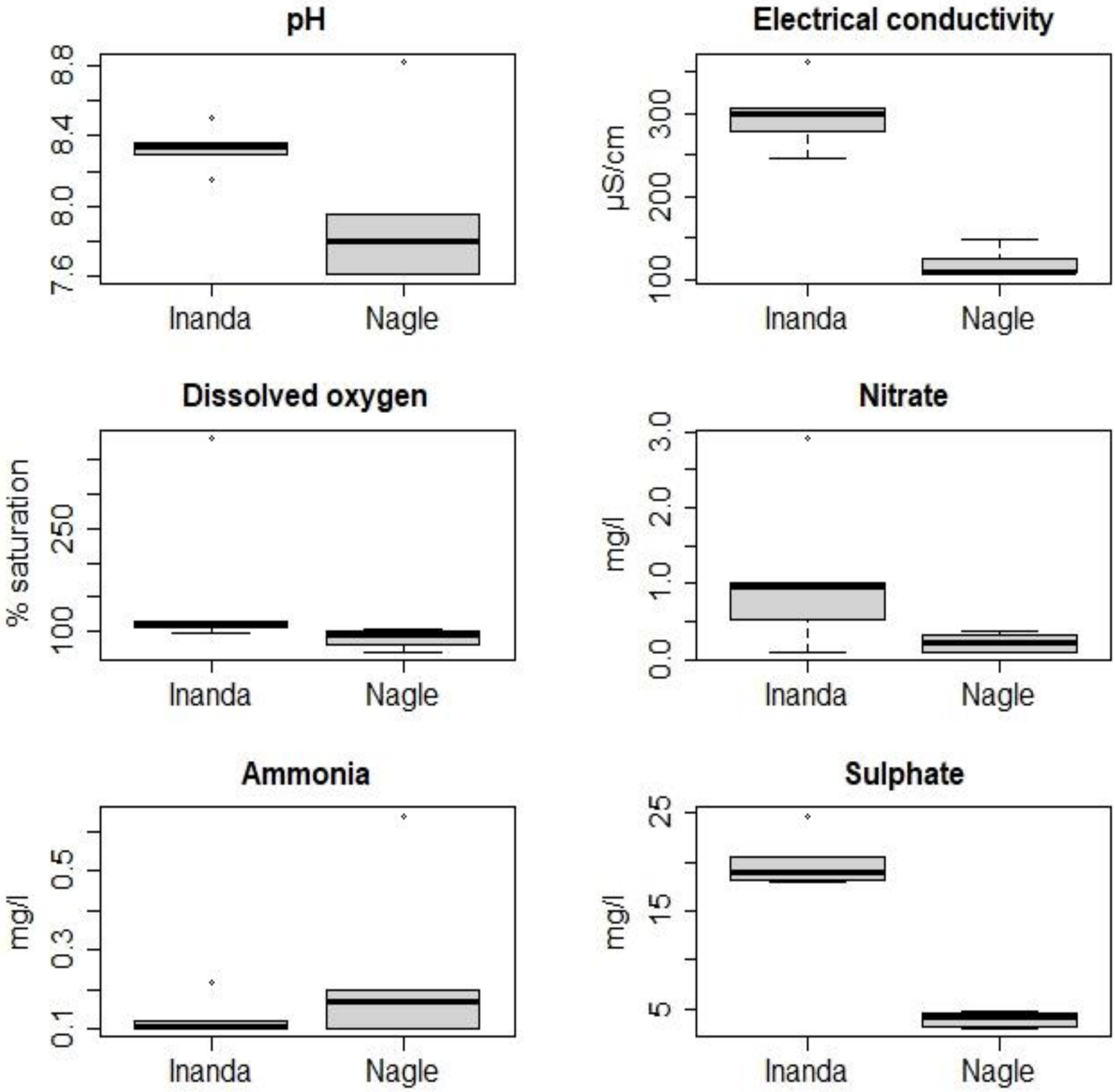

Figure 3.2. Box and whisker plots representing the range in physical parameters and nutrient concentrations recorded at the Nagle and Inanda dams during summer and winter seasons in the 2020 and 2021 survey. The box shows the 25th percentile and the 75th percentile, while the line inside the box is the median of the concentrations.

3.3.2. Metals

Metal concentrations recorded in the water of the Nagle and Inanda dams are presented in Figure 3.2. The Al concentrations recorded in the water at the Nagle and Inanda dams ranged from 0.100 mg/l to 1.289 mg/l and 0.01 mg/l to 0.856 mg/l, respectively. The Nagle and Inanda dams had a mean Al concentration of 0.30 ± 0.48 and 0.26 ± 0.31 mg/l, respectively (Table 3.1). There was no significant difference in Al concentrations in the water between the two dams ($p>0.05$), and no seasonal variation was observed within each dam. The Al concentrations in sediment has shown no significant difference between the two dams ($p>0.05$), with concentration ranging from 3063.75 to 200000 mg/kg and 121875 to 300000 mg/kg for the Nagle and Inanda dams, respectively (Figure 3.3). The Nagle and Inanda dams had a mean Al concentration of 122386.46 ± 80746.13 mg/kg and 200000 ± 77761.66 mg/kg, respectively (Table 3.1).

The Cu concentrations in the water at both dams was below the detectable limit (Table 3.1). The Cu concentrations recorded in the sediments at the Nagle and Inanda dams ranged from 200 to 518.7 mg/kg and 425 to 587.5 mg/kg, respectively (Figure 3.3). The Nagle and Inanda dams had a mean Cu concentration of 341.67 ± 139.4 mg/kg and 517.71 ± 147.92 mg/kg, respectively. There was no significant difference in Cu concentrations in between the two dams ($p>0.05$). There was a seasonal variation in Cu concentrations in Inanda Dam ($p<0.05$).

The Fe concentrations recorded at the Nagle and Inanda dams ranged from 0.068 to 2.04 mg/l and 0.025 to 1.27 mg/l, respectively (Figure 3.2). The Nagle and Inanda dams recorded a mean Fe concentration of 0.53 ± 0.77 mg/l and 0.38 ± 0.49 mg/l, respectively (Table 3.1). There was no significant difference in Fe concentrations in between the two dams ($p>0.05$). There was no seasonal variation in Fe concentrations within each dam. Moreover, Fe concentrations in the sediments showed no significant difference ($p>0.05$), ranging 6468.75 to 506250 mg/kg and 281250 to 665625 mg/kg, respectively (Figure 3.3). The Nagle and Inanda dams had a mean Fe concentration of 295349 ± 190078.9 mg/kg and 441666.7 ± 162435.9 mg/kg, respectively

The Mn concentrations recorded at the Nagle and Inanda dams ranged from 0.025 to 0.066 and 0.025 to 0.074 mg/l, respectively (Figure 3.2). The Nagle and Inanda dams recorded a mean Mn concentration of 0.04 ± 0.022 mg/l and 0.05 ± 0.02 mg/l, respectively (Table 3.1). There was no significant difference in Mn concentrations in between the two dams ($p>0.05$). Moreover, Mn concentrations showed no significant difference ($p>0.05$), ranging from 203.13 to 17812.5 mg/kg and 5750 to 8906.25 mg/kg, respectively (Figure 3.3). The Nagle and Inanda

Dam had a mean Mn concentration of 7986.98 ± 6229.88 mg/kg and 9119.79 ± 2607.56 mg/kg, respectively. There was no seasonal variation in Mn concentrations in each dam ($p>0.05$).

The Zn concentrations in the water at both dams were below the detectable limit (Table 3.1). The Zn concentrations recorded at the Nagle and Inanda dams ranged from 565.63 to 1718.75 mg/kg and 662.5 to 5656.25 mg/kg, respectively (Figure 3.3). The Nagle and Inanda dams recorded a mean Zn concentration of 705.73 ± 513.3 mg/kg and 1870.83 ± 1970.86 mg/kg, respectively. There was no significant difference in Zn concentrations in between the two dams ($p>0.05$). There was no seasonal variation in Zn concentrations within each dam ($p>0.05$). Mean metals found in water and sediments at both the Nagle and Inanda dams were as follows; Fe>Al>Mn>Zn>Cu.

3.3.2.1. Inter-metal relationships

The Fe and Mn showed a positive correlation ($r=0.763$, $p>0.05$) and ($r=0.661$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Al and Mn showed a positive correlation ($r=0.448$, $p>0.05$) and ($r=0.529$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Fe and Al showed a negative correlation ($r=-0.229$, $p>0.05$) at the Nagle Dam. Moreover, Fe and Al showed a positive correlation ($r=0.986$, $p>0.05$) at the Inanda Dam.

The inter-metal correlation coefficients recorded in sediments in the Nagle and Inanda dams is presented in Table 3.2. The Al and Zn showed a negative correlation ($r=-0.299$, $p>0.05$) and ($r= -0.083$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Cu and Al showed a positive correlation ($r=0.100$, $p>0.05$) and ($r=0.347$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Fe and Mn showed a positive correlation ($r=0.435$, $p>0.05$) and ($r=0.671$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Al and Fe showed a positive correlation ($r=0.233$, $p>0.05$) and ($r=0.960$, $p<0.05$) at the Nagle and Inanda dams, respectively. The Fe and Cu showed a positive correlation ($r=0.811$, $p>0.05$) and ($r=0.463$, $p>0.05$) at the Nagle and Inanda dams, respectively (Tables 3.2).

The Cu and Mn showed a positive correlation ($r=0.603$, $p>0.05$) and ($r=0.533$, $p>0.05$) at Nagle and Inanda dams, respectively. The Cu and Zn showed a positive correlation ($r=0.496$, $p>0.05$) and ($r=0.745$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Mn and Fe showed a positive correlation ($r=0.453$, $p>0.05$) and ($r=0.671$, $p>0.05$) at the Nagle and Inanda dams, respectively. The Zn and Mn showed a positive correlation ($r=0.092$, $p>0.05$) and ($r=0.277$, $p>0.05$) at the Nagle and Inanda dams, respectively (Tables 3.2). The Fe and Zn showed a positive correlation (r=0.775, p>0.05) at the Nagle Dam. The and Zn showed a negative correlation (r=-0.054, p>0.05) at the Inanda Dam (Table 3.2).

*Table 3.2. Inter-metal relationships recorded in sediment in the Inanda Dam and Nagle Dam (highlighted in grey) during the 2020 and 2021 survey. * Correlation significant at p<0.05*

Metals	Aluminium	Copper	Iron	Manganese	Zinc
Aluminium	1	0.110	0.233	0.060	-0.299
Copper	0.347	1	0.811	0.603	0.496
Iron	0.960*	0.463	1	0.435	0.775
Manganese	0.495	0.533	0.671	1	0.092
Zinc	-0.083	0.745	-0.054	0.277	1

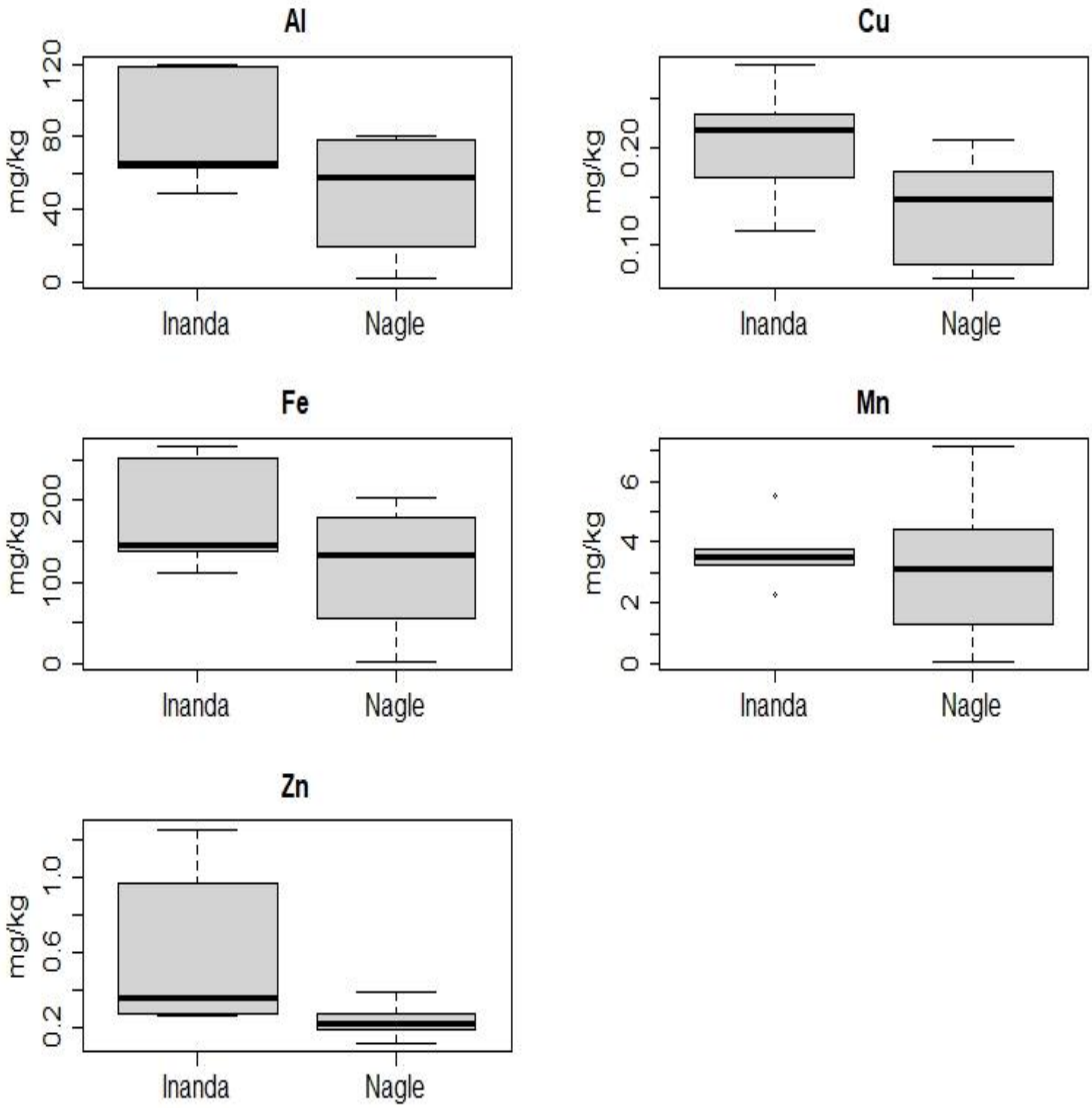

Figure 3.3. Box and whisker plots representing the range in aluminium, copper, iron, manganese, and zinc concentrations recorded in the sediments of the Inanda and Nagle dams during the winter and summer seasons in the 2020 and 2021 survey. The box shows the 25th percentile and the 75th percentile, while the line inside the box is the median of the concentrations.

3.4. DISCUSSION

3.4.1. Physical parameters

3.4.1.1. pH

pH is an indicator of acidity or alkalinity of water. Growth, metabolism and survival of aquatic organisms including fish is ideal within a pH range of 6.5 to 8.5 (Rangeti and Dzwairo 2021). pH is generally influenced by rain run-off that deposit inorganic matter possibly from abandoned mines seeping into the river (Iqbal et al. 2004). In the present study, the pH from the Nagle Dam was slightly alkaline and was within the water pH guideline stipulated by DWAF (1996) for aquatic ecosystems. Slight alkaline pH in the Nagle Dam may possibly attributed to agricultural run-off and wastewater discharge. The Inanda Dam recorded alkaline pH and was within the water pH guideline stipulated by DWAF (1996) for aquatic ecosystems. The alkaline pH in the Inanda Dam can possibly be due to agricultural run-off in around Inanda area, waste discharge from municipality treatment plants and industrial effluent deposited by the uMsunduzi River and other polluted tributaries. The alkaline status of river waters may be partly attributed to the disposal of industrial and domestic wastewater and higher photosynthetic activities by the macrophytes (Singh et al. 2017). The neutral and alkaline pH often show mesotrophic and eutrophic state of natural waters (Dirican 2015). The alkaline pH in the uMgeni River is often associated with microbial processes (Singh and Lin 2015). Ahipathy and Puttaiah (2006) highlighted that slight alkaline pH is preferable in waters because it removes metals as carbonate or bicarbonate precipitates.

3.4.1.2. Temperature and dissolved oxygen

Temperature is an important parameter that determine the overall health of an ecosystem. It affects the growth, metabolism, survival, and behaviour of the aquatic organisms. However, different aquatic species including fish have different tolerance levels to water temperature (Caissie 2006; Zhu et al. 2019). Hydropower plants, industrial thermal pollution, and geomorphology of the basin are among other factors that affect the water temperature (Jurgelėnaitė et al. 2018). Temperature has an impact on the DO in a water body (Gaber et al. 2013). In the present study, water temperature showed no significant difference between the two dams. Although DWAF (1996) has not stipulated the water temperature guideline, fish mortalities can occur when the water temperature exceed desired thermal tolerance levels of the fish species (Zhu et al. 2019). Prokešová et al. (2015) highlighted that *C. gariepinus* early life thermal tolerance range was between 18.9- 33.7℃, beyond which can be lethal. So, the temperature range of both the Nagle and Inanda dams is within the desired water temperature tolerance for *C. gariepinus*.

The DO are an important water parameter in aquatic ecosystems, essential for the functioning of aquatic fauna and flora. Although affected by temperature, DO can fluctuate due to hydrological changes and biological processes such as respiration, photosynthesis, and decomposition of organic matter (Rajwa-Kuligiewicz et al. 2015). Moreover, decreased DO can be associated with huge organic loadings into rivers and dams. In the present study, DO levels in the Inanda Dam were high as compared to that of the Nagle Dam and had exceeded the DO water guideline stipulated by DWAF (1996) for aquatic ecosystems. Post et al. (2018) suggested that water temperature is a primary regulating parameter that determines how much DO water can hold. Cox (2003) and Dikole (2014) further suggested that DO is inversely dependent on temperature, increasing water temperature reduces DO in the water, vice versa. The increased DO observed at the Inanda Dam may possibly be attributed to the decreased temperature.

3.4.1.3. Electrical conductivity

Electrical conductivity (EC) is an indirect indicator of pollution, it presents a close relationship with dissolved salts and salinity in natural waters (de Sousa et al. 2014). The EC is generally affected by the geology of the area in which the rivers and stream flow (Bhateria and Jain 2016). In the present study, there was a significant difference in EC in between the two dams, the Inanda Dam having a significantly higher EC than the Nagle Dam. However, DWAF (1996) have not stipulated a guideline for EC for aquatic environments. Possible drivers of high EC in the Inanda dam may possibly be attributed to domestic waste from the informal settlements and sewage effluent from wastewater treatment plants. High EC is often associated with domestic waste and untreated sewage due to an increase in chlorine ion concentrations and the enrichment of electrolytes (Prabu et al. 2011).

3.4.2. Nutrients

3.4.2.1. Nitrogen

Nitrogen (N_2) is the most abundant chemical element in the Earth’s atmosphere and most important element in biomolecules essential for most life forms (Padhye 2017). Inorganic nitrogen is present in three major forms (ammonium, nitrate, and nitrite), which can be assimilated by microalgae into amino acids and other biomolecules (Wang et al. 2021). The

NH_3, NO_2 and NO_3 forms can be bioavailable through N_2 fixing bacteria and biodegradation of organic matter. Ammonia is one of the widespread toxicants in most developing nations as a result of both natural and anthropogenic sources (Alonso and Camargo 2011). South Africa has the largest market for pesticides in the Sub-Saharan Africa with approximately 60% of pesticides sold in most African countries (Adeyinka et al. 2019). In the present study, NH_3 concentrations showed no significant difference in between the two dams and were within the TWQR guideline for both dams. Ammonia may be removed from water by macrophytes, algae and bacteria which assimilate it as a source of N_2 (Camargo and Alonso 2006). Moreover, concentrations of NH_3 are generally dependent on pH and water temperature (Camargo and Alonso 2006). Moreover, elevated pH tends to convert less toxic compound ammonium (NH_4^+) into highly toxic unionized NH_3 (Rangeti and Dzwairo 2021). Olaniran et al. (2014) also recorded low concentrations of NH_3 and not exceeding the water guideline at the uMgeni River.

Nitrite (NO_2) is one of the most widespread compounds of inorganic N_2 and an important nutrient in the aquatic environment. Elevated concentrations of NO_2 can have deleterious effects in aquatic environments (Olaniran et al. 2012). It can be produced through the nitrification process or dissimilatory nitrate reduction to NH_4^+ in water and sediments (Raimonet et al. 2015). Moreover, it can also be produced during the biodegradation of industrial or domestic wastes and fertilizers (Aydın et al. 2005). In the present study, the Inanda Dam had considerable NO_2 concentrations and DWAF (1996) has no stipulated guideline for NO_2 for aquatic environments. The NO_2 was below the detectable limit at the Nagle Dam. Possible drivers of NO_2 in the Inanda Dam include effluent from municipality treatment plants and intensive agricultural activities. Singh and Lin (2015) recorded considerable NO_2 concentrations in the uMgeni River ranging from 1.54 to 1.58 mg NO_2/L during the winter and summer surveys.

Nitrate (NO_3) is another form of inorganic N_2, essential nutrient for living organisms and forms building blocks for molecules (Wang et al. 2016). It originates from soil organic N_2, fertilizers, manure, and unregulated sewage waste. Under aerobic conditions, NH_3 can be oxidized to NO_3 and then to NO_2 by a bacteria (Cox 2003). In excessive concentrations, it has potential to disrupt endocrine function in aquatic organisms (Raimonet et al. 2015). In the present study, NO_3 concentrations showed no significant difference in between the two dams. DWAF (1996) has no stipulated guideline for NO_2 for aquatic environments. Jaji et al. (2007) suggested that NO_3 is seldom present in waters because it is generally assimilated by aquatic plants. Olaniran et al.

(2014) recorded considerable NO_3 concentrations in the uMgeni River ranging from 1.56 to 1.67 mg NO_3/L during both summer and winter surveys.

The Inanda Dam had a significantly higher total inorganic N_2 as compared to the Nagle Dam, however, both dams exhibited mesotrophic conditions for total inorganic N_2. The Inanda Dam is located on the lower reaches of the uMgeni River, which is mostly affected by industrial waste, wastewater from municipality plants and fertilizer run-off. The Nagle Dam also exhibited mesotrophic conditions for total inorganic N_2 (DWAF 1996). Mesotrophic conditions in the Nagle Dam may be attributed to the fertilizer run-off from the commercial and subsistence farming and livestock farming occurring in the vicinity of the river. Agunbiade and Moodley (2014) suggested that agricultural run-off and municipality wastewater are the major drivers of pollution in the uMgeni River.

3.4.2.2. Phosphate

Phosphate (PO_4) occurs naturally in streams due to weathering of phosphate containing rocks. However, human influences have altered its natural cycle due to increased pollution. The global evolution of industries and agricultural activities have significantly contributed to the increased production of PO_4 wastes (Hashim et al. 2019). Excess concentrations of PO_4, influenced by pH can induce the immobilization of heavy metals by converting unstable ions into stable ones (Han et al. 2020). In freshwater ecosystems, phosphorus has often been identified as a limiting nutrient for algal growth (Camargo and Alonso 2006). In the present study, PO_4 concentrations recorded at the Inanda Dam were significantly higher than that of the Nagle Dam. Although DWAF (1996) have not stipulated a water guideline for PO_4 but the Inanda Dam exhibited eutrophic conditions and the Nagle Dam mesotrophic conditions. Increased PO_4 concentrations are often linked with run-off from agricultural activities along catchments (Kleinman et al. 2011). Olaniran et al. (2014) and Singh and Lin (2015) recorded very high PO_4 concentrations in the uMgeni River, which were deemed unsafe for aquatic life. Eutrophic conditions recorded in the Inanda Dam may possibly be attributed to the wastewater discharge from municipality treatment plants. Rangeti and Dzwairo (2021) highlighted that the DWWTP contributes 50% soluble phosphate and 15% total phosphate into the Inanda Dam.

3.4.2.3. Sulphate

Sulphate (SO_4) is an anion of sulfuric acid, it is released by aquatic, terrestrial and geological processes. It is one of the most abundant and important chemical compounds in freshwater ecosystems (Jedrysek 2005). Sulphates are mixed in rivers, partly absorbed by plants and reduced by anaerobic bacteria (Vokal-Nemec et al. 2006). Increasing SO_4 concentrations in water is generally influenced by biogeochemical processes of carbon, nitrogen, and phosphorus. Major sources of SO_4 include waste from textile companies, solid waste from processing plants, acid mine drainage and fertilizer run-off from agricultural activities (Zak et al. 2020). In the present study, the Inanda Dam had a considerably higher sulphate concentrations as compared to Nagle Dam. Possible drivers of SO_4 in the Nagle Dam include run-off from the small-scale land cultivation and livestock farming. Although DWAF (1996) have not stipulated a water guideline for SO_4, high concentrations of SO_4 recorded at the Inanda Dam remain a cause for concern. Increased SO_4 concentrations may possibly be linked to the discharge of wastewater from municipality treatment plants and agriculture run off into the Inanda Dam. Zak et al. (2020) suggested that SO_4 concentrations in rivers and dams generally range from 0 to 630 mg SO_4/L. Olaniran et al. (2014) recorded considerably higher SO_4 concentrations in the uMgeni River ranging from 549.81 to 567.86 mg SO_4/L during both winter and summer surveys. Olaniran et al. (2014) suggested that acid-sulphate soils around the river can discharge high concentrations of SO_4^{2-} complexes under the influence of electrical conductivity.

3.4.3. Metals

3.4.3.1. Aluminium

Aluminium (Al) is the 3rd most abundant metal constituting an average 8% of minerals but has no known biological function (Gensemer and Playle 1999). It is very versatile due to its characteristics such as lightweight, electroconductivity and long-life. Aluminium is a strongly hydrolyzing metal and insoluble in the neutral pH. It is strongly mobilized as a result of acid mine drainage (AMD) and acid precipitation (Bartoli et al. 2012). In the present study, Al showed no significant difference between the two dams in both seasons. The Al solubility and toxicity is highly dependent on water pH (Senze et al. 2015). The solubility of Al is considered to increase with decreasing pH (Neal et al. 2011). Temperature and salinity are among other factors affecting Al solubility in aquatic ecosystems. In alkaline pH, Al can easily be absorbed by bottom sediments in the form of metastable compounds and can further be released should the pH drop, as it is inversely correlated with pH (Crafford and Avenant-Oldewage 2010; Senze et al. 2015). In the present study, a significant concentration was observed in sediment at both dams. However, the Inanda Dam recorded considerably higher Al concentrations in sediments as compared to the Nagle Dam in both seasons. The Inanda Dam receives waste from different tributaries, depositing waste from wastewater treatment plants, industries, and waste from the unregulated sand mining activities (Padrilah et al. 2018). These results are comparable with those observed by Cochiorca et al. (2018) from the Salnic and Trotus rivers draining a catchment characterized by mining activities in Romania. Moreover, Poshtegal and Mirbagheri (2019) reported similar concentrations in the Zarrineh River generally affected by illegal and legal mining activities.

3.4.3.2. Copper

Copper (Cu) is an essential element found in small quantities for metabolic processes in living organisms and can be easily assimilated by organisms. It is also essential to the function of at least 30 different enzymes and 20 major proteins. When present in high quantities, it can be detrimental when consumed (Padrilah et al. 2018). In the present study, Cu concentrations in water in both dams were the below detectable limit. However, notable concentrations were in sediments at both dams. Copper solubility is highly dependent on pH, it is soluble in acidic conditions and precipitate as copper hydroxides in alkaline conditions (DWAF 1996). Industrial effluent, waste from municipality treatment works and agriculture run-off are possible drivers of Cu in the Inanda Dam, fertilizer run-off and animal waste in the Nagle Dam. Dikole (2014) highlighted that uMgeni business park has steel industries that contribute significant amounts of Cu in the uMgeni River. Copper toxicity in aquatic organisms is often associated with changes in pH, temperature, and water hardness (Bartoli et al. 2012). However, it's valency state can shift between Cu^{2+} to Cu^{+1} through the Fenton reaction and facilitate the production of reactive oxygen species (ROS) (Wuana and Okieimen 2011). These results are comparable with those observed by Dikole (2014) recording significant Cu concentrations in the sediments in the Inanda Dam ranging from 107.7 to 117.7 mg/kg.

3.4.3.3. Iron

Iron (Fe) is the second most abundant metal and 4th in the Earth's crust. It is an essential element in living organisms and can be found in two reduced forms which are the ferrous (Fe^{2+}) and oxidized (Fe^{3+}) states. Naturally, Fe is released during the rock weathering process controlled by a variety of physico-chemical properties such as precipitation, temperature, soil composition and hydrology (Xing and Liu 2011). In the present study, Fe concentrations showed no significant difference in between the two dams. Iron is generally present in surface waters as salts (Fe^{3+}) when the pH is above 7 (Hussain et al. 2017). It is generally insoluble in water and found in low concentrations in freshwater environments. The presence of freshwater algae can affect the levels of Fe in surface water (DWAF 1996). Kritzberg et al. (2014) suggested that Fe interactions with organic matter maintains Fe suspended in water. The Inanda Dam recorded considerably higher concentrations of Fe in the sediment as compared to the Nagle Dam. Possible drivers of Fe in the Inanda Dam include waste from municipality treatment plants, landfills, and nutrient run-off. Similar results were recorded by Moodley et al. (2014) at the Palmiet River (one of the tributaries of the uMgeni River in winter and summer surveys. Van Ha et al. (2011) suggested that low pH and anaerobic conditions can significantly influence the amount of Fe released from the sediments.

3.4.3.4. Manganese

Manganese (Mn) is a naturally occurring metal found in the Earth's crust and considered as an essential element in living organisms. It is not as toxic as the other metals but can be very detrimental at high concentrations and can cause a wide variety of deleterious effects on living organisms (Genc et al. 2009). Manganese has been used as an antiknock gasoline additive in most countries. Major sources of Mn include mining activities, agricultural and industrial waste. It is mainly used in fireworks, ceramics, paint, cosmetics, and organic pesticides (Ren et al. 2015). In the present study, Mn concentrations in water showed no significant difference in between the two dams and was within the Mn TWQR guideline for aquatic environments. pH and redox reactions are some of the factors affecting the concentrations of Mn in the water column (Hou et al. 2020). Dikole (2014) suggested that pH between 5 and 8 can result in low concentrations of Mn. Wastewater from municipality treatment plants and industrial waste are major potential drivers of Mn in the Nagle and Inanda dams. Hasan et al. (2011) suggested that municipality wastewater is the biggest contributor of Mn particularly in industrialized and urbanized areas. Moreover, the Inanda Dam recorded considerably higher concentrations of Mn as compared to the Nagle Dam in both seasons. Li et al. (2014) also recorded high concentrations of Mn (>460 mg/kg) in the sediments of Yellow River located in the vicinity of a local mine. Moreover, Varbanov et al. (2021) recorded high concentrations of Mn in the Iskar River located in the vicinity of treatment works and further receive waste from its three polluted tributaries.

3.4.3.5. Zinc

Zinc (Zn) is an essential metal playing an important role in biological systems in animals and humans. It is required for development, growth, tissue repair and can activate many enzymes which are involved in protein synthesis and metabolic activities (Andrade et al. 2015). However, it can be detrimental when present in large quantities (Gu and Lin 2010; Li et al. 2016). In the present study, Zn concentrations was below the detectable limit at both dams in both seasons. The Inanda Dam had considerably higher Zn concentrations in sediments as compared to the Nagle Dam. The pH strongly influence the speciation and solubility of Zn (Kritzberg et al. 2014). The possible drivers of Zn in the Inanda Dam include run-off from local farming, sewage effluent and industrial effluent. Moreover, domestic waste from the informal settlements and run off from small scale farming activities are possible drivers of Zn in the Nagle Dam. Hüffmeyer et al. (2009) suggested that the use of Zn in cosmetic products contributes significant amounts of Zn in domestic waste. In alkaline conditions, Zn precipitate and get suspended into the sediments and occur as a carbonate, sulphide or hydroxide as it stabilizes in the sediments (Callender and Rice 2000). Similar results were observed by Dikole (2014) recording considerably high Zn concentrations in the sediments in the Inanda Dam range between 449.9 to 485 mg/kg. Iloms et al. (2020) recorded high concentrations of Zn in the Vaal River generally affected by wastewater treatment works.

CHAPTER 4

Histopathology-based health assessment of *Clarias gariepinus* from the Nagle and Inanda dams

4.1. INTRODUCTION

Aquatic environments are the most fragile ecosystems with high susceptibility to pollution (Farombi et al. 2007; Carpenter et al. 2011). Fish are one of the most widely distributed organisms in aquatic environments and their health may reflect the overall health of an ecosystem (Zeitoun and Mehana 2014). Fish may provide an integrative insight into the status of their environment over longer periods of time (Plessl et al. 2017). Pollutants found in the aquatic environment are taken up by aquatic organisms either directly through water uptake, gills or indirectly through their diet (Obasohan et al. 2010). In this regard, fish species are accurate indicators of overall environmental conditions and can provide a general health status of an aquatic ecosystem (Crafford and Avenant-Oldewage 2009). However, comprehensive knowledge of taxonomy, physiology and habitat requirements of fish are important pre-requisites for using fish as biological indicators (Schwaiger et al. 1997). The toxic effects of contaminants on fish are multidirectional and can be manifested by numerous changes in the physiological and chemical processes of their body systems (Martinez et al. 2004).

A variety of approaches have been used to evaluate the effects of contaminants on the health of fish populations. Some of the classical approaches include the use of fish zone patterns to determine the fish spatial changes along the course of the river (Chovanec et al. 2003). However, the histopathology-based assessment has proven to be an ideal approach for both short- and long-term toxic effects (Velkova-Jordanoska and Kostoski 2005). One of the greatest advantages of histopathology assessment is the examination of specific target organs such as a kidney, liver and gills because of their role in vital functions of the fish body (Martinez et al. 2004). Histopathology is routinely used for stage development identification, sex verification, documentation of the presence of intersex, tumours, parasites, abnormalities and quantifying atresia (Annabi et al. 2013). However, fish migrations may reduce the effectiveness of the bio-indication approach. They make it difficult to discuss other anthropogenic degradations of aquatic ecosystems and identify the exact source of pollution (Chovanec et al. 2003).

The liver, gills, and gonads are regarded as target organs due to their important functions in fish (Kostić et al. 2017). The histopathology of these target organs have been regarded as the most reliable indication of health impairment and have been successfully used in bio-monitoring programmes (Yancheva et al. 2016). Gills are the first organs to be affected due to their constant contact with water environment, sensitivity to chemicals, physical alterations and their ability to serve as the main route for contaminants entering the fish body (Andrade-Porto et al. 2018). Liver is a primary site for biotransformation and detoxification, hence, one of the main target organs (Agunbiade and Moodley 2014). Gonads are responsible for fish reproduction and are target organs for pollutants thus impairing the reproductive capacity (Chovanec et al. 2003). The liver, gills, and gonads are ultimately regarded as the most important organs in environmental biomonitoring and histopathologic assessment studies (Yancheva et al. 2016; Bengu et al. 2017; Modley et al. 2019).

The histopathology-based assessment has proven to provide insight and early warning signal of environmental risk and it can be used in acute, chronic and *in situ* studies (Van der Oost et al. 2003). It identifies morphological alterations, combines antagonistic and synergistic effects of pollution, shows effects on target organs manifested over short and long term (Van der Oost et al. 2003). However, limitations associated with the histopathological approach include that the approach does not show a clear cause-effect relationship for *in situ* studies and requires expertise to avoid wrong interpretation of results (Ruiz-Picos et al. 2015).

The uMgeni River passes through agricultural and industrial activities (mostly the lower reaches of the river due to proximity to metropolitan areas), urban and rural areas (Gakuba et al. 2015). As a result, there has been deterioration of water quality which has even attracted media attention due to episodic fish mortalities. Therefore, the health of inhabitant fish in this river system remains a cause for concern. This chapter aims to employ various fish health indices and histopathology-based assessment to evaluate the health status of *C. gariepinus* from the Inanda and Nagle dams. Due to the record of pollution level (Agunbiade and Moodley 2014; Banda and Kumarasamy 2020) in the lower uMgeni River, it was hypothesised that the health of *C. gariepinus* population from the Inanda Dam will be poorer compared to populations from the Nagle Dam.

4.2. MATERIALS AND METHODS

4.2.1. Fish sampling

Fish was collected from both Nagle (n=18) and Inanda (n=28) dams during winter and summer seasons using both an electro-shocker and gill nets (25m long and 2m deep). The collected fish were examined for ecto-parasites prior to processing, they were placed in aerated tanks to maintain the oxygen content and transported to the field lab for further processing and analyses. Ethical clearance was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/019/018).

Figure 4.1. Fish sampling using gill nets

4.2.2. Fish processing

The fish processing was done in the field at the Inanda and Nagle dams. Sampled fish were euthanized by severing the spinal cord at the back of the head. After fish processing and macroscopic health assessment, a piece of liver, middle gill arch, and gonads (ovaries and testis, separately) were fixed in 10% neutral buffered formalin (NBF) and preserved prior to the histopathology assessments. Thereafter, the fish was weighed, measured the total length (the length from the head to the end of the caudal fin) (TL) and standard length (the length from the head to before the caudal fin) (SL). The fish weight (g) and total length (mm) were used to calculate the Fulton's Condition Factor (K) using the following formula:

$$CF=\frac{W\ (g)*100}{L(mm)^3}\text{..Equation 6.1}$$

Thereafter, the fish length and weight relationships were measured using the following formula:

$$W = aL^b\text{..Equation 6.2}$$

$$\text{Log } W = \log a + b * \log L\text{..Equation 6.3}$$

Weight (g), Total length (mm), a (intercept), b (b< 3 negative allometric; b=0 isometric; b> 3 positive allometric) (Ak et al. 2009).

Thereafter, all fish samples were dissected to harvest the following organs: liver, spleen, gonads (females and males separately), gills and the brain tissue. The liver and gonads were weighed for the hepatosomatic and gonadosomatic indices, respectively calculations using the following formula:

a) Organosomatic index= $\frac{\text{organ weight (g)}}{\text{fish weight (g)}} * 100$

4.2.3. Macroscopic assessment

The level of severity on both external and internal organs was assessed following the protocol by Adams et al. (1993). Organs were assigned numerical values according to the severity of abnormalities with 0 being normal and 30 being abnormal. Examined organs include but not limited to liver, spleen, gonads, kidney, opercula, gills, skin, eyes, fins, and mouth.

4.2.4. Tissue processing

The samples were dehydrated through a series of ethanol 50% (30 mins), 70% (45 mins), 80% (20 mins), 96% (20 mins), 99% (45 mins) and 100% (45 mins) and were cleared through a series of xylene (45 mins; 45 mins). Samples were infiltrated through the increasing concentrations of Tissue-Tek® III wax at 60ºC for 30 mins, 30 mins and 45 mins (Bancroft and Gamble 2008). Thereafter, samples were embedded in Tissue-Tek® III wax blocks. Each block was sectioned at 4-5µm using rotary microtome, mounted on glass microscope slide and air dried at 60ºC. Haematoxylin and eosin staining was performed on dried samples following the Bancroft and Gamble (2008) protocol. Thereafter, the stained sections were mounted with cover slips using Entellan®.

4.2.5. Histopathologic assessment

The histopathological assessment was done using the compound microscope to identify alterations in the target organs. The organ alterations were assessed following the methods set out by Bernet et al. (1999) which was later modified by Agamy (2012). Histopathological alterations were categorized into four reaction patterns which are i) circulatory disturbances, ii) regressive, iii) progressive and iv) inflammation. Each reaction pattern shows different histological characteristics and are very specific in the areas in which they affect. Thereafter, each alteration was identified and given an important score (how the lesion affects the organ function and the fish's ability to survive) (1-6). The score values were assigned based on the extent of the lesion (1-3). Furthermore, the important scores and score values determined during the assessment were used in calculating the organ index (I_{org}). The Iorg values were used to categorize the severity of the alteration following the classification system developed by Zimmerli et al. (2007):

a) Histological index <10; normal tissue; no pathological changes.

b) Histological index 11–20; slight modifications are present.

c) Histological index 21–30; moderate modifications.

d) Histological index 31–40; pronounced modifications.

e) Histological index >40; severe alterations.

The scoring values will also be used to categorize the reaction patterns.

4.2.6. Statistical analysis

R-commander software was used for data analysis. Normality assumption was tested using the Shapiro-Wilk test and equality of variances was tested using the Levene's test. Based on the results from assumption tests, independent sample t-test or Mann- Whitney test was used to test the differences in condition factor, hepatosomatic index, gonad-somatic index, organ index, and health assessment index for *Clarias gariepinus* between the Nagle and Inanda dams. The Simple Linear Regression was used to test the fish length-weight relationships between *C. gariepinus* from the Nagle and Inanda dams to determine the population growth. Statistical significance was set at $p < 0.05$.

4.3. RESULTS

4.3.1. Fish health indices

4.3.1.1. Condition Factor (CF)

The condition factor (CF) of fish from the Nagle and Inanda dams are presented in Figure 4.2. Fish from the Nagle Dam had a mean CF of 1.39 ± 0.24 and 1.19 ± 0.46 during the winter and summer seasons, respectively. Fish from the Inanda Dam had a mean CF of 2.07 ± 0.78 and 1.98 ± 0.94 during the winter and summer seasons, respectively. The CF of fish from the Nagle Dam ranged from 0.76 to 2.32 and from the Inanda Dam ranged from 0.68 to 3.78 (Figure 4.2). There was a significant difference in CF of fish between the Nagle and Inanda dams ($p<0.05$) (Figure 4.2). The CF of female populations from the Nagle Dam ranged from 0.76 to 1.65 and from the Inanda Dam ranged from 0.97 to 2.91 (Figure 4.2). The CF of male populations from the Nagle Dam ranged from 0.84 to 1.59 and from the Inanda Dam ranged from 0.68 to 3.87 (Figure 4.2). There was no significant difference in CF of male and female populations from the Nagle and Inanda dams ($p>0.05$). There was no seasonal variation in CF of fish within each dam ($p> 0.05$) (Figure 4.2).

4.3.1.2. Fish weight-length relationships

Fish weight-length relationships of fish from the Nagle and Inanda dams are presented in Table 4.1. All fish from the Inanda Dam exhibited a negative allometric pattern ($b<3$) (Table 4.1). Combined males and females and female populations from the Nagle Dam exhibited negative allometric pattern ($b<3$). Male populations from the Nagle Dam showed a positive allometric growth pattern ($b>3$) (Table 4.1). The weight-length relationships of fish from the Nagle Dam were as follows; females ($W=0.066L^{2.568}$), males ($W=0.00967L^{3.03}$) and for combined female and males ($W=0.0000681L^{2.718}$) (Table 4.1). The weight-length relationships of fish from the Inanda Dam were as follows; females ($W=0.021L^{2.926}$), males ($W=0.274L^{2.262}$) and for combined female and males ($W=0.000645L^{2.389}$) (Table 4.1).

4.3.1.3. Hepatosomatic Index (HSI)

The hepatosomatic index (HSI) of fish from the Nagle and Inanda dams are presented in Figure 4.2. The HSI of fish from the Nagle and Inanda dams ranged from 0.05 to 2.75% and 0.04 to 1.95%, respectively (Figure 4.2). Fish from the Nagle and Inanda dams had a mean HSI of 1.13 ± 0.60% and 0.88 ± 0.58%, respectively. There was no significant difference in HSI of fish between the Nagle and Inanda dams ($p>0.05$). There was no seasonal variation within each dam ($p>0.05$) (Figure 4.2).

4.3.1.4. Gonadosomatic Index (GSI)

The gonadosomatic index (GSI) of females and males from the Nagle and Inanda dams are presented in Figure 4.3. Females from the Nagle and Inanda dams had a GSI ranging from 0.22 to 9.93% and 0.0002 to 14.34%, respectively (Figure 4.3). Females from the Nagle and Inanda dams had a mean GSI of 4.93 ± 3.48% and 2.98 ± 5.68%, respectively. There was a significant difference in GSI of females between the Nagle and Inanda dams ($p<0.05$). There was no seasonal variation in GSI of females within each dam ($p>0.05$) (Figure 4.3). Males from the Nagle and Inanda dams had a GSI ranging from 0.00077 to 0.71% and 0.00031 to 1.57%, respectively (Figure 4.3). Males from the Nagle and Inanda dams had a mean GSI of 0.38 ± 0.31% and 0.27 ± 0.39%, respectively. There was no significant difference in GSI of males between the Nagle and Inanda dams ($p>0.05$) (Figure 4.3). There was seasonal variation in GSI of males from the Inanda Dam ($p<0.05$) and there was no seasonal variation in GSI males from the Nagle Dam ($p>0.05$) (Figure 4.3).

4.3.1.5. Macroscopic assessment

The skin of fish from both dams and both winter and summer seasons showed mild to moderate abnormalities. The liver and gills of fish from the Inanda Dam showed severe abnormalities as compared to that of the Nagle Dam. The spleen of fish from both dams showed mild to moderate abnormalities and kidney of fish from the Nagle Dam showed no abnormalities whereas the one from the Inanda Dam showed mild abnormalities from both winter and summer seasons. The HAI of fish populations from the Nagle and Inanda dams is presented in Figure 4.4. There was no significant difference in HAI of fish between the Nagle and Inanda dams ($p>0.05$) (Figure 4.4). There was no seasonal variation in HAI of fish within each dam ($p>0.05$) (Figure 4.4).

Table 4.1. Length-weight relationships of Clarias gariepinus recorded at the Nagle and Inanda dams during winter and summer seasons in the 2020 and 2021 surveys.

Site	Sex	n	a	b	R^2	Growth pattern
Nagle Dam	Female	10	0.066	2.568	0.983	Negative allometric
	Male	8	0.00967	3.03	0.980	Positive allometric
	Combined	18	0.0000681	2.718	0.972	Negative allometric
Inanda Dam	Female	6	0.021	2.926	0.895	Negative allometric
	Male	22	0.874	2.262	0.867	Negative allometric
	Combined	28	0.932	1.242	0.843	Negative allometric

N= sample size; a= intercept, b= slope and R^2 (adjusted)= correlation coefficient

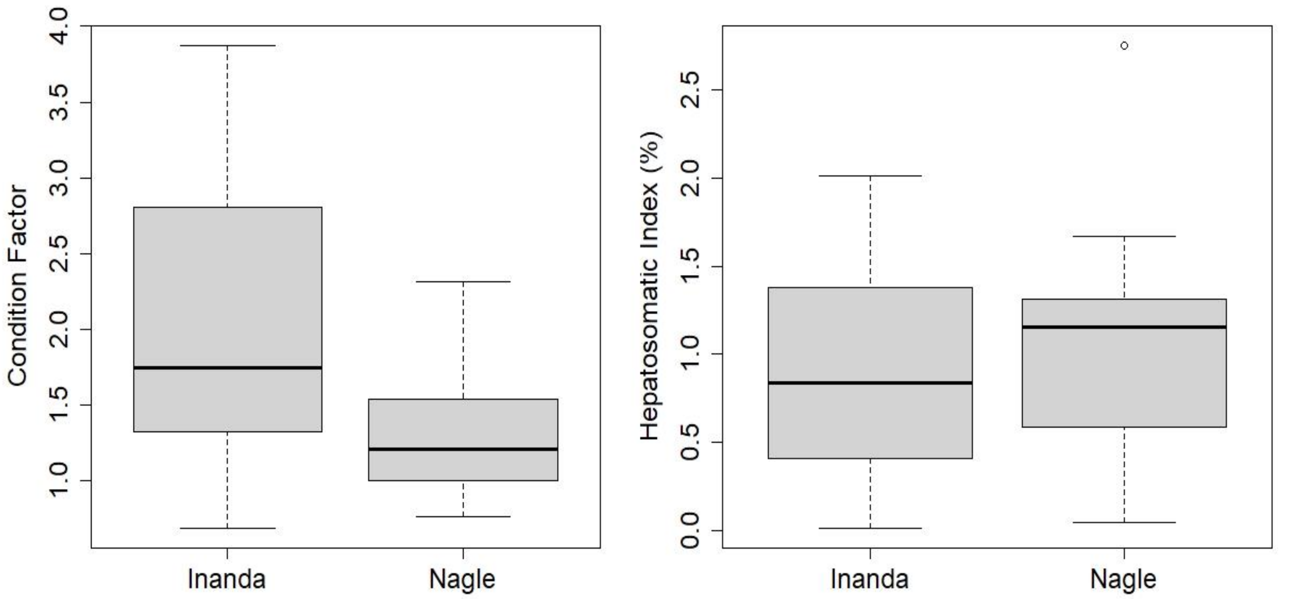

Figure 4.2. Box and whisker plots representing the range in condition factor and hepatosomatic index of Clarias gariepinus recorded at the Nagle and Inanda dams during summer and winter seasons in the 2020 and 2021 surveys. The box shows the 25th percentile , while the line inside the box is the median of each index.

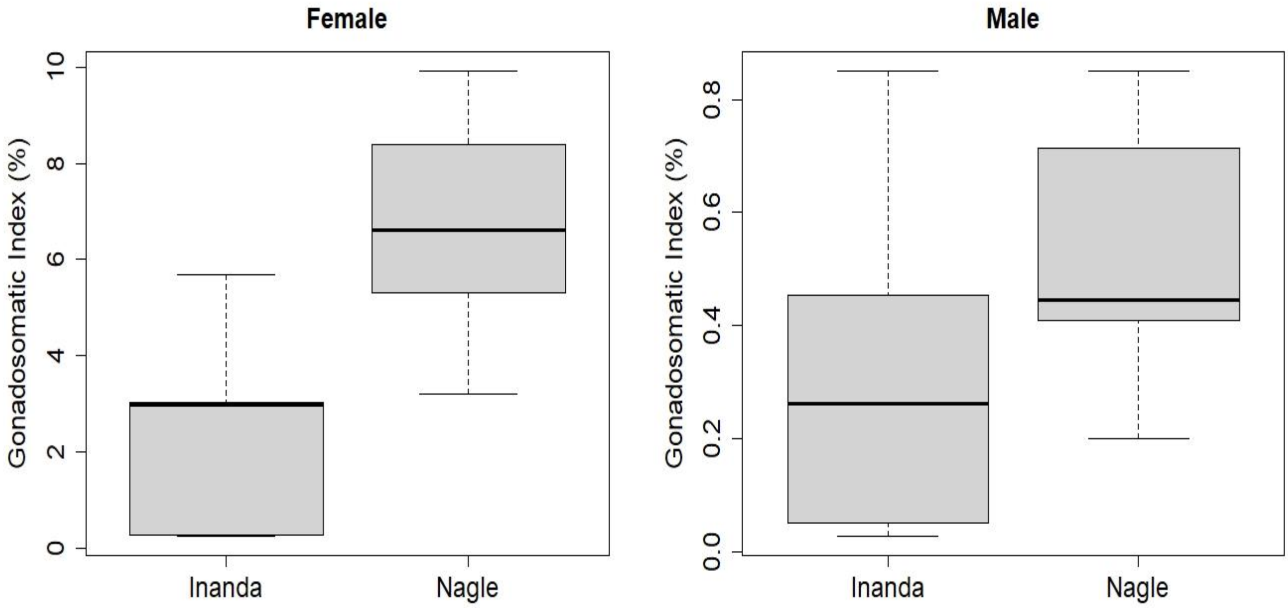

Figure 4.3. Box and whisker plots representing the range in gonadosomatic index of Clarias gariepinus recorded at the Nagle and Inanda dams during the winter and summer during 2020 and 2021 surveys. The box shows the 25th percentile and the 75th percentile, while the line inside the box is the median of each index.

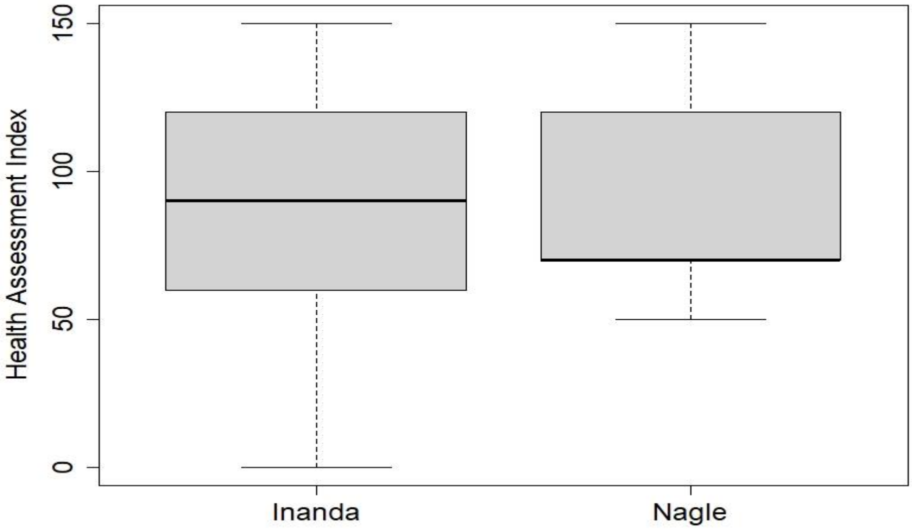

Figure 4.4. Box and whisker plot representing the range in health assessment index of Clarias gariepinus recorded at the Nagle and Inanda dams during the winter and summer seasons in the 2020 and 2021 surveys. The box shows the 25th percentile and the 75th percentile, while the line inside the box is the median of the index.

4.3.2. Histopathology assessments

4.3.2.1. Gills

Epithelial lifting, oedema, hyperplasia, and leukocyte infiltration were the most prevalent pathologies in the gills of fish from the Inanda Dam whereas lamella fusion, leukocyte infiltration and epithelial hyperplasia were common at the Nagle Dam (Table 4.2). Leukocyte infiltration was prevalent at both dams with 83% being recorded at the Nagle Dam and 77% at the Inanda Dam. Epithelial hyperplasia showed a prevalence of 83% at the Nagle Dam and 70% for the Inanda Dam population. The Inanda Dam population exhibited relatively higher prevalence for mucus cell hyperplasia compared to Nagle Dam population. A prevalence of 10% and 30% were observed for chloride cell hyperplasia for the Nagle and Inanda dams, respectively (Table 4.2). Nuclear degeneration, pillar cell rupture and necrosis were among the regressive changes reported in fish from both dams, with higher prevalence being observed for the Nagle Dam population (Table 4.2). Other regressive alterations which were common at both dams included fusion of lamella and epithelial lamella. The Inanda Dam population has shown a higher prevalence of alterations with higher degree of severity compared to the Nagle Dam. This was also supported by the gill index which was 16 ± 6.57 for the Nagle Dam population and 19.85 ± 5.97 for that from the Inanda Dam. The gills index was less than 20 for both the Nagle and Inanda dams' populations which classify the histological alterations as slight for both populations (Table 4.6). The degree of severity for gill alterations have shown a great variability within each population, however, there was no significant difference between the two populations ($p>0.05$).

Table 4.2. Percentage prevalence of histological alterations in Clarias gariepinus gills recorded at the Nagle and Inanda dams during the summer and winter seasons in the 2020 and 2021 surveys.

Pattern	Alterations	Nagle Dam	Inanda Dam
Circulatory	Haemorrhages	30	55
	Aneurysm	35	60
	Intracellular oedema	33	65
Regressive	Complete fusion of lamella	10	46
	Partial fusion of lamella	67	62
	Epithelial lifting	45	80
	Shortening of secondary lamella	17	8
	Rupture of pillar cells	50	70
	Nuclear degeneration	10	30
	Necrosis	10	25
Progressive	Epithelial hyperplasia	83	70
	Mucus cell hyperplasia	30	55
	Chloride cell hyperplasia	10	30
	Mucus cell hypertrophy	20	55
Inflammatory	Leukocyte infiltration	83	77

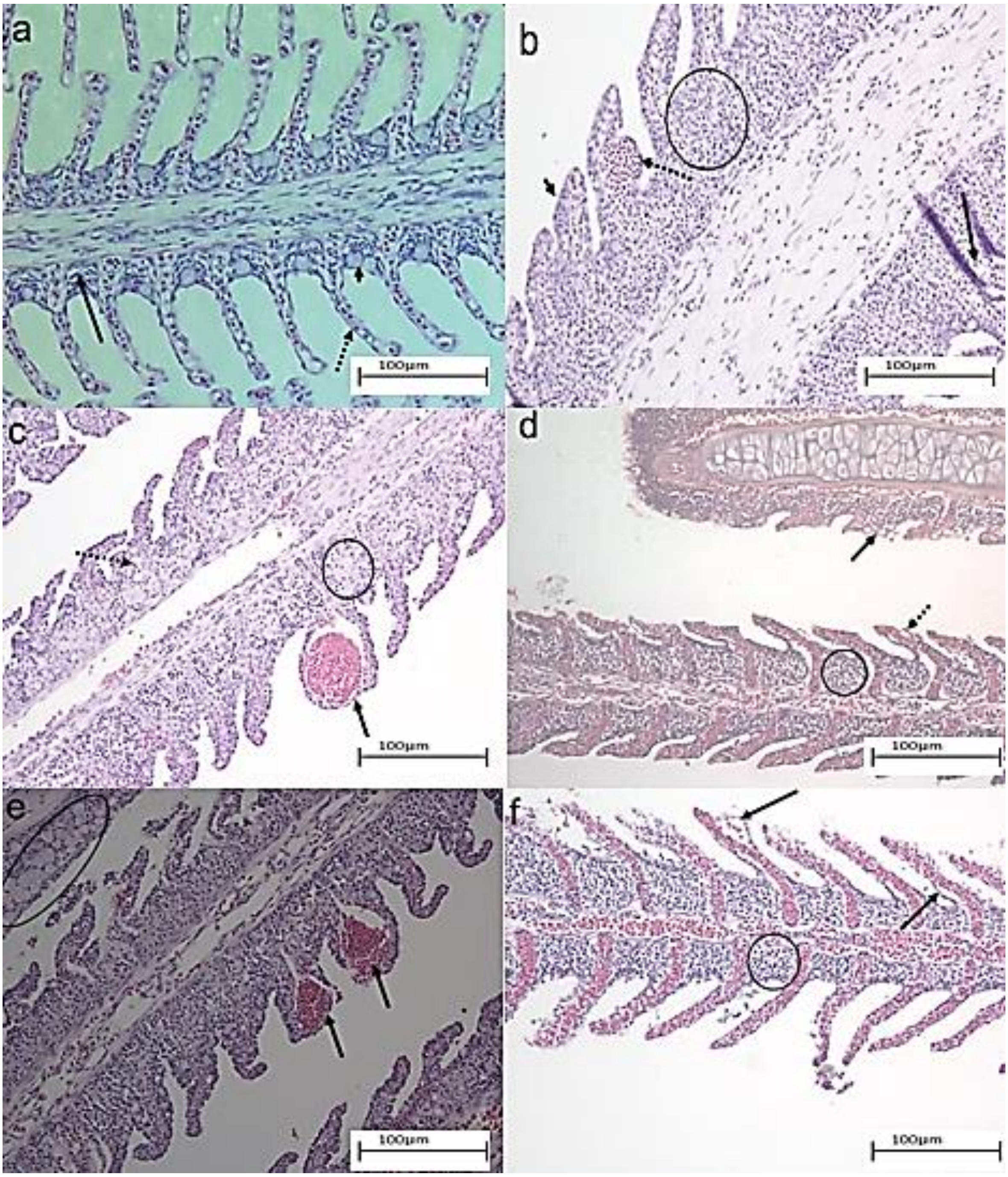

*Figure 4.5. **a.** Normal primary lamella (arrow), normal secondary lamella (dotted arrow), mucus cell (short arrow), **b.** Haemorrhage/aneurism (dotted arrow), epithelial hyperplasia (short arrow and encircled), mucus cells hyperplasia (arrow) **c.** Oedema (encircled), mucus cells hyperplasia (dotted arrow), aneurism resulting in pillar cell rupture and epithelial lifting (arrows), **d.** Chloride cell hyperplasia (arrow), oedema and epithelial lifting (dotted arrow), epithelial hyperplasia (encircled); **e.** Mucus cells hyperplasia (encircled), and aneurism, epithelial lifting and pillar cell rupture (arrows); **f**. Infiltration of leukocytes, epithelial lifting (arrow), hyperplasia (encircled). Haematoxylin and Eosin staining (H&E). Scale bar: 100 µm.*

4.3.2.2. Liver

Most pathologies have shown a higher prevalence for the Inanda Dam population compared to that from the Nagle Dam (Table 4.3) with sinusoid congestion and melanomacrophages centers (MMCs) showing relatively higher prevalence in both populations. Congestion of sinusoids were the most prevalent circulatory alterations at the Nagle and Inanda dams exhibiting prevalence of 80% and 90%, respectively. The MMCs were the most prevalent regressive alterations with 100% and 70 % prevalence at the Nagle and Inanda dams, respectively. Moreover, hepatocellular, and nuclear pleomorphism, and nuclear degeneration which has resulted in necrosis were prominent at the Inanda Dam with prevalence of ranging from 45 to 60% whereas prevalence ranging from 20 to 40% were observed at the Nagle Dam (Table 4.3). Similarly, hydropic degeneration was more prevalent for Inanda Dam population compared to that from the Nagle Dam (Table 4.3). Moreover, 60% prevalence of hypertrophied hepatocytes was evident in the Inanda Dam population whereas the Nagle Dam population exhibited a prevalence of 40% (Table 4.3). Some hepatocytes hyperplasia was observed for both populations. Leukocyte infiltrations prevalence was 56 % and 40% at the Nagle and Inanda dams, respectively (Table 4.3). Steatosis was only observed in the Inanda Dam population with a prevalence of 40% (Table 4.3). Observed liver pathologies have shown a considerable variation on the degree of alteration for both populations, however, there was no significant difference ($p>0.05$) on the liver index between the two populations. A liver index of 11.11 ± 3.76 and 9.6 ± 5.56 was observed for the Inanda Dam and Nagle Dam populations, respectively (Table 4.6).

Table 4.3. Percentage prevalence of histological alterations in the Clarias gariepinus liver recorded at the Nagle and Inanda dams during the summer and winter seasons in the 2020 and 2021 surveys.

Pattern	Alteration	Nagle Dam	Inanda Dam
Circulatory	Haemorrhages	40	70
	Congestion of sinusoids	88	90
Regressive	Melanomacrophages centres	100	70
	Fatty vacuolization	11	30
	Steatosis	0	40
	Nuclear degeneration	20	45
	Hepatocellular pleomorphism	40	60
	Hydrophic degeneration	30	60
	Necrosis	20	50
Progressive	Hepatocyte’s hyperplasia	34	44
	Hepatocyte hypertrophy	40	60
Inflammatory	Leukocyte infiltration	56	40

4.3.2.3. Testes and ovaries

Interstitial hyperplasia was more prevalent in a male population from the Inanda Dam compared to that from the Nagle Dam, recording 100% and 50%, respectively (Table 4.4). Moreover, 100% MMCs prevalence was observed in male populations from the Nagle Dam with 67% recorded for Inanda Dam population (Table 4.5). In contrast, leukocyte infiltration was more prevalent in the testes of population from the Inanda Dam. Most fish showed normal progression of ovary development for both populations. *Clarias gariepinus* ovaries from both dams showed 100% prevalence of perifocullar hyperplasia with 100% and 75 % prevalence of degeneration of perifocullar wall being observed at the Nagle and Inanda dams’ populations, respectively. Ovary oedema and decreased yolk formation showed relatively higher prevalence for the Nagle Dam than the in population from the Inanda Dam (Table 4.5). Although the populations showed signs of oocyte atresia, the prevalence was less than 50% at each dam and no substantial difference was observed between the two populations (Table 4.5).

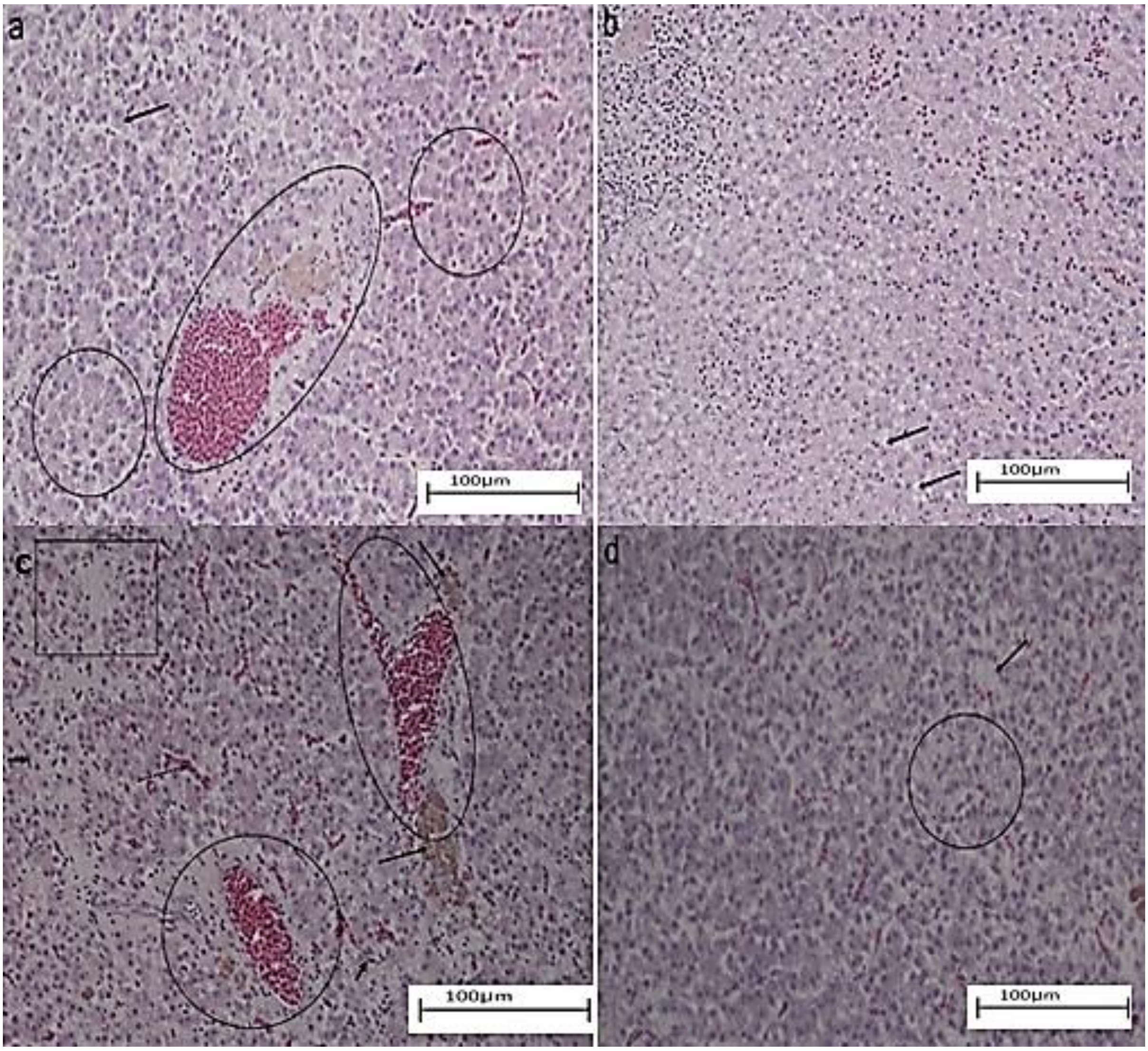

Figure 4.6. ***a.*** *Hypertrophied hepatocyte (arrow), hepatocellular pleomorphism accompanied by nuclear degeneration and necrosis (circle), hepatic portal triad (oval);* ***b.*** *Scattered steatosis (arrow), leukocyte infiltration;* ***c.*** *Central vein (oval), Melanomacrophages (arrow), congestion of the sinusoids (dotted arrow), hepatocellular and nuclear pleomorphism with necrosis (square), Kupffer cells (short arrows);* ***d.*** *Hydropic degeneration (arrow), hepatocellular pleomorphism and hypertrophied hepatocytes. Haematoxylin and Eosin staining (H&E). Scale bar: 100 µm.*

Table 4.4. Percentage prevalence of histological alterations in Clarias gariepinus testes recorded at the Nagle and Inanda dams during the summer and winter seasons in the 2020 and 2021 surveys.

Alterations	Nagle Dam	Inanda Dam
Pigmented macrophages	100	67
Interstitial cell hyperplasia	50	100
Leukocyte infiltration	40	55
Interstitial fibrosis	0	0

Table 4.5. Percentage prevalence of histological alterations in Clarias gariepinus ovaries recorded at the Nagle and Inanda dams during the summer and winter seasons in the 2020 and 2021 surveys.

Alterations	Nagle Dam	Inanda Dam
Pigmented macrophages	14	75
Perifollicular cell hyperplasia	100	100
Decreased yolk formation	57	50
Ovary oedema	57	50
Cortical alveoli	57	0
Degeneration of perifollicular wall	100	75
Oocyte’s atresia	29	25
Cystic perifollicular cells	14	0

Table 4.6. Gill and liver indices of Clarias gariepinus recorded at the Nagle and Inanda dams during the summer and winter seasons in the 2020 and 2021 surveys.

Organ index	Nagle Dam	Inanda Dam
Gill index	16 ± 6.57	19.85 ± 5.97
Liver index	11.11 ± 3.76	9.6 ± 5.56

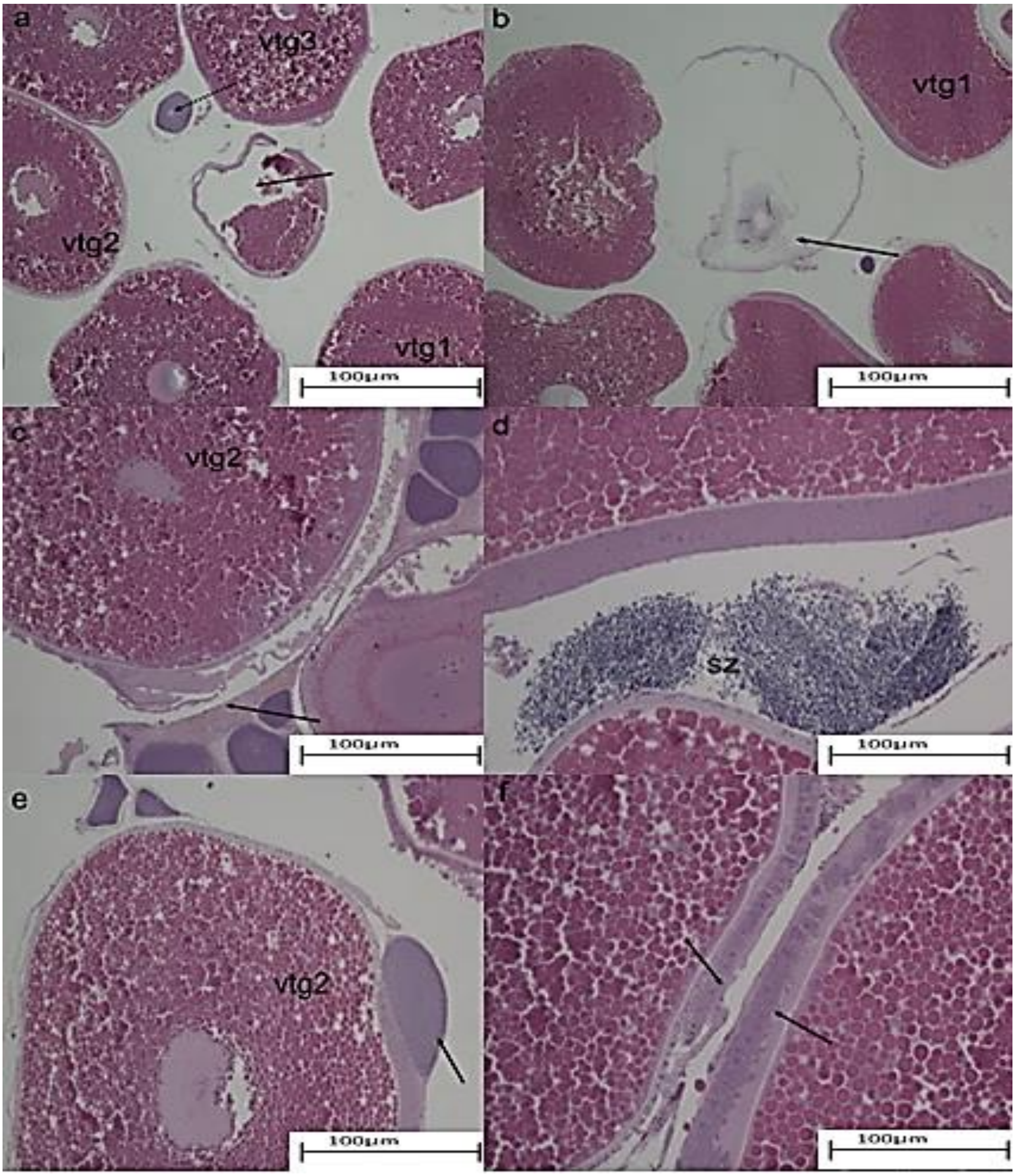

Figure 4.7. ***a.*** *Primary growth oocyte (dotted arrow), primary vitellogenic oocyte (vtg1), secondary vitellogenic oocyte (vtg2), tertiary vitellogenic oocyte (vtg3), oocyte atresia resulted from ovarian oedema (arrow);* ***b.*** *Atretic follicle (arrow);* ***c.*** *Melanomacrophages centres (arrows);* ***d.*** *The presence of spermatogenic cells in the ovaries;* ***e****. Ovarian wall cyst (arrows);* ***f.*** *Perifollicular hyperplasia (arrows). Haematoxylin and Eosin staining (H&E). Scale bar: 100 µm.*

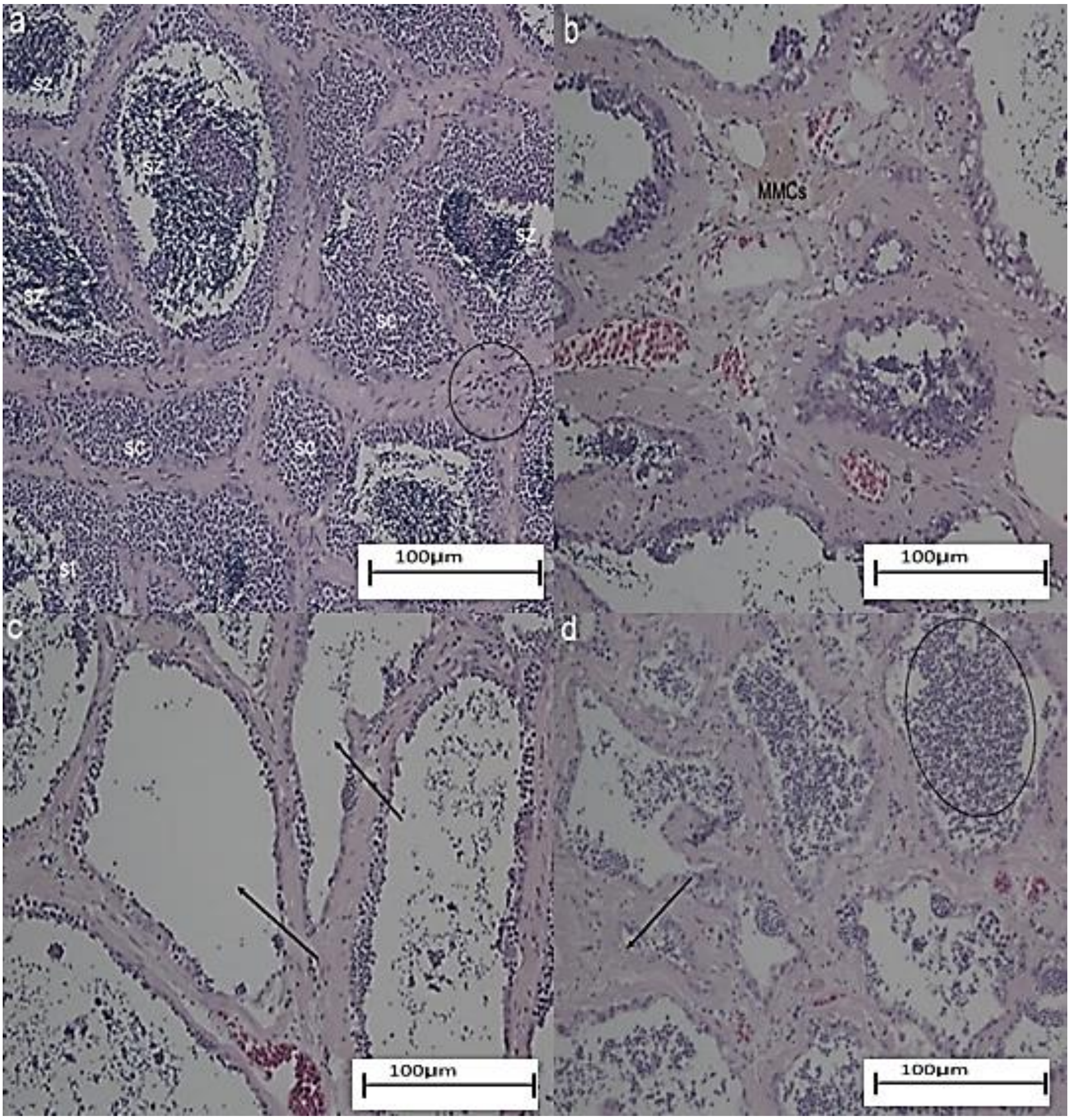

*Figure 4.8. **a.** Normal testis showing spermatocytes (sc), spermatozoa (sz) and spermatids (st), interstitial cell hyperplasia (encircled); **b**. Melanomacrophage centres and leukocyte infiltration; **c.** Spermatogenic atresia (arrows); **d.** Sertoli cell (arrow), spermatocytes and spermatozoa (encircled). Haematoxylin and Eosin staining (H&E). Scale bar: 100 µm.*

4.4. DISCUSSION

4.4.1. Fish health indices

4.4.1.1. Condition factor

The CF is often used to express the overall well-being and reflects the biotic and abiotic factors interactions with the physiological condition of fish populations (Lizama and Ambrosio 2002). The CF indicates the status of sexual maturity, food availability, age, sex, and the state of the environment (Mir et al. 2012). The CF range of 1 to 2 is regarded as ideal (El-Naggar et al. 2016). In the present study, fish from the Nagle Dam exhibited condition factor ranging from 0.76 to 1.65 and at the Inanda Dam ranging from 0.68 to 3.87 which was regarded as good. Factors that can influence conditions factor include food availability, water quality, nutrition and diseases (Jan and Jan 2017). Both the dams showed mesotrophic conditions which signifies sufficient food availability at both dams. Therefore, good condition of fish may be possibly attributed to mesotrophic conditions at both the Nagle and Inanda dams. The CF of *C. gariepinus* in the present study may also be explained by the fact that *C. gariepinus* is an omnivorous and opportunistic feeder, which feeds on a wide variety of food available (Tesfahun 2018). Anene (2005) recorded a CF ranging from 4.3 to 5.27 in four Cichlids species at a polluted man-made lake and Kumolu-Johnson and Ndimele (2011) recorded a condition factor of nine fish species ranging from 0.91 and 8.41 in polluted Ologe lagoon, Nigeria. Verma and Prakash (2019) recorded a CF ranging from 1.26 to 1.73 for *Mystus vittanus* exposed to heavy metal pollution. Although the CF is sometimes affected by factors such as sex, seasons and nutrition, pollution in the uMgeni River remains an environmental problem. The level and effects of pollution in the uMgeni River highlighted by Banda and Kumarasamy (2020), Agunbiade and Moodley (2014), Olaniran et al. (2014) may not be overlooked nor dismissed.

4.4.1.2. Fish weight-length relationships

Weight-length relationships are of significant importance in fisheries assessment as they give information on age at maturity, stock composition, mortality, growth phase, and production (Fafioye and Oluajo 2005). In the present study, all *C. gariepinus* from the Inanda Dam, females, and combination of females and males from the Nagle Dam showed a negative allometric growth pattern ($b<3$). Male populations from the Nagle Dam showed positive allometric pattern ($b>3$). The b-values in fish populations may also be affected by biological, geographical, and environmental factors and can vary significantly due to sex, stomach content and gonad development (Sekitar et al. 2015). Coinciding with this findings, Dan-Kishiya

(2013) and Imam et al. (2021) reported a negative allometric growth pattern in all the Cichlid species at a polluted local reservoir and Kano Dam in Nigeria, respectively. In contrast, Hossain (2010) reported a positive growth pattern in four cyprinid fish populations at a polluted river in Bangladesh. All fish populations from the Inanda Dam, female and combined male and female populations from the Nagle Dam grow faster in body length than in body weight. Male populations from the Nagle Dam grows faster in body weight than in body length.

4.4.1.3. Hepatosomatic Index (HSI)

The HSI is used in fisheries research as an indicator of energy reserves and metabolic activity in the fish liver. It is useful in detecting deleterious effects of environmental stressors (Sabarudin et al. 2017). Factors such as physiological development, age, sex, seasonal cycles, food availability, presence of different contaminant mixtures and parasites can affect the HSI (Yang and Baumann 2006; Nunes et al. 2011; Traven et al. 2013). Increased exposure to environmental contamination may result in liver weight reduction and a high HSI can result in the activation of biotransformation enzymes (Murali et al. 2017). In the present study, fish from the Inanda Dam showed low HSI compared those from the Nagle Dam. However, 43% of the fish from the Inanda Dam exhibited an HSI ranging from 1.03 to 2.01%.

A low HSI may be attributed to fish stress due to exposure to increased environmental contamination and possibly decreased lipid storage (Liebel et al. 2013). Moreover, a high HSI is an indication of an enhanced detoxification process in response to the increased exposure to contaminants (Traven et al. 2013). Datta-Munshi and Dutta (1996) suggested that an ideal HSI of Osteichthyes is generally between 1% and 2%. Mdegela et al. (2010) reported a high HSI in *C. gariepinus* exposed to sewage effluent compared to fish collected at a non-polluted site. Al-Ghais (2013) also reported a high HSI in *Mugil cephalus* and *Zosterisessor ophiocephalus* from a reservoir receiving wastewater effluent from municipality plants. Although it has been successfully used in distinguishing fish population from dissimilar water quality, the HSI was not conclusive about discriminating the health of *C. gariepinus* populations between the Nagle and Inanda dams.

4.4.1.4. Gonadosomatic Index (GSI)

The GSI is a useful tool to measure reproductive strains of fish and it gives details on reproductive status by identifying spawning seasons (Kiran 2015). The GSI also expresses the state of gonadal growth from the regressed to full maturity state and accounting for less than

0.1-20% of body weight (Corsi et al. 2003). In the present study, the GSI of females from the Nagle Dam was higher than that of female fish from the Inanda Dam. According to Yalcin et al. (2001) *C. gariepinus* populations spawn during the summer period. Moreover, Romanova et al. (2018) highlighted that *C. gariepinus* is one species that can spawn several times in one season. Therefore, the high GSI values of female population from the Nagle Dam maybe an indication that fish were ready to spawn during the period of sampling (October 2020 and March 2021).

The male fish GSI from both he Nagle and Inanda dams were relatively low, all exhibiting GSI values below 1% and gonad weight ranging from 0.01g to 38.15g with the body weight ranging from 0.13 to 5.35 kg. A low GSI values can also be attributed to environmental contamination stress, where fish is unable to allocate energy in gonadal maturation thus delaying the breeding season (Kaur et al. 2018). Moreover, a low GSI can also be attributed to regressive histological changes such as degeneration and arrest of spermatogenesis (Kaptaner et al. 2016). El-Naggar et al. (2016) recorded a high *C. gariepinus* female GSI and a low male GSI 2.50-12.17% and 0.47-1.29%, respectively. Marchand et al. (2008) recorded a GSI of below 1 for both *Oreochromis mossambicus* and *C. gariepinus* female 0.12% and male populations 0.07% from the Albasini Dam polluted with dichloro-diphenyl-trichloroethane (DDT). Although GSI variability can in most cases be influenced by seasonal changes, the pollution effect on the observed trend of GSI values for the Inanda Dam population may not be dismissed.

4.4.2. Histopathologic assessments

4.4.2.1. Gills

Fish gills play an important role in osmoregulation, acid-base balance, respiration, and excretion. They are always in constant contact with the external environment making them vulnerable to environmental contaminants (Camargo and Martinez 2007). They are good and efficient tools for biomonitoring (Nascimento et al. 2012). In the present study, alterations such epithelial lifting, hyperplasia of the secondary lamella, intracellular oedema, and leukocyte infiltration were prevalent in *C. gariepinus* gills from both dams, with most pathologies showing relatively higher prevalence in the Inanda Dam. Moreover, the magnitude of severity of these alteration showed great variation with the Inanda Dam population showing severe anomalies compared to the Nagle Dam population. Shahid et al. (2020) and Nascimento et al. (2012) observed epithelial lifting, hyperplasia of the lamella, and leukocyte infiltration in

Ictalurus punctatus from polluted areas in the River Chenab and in three different fish species in an eutrophic river in Brazil, respectively. Alterations such as epithelial lifting, hyperplasia and intracellular oedema can be an indication of a defense mechanism from contaminants by creating an increased distance between the external environment and blood, they also result in impairing oxygen uptake (Camargo and Martinez 2007). Barišić et al. (2015) recorded a highest prevalence of intracellular oedema in *Squalius vardarensis* from a heavily polluted Bregalcina River. Epithelial lifting may also be a result of fluid infiltration between epithelial and basement membrane (Shahid et al. 2020).

Hyperplasia of epithelial cells was more prevalent at the Nagle Dam as compared to the Inanda Dam. Zeitoun and Mehana (2014) suggested that hyperplasia characterized by cellular proliferation in the interlamellar region of the respiratory lamellae can result in decreased surface area making gaseous exchange difficult. Partial fusion of secondary lamella was moderately prevalent at both dams, with complete fusion being observed only in fish from the Inanda Dam. Complete and partial fusion of the secondary lamella may result in a decrease in free gas exchange and thus, affecting general fish health (Dane and Şişman 2015). Aneurysms, hemorrhages, and oedema was more prominent in the Inanda Dam population than in fish from the Nagle Dam. Aneurysms and hemorrhages are mostly accompanied by a pillar cell system collapse and as a result releasing large quantity of blood into the lamella (Cengiz 2006). Corroborating with the findings of the present study, Dane and Şişman (2015) recorded aneurysm in *Capoeta capoeta* exposed to wastewater effluent. Leukocyte infiltration was observed in fish from both dams. The infiltration of leukocyte may reflect an immunological response of fish to environmental contaminants (Koca et al. 2005). Although all the mentioned gill alterations were observed in fish from both dams, there was a variation of the degree of severity for these abnormalities with the Inanda Dam population showing relatively higher severity. However, the gill index exhibited slight histological alteration in fish from both dams. Coinciding with these results, Paulino et al. (2012) recorded a gill index of <20 in *Prochilodus lineatus* exposed to atrazine whereas McHugh et al. (2011) recorded a gill index of <10 for *Hydrocynus vittatus* from the Pongolapoort Dam which was polluted by DDT.

4.4.2.2. Liver

Fish liver is an important organ in the biochemical transformation of pollutants through the detoxification process and site for metabolism. The liver is very susceptible to toxicants and liver enzyme leakages into the blood may be an indication of exposure to contaminants (Chovanec et al. 2003). The contaminants entering the water bodies can affect the fish metabolic capacity (Chavan and Muley 2014). In the present study, alterations such as MMCs, congestion of sinusoids, hepatocellular and nuclear pleomorphism, hepatocytes hypertrophy, necrosis, hydropic degeneration and leukocyte infiltration were prevalent in *C. gariepinus* liver from both dams. Agbohessi et al. (2015) and Troncoso et al. (2012) observed fatty vacuolization, dilatation of sinusoids, nuclear pleomorphism and hypertrophied cells in fish from a polluted environments. Hyperplasia was more prevalent in fish from the Inanda Dam and it is sometimes regarded as a compensatory action to interstitial cell death (Xia et al. 2000). Hyperplasia and hypertrophy are associated with the hepatocellular pleomorphism which was observed at both dams with the Inanda Dam population exhibiting the higher prevalence and the degree of alteration. Hepatocellular pleomorphism is described as an initial toxic lesion due to environmental contaminants (Fernandes et al. 2008). The presence of MMCs in fish liver may be an indication of a recycling of endogenous material from damaged cells (Murali et al. 2017). However, the presence of MMCs is known to be normal but increased concentration may be attributed to pollutants exposure (Agbohessi et al. 2015).

Leukocyte infiltration was moderately prevalent at both dams. Valon et al. (2013) observed leukocyte infiltration and hyaline degeneration in the hepatic tissue of *C. gariepinus* exposed to heavy metal pollution. Hadi and Alwan (2012) recorded congestion of sinusoids, hepatocellular hypertrophy, cytoplasm vacuolization, and cellular necrosis in *Tilapia zilli* exposed to Al. Fatty vacuolization was less prevalent in *C. gariepinus* liver from both dams. Van Dyk et al. (2007) found dominance of fatty vacuolization in the liver of *O. mossambicus* exposed to Cd and Zn. The liver index from both dams were <20 which indicated slight histological alterations. McHugh et al. (2011) recorded a liver index of <10 which indicate normal to slight alterations in *H. vittatus* from the Pongolapoort Dam polluted with DDT. Nero et al. (2006) recorded a liver index of <20 which indicate slight alterations in *Perca flavescens* exposed to naphthenic acid toxicity. Liver histological alterations such as congestion of sinusoids, hyperplasia, hepatocellular pleomorphism and hypertrophy were observed in fish inhabiting a water body receiving wastewaters (Kaur and Dua 2014). The uMgeni River receives partial and treated wastewater from the Northern WWTP, the plant that treats 92% of industrial and 8% of domestic waste, in which the final effluent is discharged into the river

(Olaniran et al. 2012). The pollutants as some of the drivers for the increased liver alterations in the Inanda Dam may not be ruled out.

4.4.2.3. Ovaries and testes

Gonads (testes and ovaries) are very important organs that are responsible for reproduction, working closely with reproduction hormones. Disturbances in gonad activities can result in reduced reproduction ability (Liebel et al. 2013). In the present study, the ovaries of *C. gariepinus* from both dams showed dominance of perifollicular degeneration, perifollicular hyperplasia, decreased yolk formation and cortical alveoli with oocytes' atresia prevalence being low in populations from both dams. Atresia is an indication of nucleus disintegration and vitelline envelope breakdown due to exposure to environmental contaminants (Blazer 2002). Gaber et al. (2013) recorded ovary alterations such as oocyte atresia, uptake of yolk material by perifollicular cells, ooplasm disorganization and nucleus fragmentation in *C. gariepinus* exposed to heavy metal pollution. Tyor and Pahwa (2017) recorded prevalence of oocyte atresia in the polluted Yamuna River, India. Melanomacrophages centers were more prevalent in the ovaries and testis of the *C. gariepinus* population from Inanda Dam compared to those from the Nagle Dam. Melanomacrophages centers indicate the recycling of endogenous material from damaged cells (Murali et al. 2017). However, Blazer (2002) highlighted that oocytes' atresia may progress to structure similar to the MMCs.

Interstitial hyperplasia was more prevalent in male populations from the Inanda Dam compared to that from the Nagle Dam whereas pigmented macrophages were more prevalent in male populations from the Nagle Dam. Hyperplasia is sometimes regarded as a compensatory action to interstitial cell death (Xia et al. 2000). Weber et al. (2003) observed alterations such as testicular necrosis and Sertoli cell death in the testis of *Danio rerio* adults due to exposure to endocrine disrupting chemicals (EDCs). Gaber et al. (2013) observed the presence of inter and intra-tubular vacuoles in *C. gariepinus* testes exposed to heavy metal pollution. Kaptaner et al. (2016) observed alterations such as degeneration of germ cells, interstitial fibrosis, MMCs and seminiferous tubules in *Alburnus tarachi* exposed to EDCs and heavy metals. Although recorded alterations in the ovaries and testes of fish from both dams were slight to moderate, it is evident that the emergent pollution effects on the health of inhabitant aquatic biota. Hence, the level of pollution in the uMgeni River still remains a cause for concern.

CHAPTER 5

Acetylcholinesterase (AChE) activity and vitellogenin (VTG) as biomarkers

5.1. INTRODUCTION

Biomarkers are defined as changes in biological response ranging from molecular through to physiological responses that can be attributed to exposure to toxic effects of environmental chemicals (Hutchinson et al. 2006). Biomarkers are very important as they are sensitive hence, provide early warning signals for exposure to contaminants (Cattaneo et al. 2011). Biomarkers of contamination are significant in fish toxicity tests and for field monitoring of aquatic pollution and can provide an important linkage between lab toxicity and field assessment (Van der Oost et al. 2003; Haluzová et al. 2011). However, key criteria such as specificity, reproducibility, sensitivity and applicability must be considered when selecting appropriate biomarkers (Zelikoff et al. 2002). Moreover, the integrative use of biomarkers has advanced the understanding of fish ecotoxicology both in field and laboratory studies (Hutchinson et al. 2006).

According to Schlenk (1999) and World Health Organization (WHO) WHO (2003) biomarkers are classified into three separate groups that correspond to three major parameters useful to conduct ecological risk assessment. The classification of biomarkers include; biomarker of exposure (interaction between a xenobiotic and a target cell within an organism is measured), biomarker of effect (measurable biochemical, physiological or behavioral alteration within an organism) and biomarker of susceptibility (indication of the inherent or acquired ability of an organism to respond to xenobiotic exposure) (Van der Oost et al. 2003). Biomarkers provide a realistic stress pattern in pollution-stressed biota from both endogenous and exogenous (Hara et al. 2016). In this regard, traditional toxicity tests and chemical sensors cannot provide integrated information related to toxic events. Therefore, the inclusion of other levels of biological organizations such as biochemical and tissues could be significant when included in the process of assessments (Chovanec et al. 2003).

The use of biomarkers has several advantages that makes them a powerful tool to assess the health status of organisms and provide early-warning signs of environmental risks, they can be used after exposure to dietary, environmental and occupational sources and they may provide insight to the potential mechanisms of contamination effect (Van der Oost et al. 2003; Hook et al. 2014). However, like any other integrative biomonitoring approach, biomarkers have limitations that can interfere with the actual responses from environmental toxicants. In this regard, other non-pollution related factors such as age, sex, nutritional status, reproductive and developmental status, changes in the ambient temperature can complicate the interpretation of the biomarker responses (Van der Oost et al. 2003; De Andrade et al. 2004). In lab studies, complex behaviors such as spawning, and migrations are impossible to integrate whereas in the field, animals may migrate and miss the polluted hotspots (Hook et al. 2014).

Biomarker approaches have been used over the last few decades and proven to be very useful in providing information about environmental contamination and responses by fish species (Cattaneo et al. 2011). Moreover, carefully chosen biomarkers may be a good approach in identifying an early response to environmental contaminants. Biomarkers such as VTG induction and AChE inhibition have been successfully used in the past in analyzing contaminant exposure to aquatic organisms (Pretto et al. 2010). Vitellogenin is an important egg-yolk precursor in female reproduction process but it can be present in male fish due to exposure to environmental contaminants (Hara et al. 2016). Acetylcholinesterase is an enzyme in the nervous system, terminating nerve impulses by catalyzing hydrolysis of the neurotransmitter acetylcholine into acetate and choline and its inhibition has been linked to exposure to organophosphorus pesticides and carbamate insecticides (Mdegela et al. 2010; Pretto et al. 2010). In this regard, the multi-biomarker approach allows a better understanding of stress responses due to different pollution exposure (Hook et al. 2016).

The agricultural activities are some of the primary drivers of pollution in the uMgeni River due to proximity to the rural settlements, cultivation and livestock farms (Namugize et al. 2018). Moreover, the Northern WWTP located along the uMgeni River is one of the five biggest sewage plants in Durban. It treats 92% of industrial waste and 8% of domestic wastewater in which the final effluent is discharged into the uMgeni River (Olaniran et al. 2012). The run-off from the aforementioned land use activities have potential to contain oestrogenic environmental compounds that may be detrimental to fish inhabitants of uMgeni River. This chapter aims to report on the measure of the AChE inhibition in brain tissue and VTG induction in male liver to assess the exposure to pesticides and oestrogenic contaminants at the Inanda and Nagle dams. It was hypothesized that both the Nagle and Inanda dams' populations will exhibit AChE inhibition as both river reaches are draining agricultural land, and the Inanda Dam population will show higher inhibition due to additional contaminants. Another hypothesis is that the Inanda Dam population will show higher VTG induction due to the effluent discharge from industrial area in Cato Ridge and Darvill WWTP.

5.2. MATERIALS AND METHODS

5.2.1. Fish sampling and processing

Fish was samples were collected from both Nagle (n=18) and Inanda (n=28) dams during high and low flow seasons using both an electro-shocker and gill nets (25m long and 2m deep). The collected fish were examined for ecto-parasites prior to processing, they were placed in aerated tanks to maintain the oxygen content and transported to the field laboratory for further processing and analyses. Brain tissue for AChE analysis and male liver for VTG analysis were cut out and kept in dry ice and later stored at -80℃. Ethical clearance was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/019/018).

5.2.2. Acetylcholinesterase assay

Acetylcholinesterase activity of fish brain tissue was measured in duplicate samples, with an Enzyme-linked immunosorbent assay (ELISA) kit (Elabsience, catalogue number: E-BC-K174-M) that is based on the Ellman et al. (1961) method and as later modified by (Villescas et al. 1981). Approximately 100 mg of brain tissue was homogenized in a 0.1 mol/L lysis buffer, pH of 7.5 and centrifuged at 10000 rmp for 10 minutes. Thereafter, 20 µl of sample, 170 µl of reagent 3 working solution, and 10 µl of reagent 4 working solution were added to each well (reagents 3&4 as per the assay kit). It was then mixed with a multiplate reader for 5 seconds and changes in absorbance were measured at 412 nm within 5 minutes. Protein concentrations was assayed using the Bicinchoninic acid (BCA) protein assay kit (ThermoScientific, catalogue number: 23225) and AChE activity was expressed in U/mg protein, where 1 unit is the amount of AChE enzyme needed to catalyse the production of 1 nmol 5-mercapto-nitrobenzoic acid (TNB) per minute. The kit used has a published intra-assay coefficient of variance (CV) of 4.7%, an inter-assay CV of 9.3%, and a recovery rate is 104%

5.2.3. Vitellogenin assay

Vitellogenin induction of male liver tissue was measured in duplicate samples, with an ELISA kit (Bioassay Technology Laboratory, catalogue number: E0020Fi). Approximately 100 mg of liver tissue was homogenized in a 0.1 mol/L phosphate buffer (PBS), pH of 7.4 and centrifuged at 3000-4000 rmp for 20 minutes. All reagents, standards and samples were prepared before the use of the assay. Thereafter, 50 µl of the prepared standard, controls and 40 µl of pre-diluted samples were added into the allocated wells, and 10 µl of anti-VTG antibody was added to sample wells only. Thereafter, 50 µl of streptavidin-HRP was added to sample and standard wells. The wells were covered and incubated at 37℃ for 1 hour. After incubation, the contents of the wells were washed five times with the 350 µl wash buffer for 1 minute for each wash. The plate was then blotted into absorbent paper towels. Thereafter, 50 µl of substrate A and substrate B was added into each well consecutively, allowed to incubate for 10 minutes at 37℃ in the dark. To terminate the reaction, 50 µl of stop reaction was added to each well. Color reaction was measured at 450nm within 10 minutes with a multiplate reader. The VTG concentration was calculated with the standard curve equation and was expressed in µg/ml. The kit used has a published intra-assay CV of <8%, an inter-assay CV of <10%.

5.2.4. Statistical analysis

R-commander software was used for data analysis. Normality assumption was tested using the Shapiro-Wilk test and equality of variances was tested using the Levene's test. Based on the assumptions' tests, independent sample t-test or Mann-Whitney test was used to test the differences in AChE activity and VTG induction in *C. gariepinus* from the Nagle and Inanda dams. The relationship between fish size and AChE activity, and fish size and VTG induction will be evaluated using correlation analysis. Statistical significance was set at $p < 0.05$.

5.3. RESULTS

5.3.1. Acetylcholinesterase inhibition

The AChE activity in *C. gariepinus* from the Nagle and Inanda dams is presented in Figure 5.1. Acetylcholinesterase activity in fish populations from Nagle and Inanda dams ranged from 217 to 5406.04 U/mg protein and from 246 to 3526.76 U/mg protein, respectively (Figure 5.1). About 16% of the population from the Inanda Dam had a considerably low AChE activity ranging from 246 to 375.62 U/mg protein. Moreover, 26% of the population from the Nagle Dam had a considerably low AChE activity ranging from 217.17 to 421.88 U/mg protein. There was no signification difference on AChE activity in populations from the Nagle and Inanda dams ($p>0.05$) (Figure 5.1). Populations from the Nagle Dam had a mean AChE activity of 2620.49 ± 1955.17 U/mg protein in winter and 1496.31 ± 1011.3 U/mg protein in summer seasons. Populations from the Inanda Dam had a mean AChE activity of 1981.50 ± 775.29 U/mg protein in winter and 1893.66 ± 775.29 U/mg protein in summer seasons. There was no seasonal variation on AChE activity for populations in each dam ($p>0.05$) (Figure 5.1). No relationship ($r=-0.016$ and $p>0.05$) was observed between the fish body weight and AChE activity for the Inanda Dam population whereas a poor negative relationship ($r=-0.264$ and $p>0.05$) was recorded for the Nagle Dam population.

5.3.2. Vitellogenin induction

The VTG levels recorded in *C. gariepinus* males from the Nagle and Inanda dams are presented in Figure 5.1. The VTG concentrations in males from the Nagle and Inanda dams ranged from 48.56 to 109.67 µg/ml and from 14.94 to 131.89 µg/ml, respectively (Figure 5.1). About 63% of male populations from the Inanda Dam had significantly higher VTG concentrations ranging from 52.44 to 131.89 µg/ml with 27% of the population having lower concentrations ranging from 14.94 to 45.78 µg/ml. Moreover, about 75% of male fish from the Nagle Dam recorded significantly higher VTG concentrations ranging from 50.50 to 109.89 µg/ml with 25% having lower concentrations ranging from 48.56 to 49.94 µg/ml. There was no signification difference for VTG concentrations in male populations from the Nagle and Inanda dams ($p>0.05$) (Figure 5.1). Male fish from the Nagle Dam had a mean VTG level of 69.39 µg/ml in winter and 68.94 µg/ml in summer. Male populations from the Inanda Dam recorded a mean VTG for 76.75 µg/ml in winter and 60.47 µg/ml in summer. There was no seasonal variation in VTG concentrations in male populations from each dam ($p>0.05$) (Figure 5.1). Male fish from the Nagle Dam with bigger body weight recorded significantly high VTG concentrations ($r=0.759$, p<0.05). In contrast, there was no relationship between VTG concentrations and fish weight for populations from the Inanda Dam (r=-0.149, p>0.05).

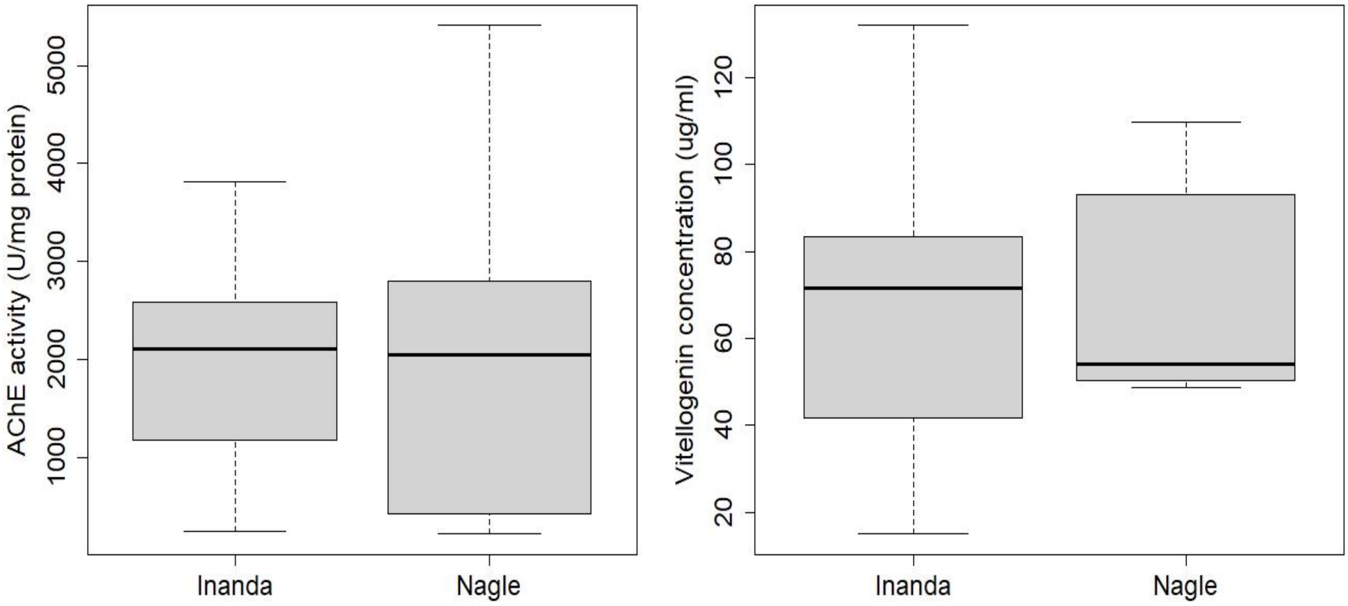

Figure 5.1 Box and whisker plots representing the range in acetylcholinesterase brain activity and vitellogenin induction in male fish liver of Clarias gariepinus recorded at the Nagle and Inanda dams during the winter and summer seasons in the 2020 and 2021 surveys. The box shows the 25th percentile and the 75th percentile, while the line inside the box is the median of the concentrations.

5.4. DISCUSSION

5.4.1. Acetylcholinesterase (AChE) inhibition

Acetylcholinesterase is a primary target for OPs and carbamates and has been extensively used as an indicator of environmental exposure in fish. Moreover, heavy metals such as but not limited to Al, Cd, Cu, and Pb, are classified as metalloestrogens due to their ability to interfere in the action of oestrogenic hormones (Paschoalini et al. 2019). Major sources of pesticides into water bodies include wastewater effluents and runoff from agricultural activities (Kist et al. 2012). Despite the Inanda Dam receiving additional contaminants from the polluted uMsunduzi, uMngcweni and Sikelekehleni rivers, the mean activity of AChE has shown no difference between Inanda and Nagle dam populations. However, the AChE activity showed a great variability within each population with the lower level being recorded in Inanda Dam population. The contaminant effect on aquatic biota may be influenced by various factors such as size and age (Santana et al. 2021).

Üner et al. (2006) reported a significant decrease in AChE activity in the brain of *Oreochromis niloticus* exposed to diazinon and Mdegela et al. (2010) recorded a 50% reduction AChE brain activity in *C. gariepinus* exposed to pesticides. Sabullah et al. (2015) highlighted that AChE inhibition due to heavy metals may have resulted from the attraction of metal ions to negatively charged amino acid side chains containing carboxyl groups causing changes in the active site. Pan et al. (2017) recorded a decreased AChE activity in *Danio rerio* exposed to Cd whereas Sabullah et al. (2015) recorded more than 70% activity loss in *Periophthalmodon schlosseri* exposed to Cd, Cr, Pb, Cu, and Zn. Although AChE activities showed no difference between the two dams, the pollution effect at the Inanda Dam may not be dismissed, particularly due to the fact that this population is the one that showed lowest activity.

The AChE activity in the fish brain prevents unceasing nerve firing, which is important in the functioning of the neuromuscular and sensory systems (Olivares-Rubio and Espinosa-Aguirre 2020). The AChE inhibition occurs as a result of phosphorylation of the serine residue in the enzyme's active site resulting in a depolarized post-synaptic membrane and synaptic transmission failure (Kist et al. 2012; Pereira et al. 2012). Intrinsic factors such as fish size and age may also have an influence the AChE activity (Durieux et al. 2011). Bigger fish generally have a larger surface area to volume ratio (SA:V) and may assimilate and detoxify contaminants at a faster rate (Santana et al. 2021). However, fish populations from Inanda Dam exhibited no relationship between fish size and AChE activity whereas fish from Nagle Dam had a weak relationship between fish size and AChE. These results coincide with Rodríguez-Fuentes et al. (2016) that recorded an inverse relationship between fish size and AChE activity in *Gambusia yucatana* in a polluted Peninsula River, Mexico and Fajardo and Ocampo (2018) in *Glossogobius giurus* in a polluted lagoon lake, Philippines.

5.4.2. Vitellogenin (VTG) induction

Vitellogenin is an egg producing hormone which is primarily secreted by female vertebrates However, it can be induced in males due to exposure to oestrogenic compounds and the use of VTG in toxicology studies has proven to be a useful tool to monitor oestrogenic exposure in fish (Tyler et al. 1996; Hook et al. 2014). Vitellogenin induction is a sign of feminization in male fish (Christiansen et al. 2000). Feminization of male fish in freshwater ecosystems were observed due to increased pollution from sewage treatment plants and agricultural activities (Archer et al. 2017; Horak et al. 2021). Males in the present study have shown considerable levels of VTG in both the Nagle and Inanda dams. The Inanda Dam receives water from tributaries draining water from catchments characterised by industrial activities and wastewater treatment plants. However, VTG levels have shown no variation between the Inanda and Nagle dam populations. The Nagle Dam population shows signs of oestrogenic effect, which implies that there could be sources of oestrogens upstream. The highest induction was observed in the Inanda Dam males which implies that the degree of induction may be significantly higher in this population. Organochlorine pesticides (OCPs) are known to induce oestrogenic effect in vertebrate. An increased concentration of OCPs was reported at the uMgeni-uMsunduzi confluence (Adeyinka et al. 2018). Therefore, the recorded VTG concentrations in this study may possibly be linked to the high concentrations of OCPs. However, the release of pesticides by the local farming activities, treated and partially treated wastewater effluent discharge by the Darvill WWTP in Pietermaritzburg and Umgeni wastewater treatment plants in Camperdown cannot be overlooked.

Increased VTG in male fish can result in kidney failure, decreased testicular growth and spermatogenesis, changes in sexual maturity, and suppressed immune system (Solé et al. 2001; Vega-López et al. 2006). These results coincide with Okoumassoun et al. (2002) that recorded significantly higher concentrations in *Sarotherodon melanotheron* males exposed to OCPs in the Oueme River, Benin and Rodas-Ortíz et al. (2008) in *O. niloticus* from two lakes polluted with OCPs in Chiapas, Mexico. Moreover, Yang et al. (2016) recorded a significant increase in VTG concentrations in *D. rerio* males exposed to high concentrations of bisphenol A. In addition, VTG heavy metals are considered as metalloestrogens due to their ability to disrupt the oestrogenic function (Paschoalini et al. 2019). de Alkimin and Fracácio (2020) highlighted that VTG is a Zn-binding protein in some fish species and can induce VTG in male fish. In that regard, the effect of heavy metals in VTG induction in males from both dams may not be dismissed. Moreover, VTG concentrations recorded in males from both dams is an indication of the possibility of the presence of oestrogens in the uMgeni River.

CHAPTER 6

Summary and Conclusion

The uMgeni River is one of the largest freshwater ecosystems in KwaZulu-Natal. It plays a significant role in the province's economy and supports livelihoods. Recent studies have suggested that the river is of poor water due to anthropogenic activities occurring along the catchment. (Agunbiade and Moodley 2014; Olaniran et al. 2014; Namugize et al. 2018; Chetty and Pillay 2019; Banda and Kumarasamy 2020). However, there are no fish health research studies identified on the river. In that regard, the study aimed at evaluating the effects of water pollution on the health status of *C. gariepinus* from the Nagle and Inanda dams both located in the uMgeni River.

Physical parameters except DO recorded at the Inanda Dam were within the DWAF (1996) stipulated guidelines for aquatic ecosystems. The Inanda Dam recorded significantly high concentrations of phosphate as compared to the Nagle Dam. There was a significant difference in sulphate concentrations in between the two dams. The Inanda Dam exhibited mesotrophic conditions for total inorganic N_2 and eutrophic for PO_4 concentrations. The Inanda Dam is located at the lower reaches of the uMgeni River, that is mostly affected by waste from polluted river tributaries, waste from municipality treatment plant and run-off from agricultural operations. Moreover, The Nagle Dam exhibited mesotrophic condition for total N_2 and PO_4 concentrations. These results are possibly linked to the increased small-scale commercial and subsistence cultivation and livestock farming occurring along the Nagle Dam.

Aluminium, Fe, and Mn were recorded in the water column with Cu and Zn being below detection level. However, sediment exhibited notable concentrations for all analysed metals. Metal concentrations have shown no seasonal variation in both mediums for both dams, however, some metals have shown a significant difference between the two dams in sediment. There was no significant difference in metals concentrations recorded in the sediments between the two dams ($p>0.05$). Neal et al. (2011) highlighted that only a portion of free metals ions stay in the water column, the rest gets deposited in the bottom sediment. The results of the present study supported that hypothesis. The difference in metal concentrations recorded in the water and sediments may be attributed to the pH of the water at both dams that resulted in metals precipitating and sinking into the sediments. Sediments are sinks and sources of contaminants and are important in assessing environmental contamination (Neal et al. 2011). Moreover, effects of heavy metals in sediments may be profiled in *C. gariepinus* since it's a bottom-dwelling feeder often foraging on sediments thus can result in exposure to metals present in the bottom sediments.

The cumulative effects of different land uses occurring in the vicinity of the uMgeni River are affecting the water quality of the Inanda Dam and should be viewed with concern. However, water quality monitoring based on physico-chemical analyses have been used for a while in most African countries but has received criticism mainly because it is time consuming, costly and only provides water analyses of the time of sampling without taking into account temporal variations (Mangadze et al. 2019)

Clarias gariepinus from both dams exhibited a CF of more than 1, indicating good condition. In contrast, all *C. gariepinus* from the Inanda Dam and female populations and combined male and females indicated a negative allometric growth pattern. Male populations from the Nagle Dam exhibited a positive allometric growth pattern in the length-weight relationship analysis. Good condition can be linked to the fact that *C. gariepinus* is an omnivorous and opportunistic feeder and can feed on a variety of food available. Furthermore, both dams recorded mesotrophic conditions, indicating availability of food. The hepatosomatic index of *C. gariepinus* from the Inanda Dam was below 1 which was attributed to fish stressed from environmental contamination. The HSI from the Nagle Dam was above 1 which was attributed to enhanced detoxification processes in response to the increased exposure to contaminants. The GSI of all *C. gariepinus* males was relatively low at both dams, suggested that male fish were not ready to spawn during the sampling period. The GSI of 60% *C. gariepinus* females from the Nagle Dam was above 2%, having bigger gonad weight relative to body size. In contrast, GSI of 60% of *C. gariepinus* from the Inanda Dam was low, indicating that they were still immature and not ready to spawn. Fish health indices are mainly affected by sex, seasons, and nutrition but the potential effects of pollutants in the uMgeni River must be viewed with concern.

The histopathology-based assessment can be used in acute, chronic and *in situ* studies (Van der Oost et al. 2003), and has proven to provide insight and early warning signal of environmental risk in this study. Prominent histological alterations were observed in the *C. gariepinus* gills from both dams, complete fusion of the secondary lamella was only observed in populations from the Inanda Dam. However, populations from both dams had gill indices of less than 20, indicating slight histological alterations following the guidelines set out by per Zimmerli et al. (2007). The Nagle Dam population exhibited a gill index of 16 ± 6.57 with Inanda Dam population showing a gill index of 19.85 ± 5.97. Liver alteration such as nuclear degeneration was observed in populations from the Inanda Dam but not in the Nagle Dam. Moreover, hepatocellular hyperplasia was observed in populations from the Nagle Dam but not the Inanda Dam. The Nagle Dam population exhibited a liver index of 11.11 ± 3.76 with the Inanda Dam population showing a liver index of 9.6 ± 5.56. Ovaries of *C. gariepinus* from both dams exhibited prevalence of MMCs, hyperplasia, and the degeneration of the perifollicular wall. *Clarias gariepinus* testes from both dams only showed the prevalence of MMCs and interstitial hyperplasia. Although there are other factors that can induce histopathologic alterations in fish tissues, exposure to environmental contamination as one of the drivers may not be dismissed.

Biomarker approaches have been used over the last few decades and proven to be very useful in providing information about environmental contamination and responses by fish species (Cattaneo et al. 2011). Acetylcholinesterase activity has shown no significant difference between the two populations. However, few of the Nagle Dam fish specimens exhibited substantially high activities. Notable VTG concentrations were recorded in the liver of male *C. gariepinus* at both dams. However, no significant difference was observed between the two populations. Despite insignificant difference between the populations, some *C. gariepinus* specimens exhibited substantially higher concentrations in the Inanda Dam. It is evident that there are signs of estrogenic effect in *C. gariepinus* populations from the Inanda and Nagle dams. *Clarias gariepinus* is known by its resistance to pollution effect, therefore, it is likely that other species are severely affected. Further exploration of pollution effect on fish health are recommended on other native species which are known to be pollution sensitive. Moreover, multi-biomarker approach should be employed to get more insight on the effects of anthropogenic estrogens on fish species in the uMgeni River system.

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APPENDIX

Table 1: Biometric data of *Clarias gariepinus* recorded during the summer season in the 2021 survey at the Inanda Dam.

Sample site	Fish #	Season	Species	Sex	Fish weight (g)	Total length (cm)	Standard length (cm)	Liver weight (g)	Gonad weight (g)
Inanda Dam	1	S	*Clarias gariepinus*	F	2310	63,5	43	24,2	331,2
Inanda Dam	2	S	*Clarias gariepinus*	M	2300	64,4	45	1	0,6
Inanda Dam	3	S	*Clarias gariepinus*	M	3930	51,5	64,5	27,9	2,2
Inanda Dam	4	S	*Clarias gariepinus*	M	1010	48,5	44,5	16,4	0,3
Inanda Dam	5	S	*Clarias gariepinus*	M	890	37	31,3	12,9	0,6
Inanda Dam	6	S	*Clarias gariepinus*	F	4102	77,4	68,5	48	124,5
Inanda Dam	7	S	*Clarias gariepinus*	M	2615	65,5	47,5	40,5	0,4
Inanda Dam	8	S	*Clarias gariepinus*	M	945	44,3	29	14	0,1
Inanda Dam	9	S	*Clarias gariepinus*	M	780	49	30,1	7,4	0,3
Inanda Dam	10	S	*Clarias gariepinus*	M	1140	51,1	45,5	8,7	0,3
Inanda Dam	11	S	*Clarias gariepinus*	M	3750	82	62,8	65,2	13,4
Inanda Dam	12	S	*Clarias gariepinus*	M	770	49	48,3	15,5	0,4

Sample site	Fish #	Season	Species	Sex	Fish weight (g)	Total length (cm)	Standard length (cm)	Liver weight (g)	Gonad weight (g)
Inanda Dam	13	S	*Clarias gariepinus*	M	266	30,4	26,4	1,08	0,1
Inanda Dam	14	S	*Clarias gariepinus*	M	350	36	22,5	2,4	0,01
Inanda Dam	15	S	*Clarias gariepinus*	F	495	30,5	32,5	3,6	1,2
Inanda Dam	16	S	*Clarias gariepinus*	F	120	26,7	23,1	0,5	0,001

Table 2: Biometric data of *Clarias gariepinus* recorded during the winter season in the 2020 survey at the Inanda Dam.

Sample site	Fish #	Season	Species	Sex	Fish weight (g)	Total length (cm)	Standard length (cm)	Liver weight (g)	Gonad weight (g)
Inanda Dam	1	W	*Clarias gariepinus*	M	2900	71,5	59,5	30	18
Inanda Dam	2	W	*Clarias gariepinus*	M	3450	87	63	55	54
Inanda Dam	3	W	*Clarias gariepinus*	M	400	25	23	0,055	0,05
Inanda Dam	4	W	*Clarias gariepinus*	M	390	25,5	24,8	3,24	3,32
Inanda Dam	5	W	*Clarias gariepinus*	M	323	24,3	23,5	0,34	0,001
Inanda Dam	6	W	*Clarias gariepinus*	F	350	25	24	0,31	0,01
Inanda Dam	7	W	*Clarias gariepinus*	M	4845	74	63,5	63,67	36,15
Inanda Dam	8	W	*Clarias gariepinus*	M	1455	47	45	18,18	3,92
Inanda Dam	9	W	*Clarias gariepinus*	F	1545	52,5	49,5	13,09	4,4
Inanda Dam	10	W	*Clarias gariepinus*	M	775	47	46,5	4,47	4,26
Inanda Dam	11	W	*Clarias gariepinus*	M	220	20,9	20	0,41	0,66
Inanda Dam	12	W	*Clarias gariepinus*	M	230	21,5	20	0,32	0,01

Table 3: Biometric parameters of *Clarias gariepinus* recorded during the winter season in the 2020 survey at the Nagle Dam.

Sample site	Fish #	Season	Species	Sex	Fish weight (g)	Total length (cm)	Standard length (cm)	Liver weight (g)	Gonad weight (g)
Nagle Dam	1	W	*Clarias gariepinus*	M	5345	73	69,5	68,67	38,15
Nagle Dam	2	W	*Clarias gariepinus*	F	275	26,5	25,5	1,61	15,80
Nagle Dam	3	W	*Clarias gariepinus*	M	130	25	23,5	0,66	0,001
Nagle Dam	4	W	*Clarias gariepinus*	F	2510	64,3	61,3	28,16	215,57
Nagle Dam	5	W	*Clarias gariepinus*	F	1835	53	49,2	24,1	182,17
Nagle Dam	6	W	*Clarias gariepinus*	F	540	35,2	34	5,76	26,3
Nagle Dam	7	W	*Clarias gariepinus*	M	550	35,9	34,1	8,25	2,45
Nagle Dam	8	W	*Clarias gariepinus*	F	485	33,5	31,9	5,79	15,52

Table 4: Biometric parameters of *Clarias gariepinus* recorded during the summer season in the 2021 survey at the Nagle Dam.

Sample site	Fish #	Season	Species	Sex	Fish weight (g)	Total length (cm)	Standard length (cm)	Liver weight (g)	Gonad weight (g)
Nagle Dam	1	S	*Clarias gariepinus*	F	30	11,3	10,9	0,5	0,3
Nagle Dam	2	S	*Clarias gariepinus*	F	2400	61	53,5	31,2	21,8
Nagle Dam	3	S	*Clarias gariepinus*	M	3285	73,4	65	14,4	27,9
Nagle Dam	4	S	*Clarias gariepinus*	M	3400	84,3	74,1	54,6	15,2
Nagle Dam	5	S	*Clarias gariepinus*	F	3260	73,3	64,4	42,9	266,2
Nagle Dam	6	S	*Clarias gariepinus*	M	2260	71	59,5	21,4	4,5
Nagle Dam	7	S	*Clarias gariepinus*	F	2140	66	59	21,3	141,5
Nagle Dam	8	S	*Clarias gariepinus*	M	3480	81	72,6	95,7	14,2
Nagle Dam	9	S	*Clarias gariepinus*	M	130	26,2	24	0,7	0,01
Nagle Dam	10	S	*Clarias gariepinus*	F	1550	61,5	58,8	0,8	3,5

Table 5: Vitellogenin concentrations of *Clarias gariepinus* male populations recorded during the winter season in the 2020 survey at the Inanda Dam.

Sample site	Season	Absorbance 1	Absorbance 2	Average absorbance
Inanda Dam	W	0,249	0,24	0,2445
Inanda Dam	W	0,186	0,101	0,1435
Inanda Dam	W	0,212	0,131	0,1715
Inanda Dam	W	0,272	0,301	0,2865
Inanda Dam	W	0,267	0,293	0,28
Inanda Dam	W	0,169	0,213	0,191
Inanda Dam	W	0,189	0,171	0,18
Inanda Dam	W	0,159	0,156	0,1575
Inanda Dam	W	0,103	0,07	0,0865
Inanda Dam	W	0,129	0,134	0,1315

Table 6: Vitellogenin concentrations of *Clarias gariepinus* male populations recorded during the summer season in the 2021 survey at the Inanda Dam.

Sample site	Season	Absorbance 1	Absorbance 2	Average absorbance
Inanda Dam	S	0,09	0,066	0,078
Inanda Dam	S	0,078	0,11	0,094
Inanda Dam	S	0,281	0,212	0,2465
Inanda Dam	S	0,209	0,183	0,196
Inanda Dam	S	0,244	0,143	0,1935
Inanda Dam	S	0,165	0,187	0,176
Inanda Dam	S	0,256	0,212	0,234
Inanda Dam	S	0,202	0,164	0,183
Inanda Dam	S	0,149	0,099	0,124
Inanda Dam	S	0,114	0,077	0,0955
Inanda Dam	S	0,084	0,068	0,076
Inanda Dam	S	0,199	0,199	0,199

Table 7: Vitellogenin concentrations of *Clarias gariepinus* male populations recorded during the winter and summer seasons in the 2020 and 2021 surveys at the Nagle Dam.

Sample site	Season	Absorbance 1	Absorbance 2	Average absorbance
Nagle Dam	W	0,243	0,25	0,2465
Nagle Dam	W	0,112	0,166	0,139
Nagle Dam	W	0,179	0,094	0,1365
Nagle Dam	S	0,134	0,146	0,14
Nagle Dam	S	0,232	0,145	0,1885
Nagle Dam	S	0,177	0,107	0,142
Nagle Dam	S	0,231	0,259	0,245
Nagle Dam	S	0,141	0,16	0,1505

S= Summer, W=Winter

Table 8: Acetylcholinesterase brain activity and protein concent of *Clarias gariepinus* recorded in the winter season in the 2020 survey at the Inanda Dam.

Sample site	Season	330 s (A2)	30 s (A1)	(A2-A1)	Protein content (ug/ml)
Inanda Dam	W	0,658	0,49	0,168	1,097
Inanda Dam	W	3,67	2,561	1,109	1,288
Inanda Dam	W	2,596	1,183	1,413	1,643
Inanda Dam	W	1,99	0,792	1,198	1,137
Inanda Dam	W	1,516	0,656	0,86	0,958
Inanda Dam	W	0,801	0,424	0,377	1,258
Inanda Dam	W	3,522	2,158	1,364	1,343
Inanda Dam	W	3,233	1,67	1,563	1,473
Inanda Dam	W	2,276	1,089	1,187	1,334
Inanda Dam	W	1,541	0,786	0,755	1,128
Inanda Dam	W	1,709	0,612	1,097	0,967

Table 9: Acetylcholinesterase brain activity of *Clarias gariepinus* recorded in the summer season in the 2021 survey at the Inanda Dam.

Sample site	Season	330 s (A2)	30 s (A1)	(A2-A1)	Protein content (ug/ml)
Inanda Dam	S	1,777	1,096	0,681	1,420
Inanda Dam	S	0,482	0,268	0,214	1,396
Inanda Dam	S	3,09	1,592	1,498	1,383
Inanda Dam	S	3,593	2,394	1,199	0,817
Inanda Dam	S	0,826	0,333	0,493	0,598
Inanda Dam	S	0,244	0,162	0,082	0,817
Inanda Dam	S	0,328	0,218	0,11	0,977
Inanda Dam	S	2,257	1,121	1,136	0,789
Inanda Dam	S	1,896	0,935	0,961	1,293
Inanda Dam	S	2,519	1,151	1,368	1,493
Inanda Dam	S	3,036	1,354	1,682	1,080
Inanda Dam	S	1,192	0,612	0,58	1,324
Inanda Dam	S	3,462	2,598	0,864	1,191
Inanda Dam	S	3,845	2,37	1,475	1,890

Table 10: Acetylcholinesterase brain activity of *Clarias gariepinus* recorded in summer and winter seasons in the 2020 and 2021 surveys at the Nagle Dam.

Sample site	Season	330 s (A2)	30 s (A1)	(A2-A1)	Protein content (ug/ml)
Nagle Dam	W	3,713	2,417	1,296	1,597
Nagle Dam	W	3,509	1,715	1,794	0,928
Nagle Dam	W	2,584	1,094	1,49	1,195
Nagle Dam	W	0,486	0,261	0,225	1,307
Nagle Dam	W	1,897	1,023	0,874	0,890
Nagle Dam	W	3,167	1,65	1,517	0,688
Nagle Dam	W	0,705	0,529	0,176	1,301
Nagle Dam	S	1,549	0,73	0,819	0,768
Nagle Dam	S	1,095	0,547	0,548	1,376
Nagle Dam	S	0,419	0,286	0,133	0,866
Nagle Dam	S	1,107	0,704	0,403	0,990
Nagle Dam	S	0,486	0,38	0,106	1,196
Nagle Dam	S	1,371	0,665	0,706	0,807
Nagle Dam	S	0,619	0,422	0,197	1,5266
Nagle Dam	S	0,96	0,418	0,542	1,0036
Nagle Dam	S	1,737	0,699	1,038	0,9076
Nagle Dam	S	0,902	0,352	0,55	1,3176
Nagle Dam	S	1,433	0,517	0,916	1,0656
Nagle Dam	S	1,561	0,518	1,043	0,835

S= Summer, W=Winter

Table 11: Metal concentrations recorded in the water in the Nagle and Inanda dams during winter and summer seasons in the 2020 and 2021 surveys.

Sample site	Season	Aluminium (mg/L)	Copper (mg/L)	Iron (mg/L)	Manganese (mg/L)	Zinc (mg/L)
Nagle Dam	W	0,100	<0,010	0,097	0,025	<0,025
Nagle Dam	W	0,100	<0,010	0,068	0,025	<0,025
Nagle Dam	W	0,100	<0,010	0,651	0,025	<0,025
Nagle Dam	S	0,100	<0,010	0,195	0,054	<0,025
Nagle Dam	S	1,289	<0,010	0,134	0,031	<0,025
Nagle Dam	S	0,100	<0,010	2,04	0,066	<0,025
Inanda Dam	W	0,1	<0,010	0,025	0,025	<0,025
Inanda Dam	W	0,1	<0,010	0,623	0,074	<0,025
Inanda Dam	W	0,362	<0,010	1,27	0,062	<0,025
Inanda Dam	S	0,856	<0,010	0,04	0,025	<0,025
Inanda Dam	S	0,154	<0,010	0,044	0,025	<0,025
Inanda Dam	S	0,01	<0,010	0,282	0,072	<0,025

S= Summer, W=Winter

Table 12: Metal concentrations recorded in the sediments in Nagle and Inanda dams during winter and summer seasons in the 2020 and 2021 survey.

Sample site	Season	Aluminium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Nagle dam	W	153125	325	353125	10968,75	534,375
Nagle dam	W	3068,75	200	318750	203,125	281,25
Nagle dam	W	134375	437,5	6468,75	6062,5	1718,75
Nagle dam	S	46875	162,5	137500	3312,5	459,375
Nagle dam	S	196875	406,25	506250	17812,5	675
Nagle dam	S	200000	518,75	450000	9562,5	565,625
Inanda dam	W	121875	506,25	346875	9437,5	1053,125
Inanda dam	W	156250	287,5	281250	5750	662,5
Inanda dam	W	165625	425	375000	8687,5	743,75
Inanda dam	S	296875	587,5	665625	8906,25	2418,75
Inanda dam	S	300000	587,5	628125	13750	690,625
Inanda dam	S	159375	712,5	353125	8187,5	5656,25

S= Summer, W=Winter

Table 13: Nutrient concentrations recorded in Nagle and Inanda dams during winter and summer seasons in the 2020 and 2021 survey.

Sample site	Season	Ammonia (mg NH_3/L)	Nitrite (mg NO_2/L)	Nitrate (mg NO_3/L)	Phosphate (mg P/L)	Sulphate (mg SO_4/L)
Inanda dam	W	0.10	<0.10	2.91	154	19.5
Inanda dam	W	0.10	0.95	0.53	<15.0	24.7
Inanda dam	W	0.12	1.69	1.01	17.9	18.1
Inanda dam	S	0.11	0.77	0.99	<15.0	18.2
Inanda dam	S	0.10	<0.10	0.93	<0.15	20.5
Inanda dam	S	0.22	<0.10	0.10	70.2	18.0
Nagle dam	W	0.10	<0.10	0.31	19.7	2.98
Nagle dam	W	0.10	<0.10	0.10	<15.0	3.12
Nagle dam	W	0.20	<0.10	0.28	<15.0	4.47
Nagle dam	S	0.64	<0.10	0.10	<15.0	3.74
Nagle dam	S	0.19	<0.10	0.38	<15.0	4.71
Nagle dam	S	0.15	<0.10	0.18	16.1	4.50

S= Summer, W=Winter