Assemblage-based monitoring of the uMsunduzi River, using biotic and abiotic lines of evidence

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Submitted in fulfilment of the academic requirements for the degree of

Master of Science

in the Discipline of Ecological Sciences

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg Campus

2024

ABSTRACT

Globally, urbanisation and industrialisation activities result in a multitude of impacts on freshwater ecosystems. Generally, frequent pollution of rivers from industrial effluents and sewage channels has caused the deterioration of the water quality of rivers. The availability of healthy, clean, and good-quality drinking water is a matter of concern in urban areas, particularly in developing countries such as South Africa. The instream physical barriers that lack fish ladders, development of industrial complexes adjacent to the river banks, extensive agricultural activities, abstraction of water for domestic and industrial use, and ageing sewage networks, as well as periodic industrial spills, have worsened the ecological well-being of the two socio-economically important rivers in the KwaZulu-Natal Province, South Africa, the uMsunduzi River a tributary of the uMngeni River. The present study evaluated the recovery of the uMsunduzi River, and efforts were made to improve fish population structures following a fish kill event using biotic and abiotic lines of evidence. It was hypothesised that water quality characteristics and biological communities would exhibit improvement since the occurrence of the spill. Abiotic lines of evidence included water quality and habitat characteristics, whereas biotic lines of evidence involved fish communities and fish population attributes of the indigenous KwaZulu-Natal yellowfish (*Labeobarbus natalensis*), macroinvertebrates, and benthic diatoms. The research was carried out quarterly from 2022 to 2023 at selected sections of the uMsunduzi system, with sites for fish collection upstream of the spill point and re-introduction sites downstream of the spill point. After the mixed product spill and fish kill in the Baynesspruit and uMsunduzi Rivers, nine sites were considered for fish community assessment and ecological monitoring. This included how assemblages respond to a wide range of environmental variables and for fish using the Fish Response Assessment Index (FRAI). The results showed that the uMsunduzi River is heavily polluted, and the stressors impact water quality, fisheries and habitat availability. The multivariate analyses indicated that the anthropogenic stressors that drive the ecological well-being and fish community structures of the uMsunduzi River could be related to changes in water quality and instream habitat modifications. The identified environmental modifications can be directly linked to human activities and flow modifications along the uMsunduzi system.

Assessment of attributes of fish communities along the uMsunduzi catchment, with a focus on the KwaZulu-Natal yellowfish as an indicator species, showed a declining state of fish communities in terms of species richness and abundance. The poor fish communities include the fish collection sites upstream of the spill point selected for fish collection. The downstream site, further away from the city, showed some improvement in fish communities. The poor water quality and fish stock for collection sites highlight poor water quality and the impact of stressors on fish communities. The outcomes of this study can contribute to the holistic management, monitoring, and rehabilitation strategies for the uMsunduzi River after a fish kill event. The pre-spill fish data from (Dlamini et al. 2019) showed fish to be slowly recovering to attain the pre-spill abundances. Furthermore, biological stressors should be minimised, particularly inputs of toxic industrial effluents from industrial complexes, sewage from poorly maintained sewage systems and instream physical barriers that fragment fish populations and reduce environmental flows required by indigenous fishes. The development of evidence-based rehabilitation strategies is recommended to attain ecological health for the uMsunduzi River. Removing obsolete instream barriers and incorporating fishways on operational barriers is recommended to allow the movement of migratory fish between upstream and downstream niches. Moreover, inter-system translocation of the KwaZulu-Natal yellowfish from adjacent catchments with healthy breeding populations, such as the uMngeni River, should be collectively decided by the relevant stakeholders to improve population attributes of the yellowfish in the uMsunduzi River. The fisheries management could

benefit local subsistence fishermen and improve river conditions. Before this, the genetics of the species need to be determined so as not to jeopardise the genetic fitness or resilience of the existing population. The existing legislative framework for environmental protection should be enforced for industrial and municipal operations to render minimal degradation to freshwater ecosystems. Conservancies should be established to strategically manage and protect freshwater resources and potential fisheries along the uMsunduzi system.

PREFACE

The data described in this thesis were collected in the uMsunduzi River, Pietermaritzburg, Republic of South Africa, from February 2022 to November 2023. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof Colleen T. Downs and Dr Matthew J. Burnett.

This thesis, submitted for the degree of Master of Science in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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Lwandile Ngozi

January 2024

I certify that the above statement is correct, and as the candidate's supervisor, I have approved this thesis for submission.

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Prof Colleen T. Downs

Supervisor

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DECLARATION 1 - PLAGIARISM

I, Lwandile Ngozi, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

PUBLICATION 1- not submitted

A systematic review of global and regional fish kills

Ngozi, L., Burnett, M., Downs, C.T.

Author contributions:

LN conceived paper with CTD and MB. LN collected and analysed data, and wrote the paper. CTD and MB contributed valuable comments to the manuscript.

PUBLICATION 2- not submitted

Evaluating diatoms, macroinvertebrates and water quality following a severe pollution event in the uMsunduzi River, KwaZulu-Natal

Ngozi, L., Burnett, M., Downs, C.T.

Author contributions:

LN conceived the paper with CTD and MB. LN collected and analysed data, and wrote the paper. CTD and MB contributed valuable comments to the manuscript.

PUBLICATION 3- not submitted

Pre, post and present ecological conditions in an environmentally and economically important river: the uMsunduzi River, Pietermaritzburg, South Africa Ngozi, L., Burnett, M., Downs, C.T.

Author contributions:

LN conceived the paper with CTD and MB. LN collected and analysed data, and wrote the paper. CTD and MB contributed valuable comments to the manuscript.

PUBLICATION 4- not submitted

Conditions of freshwater ecosystems need consideration in the application of telemetry techniques: Lessons from using telemetry to monitor fish in an urban river

Ngozi, L., Burnett, M., Downs, C.T.

Author contributions:

LN conceived the paper with CTD and MB. LN collected and analysed data, and wrote the paper.

CTD and MB contributed valuable comments to the manuscript.

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Lwandile Ngozi

January 2024

ACKNOWLEDGEMENTS

I would like to extend my sincerest gratitude to Prof CT Downs and Dr MJ Burnett, who are my supervisors, for financial assistance, guidance, invaluable comments on my draft chapters, and for covering costs for me to attend four conferences (SASAqS, ZSSA Conference 2023, PRIS 2023 and the Conservation Symposium), during the 2023 academic year. Thanks to Dr MJ Burnett, Dr Streicher and Prof CT Downs for proofreading and editing my conference presentations and for fun conference experiences at conferences.

I want to thank postgraduate students and interns from the Downs lab for assisting with fieldwork and moral support during the course of the present research. Special thanks to the A team and the Downs BUHA lab for help with fieldwork and social support. Thanks to GroundTruth Water, Wetlands and Environmental Engineering consultants, in particular Juan Tedder, Lindelani, and Ayanda Lepheyana, for the macroinvertebrate collection, and field macroinvertebrate identification crash courses they offered me! Kyle Johnstone, Catherine Meyers, and Carla Hardman are thanked for helping with mapping out the survey locations and providing access to previous reports. I would like to thank Dr. Abie Khan and Jennifer Pillay for always organising access to the mid Baynesspruit site through their facilities to collect water samples, and also allowing us to mount one of our base stations on the rooftop of their factory to communicate with probes deployed along the Baynesspruit.

Special thanks to the National Research Foundation (ZA), the University of KwaZulu-Natal, the Willowton Group, and GroundTruth for providing financial support and the Ford Wildlife Foundation for vehicle support to conduct fieldwork for the present study.

I extend my heartfelt gratitude to my great-grandma for her love and support. Thanks to my friends for their support, especially to Ms N Ngcobo, Mr N Makhathini, Ms P Madikizela and Ms Thobeka Zulu for occasionally providing home-cooked meals when we were writing up. Much of the data analyses required for the present study would not be possible without the help of Dr Burnett, Dr Bitani and Ms T Mthimunye! Many thanks to Dr Senoge, Nolwazi Ngcobo, Ntando Makhathini, Realene Sappor, Aphelele Langa, Luyanda Hlamaphi, Nomthandazo Xhatshwayo, Sanele Xaba, Joy Gumede, Zain Armien, and Glen Ndlovu for assisting with fieldwork.

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CHAPTER 1

Introduction

1.1 Background

Water is a strategic and valuable natural resource that supports human livelihoods and realises long-term sustainable development goals (Mbao et al. 2020; Lynch et al. 2020). The sources of fresh water in South Africa are mountain streams, wetlands, lakes, springs, wells and rivers; however, the water gets polluted as a result of residential, municipal, and industrial activities (Gemmel and Schmidt 2013; Matongo et al. 2015). Seasonal fluctuations in rains and anthropogenic impacts challenge the realisation of sustainable development goal 6 (SDG6) of clean water and sanitation for all (DWS 2022). The continued pollution of socio-economically important rivers and increased occurrence of fish kills in South African rivers suggests that management and conservation have been poor (Tempelhoff 2009; Gemmel and Schmidt 2013; Zampatti et al. 2022). Numerous abiotic and biotic indices have been extensively used to monitor and manage rivers to conserve aquatic biodiversity to address this (Karr 1981; Evans et al. 2022)..Abiotic indices involve water quality and chemistry to ascertain the dissolved and suspended substances of organic and mineral origin (Gemmel and Schmidt 2013). Biotic indices include the response of aquatic biota at various biological levels, particularly freshwater invertebrates and fish communities (Agboola et al. 2020a,b; Burnett et al. 2020, 2021). The deterioration of water quality of economically important rivers is of major concern, as impacted by increasing use for human needs and the ill effects of industrial activities (Tempelhoff 2009; Thiem et al. 2022).

Pietermaritzburg, which is the capital city of the KwaZulu-Natal Province, South Africa, has seen a rapid increase in population growth because of industrialisation and rapid urbanisation. The land used for residential areas was 406 ha in 1989 and 920 ha in 2009, with cultivated land and open green spaces shrinking, the overall water demand has been increasing with increased refuse dumping, agricultural runoffs and industrial effluents (Pole 2002; Hlatshwayo 2012; Matongo et al. 2015). Pietermaritzburg has a large number of industrial complexes which use large volumes of water in their manufacturing and supporting operations (Pole 2002). The rapid industrialisation rate lacks innovative strategies to contain by-products from industrial processes, creating many environmental threats (Pole 2002). Particularly, the present way of disposing of untreated industrial effluents in sewage channels and natural water bodies results in serious water contamination of socio-economically important rivers for water resource provision (Tempelhof 2009; Koehn 2021). A significant amount of wastewater is discharged daily into the water bodies by the different industries in South Africa (Matongo et al. 2015). These effluents contain toxic chemicals, toxic ions, and metallic compounds (Gemmel and Schmidt 2013). Industrial inputs negatively impact the well-being of aquatic organisms and water quality, as well as the local fishermen who depend on the local rivers for income benefits and to provide food resources for their families (Tempellhoff 2009; Evans et al. 2022).

1.2 River connectivity

Connected river systems allow for the movement of aquatic organisms between habitats at different stages of their life cycle, exchange of energy, nutrients and sediments, as well as organic matter, throughout the system (Holmlund and Hammer 1999; O'Brien et al. 2019). Flow modification resulting from river engineering for water appropriation for human use is amongst the greatest threats to freshwater ecosystems (Grill et al. 2019; Thiem et al. 2022) and has altered natural river connectivity around the globe, with instream barriers lacking fish ladders for fish migration (Dudgeon et al. 2004; Grill et al. 2019). Instream barriers impact natural aquatic systems by changing sedimentation processes, flooding, channelisation, and available habitats (Holmlund and Hammer 1999; O'Brien et al. 2019). They further interfere with the migratory pathways of facultative migratory fish between spawning and feeding sites and nutrient processes (Hanzen et al. 2020; Burnett et al. 2021).

1.3 Abiotic indices

1.3.1 Water quality

Water quality describes water's chemical, physical, and biological properties, which are driven by the suspended or dissolved substances of organic and mineral origin (DWAF 1996a; Sutadian et al. 2016; Yan et al. 2022). Monitoring a system's water quality is pivotal to protecting the ecosystem's ecological integrity and sustainable use of ecosystem services (DWAF 1996; Kumar et al. 2019). When monitoring water quality, core physicochemical parameters of water are measured. These include temperature, dissolved oxygen, salts, pH, total dissolved solids, electrical conductivity, and nutrients such as phosphate, nitrite, and nitrate, toxic and nontoxic substances (DWAF 1996; Dickens and Graham 1998; Darko et al. 2022). The change in demographics and extensive agricultural practices to meet the growing food demand can significantly alter water quality, driving the deterioration of the ecological integrity of a riverine ecosystem (Selemani et al. 2018; Agboola et al. 2020a; Darko et al. 2022). Modifications in water quality directly impact the response of aquatic fauna, such as fish and macroinvertebrates; therefore, continuous monitoring of changes in water quality has implications for the management of healthy aquatic ecosystems and informs decisions for fisheries management (Ramesh et al. 2018; Agboola et al. 2020b; Karr et al. 2021). Sustained poor water quality makes conditions conducive for hardy and pollution-tolerant fish species, which are mostly invasive and can tolerate pollution to a certain extent, driving declines in indigenous fishes (Dudgeon 2014; Burnett et al. 2023).

1.3.2 Habitat

The impacts of anthropogenic stressors mostly result in deteriorated water quality but also drive physical modifications in aquatic habitats (Kleynhans 1999, 2007). This makes habitat integrity an important factor for the survival of aquatic organisms in an ecosystem, as they require various biotopes between seasons and life stages (Skelton 2001; James and King 2010). The importance of habitat availability for the well-being of aquatic organisms makes it an important factor to consider when managing fisheries and monitoring the health of ecosystems in which they occur (James and King 2010; Ramesh et al. 2018; Evans et al. 2022). Habitat is defined as the physical and chemical features that individual fish, populations, or communities require to propagate successfully (La and Cooke 2011). Various niches are set apart by their physical, chemical, and biological properties, including basic life requirements such as food and water resources and available cover features (Impson et al. 2008; La and Cooke 2011; O'Brien et al. 2019). To ascertain the impacts of depth and river gradient of fish community structures, the substrate type (silt, mud, sand, gravel, cobbles, boulders, and bedrock), various cover features (undercutting banks, roots, marginal vegetation, overhanging vegetation, depth, and substrate) and types of flow (still marginal, deep pool, shallow pool, deep glide, shallow glide, run, riffle and rapids) were measured all which allow for the river to be categorised as either in the upper, middle and lowers zones (James and King 2010; Kleynhans 2007).

1.4 Biotic indices

1.4.1 Diatoms

Diatoms are microscopic, unicellular organisms that occur in high abundance in freshwater ecosystems (Taylor et al. 2007; Salinas-Camarillo et al. 2021; Costa and Schneck 2022). Their photosynthetic ability allows them to fix enormous amounts of carbon dioxide (Falkowski et al. 2004). In addition, the diatoms provide food resources for the high trophic levels, such as the macroinvertebrates and fish (Lobo et al. 2016). Diatoms are particularly valuable in freshwater monitoring studies since the different taxa exhibit different sensitivities to environmental changes (Taylor et al. 2007; Mbao et al. 2020). Studies on diatoms have accumulated over the years, such that the baseline data for taxa that are sensitive and those that are tolerant to pollution is well established, making diatoms relevant to the monitoring of rivers and the development of water resource protection strategies (Taylor et al. 2007; Mbao et al. 2020). Moreover, understanding the diatom biology of attaching to substrates has allowed standardised and proper collection methods (Mbao et al. 2022).

1.4.2 Macroinvertebrates

Macroinvertebrates are ubiquitous and play various roles, such as the cycling of organic matter and mixing of sediments (Kenney et al. 2009; Sharma and Behera 2022). Macroinvertebrates are important food sources for animals that are higher on the food chain (Sharma and Behera 2022). When they die and decay, nutrients are reused by aquatic flora and fauna (Hauer and Resh 2017; Sharma and Behera 2022). The persistence of macroinvertebrates is threatened by changes in biotopes associated with pollution, erosion, and siltation (Dudgeon 2000; Raphahlelo et al. 2022), and their responses can be used to monitor the impact of stressors (Dickens and Graham 2002; Ojija and Laizer 2016; Ngamalana et al. 2023). Macroinvertebrate taxa vary in their degree of tolerance; a high abundance of pollution-tolerant taxa signifies the extent of the impact of pollution (Dickens and Graham 2002; Taylor et al. 2007). Macroinvertebrate orders such as mayflies (Ephemeroptera), stoneflies (Plecoptera), aquatic snails (Mollusca), beetles (Coleoptera), biting midges (Chironomids), caddisflies (Trichoptera), and leeches (Hirudinea) are used when assessing the ecological health of a river (Dickens and Graham 2002; Sharma and Behera 2022).

1.4.3 Fish

The acute response of fish to environmental changes has made them useful as key indicators when assessing the ecological state of aquatic ecosystems (Karr 1981; Ramesh et al. 2018; Burnett et al. 2020, 2024). However, studies that look at a single level of biological organisation may not always have sufficient data for thoroughly monitoring ecological impact, so it has become widely accepted to use different levels of biological organisation (Wepener 2008; Marin et al. 2023). The response of organisms to anthropogenic stressors can be observed at different levels of biological organisations, from sub-cellular levels to organism, population, community, and eventually at the ecosystem level (Dickens and Graham 2002; Taylor et al. 2007; Evans et al. 2022). Lower levels of biological organisation respond swiftly to stressors and give a better understanding of cause-and-effect pathways, while the higher-level responses highlight broader ecological implications of environmental and anthropogenic stressors (La and Cooke 2011; Koehn 2021; Evans et al. 2022).

1.4.4 Fish communities

Healthy aquatic ecosystems host diverse biotic communities with high abundances (Karr 1981; Chakona et al. 2022; Evans et al. 2022). Fish communities are beneficial when assessing the ecological state of an aquatic ecosystem as they are relatively easy to identify and give information on both species composition and abundance, which can further be linked to the effects of stress and toxicity (Karr 1981; Kleynhans 1999, 2007; Desai et al. 2021; Evans et al. 2022). Fish communities can be defined or classified in various ways depending on the aims of the study and the characteristics of interest of the fish communities (Jackson et al. 2001). One approach to evaluate fish communities is to use multivariate statistical approaches. The multivariate statistics not only summarise and predict community trends, but they also identify fish assemblages and the impact of environmental perturbations in fish communities (O'Brien et al. 2009; Burnett et al. 2018; Desai et al. 2021; Evans et al. 2022; Wepener and O'Brien 2022).

Another approach to studying riverine fish communities is using the Fish Response Assessment Index (FRAI) (Kleynhans 1999, 2007; Wepener et al. 2011; Desai et al. 2021; Evans et al. 2022). The FRAI is regularly used to monitor the health of rivers in southern African freshwater ecosystems by assessing the response of fish assemblages to changes in habitat, water quality, and impacts of alien fishes (Kleynhans 2007; Wepener et al. 2011; Evans et al. 2022). The responses of different fish species to available biotopes and water quality are the variables used when assessing the ecological health of the system using FRAI and multivariate statistics to ascertain the drivers of changes in fish communities (Kleynhans 2007; Evans et al. 2022). The FRAI uses information from these environmental variables, together with existing databases (FBIS/PESEIS) of the intolerance and preference ratings for a variety of southern African freshwater fish species, to determine changes in fish assemblages from the expected natural state and the driving factors for these changes (Kleynhans 1999, 2007; FBIS 2023). Metric categories assessed in FRAI include habitat availability (velocity-depth classes), flow modification, migration, cover, physicochemical metric, and introduced species (Kleynhans et al. 2005; Kleynhans 2007; Desai et al. 2021; Evans et al. 2022).

1.4.5 Fish populations

The KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) forms part of the Cyprinidae family and is one of seven different yellowfish species (*Labeobarbus* spp.) recognised in southern Africa (Skelton 2001; Impson et al. 2008). It is endemic to KwaZulu-Natal and occurs in major catchments that host socio-economically important rivers, from the Mtamvuna River located at the Eastern Cape border to the Mkuze River in northern KwaZulu-Natal (Skelton 2001; Stobie et al. 2018; Desai et al. 2021; Evans et al. 2022). It is the most widespread yellowfish species in KwaZulu-Natal (Karssing 2007; Fouchy et al. 2019). Importantly, recent studies suggest that the species is more genetically diverse and presently describe that the species can be split further between catchment and sub-catchments, as in the case of the uThukela catchment (Stobie et al. 2018). *Labeobarbus natalensis* can be found in a range of different habitats but prefers habitats in the middle reaches of rivers that have a combination of deep pools and fast-flowing rapids and riffles and is especially selective during reproduction season (Karssing 2007; Jacobs 2017).

Labeobarbus natalensis spawn in fast-flowing rapids of high oxygen content over a gravel and cobble substrate. Mature breeding adults, sub-adults, and juveniles migrate seasonally upstream in search of suitable spawning and feeding niches (Skelton 2001; Karssing 2007; Burnett et al. 2021). *Labeobarbus natalensis* are potamodromous, migrating seasonally upstream in search of suitable spawning areas as the larvae are unable to burrow in silt-covered gravel and are susceptible to predation and displacement by current (Impson et al. 2008; Burnett et al. 2020, 2021). However, impenetrable barriers impede migration, especially into the upper reaches (Wright and Coke 1975a; Karssing 2007). Despite the relatively tolerant nature of *L. natalensis*, it is still vulnerable to anthropogenic impacts associated with land use changes, such as chronic pollution, siltation, and increased water abstraction and has been heavily impacted by acute events causing mass fish kills (Karssing 2007; Burnett et al. 2021). Instream dams and weirs throughout KwaZulu-Natal promote siltation that would otherwise wash away with flows, particularly silt resulting from agricultural runoffs (Karssing 2007).

Labeobarbus natalensis is also vulnerable to illegal fishing, particularly during spawning runs (Karssing 2007), and hybridisation with translocated OrangeVaal smallmouth yellowfish (*Labeobarbus aeneus*) in the upper Thukela catchment (Karssing 2007; Stobie et al. 2018). As noted by Karssing (2007), inter-basin water transfers and direct stocking for angling purposes may promote intraspecific hybridisation with genetically distinct *Labeobarbus* forms from different river systems (Impson et al. 2008; Stobie et al. 2018). There is now some genetic evidence of intraspecific hybridisation between *L. natalensis* populations in the Thukela and uMngeni catchments (Stobie et al. 2018). Another source of concern is the increasing threat by alien fish (e.g., largemouth bass (*Micropterus salmoides)*, trout (*Salmo trutta*), and common carp (*Cyprinus* *carpio*)) on native fish like *L. natalensis* (Burnett et al. 2023). Likewise, extralimital species, such as the *L. aenues*, compete directly with *L. natalensis* for food and habitat and prey on *L. natalensis* juveniles (Karssing 2007; Impson et al. 2008; Stobie et al. 2018). *Labeobarbus natalensis* is presently regarded as a species of least concern (Cambray et al. 2017); however, the species may be in decline because of the continual anthropogenic impacts on the aquatic ecosystems and fragmentation of the population because of barriers, such that regular surveys are recommended to monitor the state of the *L. natalensis*, particularly in areas where the species is susceptible to human activities like uMsunduzi and uMngeni catchments (Skelton 2001; Karssing 2007; Burnett et al. 2021).

1.5 Study area

The uMsunduzi River is a working urban river faced with many anthropogenic impacts. This makes the uMsunduzi River an ideal system for studying the responses of aquatic organisms to anthropogenic impacts. The uMsunduzi River flows through the capital city of KwaZulu-Natal Province, and is an important tributary of the uMngeni River, a strategic water resource for uMgungundlovu and eThekwini metropolitans, supporting more than four million residents (Matongo et al. 2015). The uMsunduzi system comprises a variety of land uses along the water column as it drains two-thirds of the metropolitan region.

The main uMsunduzi River flows through agricultural, domestic, municipal, and industrial zones to its confluence with the uMngeni River between Nagle and Inanda Dams. The land use activities along the uMsunduzi River provide a basic understanding of sources for anthropogenic stressors and their impacts along the course of the river as it flows to join the uMngeni River. The uMsunduzi River receives runoffs from municipal and industrial operations and periodic sewage inputs from dysfunctional sewers (Dickens and Graham 1998; Dickens and Graham 2002). The major wastewater treatment plant for the Msunduzi district releases its final treated effluent into the uMsunduzi River. The Darvill wastewater treatment plant receives wastewater from hospitals and domestic, commercial, and industrial sources (Gemmel and Schmidt 2013).

In addition to the already existing anthropogenic threats, in August 2019, a chemical spill occurred when a suspected valve burst on a holding tank for fatty acids, subsequently bringing down crude oil and caustic soda tanks (Groundup 2019). Approximately 240 tons of the mixed product effluent was left to flood the Baynesspruit tributary when (SABC 2019). 1.6 million Litres of the effluent entered the main stem of the uMsunduzi River via the Baynesspruit-uMsunduzi confluence and devastated aquatic life in the 82 km stretch of the river up to Inanda Dam (Environmental Justice Atlas 2020). The spill left a trail of destruction in aquatic life, with at least 50 tons of dead fish collected and livestock deaths reported by local farmers (eNCA 2019; Mail and Guardian 2019). In efforts to monitor the recovery from the spill and aid rehabilitation, we evaluated fish communities, diatom and macroinvertebrates assemblages at selected sections of the uMsunduzi River. Local KwaZulu-Natal yellowfish specimens from upstream breeding fish populations in the uMsunduzi system were sourced, fitted with internal pit tags, and translocated to re-introduction sites impacted by the spill. When yellowfish of substantial size were sourced from healthy upstream populations, external radio tags were administered through a surgical procedure (Thorstad et al. 2013; Burnett et al. 2020) and translocated into downstream sites, with the premise that adults would improve the population structures of impacted sites. The efforts were directed towards a post-spill biomonitoring program to track the natural recovery of the uMsunduzi

River and provide a comprehensive indication of the habitat conditions from which the behaviour of fish would be used.

1.6 Life history traits and distribution of *Labeobarbus* species

Yellowfish (*Labeobarbus* spp.) are endemic and widespread across the rivers of Africa, distributed over a wide range and occupying large African rivers and lakes where they use various niches across the distribution range (Impson et al. 2008; Burnett et al. 2018). Yellowfish are known to occur in large catchments in Africa, such as the Nile River, Niger River, Zambezi River, Congo River, the Great Rift Valley, and in lakes and rivers of Southern Africa (Skelton 2001; Impson et al. 2008). In the latter, they occur in the streams in KwaZulu-Natal, Orange River, and the Olifants River (Impson et al. 2008; Burnett et al. 2021; Evans et al. 2022). Yellowfish comprise 80 well-established and large African cyprinid lineages (Skelton 2001; Impson et al. 2008). The taxonomy of yellowfish in the southern African region has evolved since the early 1900s, and only six yellowfish species are recognised for the region at present, with a majority of previously recognised species lumped into existing lineages because of subtle differences in their characteristics and well-established intra-species morphological diversity, particularly the lip forms owing to diverse feeding strategies (Skelton 2001; Impson et al. 2008; Burnett et al. 2018, 2021).

The prominent characteristics of yellowfish include generous size, prolonged lifespan, and life history traits that indicate ecosystem well-being (Impson et al. 2008; Burnett et al. 2021). The yellowfish can grow up to 300 mm total length and attain a mass of above 3 kg (Skelton 2001; Impson et al. 2008); however, selected individuals of certain species can obtain a length of a metre and a maximum body mass of 30 kg (Skelton 2001). Yellowfish are spindle-shaped with shortbased fins and dorsal fins with simple bony and spiny anterior rays. The scales of yellowfish are strong and well-developed, comprising parallel striations along with the lateral line that runs from head to tail (Skelton 2001). The lateral line of *Labeobarbus* spp. often comprises scales from the range of 35-45, mostly with 16 scales around the caudal peduncle (Skelton 2001). The mouth of yellowfish is mostly subterminal with variable lip forms, from large fleshy lips for grubbing between cobbles to thin keratinised lips for scrubbing food off the rocks (Skelton 2001; Impson et al. 2008). They lack jaw teeth but have strong pharyngeal teeth in three rows that vary from heavy, rounded, crushing teeth to hooked teeth for taking soft food from mouth to intestine (Skelton 2001; Impson et al. 2008).

During breeding, yellowfish males and females are known to depict subtle physical differences; both sexes turn deep, brazen gold and develop small pimple-like nuptial tubercles on the head and sometimes all over the body (Skelton 2001; Dlamini 2019). Most yellowfish species are potamodromous migrators, and they migrate seasonally to headwaters for suitable spawning niches with water of high oxygen content, gravel beds, and cobble substrate for protecting eggs and providing refuge for fry to avoid predators (Impson et al. 2008; Dlamini 2019; Burnett et al. 2021). Juveniles of most *Labeobarbus* spp. shoal in a small school in peripheral waters and are often silvery with dark blotches that fade away as they mature (Skelton 2001). *Labeobarbus* spp. typically exhibit slow growth rates and are long-lived, with most species reaching 100 mm full length at 1 year and 300 mm full length at 5 years (Skelton et al. 2001; Burnett et al. 2021). The maximum life expectancy for yellowfishes varies with species ranging from 10 - 20 years of age, with females living longer than males (Skelton et al. 2001; Paxton et al. 2013; Burnett et al. 2021).

Yellowfish have omnivorous feeding habits, and their diet comprises invertebrates, diatoms, and detritus; however, some cyprinid lineages at larger sizes exhibit piscivorous feeding habits, particularly the Orange-Vaal largemouth yellowfish *L. kimberleyensis* (Skelton 2001; Impson et al. 2008).

Yellowfish thrive in fast, deep pools, rapids with boulders, and deep turbid waters and prefer habitats with diverse niches to accommodate their reproductive and feeding needs (Skelton 2001; Impson et al. 2008; Burnett et al. 2021). Yellowfish are amongst the targeted species by anglers for recreation, subsistence fishing, and the commercial fish industry in southern Africa (Skelton 2001; Impson et al. 2008; Brand et al. 2009). Amongst the well-recognised *Labeobarbus* species in the southern region are the Vaal-Orange largemouth yellowfish (*L. kimberleyensis*), Vaal-Orange smallmouth yellowfish (*L. aeneus*), Clan Willian yellowfish (*L. capensis*), Lowveldlarge scale yellowfish (*L. marequensis*), bushveld small scale yellowfish (*L. polylepsis*), upper Zambezi yellowfish (*L. godringtonii*) as well as the KwaZulu-Natal yellowfish (*L. natalensis*) (Skelton 2001). Cyprinid species are useful ecological indicators because of their life history traits and acute response to environmental changes relevant to stressors of water resources (Burnett et al. 2021). Cyprinid species have been translocated outside their natural ranges through stocking for recreational purposes and inter-basing water transfer schemes (Skelton 2001; Impson et al. 2008; Brand et al. 2009).

1.6.1 *Labeobarbus natalensis* as a flagship species to indicate river health

The KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) (Castelnau 1861) are among the well-studied *Labeobarbus* species, endemic and widespread in KwaZulu-Natal streams (Skelton et al.

2001; Impson et al. 2008; Dlamini 2019; Burnett et al. 2021). *Labeobarbus natalensis* use a range of freshwater niches, ranging from fast deep pools, rapids with boulders as a cover feature, and deep turbid waters, whereas juveniles use shallow, marginal habitats and use instream and overhanging vegetation cover features for refuge (Skelton 2001; Dlamini 2019; Burnett et al. 2021). The males of the KwaZulu-Natal yellowfish mature as yearlings at 100 mm total length, while females mature at two years, attaining a full length of 150 mm and an estimated fecundity of 20,000 ova per female (Skelton 2001). *Labeobarbus natalensis* exhibit omnivorous feeding habits, and their diet comprises crabs, nymphs, detritus, and algae (Skelton 2001; Impson et al. 2008). During the austral summer months, when environmental conditions improve, *L. natalensis* are prompted by environmental cues to migrate to the river's upper reaches to search for food resources and suitable spawning niches (Dlamini 2019; Burnett et al. 2021). *Labeobarbus natalensis* are known to be particularly selective when spawning and prefer fast-flowing rapids with gravel and cobble substrate for attachment of fertilised eggs and the burrowing of fry post-hatching to avoid predation and displacement by high flows (Impson et al. 2008; Dlamini 2019).

Environmental cues and seasons initiate the facultative migration of *L. natalensis*, with juveniles migrating to the lower reaches of the water bodies to recruit in schools (Wright and Coke 1975a,b; Burnett et al. 2021). Instream physical and chemical barriers affect the potamodromous migration of *L. natalensis* to and from spawning sites, and the presence of impoundments and weirs influence natural flow regimes and limit access to suitable habitats for food resources and reproduction (Impson et al. 2008; O'Brien et al. 2019; Burnett et al. 2021). Furthermore, yellowfish populations face exploitation through destructive fishing methods such as illegal gill netting and poisonous fishing during fish spawning runs (Impson et al. 2008; Tempelhoff 2009). Although *L.*

natalensis exhibits some level of tolerance to stressors, their behaviour is known to be 10 to 100 times more responsive to environmental disturbances and are still susceptible to the effects of stressors through niche alteration and chemical pollution (Dlamini 2019; Burnett et al. 2021).

Poor phylogenetic relationships and inter-basin translocation of yellowfishes, as well as the existence of forms elsewhere in Africa, result in uncertainty amongst yellowfish species in Africa (Skelton 2001; Impson et al. 2008; Chakona et al. 2022). The translocation of *Labeobarbus* spp. outside of their natural home ranges further exacerbates the ecological problems, providing possibilities for hybridisation and the invasion of exotic introduced fish species (Impson et al. 2008). This necessitates comprehensive genetic studies in *Labeobarbus* spp. occurring in the KwaZulu-Natal rivers to inform inter-system translocation studies aiming to improve the recovery of impacted populations after catastrophic events.

Labeobarbus natalensis is a charismatic angling species that provides food and income benefits and a flagship species that can be used to rehabilitate and monitor recovery after catastrophic fish kill events owing to their life history traits (Impson et al. 2008; Burnett et al. 2021). The acute response to environmental changes and suitable life history traits as a flagship species, *L. natalensis* was selected as a model fish for monitoring of freshwater ecosystems, compounded with telemetry advancements that allow fish marking with pit tags and real-time tracking to quantify movement and survival of translocated and tagged fish (Burnett et al. 2020, 2024; Sonamzi et al. 2020).

1.7 Hypotheses and predictions

We hypothesised that diatoms, macroinvertebrates, and fish communities in the uMsunduzi River are impacted by stressors and can be used as lines of evidence to monitor and rehabilitate the uMsunduzi River after a chemical spill. It was predicted that the uMsunduzi River would generally exhibit poor ecological health, driven by the impacts of anthropogenic activities around the river system, and that the well-being of *L. natalensis* populations along the course of the uMsunduzi River would be compromised as a result of instream physical barriers, habitat modifications and periodic inputs of sewage and industrial effluents.

1.8 Aims and objectives

The study aimed to gather literature on the science of fish kill by monitoring the water quality and fish community structures along the uMsunduzi River, focusing on *L. natalensis* as an ecological indicator species. Furthermore, we aimed to translocate the telemeter pit and radio-tagged *L. natalensis* to understand their persistence in the impacted sites and their longitudinal movement in the system. To achieve these aims, a systematic review of fish kills was conducted, highlighting the impacts of fish kills globally. The water quality was monitored through in situ physicochemical measurements, water sample analyses, diatom assessment and macro-invertebrate evaluation. Fish communities were evaluated using the Fish Response Assessment Index (FRAI). Multivariate analyses were used to correlate environmental variables and biotic responses to determine the environmental attributes driving community shifts. Individual *L. natalensis* were sourced from yellowfish populations recruiting in tributaries and upstream locations, fitted with telemeter tags, and translocated to re-introduce the downstream sites impacted by the spill.

1.9 Structure of the thesis

The main body of this thesis is organised as manuscripts prepared for publication in peer-reviewed journal articles. The first chapter (Chapter 1) is the Introduction, which provides a literature review of the concepts covered in this study. The next four chapters (Chapter 2, 3, 4 and 5) are empirical chapters, each covering a specific objective. Each chapter is formatted according to the journal it is intended to be submitted to. Because of this thesis format, a certain degree of repetition was unavoidable, especially in the methods section. However, this is deemed to be of little concern as this format allows the reader to read each chapter separately without losing the overall context of the thesis.

The Chapters are as follows:

Chapter 2: A systematic review of global regional fish kills with an emphasis on South Africa;

Chapter 3: Evaluating diatoms, macroinvertebrates, and water quality following a severe pollution event in the uMsunduzi River, KwaZulu-Natal;

Chapter 4: Pre, post and present ecological conditions in an environmentally and economically important river: the uMsunduzi River, Pietermaritzburg, South Africa;

Chapter 5: Conditions of freshwater ecosystems need consideration in the application of telemetry techniques: Lessons from using telemetry to monitor fish in an urban river;

Chapter 6: Conclusions and recommendations.

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CHAPTER 2

A systematic review of global regional fish kills with an emphasis on South Africa

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Formatted for the *Journal of Fish Biology*

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Running header: A systematic review of regional fish kills

2.1 Abstract

Freshwater ecosystems maintain human livelihoods, and these systems are subject to a plethora of anthropogenic impacts because of ongoing human activities. The anthropogenic stressors impact the state of water quality, causing deterioration in the ecological integrity of the river and leading to fish kill events. This review aimed to determine and assess the science of fish kills, documentation and mitigation measures. A systematic approach was used to gather, assess and categorise research articles conducted across various regions over the last century to achieve this goal. Eligibility for including articles required demonstrating a direct impact of a fish kill event. A total of 183 articles found met the eligibility requirements and were included in this review. The results of this review demonstrated that fish kills are rising, and their impacts have intensified. Fish kills impact the ecological integrity of a system and limit income benefits, particularly for local fishermen. Moreover, anthropogenic stressors accounted for most (90%) fish kills documented, and only 10% of fish kills were caused by natural perturbations. The rise in fish kills because of changing demographics requires holistic, evidence-based monitoring strategies to guide effective management decisions and recovery.

Keywords: Fish mortalities; Fish die-offs; Fish indicators; Mass mortality, chronic fish kills

2.2 Introduction

Freshwater ecosystems and the associated biota are threatened by many stressors that cumulatively and synergistically impact their well-being (Holden 1996; La and Cooke 2011; Vasas et al. 2012; Wade et al. 2021; Chakona et al. 2022). Mass fish mortalities are relatively common and result from the impacts of stressors, impacting ecosystem services, causing declines in aquatic biodiversity and negatively impacting hedonic values (Stocks et al. 2022). The recent rise in fish kill frequency and deteriorating river ecological health has raised concerns regarding the management of rivers globally (Koehn 2021). Fish kills are described as mass mortalities of fish in streams, lakes, or dams caused by either natural phenomena, anthropogenic stressors, or synergistic effects of both (Meyer and Barclay 1990; La and Cooke 2011; Koehn 2021). Fish are largely associated with culture, recreation, food, and income benefits, particularly by vulnerable communities (Evans et al. 2022). Fish mortalities have serious cascading effects on the cultural, economical, and conservation values of fish and associated beneficiaries (La and Cooke 2011; Koehn 2021; Chakona et al. 2022). Fish kills typically involve indigenous and charismatic species targeted for recreational angling, food, and income benefits, so they severely impact the economic and ecological value of freshwater ecosystems (Greenbank 1945; La and Cooke 2011; Koehn 2021). Poor management and recovery from fish kills exacerbate the economic losses, particularly when the afflicted stream is important for industry operations, tourism, and water resources that support human livelihoods (La and Cooke 2011; Koehn 2021). Although the frequency and intensity of stressors and fish kills have been rising globally, management strategies remain rudimentary (La and Cooke 2011; Grant et al. 2014).

2.2.1 Fish kills and ecosystem services

The interaction between fish and people dates back to ancient times, as depicted in the hieroglyphics of ancient Egypt and the rock structures in South Africa (Hall 1997; Impson et al. 2008; Burnett et al. 2021). Historical information forms a base for understanding communities' modern cultural and financial dependence on fish (Impson et al. 2008). Freshwater ecosystems provide many vital ecosystem services to sustain human livelihoods (Evans et al. 2022). The threats to freshwater systems necessitate strategic management and conservation strategies to maintain river ecological health and conserve aquatic biota (Pimm et al. 2001; Thronson and Quigg 2008). Freshwater ecosystems must have limited impacts from stressors to maintain fundamental and demand-derived ecosystem services (Holmlund and Hammer 1999; O'Brien et al. 2019). Fundamental ecosystem services facilitate energy exchange and functionality of freshwater ecosystems (Holmlund and Hammer 1999). Demand-derived ecosystem services are associated with economy and culture, so these practices rarely prioritise the protection of nutrient and energy recycling (Holmlund and Hammer 1999; Dudgeon 2019; Lynch et al. 2020). Including biomonitoring in management provides a holistic, evidence-based approach to conserving and managing freshwater ecosystems (Wepenaar 2008; Burnett et al. 2018, 2024). Particularly to take action for sustainable development goals set forth by the United Nations and ensure optimal ecological status and welfare of riparian communities (Holmlund and Hammer 1999; Lynch et al. 2020). Fishes have been an affordable and reliable source of food since ancient times, and today, vulnerable communities still rely on fish for food and income benefits (Holmlund and Hammer 1999; Impson 2008; Koehn 2021; Stocks et al. 2022). Therefore, fish kills largely impact dependent communities and raise concerns about managing aquatic ecosystems (Thronson and Quigg 2008; Koehn 2021). The rise in frequency and intensity of fish kill events requires strategic evaluation, documentation, and further monitoring to swiftly mitigate and prevent future fish kills (Grant et al. 2014; Koehn 2021).

2.2.2 Origin of fish kills

The welfare of aquatic organisms is largely influenced by the surrounding environmental conditions and the interaction with surrounding media (Grant et al. 2014; Wade et al. 2021; Burnett et al. 2018, 2020). Aquatic habitats comprise variable niches, physicochemical, biotic, and abiotic properties which are influenced by stressors (Donaldson et al. 2008; Wepener et al. 2011; Grant et al. 2014; Burnett et al. 2021). Fish behaviour has been noted to be at least 10 to 1000 times responsive to change in the aquatic environment and has been incorporated as a line of evidence in aquatic research since the 1950s (Beitinger 1990; Wepener 2008; Burnett et al. 2024). Fish show multiple responsive stages to stressors, with the initial stage depicted by releasing stress hormones such as cortisol to achieve homeostatic biological conditions and cope with extreme conditions (Koakoski et al. 2012; Grant et al. 2014). The secondary response involves biochemical and physiological changes such as altered behaviour, finding refuge sites, and eventually, the saturation of stress-response mechanisms where an organism can no longer cope with the stress and faces the fate of mortality (Whitfield 1995; Koakoski et al. 2012; Grant et al. 2014). Different lineages in aquatic communities depict varied tolerances to stressors (Koakoski et al. 2012; Grant et al. 2014). Furthermore, distinct size classes of the same species exhibit varied tolerances to stressors. Adults and sub-adults are more tolerant of stressors than juveniles because of well-developed stressor response organ systems (Whitfield 1995; Koakoski et al. 2012; Grant et al. 2014).

The rudimentary documentation of fish kills across global regions is fuelled by a subjective definition of a kill event amongst regions, so a more objective, universal description was established to ease the diagnosis and mitigation of fish kill events globally (La and Cooke 2011; Grant et al. 2014). According to La and Cooke (2011), fish kill is a phenomenon where at least 25 dead fish occur within a square kilometre and one kilometre of lentic and lotic water bodies, respectively. Moreover, fish mortality must not form part of the natural life cycle, such as post-spawning in semelparous fish, harvest, and predation events. This universal definition of fish kills was aimed at easing the identification, mitigation, documentation and monitoring of such catastrophes globally across regions, including developing countries (La and Cooke 2011; Koehn 2021). Most existing fish kill reports are on commercial fisheries, and most are held by private societies (Thronson and Quigg 2008; Grant et al. 2014). The limited access to fish kill reports affects the implementation of management strategies, monitoring, and mitigation of fish kills to protect aquatic biodiversity and water resources (Grant et al. 2014; Lynch et al. 2020).

Fish kills may originate from natural processes; however, anthropogenic activities are responsible for major fish kill events globally (Tempelhoff 2009; La and Cooke 2011; Wepener et al. 2011). River stressors with the most negative impact are largely the by-products of commercial agriculture, industrial processes, transport, changing demographics, and climate change (Wepener et al. 2011; Wade et al. 2021). Changing demographics and expanding economies contribute to fertilisers, pesticides, chemical spills, sewage effluent, and hydropower discharge into the aquatic ecosystem (Dudgeon 2006; Dudgeon 2010). These stressors impact the ecological integrity and functional capacity of aquatic ecosystems through fish kills caused by oxygen depletion, water toxicity, thermal pollution, eutrophication, regulated flows, and harmful planktonic blooms (Raleigh et al. 1978; Tempelhoff 2009; Wepener et al. 2011; Grant et al. 2014; O'Brien et al. 2019; Burnett et al. 2021). Natural phenomena account for a large proportion of fish kills, with natural processes such as diseases, low oxygen content, acidification, gas bubble trauma, and temperature fluctuation responsible for 10% of the documented fish kill events globally (La and Cooke 2011; Grant et al. 2014).

2.2.3 Fish kills

Human activities have impacted freshwater systems since early civilization, and these impacts on freshwater ecosystems are apparent from fish mortality endpoints as a line of evidence (Moshiri et al. 1978; Poleo and Bjerkely 2000; Research Watch 2003; Borsuk 2004; Brand et al. 2009; AnsaraRoss et al. 2012; Thresher et al. 2018). The difficulty of dealing with fish kills necessitates a holistic, proactive approach, particularly record keeping of fish kill events for future inference and preventative measures (La and Cooke 2011; Grant et al. 2014). Recent advancements in telemetry have seen the incorporation of fish behaviour in freshwater monitoring (Thorstad et al. 2013; Burnett et al. 2020, 2024; Sonamzi et al. 2020). Fish response to changing environmental conditions is acute; therefore, biomonitoring can be used to monitor environmental changes in real-time (Burnett et al. 2020, 2024).

The present review aimed to gather literature on fish kill events across various regions to highlight the impacts if human activities. We then focussed mainly on fish kills in South Africa, especially those documented to have devasted the respective rivers. We relied on grey literature, newspaper articles, peer-reviewed journals and local knowledge. We predicted that fish kill events have increased in frequency and intensity.

2.3 Methods

We carried out a comprehensive literature search for documented fish kill events using the

PRISMA method (Moher et al. 2009) as well as additional searches. We compiled data from internationally peer-reviewed journals, accessible reports, and newspapers. The journals were found using Google Scholar, Scopus, and Web of Science. Newspaper articles and reports were collected from private websites by using the Google search engine. The keywords “fish kills”, “fish mortalities”, “fish die-offs”, “fish indicators” and “fish” were used when searching for fish kill reports and articles. We limited the time frame of the literature search from 1940 to the present. The selection criteria for inclusion involved access to full abstract and article, relevance, and wildlife-based research. Articles with the date of when the fish kill occurred, the severity of the fish kill according to the number of fish dead and area impacted, location of the impacted water body and area impacted, as well as the type of fishes implicated in the fish kill was considered in the analysis, whereas papers lacking such information were excluded. The journal results were classified according to major global regions, such as North America, South America, Europe, Oceania, Asia, and Africa, depending on the country where the fish kills were reported.

We used descriptive statistics to summarise the trends. We mainly used frequency assessments.

2.4 Results and Discussion

We retrieved 1200 articles. The total studies included in the review were relatively few (n = 183) (Figure 2.1). Total reports and newspaper articles included in the review (n = 78) (Figure 2.1) We found the number of fish kills increased substantially since the 1990’s (Figure 2.2).

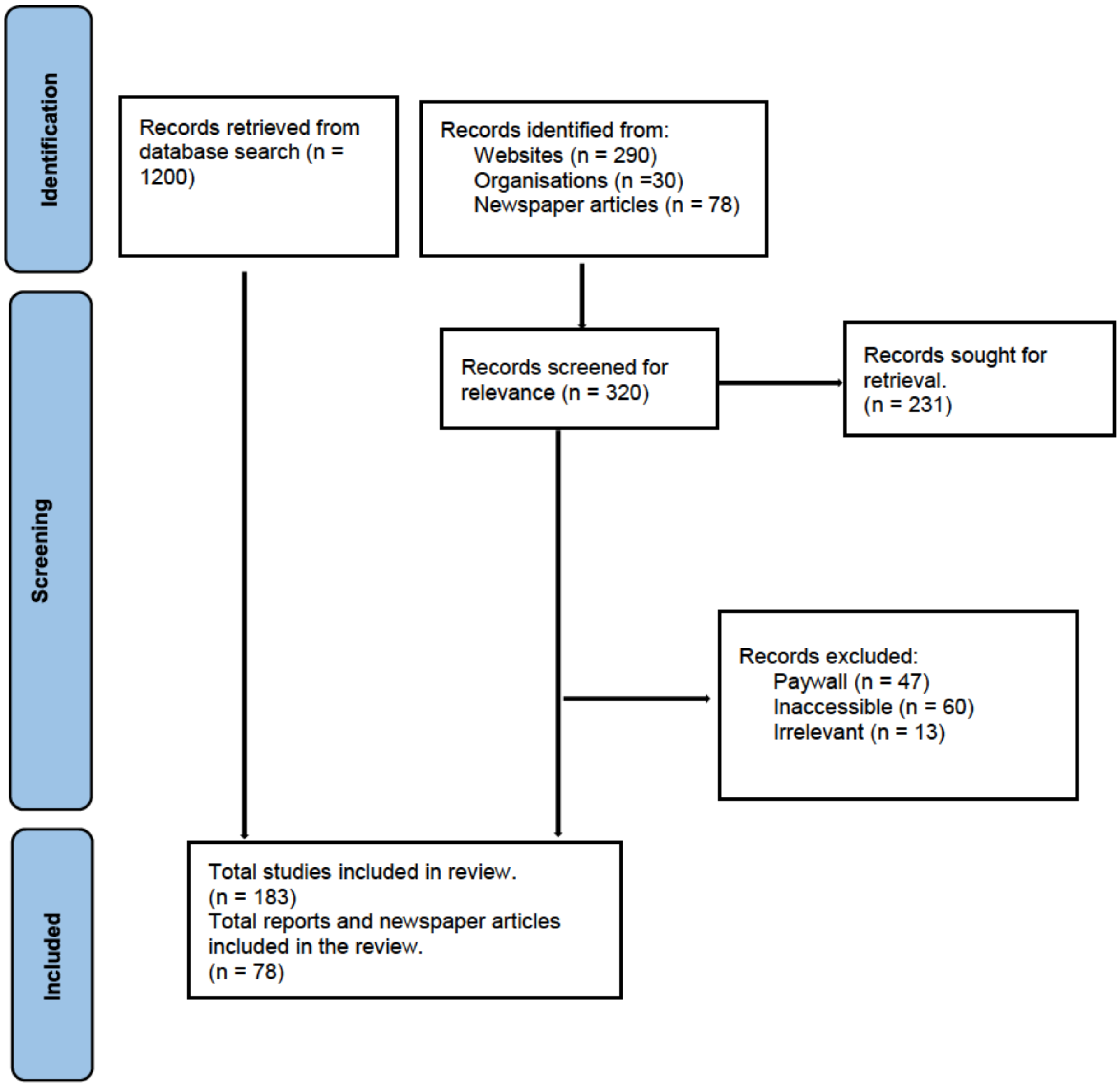

Figure 2.1: Summary of publications found and the number remaining after assessment.

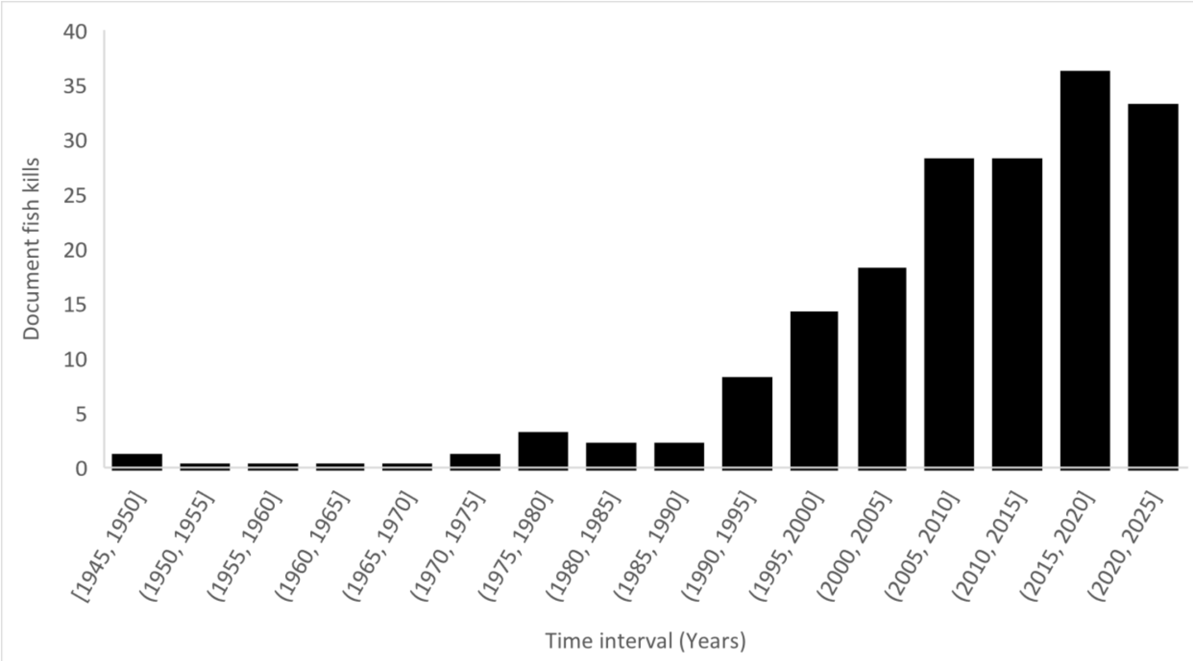

Figure 2.2: Frequency timeline of documented regional fish kill and future projections.

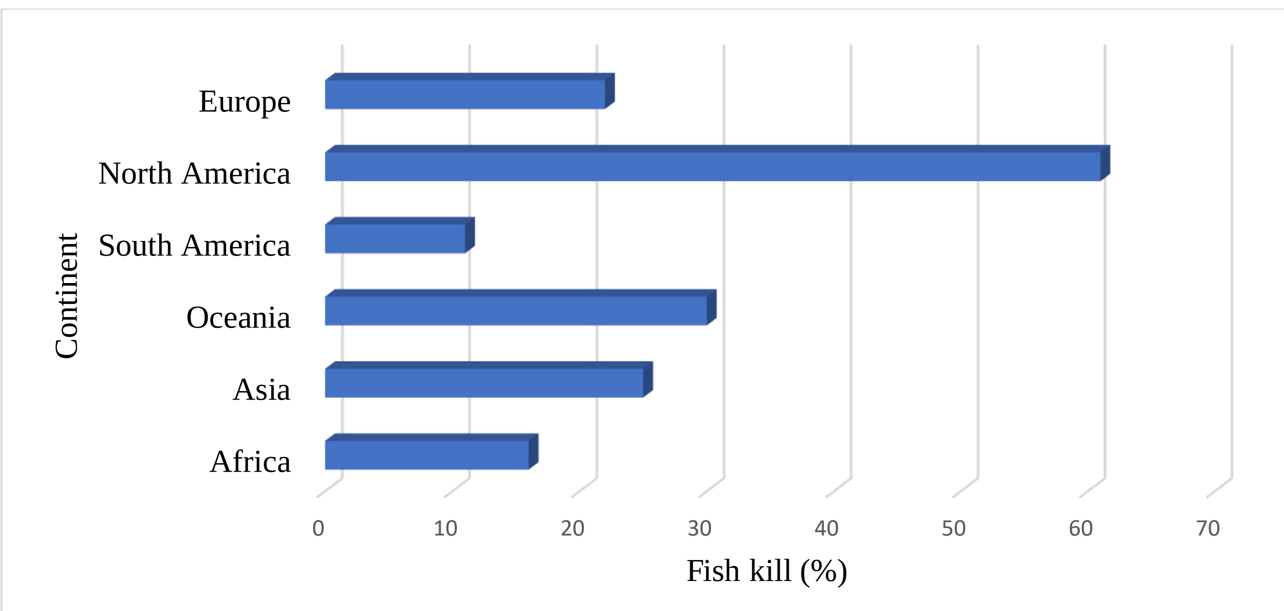

Figure 2.3: Percentage of literature review on fish kills by continent, sourced from accessible fish kill reports, grey literature and published peer-reviewed journals.

We found fish kill records exist across regions of the globe. Most of the fish kills reported were in the northern hemisphere (Figure 2.3). Although the reporting may be fragmented and reports held by private institutions, they provide a baseline for advancing fish kill management, such as the 1936 winter fish kill in the Michigan Lakes, United States of America (USA) (Greenbank 1945; Mitchel 2003). Thousands of fish were observed to pile up along the banks of the lakes, and the fish kill was attributed to low oxygen and heat exchange as the water was frozen (Greenbank 1945). During the winter of 1967, 198 million m^3 of caustic alkaline slurry from the Appalachian steam-power generation plant entered the Clinch River, Virginia, USA, when a valve burst on a fly ash holding pond (Raleigh et al. 1978). The fish kill impacted aquatic fauna severely; benthic invertebrates perished in the first 7 km stretch of the river, and index catches and species richness were reduced from 79% to 72% and 15 species to seven species, respectively (Raleigh et al. 1978). In the subsequent winter of 1970, a sulfuric acid effluent from an Appalachian steampower generating company entered the Clinch River, Virginia, USA (Raleigh et al. 1978). The incident killed 5,300 fish and severely reduced aquatic fauna in the river (Raleigh et al. 1978). The periodic wastewater leaks and algal blooms in the Bayou Texar estuary, Florida, USA, led to a 5week fish kill event, where at least 2.25 tons of fish were removed daily from the estuary (Moshiri et al. 1978). The suspected cause of fish mortality was low dissolved oxygen because of the phytoplankton blooms (Moshiri et al. 1978).

Millions of dead hairback herring (*Nematalosa erebi*) and hardyhead (*Craterocephalus eyresii*) were found along the shores of Lake Eyre, Australia, in July 1975 (Ruello 1976). The cause of this fish mortality remains an enigma; various speculations point to algal blooms and low oxygen as possible causes of fish mortality (Ruello et al. 1976). The mass mortality of approximately 3400 fish belonging to eight species was observed in the Billabong River, Australia, in January of 1980 (Brow et al. 1983). The fish kill was suspected of having resulted from acid water runoff based on elevated biotoxin aluminium levels on fish when assessed (Brown et al. 1983). The effects of golden algae (*Prymnesium parvum*) in the Pecos River, Texas, USA, were observed through the mass killing of fish in 1986 (Rhodes and Hubbs 1992). The fish kills have been observed to be frequent in the Pecos River since 1985, impacting fish community structures and diversity (Rhodes and Hubbs 1992).

Fires are beneficial to managing rangelands and removing moribund biomass; however, they impact freshwater ecosystems, particularly water chemistry and geomorphology (Bozek and Young 1994). The abundant and widespread nutrients after fires influence water chemistry and induce fish kills (Bozek and Young 1994). Mass fish mortalities were recorded in the greater Yellowstone River, Wyoming, USA, following the 1988 fires (Minshall et al. 1989; Bozek and Young 1994). In October 1991, a mass mortality of approximately 190,000 fish in Roanoke River, Virginia, USA, occurred when an accidental spill of liquid manure from a dairy farm entered the river (Ensign et al. 1997). The manure effluent impacted 9 km of the Roanoke River through its confluence with Elliot Creek, where the spill originated (Ensign et al. 1997). In 1996, the dinoflagellate *Gymnodium pulchellum* devastated the Indian River lagoonal system in Florida, USA, wiping scores of fish through algal blooms and toxic chemicals (Steidinger et al. 1998). The incidents were frequent and often implicated at least eight fish species, crabs, and shrimp (Steidinger et al. 1998).

A largemouth bass (*Microterus salmoides*) virus-induced fish mortality during the spring of 1998 in the Sardis reservoir, Mississippi, USA, involved at least 3000 adult bass of various size classes ranging from 0.5 to 3 kg (Hanson et al. 2001). A fish kill that included the freshwater wallago catfish (*Wallago attu)*, a game fish, was reported in the Panipat region of India (Parvez et al. 2006). The fish kill wiped out scores of indigenous fish and was suspected to be induced by an industrial effluent (Parvez et al. 2006).

Natural perturbations and human activities have been implicated in the 637 recorded fish kill events between 1984 and 2002 in Florida, USA, freshwater ecosystems (Hoyer et al. 2009). During the summer of 1997, a fish kill of 1,300,000 fish was observed in the Alafia River when 220 million litres of acidic process water from a phosphate plant in Mulberry, Florida, accidentally spilt into the river (Hoyer et al. 2009).

Seasonal changes induce winterkills in European regions since freshwater bodies freeze in winter (Ruuhirjavi et al. 2010). The winter fish kill in Lake Aimajarvi, Finland, which impacted an area of 8.5 km^2 and 12 fish species in 2003, was attributed to seasonal change (Ruuhirjavi et al. 2010). The combined impacts of climate change and anthropogenic stressors are detrimental to river ecological integrity (Stocks et al. 2022). Prolonged periods of low flow, followed by abrupt flooding, affect aquatic organisms negatively (Thiem et al. 2020).

Hypoxic conditions were suspected following a massive fish kill in the Murray Darling River, New South Wales, Australia, where millions of prominent flagship species such as the Murray cod (*Maccullochella peelii*), trout cod (*Maccullochella macquariensis*) and silver perch (*Bidyanus Biyanus*) were wiped out (Koehn 2021; Stocks et al. 2022; Thiem et al. 2022). The estimated cost incurred to mitigate the fish kill was 4-5.6 million Australian dollars (Koehn 2021; Stocks et al. 2022). The prolonged fishery closure limited economic productivity and revenue generating abilities (Koehn 2021; Zampatti et al. 2022). Furthermore, fish population structures were severely impacted, and a population decline below 10% of the natural capacity was reported (Koehn 2021; Stocks et al. 2022). To re-establish normal population structures and pre-kill ecological conditions, monitoring is important to guide evidence-based rehabilitation strategies, and fish kill records should be kept for future inferences to improve fish kill management (Grant et al. 2014; Zampatti et al. 2022).

Fish kill records exist in the South America region (Starling et al. 2002; Sinkels et al. 2014; Schulz and Costa 2015; Azevedo-Santos et al. 2016; Pacheco et al. 2021). The African region had a limited number of fish kill records and peer-reviewed monitoring techniques to mitigate such catastrophes, and this necessitates monitoring techniques guided by scientific knowledge in the region (Grant et al. 2014).

In 1990, a massive fish kill was observed in Lake Victoria (Ochumba 1990). The disintegration of algae in Lake Chivero, Zimbabwe, is implicated in the increased frequency of fish kills since earlier centuries (Mhlanga et al. 2005). Low dissolved oxygen was suspected to perpetuate the fish kill episodes in 2005 in Lake Chivero, where scores of the Nile tilapia (*Oreochromis niloticus*) were killed (Mhlanga et al. 2005).

More fish kills were reported in South Africa than in the rest of Africa. However, many were reported in the press rather than in peer-reviewed papers. They showed graphic photographs of the fish kills (Supplementary information Figures S2.1 - S2.4). The early available documents of fish kills in South Africa report the effects of temperature and salinity on a fish kill of more than 100,000 individuals in St Lucia Lake during July 1976 when the salinity of the lake drastically dropped beyond optimum levels, eliminating scores of freshwater cichlids (*Oreochromis mossambicus*) (Whitfield 1995). Low salinity also killed ~7000 fish in the Botriviervlei Lake, situated on the southern coast of the Western Cape, in October 1981 (Whitfield 1995). Excessive abstraction of water for farming compounded by drought-induced hypersaline conditions in the Seekoei Estuary on the coast of the Eastern Cape killed 6000 fish in April 1989 (Whitfield 1995).

The decline in water temperatures beyond optimum conditions in the Kasuka Estuary of the Eastern Cape induced a fish kill event in July 1979, where scores of freshwater cichlids (*Oreochromis mossambicus*) were wiped out (Whitfield 1995). Other fish kills were reported in the Elands River in the Crocodile River catchment in South Africa (Kleynhans et al. 1992; O'Brien et al. 2014).

Low dissolved oxygen is responsible for many fish kill events in South African streams through the suffocation of fish (Grant et al. 2014). Low dissolved oxygen may arise from inputs of treated sewage effluents and sugar mills into the rivers, such as the 1977 fish kill caused by oxygen depletion in the water (Whitfield 1995). Siltation negatively impacts freshwater ecosystems by increasing turbidity and sediment suspension, which clogs fish gills (Dudgeon 2000). According to Whitfield (1995), the fish kill events on 16 January 1995 resulted from suspended sediments on the water column, interfering with osmoregulation and leading to fish kills. In addition, the Phalaborwa barrage resulted in a decline in dissolved oxygen and silt-induced mechanical damage to the gills of freshwater cichlids (*Tilapia rendalli*) (Smit 2012). Managing the same Phalaborwe barrage to reduce siltation often results in downstream fish kills from highly turbid and deoxygenated water caused by flushing (Riddel et al. 2019). The Hartbeespoort impoundment, located at the confluence of the Crocodile-West River and Magalies River, is burdened by the highly populated areas of Pretoria, Johannesburg, and Krugersdorp to receive excessive nutrient inputs that cause algal blooms (*Macrocystis auriginosa*) and rapid growth of aquatic weeds (*Eichonia crassipes*) (DWAF 1999). The Kosmos area, along the bank of the Hartbeespoort Dam, experienced a fish kill in October 1999, originating from the effects of eutrophication and a decline in oxygen levels because of the expansion of photosynthetic phytoplankton and decomposing organic material (DWAF 1999). At least 20 dead common carp

(*Cyprinus carpio*), a fish targeted by recreational fishers, were observed during that fish kill event (DWAF 1999).

The Olifants River, running through the Kruger National Park, South Africa, is amongst the perennial rivers in South Africa, but in its current state, the flow is altered and can no longer sustain the needs of its biological components (Mail and Guardian 2005; Marr et al. 2017). Hippos (*Hippopotamus amphibius*) congregate in the remaining pools, and the accumulation and degradation of their excretory waste in their pools reduces dissolved oxygen levels, causing fish to suffocate (Mail and Guardian 2005; Smit 2021). Along the banks of the Olifants River, in August 2005, fish were affected by the altered river flow and reduced dissolved oxygen levels in the river that induced a fish kill event involving yellowfish (*Labeobarbus* spp.); cichlid (*Oreochromis mossambicus*) and catfish (*Claria gariepinus*) of approximately 500 individuals observed after scavenger birds had a fed on the dead fish (Mail and Guardian 2005).

The Vaal River, one of South Africa's most important rivers for commercial and domestic activities, has been deteriorating because of continuous industrial and sewage pollution caused by failing infrastructure and a lack of legislative enforcement (Tempelhoff 2009). The decomposition of sewage causes a decline in oxygen levels in the river, threatening aquatic biota and the communities reliant on water from the river (Tempelhoff 2009). The effects of sewage pollution led to a fish kill in 2006 along the Vaal system because of a lack of adequate oxygen, eliminating scores of indigenous yellowfish (*Labeobarbus* spp.) (Tempelhoff 2009). Similar effects of infrastructure failure were reported in the Isipingo River estuary in 2008, where cable theft cut the power supply to sewage pumps, and this resulted in large volumes of raw sewage effluent depleting oxygen in the river and causing fish mortalities (News 2008). More than 20 fish were reported dead (News 2008). The continual industrial and sewage spills within reach of the Isipingo River are causing the river to deteriorate into an unacceptable ecological state (The Mercury 2014). Periodic sewage pollution spills and semi-treated industrial effluent from a beer and potato chips factory via the stormwater canals caused thousands of fish to die in the Umdloti lagoon in 2014, as reported by residents (The Mercury 2014).

The limited capacity of the infrastructure for the Emfuleni Municipality along the Vaal River to treat sewage accordingly since a decade ago has compromised the ecological state of the river (Saturday Star 2018). Failure of sewer networks in Vereeniging and Vanderbijlpark has led to chronic pollution, resulting in fish deaths of the indigenous yellowfish (*Labeobarbus* spp.) and putting reliant communities at risk of disease (Saturday Star 2018).

The Groenvlei Lake, situated near Sedgefield, Western Cape, is a rare ecosystem, home to two endemic fish species, the estuarine round herring (*Gilchristella aestuaria*) and the Cape silverside (*Atherina breviceps*) (Cape Times 2018). The former species co-exist with well-established alien invasive fishes such as common carp (*Cyprinus carpio*) (Cape Times 2018). During early October 2018, fish started dying in small numbers, subsequently increasing in numbers and species, including bass (*Micropterus* spp.) and blue gill (*Lepomis microchirus*), observed floating along the banks of the lake in the reeds (Cape Times 2018). The principal cause of the fish kill was difficult to pinpoint since the common carp and tilapia species were not affected (Cape Times 2018). Poor water quality and alkalinity were the suspected causes of fish kill from water samples tested by the garden route municipality and SANParks (Cape Times 2018).

The uMsunduzi River is amongst the valuable tributaries of the uMngeni River, stretching 115 km across the midlands of KwaZulu-Natal Province. The rivers jointly provide water to ~4 million people between uMgungundlovu and eThekwini metropolitans (Gemmel and Schmidt

2013; Matongo et al. 2015; Dlamini 2019). In November 2018, scores of dead fish were observed floating and washed up on rocks along the riverbank in the popular hiking area of the river in Ashburton (The Witness 2018). The concerned citizens reported the catastrophe to the DuziuMngeni Conservation Trust (DUCT), who further made the matter apparent to the Msunduzi Municipality (The Witness 2018). More than 20 fish were observed floating on riverbanks and further downstream over the rocks with a foul smell of rotting fish. DUCT pollution control officers suspected a concentrated industrial effluent as the primary cause of fish kill, whilst the Msunduzi officials suspected a dramatic temperature fluctuation as the cause (The Witness 2018). Leakages from the Darvill Wastewater Treatment Works were also suspected as the cause of the fish kills following a power outage; however, it was reckoned to be further from the location of the fish kill as the release of water from the Henley Dam dilutes the sewage spills (The Witness 2018).

The collapse of the crude oil storage tanks at Willowton Oil Mills, Pietermaritzburg, left six workers injured, and moreover, fatty acid and caustic soda storage tanks released 240 tons of slimy, toxic effluent to flood the Baynesspruit that joins the uMsunduzi River a few kilometres downstream from the spill (Averda News 2019; Environmental Justice Atlas 2019; Getaway Magazine 2019; George Herald News 2019; International Spill Control Magazine 2019). As a result, ~1.6 million litres of the effluent reached the uMsunduzi River through the BaynesspruituMsunduzi confluence tributary and severely impacted the ecological integrity of the river up to 80 km downstream, causing significant deaths of aquatic fauna and flora (Business Insider South Africa 2019; Capital News 2019; DUCT 2019; Dusi Canoe Marathon 2019; ENCA News 2019; South African People News 2019). Indigenous fish such as yellowfish (*Labeobarbus natalensis*), cichlids (*Tilapia* spp. and *Orechromis* spp.), and sharptooth catfish (*Clarias* *gariepinus*) were severely affected by the pollution event causing mortalities, such that yellowfish were observed by subsistence anglers jumping out of the water before dying (African Wild Forum 2019; Mail and Guardian 2019; SABC News 2019; The Witness 2019; Trialogue Knowledge Hub 2019). Residents along the low-lying Grimthorpe Bridge area reported an apparent chemical smell, milky white foam along the river column, and oil film in the riverbanks extending toward the Table Mountain Valley (DUCT 2019; Daily Maverick 2019; Mail and Guardian 2019; IOL News 2019; Times Live News 2019). In the wake of the spill, the Willowton Group committed itself to mitigating the repercussions of the spill by appointing clean-up companies (The Witness 2019; various pers. comm.).

The frequency of fish kills has increased over the past 25 years (Figure 2.2), with extreme fish kill events wiping out millions of fish (Koehn 2021; Stocks et al. 2022). The impacts of the industrial revolution and ongoing human activities to better human livelihoods have degraded freshwater ecosystems and driven declines in species abundances, shifts in distribution ranges and even caused local extinctions (Thiem et al. 2022). Freshwater ecosystems and associated biota are under siege; the present rise in the global human population, compounded by an increase in water demand at twice the rate of population growth, points to a future with deteriorated ecological systems and intense fish kills (Grill et al. 2019; Stocks et al. 2022). Evidence-based strategies must be employed to monitor and swiftly mitigate such catastrophes, particularly in developing countries where vulnerable communities rely on freshwater resources and fish for income. The incorporation of biomonitoring has shown the advantages of understating ecological health and the impacts of pollution. Telemetry advancement for the local southern African rivers has allowed real-time monitoring and tracking of the behaviour of free-swimming fish as lines of evidence to monitor stressors and the ecological health of rivers (Burnett et al. 2020, 2024). The local government and parastatal organisations should implement the bylaws of the polluter pay principles for offenders. The offenders must be charged with hefty fines to increase polluting costs and curb impunity (Pole 2002, Neysmith 2008). The Baynesspruit, a tributary of the uMsunduzi River, has been subject to a wide range of stressors and fish kills, mainly industrial inputs and sewage effluents (Pole 2002). The rivers deteriorating ecological integrity of this river is based on the poor implementation of the bylaws of the polluter pays principle and the impunity of popular and politically inclined industries (Pole 2002; Neysmith 2008). Stakeholder engagement to forge strategies to rehabilitate the uMsunduzi and associated tributaries is pivotal to maintaining desirable aquatic ecosystems and biodiversity. However, this needs to be implemented globally to support the persistence of functioning freshwater ecosystems.

2.4.1 Conclusions

In conclusion, the intensity of fish kills and their impacts on the ecological integrity of aquatic ecosystems have increased with the upscaling of industrial activities and changes in human demographics. Intense agricultural practices to meet food demand and the tragedy of the commons negatively influence aquatic ecosystems' health. The gradual rise in fish kill events reflects a trend towards intense fish kill events that impact millions of individual fish and related aquatic biota. The water demand has been rising at twice the rate of population growth, and the abstraction of water further limits necessary flows and resources available in the system, limiting the recruitment and recovery of indigenous fish that require various niches to complete certain life stages. Fish kill occurrence can be reduced by holistic monitoring of aquatic biota and physicochemical parameters to detect thresholds of potential concern rather than relying on fish mortality bioassays. The relevant stakeholders for water provision and freshwater management should devise holistic management strategies where telemetry and biotic indices are used to monitor and understand trends over time.

2.5 Acknowledgements

We are grateful to the University of KwaZulu-Natal (ZA), the National Research Foundation (ZA, grant 98404) and the Willowton Group LTD (ZA) for funding.

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2.7 Supplementary information

Supplementary information Figure S2.1: Dense mats of green algae and dead fish at Isipingo 1826 River, Durban, South Africa, in 2014. (Source: IOL NEWS (07/2014)).

Supplementary information Figure S2.2: Photograph of fish in a fish kill in the Amanzimtoti River, South Africa, in 2015. (Source: South Coast Sun Dec/2015)

Supplementary information Figure S2.3: Photograph of piles of dead fish along the river banks of uMngeni River, Durban, South Africa, in 2022 following a fish kill. (Source: Northeglen News (08/2022))

Supplementary information Figure S2.4: Dead fish (*L. natalensis*) along the uMsunduzi River, Pietermaritzburg, South Africa, in 2019, following the impacts of a mixed chemical spill from the Willowton Group Ltd factory.

CHAPTER 3

Evaluating diatoms, macroinvertebrates, and water quality following a severe pollution event in the uMsunduzi River, KwaZulu-Natal

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Running header: Monitoring water quality to guide rehabilitation efforts and management of a river

3.1 Abstract

The recent advances in technology can monitor water quality remotely in real-time along with routine collection of water quality samples for water chemistry. Furthermore, monitoring abiotic and biotic indices to assess water quality provides a holistic approach to managing freshwater ecosystems. We monitored in situ physicochemical parameters and sent water samples ti SANAS accredited laboratories for water chemistry from 2022 to 2023 along the Baynesspruit tributary and selected section in the mainstream uMsunduzi River, Pietermaritzburg, KwaZulu-Natal, South Africa, to ascertain the recovery of water quality following a mixed product chemical spill from the Willowton Group Ltd facility in August 2019. Water quality probes were deployed at the water quality monitoring sites to send water quality data remotely to a data management system in real-time. We monitored the biotic indices quarterly to assess the water quality, including benthic diatoms and aquatic macroinvertebrates, using the South African Scoring System version 5 (SASS5). We found that water quality had improved, given the remedial assimilation of pollutants and dilution as they moved downstream, such that further downstream sites had improved significantly. The pollution-sensitive taxa (diatoms and macroinvertebrates) were starting to colonise the downstream sites, Grimthorpe (FR2) and Inkanyezini (FR3), depicting recovery in water quality. However, toxic industrial and sewage inputs compromised water quality recovery in sites near the city. It is recommended that the existing legislative framework be properly enforced to curb the input of toxic effluents from industrial operations, sewage networks be properly maintained, and conservancies be established to protect water resources. Furthermore, continual monitoring is recommended to ascertain pollution sources and manage to mitigate their impacts on water quality in the uMsunduzi River.

Keywords: Benthic diatoms, macroinvertebrates, SASS5, water quality, water chemistry

3.2 Introduction

A variety of anthropogenic stressors threaten freshwater ecosystems in various regions across the globe, which is further exacerbated by poor management practices and exploitation of resources (Dudgeon et al. 2006; Vörösmarty et al. 2010). The rapid rise in human population, changes in land use, demographics, and extreme climate further exacerbate the impact of river stressors (Dudgeon 2019; Vörösmarty et al. 2010). The main causes of stressors are water abstraction, agricultural runoffs, and inputs of sewage and industrial effluents, which affect the physicochemical properties of water. Furthermore, habitat degradation and physical barriers impact biological communities and water quality (Chapman 1996; Dudgeon et al. 2006; O'Brien et al. 2019; DWS 2020).

Globally, the water demand was reported to increase at twice the growth rate of the human population (Grill et al. 2019). The demand for water in Africa is made worse by extreme hydrological conditions; as such, many rivers are regulated by dams, reservoirs, and other structures for water storage (O'Brien et al. 2019). South Africa's 2030 vision aligns with the African Union's vision for 2063 and the United Nations Sustainable Development Goals (SDG) to advocate for the sustainable use of natural resources and protection of the environment (Lynch et al. 2016, 2020; DWS 2020, 2022). South Africa's legislative framework is enshrined in the country's constitution, such that section 27 (1) states that everyone has a right to access sufficient water, and section 27(2) obliges the state to take reasonable measures within its available resources to ensure the realization of section 27(1) (Tempelhoff 2009; DWS 2020, 2022). However, water and sanitation (SDG6) are still major global concerns (Lynch et al. 2016, 2020; Tickner 2020). The realisation of this goal in South Africa is limited by poor implementation of the legislative framework, failure of infrastructure because of lack of routine maintenance, pollution from sewage inputs and chemical effluents from industrial operations, and illegal refuse dumping (Tempelhoff 2009; DWS 2020). The ageing and poorly maintained infrastructure, compounded by an increase in human population, extensive agricultural practices, and expanding economic activities, are further facilitating the impacts of stressors causing the deterioration of river ecosystems beyond acceptable ecological states (Tempelhoff 2009; Dudgeon 2014; Tickner 2020).

Rivers support a multitude of services, such as the production of natural resources on a subsistence, recreation, and commercial level, and water quality thresholds have been established to ease the detection of stressors and swift remediation where necessary (Bartram and Balance 1996; Gemmel and Schmidt 2013; Tiyasha et al. 2020). Water quality evaluation includes acquiring quantitative and representative data on the sampled river's chemical, physical, and biological parameters (Ouyang 2005; Swamee and Tyagee 2007). A viable water quality monitoring technique identifies water quality stressors and provides baseline data to inform strategic mitigation measures (Ouyang 2005). Furthermore, long-term water quality data guide the decision-making for water resource managers and aid the development of progressive strategies for protecting aquatic ecosystems (Sutadian et al. 2016).

The need for water pollution legislative acts and water quality monitoring emerged as early as the 1940s in the United States of America (USA) to prevent the deterioration of natural water quality; however, the ancient methodologies were arbitrary and lacked chronology (Horton 1965; Barry 1970; Resh and Unzicker 1975; Strobl and Robillard 2008). Sampling sites were subjectively established through convenience and ease of access;

therefore, a reliable and consistent water quality monitoring design was lacking (Horton 1965; Resh and Unzicker 1975). Water quality sampling techniques have improved over the years, with cutting-edge physicochemical water quality collecting tools, biomonitoring techniques, and telemetry, which enhanced the reliability and objective collection of water quality data (O'Flynn et al. 2007; Thorstad et al. 2013; Meyer et al. 2019).

Biomonitoring is based on the interaction of aquatic taxa at different trophic levels and often includes benthic diatoms, macroinvertebrates as primary consumers, and fish as secondary consumers (Dickens and Graham 2002; Taylor 2007; Evans et al. 2022). Recent technological advancements prompted local developers to adopt global telemetry techniques for sampling southern African rivers (Burnett et al. 2020, 2024). Technological innovations include water quality monitoring techniques that send remotely acquired data to the data management system in real-time (O'Flynn et al. 2007; Burnett et al. 2020). The acquired data over time enables researchers to establish baseline trends of water quality parameters, which aids in the early detection of stressor impacts on the water body and guides the management of water resources to conserve aquatic organisms (Sutadian et al. 2016; Burnett et al. 2024). The water quality was severely impacted, and the aquatic fauna was compromised in the uMsunduzi River, KwaZulu-Natal, South Africa, when 1.6 million tons of the mixed product effluent from the Willowton Group Ltd factory entered the river (DUCT 2019a). This occurred when a holding tank of fatty acids collapsed because of a burst valve, dislodging two other tanks of edible oil and caustic soda to form a toxic effluent that flooded the Baynesspruit tributary and uMsunduzi River (East Coast Radio 2019). The spill had a devastating downstream impact of ~82 km into Inanda Dam and severely impaired the water quality of the uMsunduzi River and water provision to Durban (Daily Maverick 2019; The Witness

2019). We hypothesised that the water quality of the uMsunduzi River has improved since the spill. The present study aimed to monitor the water quality of the uMsunduzi River following the chemical oil spill in August 2019 (Ground up 2019) and periodic sewage inputs (Gemmel and Schmidt 2013). We collected in situ physicochemical properties, collected water samples for analyses, sampled for diatoms and assessed macroinvertebrates to monitor water quality changes quarterly. We monitored the responses after this severe oil spill to determine the recovery of the uMsunduzi River. With our findings, we suggest a rehabilitation management plan.

3.3 Methods

3.3.1 Study area

The uMngeni River in KwaZulu-Natal, South Africa, is socio-economically important, providing water for over 4 million people in the uMgungundlovu and eThekwini metropolitans. One of the most important tributaries of the uMngeni River is the uMsunduzi River, which is amongst the largest and faced with pollution as it passes through industrial, agricultural, and industrial areas (Gemmel and Schmidt 2013; Matongo et al. 2015). uMsunduzi River flows through the KwaZulu-Natal Midlands, passing through the Msunduzi district comprising an area of 634 km^2, through the capital city of Pietermaritzburg (Fig. 3.1; Matongo et al. 2015). The climate is classified as subtropical, characterised by hot and wet summers and cold, dry winters (Matongo et al. 2015). The uMsunduzi River is amongst the prominent tributaries of the uMngeni River and comprises a catchment size of 875 km^2 and 115 km tributary length (Matongo et al. 2015). Along the uMsunduzi River, there exists a variety of land use activities since it drains two-thirds of the metropolitan region, and the main river flows through agricultural, domestic, municipal, and industrial zones to the confluence with the uMngeni River between the Nagle Dam and Inanda Dam (Gemmel and Schmidt 2013). The uMsunduzi River receives runoff water from urban and peri-urban areas, communal and municipal areas that pollute the system with toxic effluents, sewage inputs, and treated wastewater from treatment plants (Dickens and Graham 1998).

Sampling sites along the uMsunduzi River were strategically selected to encompass the industrial zones, activities, and areas afflicted by catastrophic chemical oil pollution from the Willowton Group Ltd factory (Environmental Justice Atlas 2020). The sampling sites comprised three sites upstream of the chemical oil spill. These sites included the FSS Refinery (FC1) (29.633692, 30.339811), Alexandra Park (FC2) (-29.608872, 30.380418), and Musson's Weir/N3 bridge (FC3) (-29.602114802614313, 30.404199586869773). Our downstream sites were below the spill point, including Darvill Wastewater Treatment Plant (FR1) (-29.593554,30.438395); Grimthorpe Bridge (FR2) (-29.618502, 30.447223) and Inkanyezini (FR3) (-29.60699,30.55220).

3.3.2 Sampling techniques

The benthic diatoms, macroinvertebrates, water grab samples, and in situ physicochemical measurements were collected samples quarterly from 2022 and 2023 at each of our sampling sites over eight surveys. The Baynesspruit River, adjacent to the Willowton industrial complex, is an essential tributary to the uMsunduzi River. Water quality probes were submerged at strategic locations along the Baynesspruit tributary to the uMsunduzi main stem (Fig. 3.1). We collected water samples and benthic diatoms each quarter at the water quality probe locations (Fig. 3.1).

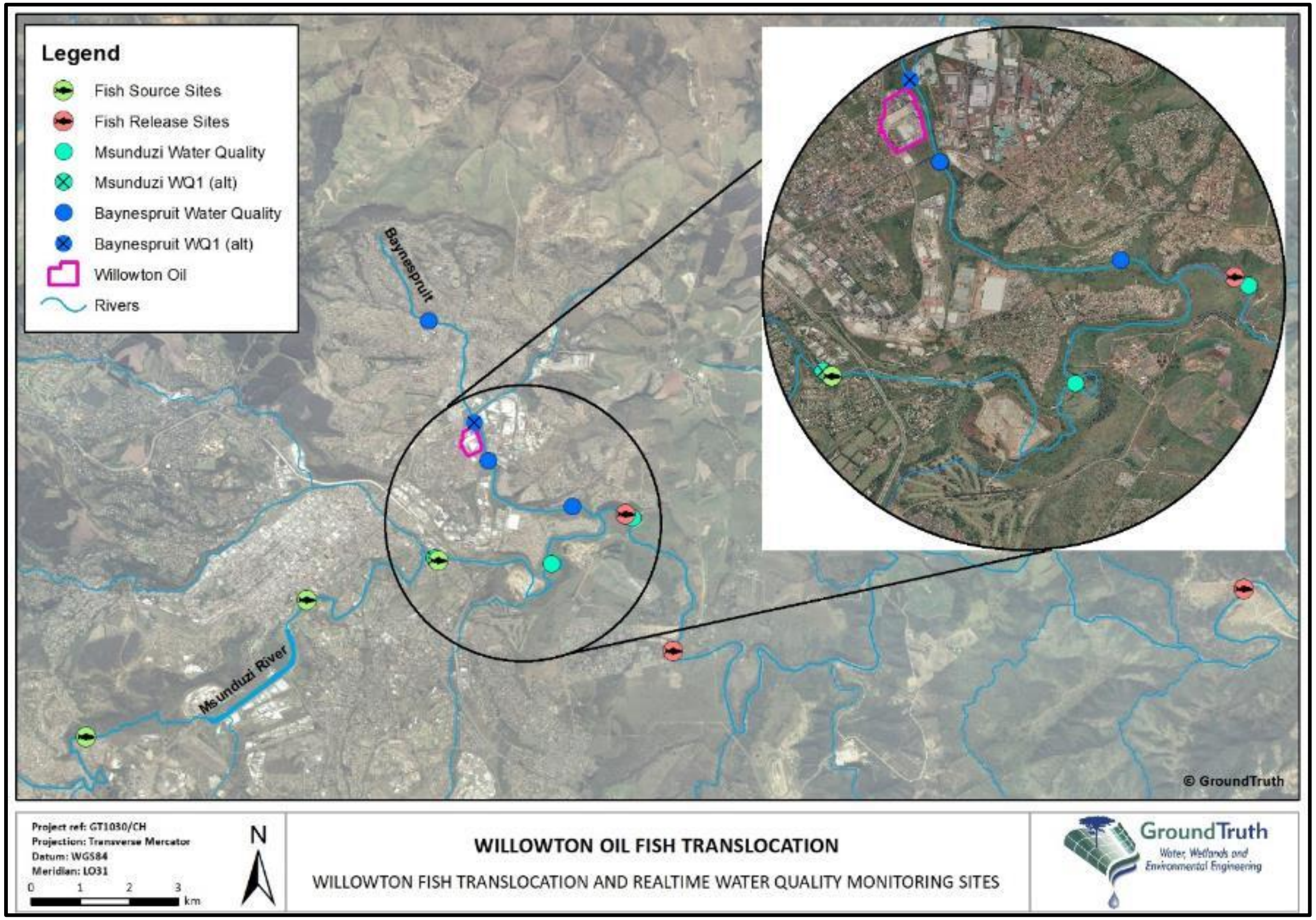

Figure 3.1: Water quality and chemistry monitoring sites along the Baynesspruit tributary and uMsunduzi River, including sites impacted and not impacted by the mixed chemical spill in the present study.

3.3.3 Water quality

We monitored in situ water quality through various sampling techniques, including *in situ* water quality readings and real-time water quality probes (Wireless Wildlife, Potchefstroom, South Africa). Various parameters of water quality were measured, such as water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity (Sal), dissolved oxygen (DO%), (DO mg/L), and pressure (mm Hg) using a calibrated water quality meter and a multiprobe handheld dissolved oxygen meter (Horiba, China).

3.3.4 Water chemistry

In addition to in situ measurements, we collected water samples using 500 ml sterilised, clear plastic bottles for water chemistry analyses at each study site. The water samples were preserved in the field at 4°C using a cooler box and transported to the SANAS accredited laboratories for biological and microbial analyses to quantify the associated water chemistry parameters relevant to stressors of the Baynesspruit and uMsunduzi systems, such as pH, nitrates, orthophosphates, total coliforms, and *Escherichia coli*.

3.3.5 Real-time water quality

We deployed water quality probes from Wireless Wildlife (Potchefstroom, South Africa) programmed to record and transmit physicochemical water quality measurements remotely to a receiver and a data management system to monitor real-time water quality in the selected sections of the Baynesspruit and uMsunduzi systems. The water quality probes were installed and submerged within wadeable areas along the river channel at six sites, viz upper Baynesspruit, mid Baynesspruit, Lower Baynesspruit, and mainstream of the uMsunduzi River at Musson's Weir (site FC3), Darvill Wastewater Treatment Plant (site FR1) and Inkanyezini (site FR3) (Fig. 3.1, Supplementary information Table S3.1). We mounted probes on at least 1 m of steel angle iron pole using a size 10 spanner with grip pliers, hammered underwater firmly on a deep bank with a rock. We covered the cord with a sprayed conduit pipe lying outside the water and camouflaged it with marginal vegetation to keep it from submerging. We used an alternative for the sites lacking suitable banks for the steel angle iron pole: a steel cage tightened with bolts and nuts on a heavy rock to secure the cage and the probe.

The probes were programmed to send data remotely in real-time to a network of relay and base stations. The probes sent the data to receivers that passed it to a data management system accessible online. In addition to the relay receivers, a portable Bluetooth base station connected to the Wireless Wildlife mobile app was occasionally used to retrieve the data from the probes and detect faulty and broken probes because of flooding.

The real-time data was useful in detecting pulses of periodic water or episodic pollution events that could have been overlooked. This was related to rainfall and reports of pollution events. Concerning aquatic fauna, real-time water quality monitoring provides context for the living conditions of taxa dependent on the Baynesspruit and uMsunduzi systems to complete their life cycles.

The water quality probes transmit data via UHF radio frequency to a receiver in the locality (Burnett et al. 2020). The receivers were either a base station or a relay station. Both stations received data from the water quality probes. The relay stations transmitted the data to a base station, and the base station sent data to a central database stored on an electronic server (the cloud). A base station was deployed near the uMsunduzi River at Newton School near Musson's Weir to detect the water quality probe deployed at Musson's Weir (site FC3), and a relay station was installed on-site at the Willowton Group Ltd factory to detect the water quality probes at the mid and lower Baynesspruit sampling sites. We later replaced the relay station at the Willowton Factory with a base station. Due to the lack of other secure locations and the fear of theft of base and relay stations, the portable Bluetooth base station, connected to the Wireless Wildlife mobile app, was used to download data from probes deployed from the other sampling sites.

3.3.6 Benthic diatoms

We collected benthic diatoms (microalgae) every quarter at sampling sites where real-time water quality probes were deployed to indicate the present state of water quality and its impact on aquatic biota. The diatoms were collected using a toothbrush to scrub them off submerged rocks into a white plastic tray, transferred to a vial, and preserved with 70% ethanol. The diatom sample vials were then labelled with the date and location and stored to be sent to SANAS-accredited laboratories for biological analyses.

Data from the diatom samples were interpreted according to the specific pollution sensitivity index (SPI) (Taylor et al. 2007) to assess the ecological health status of the uMsunduzi River at each of the sampling sites and to ultimately infer the ecological/ environmental impacts of the present state of water quality in the Baynesspruit and uMsunduzi systems. We also determined the percentage of pollution tolerant values (% PTV) for each diatom sample collected for Baynesspruit and uMsunduzi mainstream. The % PTV depicts the proportion of diatoms within a collected sample that tolerate pollution, further indicating the river system's water quality.

3.3.7 Macroinvertebrates

Aquatic macroinvertebrates represent primary consumers within river ecosystems (Dickens and Graham 2002). Assessments of aquatic macroinvertebrates were conducted quarterly from August 2022 using the South African Scoring System version 5 (SASS5, Dickens and Graham 2002), accredited to ISO 17025 standards. The SASS5 method samples aquatic macroinvertebrate taxa in selected sections of the river systems, each of which has differing sensitivities to pollution, and produces an index of the health to represent the overall ecological integrity of the system based on observed taxa (Dickens and Graham 2002).

We collected macroinvertebrates using a SASS net (mesh size 1 mm; GroundTruth, Hilton, South Africa) in selected habitats representative of all the biotopes in the river at each sampling site (Supplementary information Fig. S3.1). The marginal vegetation, rock, and gravel/sand/mud (GSM) were the sampled biotopes for macroinvertebrates. Marginal vegetation was sampled by brushing the SASS net against the overhanging vegetation and scooping into the net, rock biotope was sampled by kicking and scrubbing the sampler's feet against boulders, and the gravel/sand/mud was sampled by stamping the biotope with the sampler's feet and scooping into the net. We used a stopwatch to time the duration of sampling each biotope, which was 2 min for rock and marginal vegetation and 1 min for gravel/sand/mud. We transferred the contents of the SASS net from each biotope into plastic buckets (5 L), then carefully cleaned off the debris and used a white plastic tray to identify and count macroinvertebrate taxa. When identifying and counting macroinvertebrates from each biotope, the observer was allocated 15 min. to standardise the methods, whereas 7.5 min. was allocated for two observers. The grade for each site was determined based on macroinvertebrate abundances to give it an ecological score using a South Africa scoring system version 5 (SASS5), the average score per taxon, and the total number of families (Dickens and Graham 2002). The ecological health status of each site was graded according to ecological categories for the South-eastern Upland and North-eastern Coastal Belt ecoregions to infer the overall ecological integrity and recovery of the uMsunduzi River and recovery onwards (Dallas 2007).

3.3.8 Data analyses

We used the analysis of similarities (ANOSIM) to understand similarities between study sites. We applied cluster analysis and non-metric multidimensional scaling (NMDS) to investigate trends in macroinvertebrate assemblage composition. We used the Bray Curtis resemblance matrix to quantify the abundance contribution of each macroinvertebrate taxon to each of the study sites. We used the similarity percentage (SIMPER) to determine the taxa responsible for similarity within groups and dissimilarities between groups, as described by Agboola et al. (2020a). We used the Primer software (version 6) to run the analyses. The non-metric dimensional scaling and ANOSIM were obtained by square root transformation of the data using the Bray-Curtis index.

3.4 Results

3.4.1 Water quality probes

The study's real-time water quality monitoring component was not without technical challenges. The flooding on the Baynesspruit caused the loss of three real-time water quality probes that washed downstream. The upper Baynesspruit site had a probe washed downstream over the high-flow season of December 2022. This probe was replaced, and the replacement washed downstream during the high flow season in March 2023. Similarly, the probe at midBaynesspruit washed down. Both probes were adequately secured into the substrate and attached to a large boulder, respectively. The probe at mid-Baynesspruit was mounted on a large boulder that survived the April 2022 flooding of the uMngeni River and failed on the Baynesspruit. There is uncertainty about whether the flow fluxes in the Baynesspruit created higher and faster flows or whether the probe was stolen. However, not even the rock was found, suggesting the former. These real-time water quality probes would accumulate debris and clog from all the anthropogenic litter, including disposable nappies and plastic, flowing down the river, and this resulted in technical issues with the probes that resulted in the probes having to be returned to the manufacturers for service and improvement. Here, it was found that the extended antenna used to transmit the data to the base and relay station network allowed water through, which damaged the probes. The company then looked into a better design and reverted to short antenna probes. The short antenna probes decreased the range that a relay or base station could detect a water quality probe as now the antenna was below the surface of the water; however, this minimised water damage.

3.4.2 Water quality

The overall physicochemical parameters were consistent across seasons and sampling locations (Fig. 3.2). Conductivity results were comparable to previous monitoring surveys on the uMsunduzi and Baynesspruit (Gemmel and Schmidt 2013; GroundTruth report 2021), except towards the end of September 2022 at Mid Baynesspuit (BS2) and Musson's Weir (FC3) and the first week of November at Lower Baynesspruit (BS3) where conductivity significantly exceed chronic effect value (CEV) (Fig. 3.2); however, pH levels were overall skewed towards the alkaline end (6.8- 7.92). Despite comparatively elevated pH in the Baynesspruit, the pH of the downstream uMsunduzi site (site FR1) appeared to be unaffected and recorded slightly lower pH in comparison with the upstream sites (site FC1) (Fig. 3.2).

The total dissolved solids (TDS) concentrations for sites within the Baynesspruit ranged from 124 to 186 mg/l (Fig. 3.2; Table 3.1). Higher concentrations were generally recorded during the quarter 3 (November 2022) survey, and a value of 458 mg/l was recorded during quarter 1 of 2023, significantly exceeding the acute effect value (AEV) according to the South African Water Quality Guidelines (Table 3.1). This may be because of inputs of dissolved substances from surface run-off during rains in this quarter.

Nutrient levels, such as orthophosphate, mostly exceeded recommended threshold values ranging from 0.008 to 0.280 mg/l (Fig. 3.2). The orthophosphate concentration exceeded the recommended limits by international and local authorities in the lower Baynesspruit in quarter 4 (March 2023) (0.280 mg/l) (Table 3.1). The upstream and downstream concentrations were comparable in 2022, and in 2023, the concentration at the downstream site was elevated, which correlated with the high concentrations observed at the Baynesspruit tributary during the same sample period. Nitrate concentrations in the Baynesspruit generally exceeded the chronic effect value (CEV), whilst, in the uMsunduzi River (sites FC3, FR1), concentrations exceeded the target water quality range (TWQR) but were within acceptable limits (Table 3.1). Despite high levels in the Baynesspruit, particularly during quarter 3 (November 2022), concentrations at site FR1 appeared unimpacted by contributions from the Baynesspruit through the Baynesspruit-uMsunduzi confluence just above the sample point at site FR1 (Fig. 3.2). Nitrate concentrations in the uMsunduzi and Baynesspruit were more than double the concentrations measured during the previous surveys (Table 3.1).

Additional data for uMsunduzi sites from Umgeni Water indicated that the concentrations of suspended solids exceeded the general limit value (GLV) for wastewater discharge for both sites and surveys, ammonia marginally exceeded the TWQR at these sites in quarter 3 (November 2022), but exceeded the CEV at the upstream site (site FC3) in quarter 4 (March 2023) (Supplementary information Table S3.2). Moreover, the monthly measurements of aluminium concentrations from 2022 and early 2023 indicate that the aluminium concentrations were 10 times greater than the recommended thresholds of potential concern at sites FC3 and FR1, respectively (Supplementary information Table S3.2).

Table 3.1: Average water chemistry from monitoring at Baynesspruit and selected sections along the mainstream uMsunduzi River between the 2022 and 2023 survey years. (Bold indicates significant, TDS = Total dissolved solids).

Site	Date	pH	TDS (mg/l)	Orthophosphate (mgP/l)	Nitrates (mg N/l)	*E. coli* (MPN/100l)
FR1	11.2022	7.9	164	**1.01**	1.99	**32300**
BS3	11.2022	7.9	164	0.1	2.47	2420
BS2	11.2022	7.9	138	0.1	2.55	2420
BS1	11.2022	6.3	150.3	0.1	3.01	2420
BS2	05.2023	6.8	142	0.1	4.87	2420
BS2	03.2023	7.7	166	0.13	4.15	**9600**
BS1	03.2023	7.6	124	0.12	3.89	2420
BS3	05.2023	6.9	232	0.1	3.71	2420
BS1	05.2023	6.8	**458**	0.1	3.63	2420

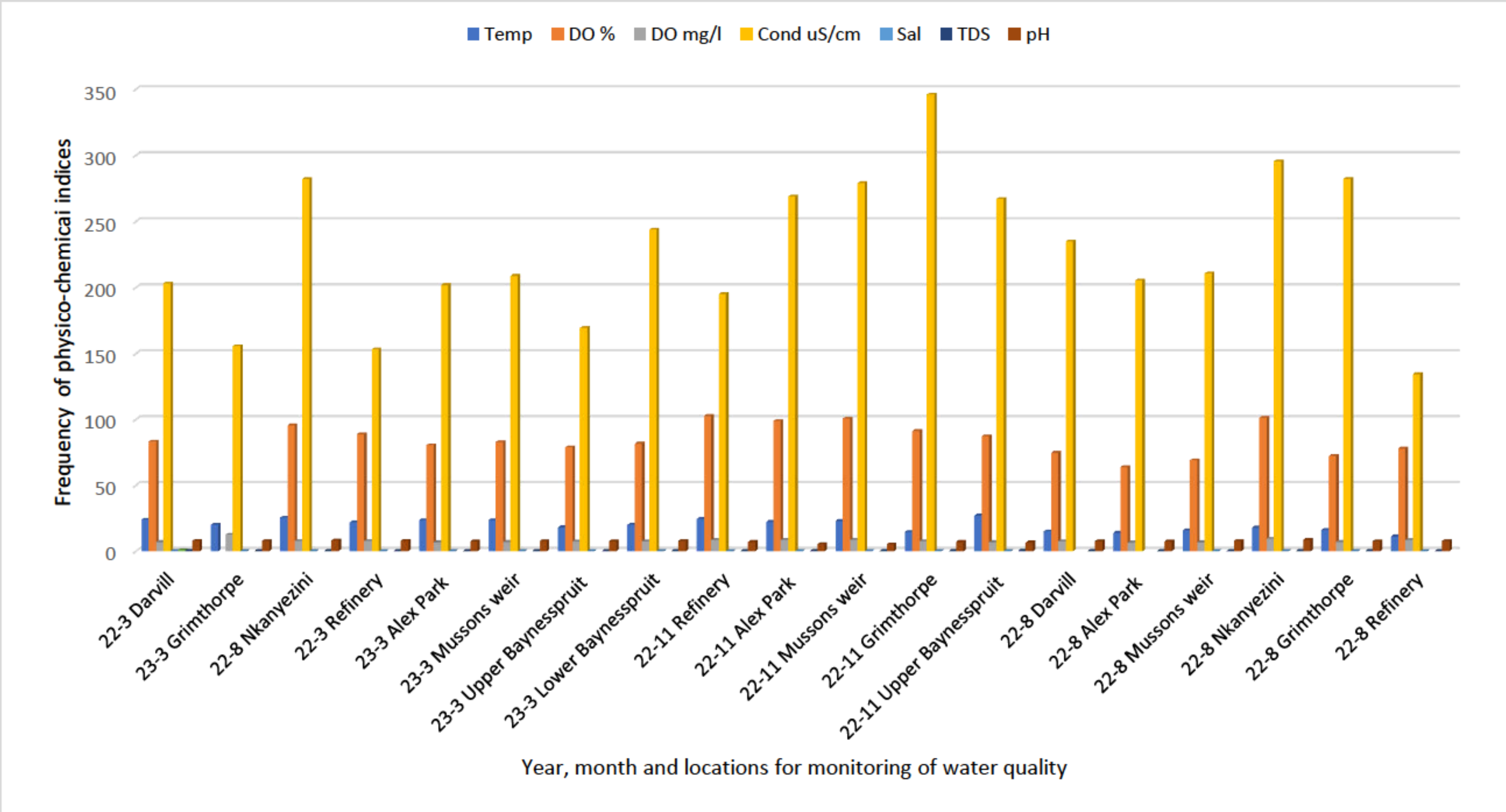

Figure 3.2: Physicochemical parameters were monitored across various seasons amongst the selected survey sites to evaluate the state of water quality in the uMsunduzi River. (Note: temp = water temperature (°C), DO = dissolved oxygen, cond = electrical conductivity, sal = salinity, TDS = total dissolved solids).

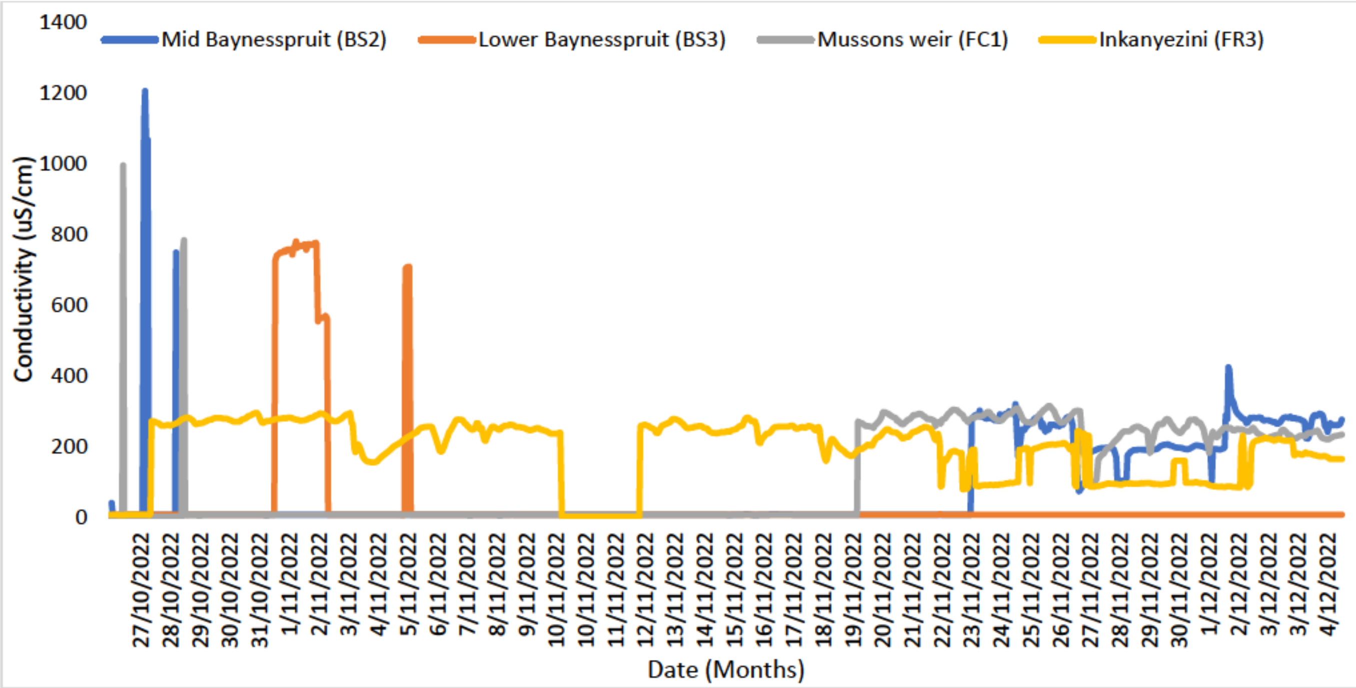

Figure 3.3: Conductivity results from real-time water quality monitoring with water quality probes at Baynesspruit and Musson's Weir (FC3).

Presently, the available real-time data is fragmented; however, it depicts the potential for ecological monitoring of stressor impacts (Fig. 3.3). The available conductivity results from the real-time water quality probes showed that conductivity fluctuated amongst the sites, generally within acceptable limits except for a few sites where it exceeded acceptable limits, indicating possible pollution (Fig. 3.3).

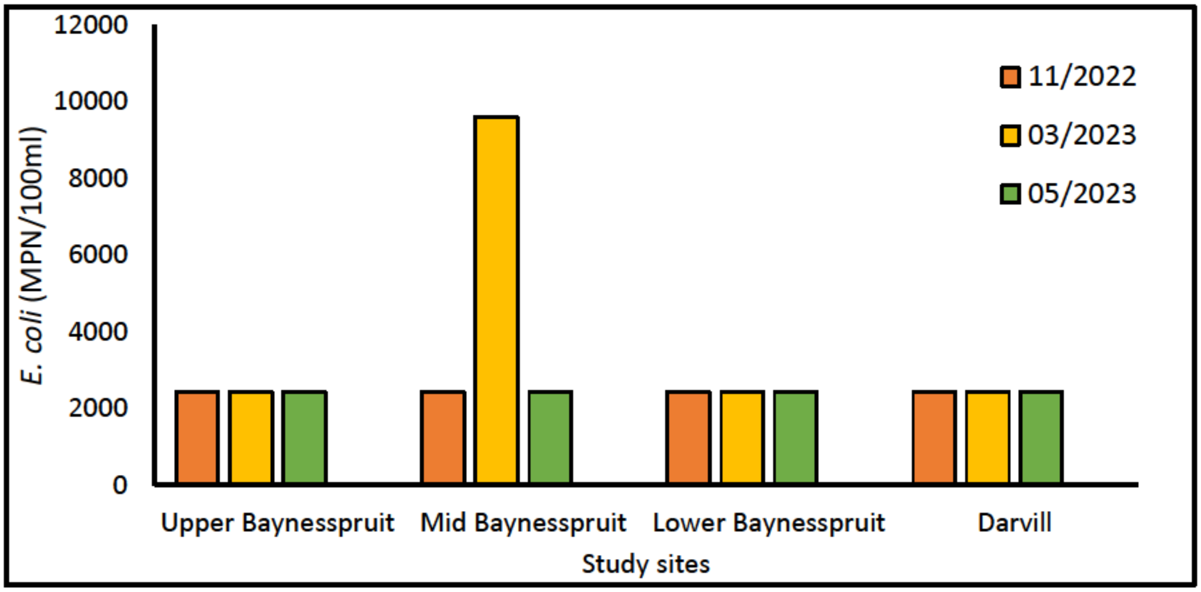

Figure 3.4: *Escherichia coli* in the Baynesspruit tributary and US Darvill water quality monitoring sites between 2022 and 2023 quarters. (Note: 0 E. coli/ 100 ml for drinking; 1-10 MPN/100ml is low risk; 11-100MPN/ 100ml is medium risk; >100MPN/ 100ml is high risk).

The *Escherichia coli* levels constantly exceeded thresholds of concern recommended by local and international authorities for drinking (O MPM/100), recreation (< 406 E. coli/100ml ppm) and irrigation (< 235 E. coli/100ml) in the Baynesspruit tributary and uMsunduzi sites (Fig. 3.4; Supplementary information Table S3.2). Amongst all quarters, the mid Baynesspruit site (BS2) showed excessive elevation in the *E. coli* levels, reaching 9600 MPN/100ml (Fig. 3.4). The upstream Darvill (FR1), *E. coli* levels for all the quarters may be influenced by the inflow of polluted waters from the Baynesspruit tributary.

3.4.3 Benthic diatoms

The number of diatom species fluctuated significantly between seasons and sampling locations, indicating varying responses to changes in water quality (Tables 3.3 and 3.3). No diatom samples were collected from the Baynesspruit during the low flow season of quarter 2 (August 2022) as the real-time water quality probes were being delivered from the manufacturers (Table 3.2). No viable diatom samples were retrieved for the downstream site (FR2) and upstream Baynesspruit site (BS1) during the high-flow season of quarter 3 (November 2022) survey (Table 3.2). The overall diatom cell counts at all the uMsunduzi sampling locations during this survey were relatively lower than the recommended amount (400 cell count) to provide reliable results (Table 3.2). This was attributed to seasonal high rainfall, and river flows that disturb sediment and substrate-attaching microalgae.

The number of recorded diatom species was generally high for each site during the quarter 4 (March 2023) survey, with sites FC2 and FR2 recording 23 and 39 species, respectively (Table 3.3). Fewer diatom species were generally recorded during the quarter 3 (November 2022) survey, with 16 to 23 species recorded, the exception being site FC2 with 32 species (Tables 3.2 and 3.3).

Diatom assemblages at both the fish collection sites (unimpacted sites) and fish release sites (impacted sites) reflected predominantly poor water quality conditions, with a few sampling locations exhibiting some recovery (Tables 3.2 and 3.3). Periodic fair water quality conditions and recovery were seen at site FC1 (the FFS Refinery), and FR3 at Inkanyezini (Tables 3.2 and 3.3). These two sites were the most distant from the mixed product spill point upstream and downstream, respectively (Fig. 3.1).

The Baynesspruit was in a seriously modified condition throughout the study (Tables 3.2 and 3.3). The mid Baynesspruit, and on an occasional occurrence at site FC3 during quarter 3 (November 2022), water quality was classified as seriously modified (Tables 3.2 and 3.3). These conditions correlated with critically high proportions (>80%) of PTV (Tables 3.2 and 3.2). Levels of PTV above 80% reflect severe levels of organic pollution (DWAF 1996). The dominance of species such as *Sellaphora seminulum* and *Gomphonema parvulum* indicates very heavy organic pollution; *G. parvulum* is also indicative of siltation. In quarter 3 (November 2022), these two species accounted for 61% and 24% of the samples in the Baynesspruit, respectively, whilst at site FC3, *Nitzschia palea* was the dominant species, which generally indicates highly polluted waters, with high levels of nutrients and dissolved salts (Table 3.1; Supplementary information Tables S3.3 - S3.5). At site FC2 during quarter 2 (August 2022), *Fistulifera saprophila¸*, a species tolerant of very heavy organic pollution, was the dominant species, making up 53% of the sample (Supplementary information Table S3.3). This species was also abundant at site FC1 during the quarter 4 (March 2023), where it accounted for 35% of the sample (Supplementary information Table S3.5). The downstream sampling locations, sites FR2 and FR3, exhibited diatom communities with an even distribution of species, although pollution-tolerant species, such as *G. parvulum*, *N. palea*, and *Navicula symmetrica*, were present. The species *Cocconeis placentula var. euglypta*, was particularly abundant at the furthest downstream site.

The diatom communities of the Baynesspruit and at both fish collection sites and fish release sites on the uMsunduzi River were dominated by pollution-tolerant taxa (Supplementary information Tables S3.3 - S3.5), which are indicative of poor water quality and point towards a eutrophic system. Across all quarters and almost all survey sites, the percentage of deformed cells was low (0 – 3.0%), except at site BS2 in the mid-Baynesspruit, which exhibited a significant percentage of deformed cells (12.5%), indicating the impact of heavy metal contamination (Table 3.2).

Table 3.2: Species richness and abundances of pollution tolerant and deformed cells in benthic diatoms sampled between quarters 2 and 3 of 2022 in the uMsunduzi system. (Note SPI = pollution sensitivity index; % PTV = percentage of pollution tolerant valves).

Survey	Site	Number of species	SPI	PTV (%)	Deformed cells (%)
Quarter 2	Refinery	32	12.4	40.0	3
August 2022	Alex Park	23	5.0	90.5	0.5
	Musson's Weir	19	7.4	46.0	0
	US Darvill	26	6.4	61.4	0.5
	Grimthorpe	33	6.1	37.3	2
	Nkanyezini	16	12.5	6.5	0.25
Quarter 3	Mid Baynesspruit	20	4.6	80.2	12.5
November 2022	Lower Baynesspruit	16	3.9	94.6	2.25
	Refinery	16	7.2	62.0	0
	Alex Park	32	8.5	31.1	3
	Musson's Weir	17	2.9	82.2	1
	US Darvill	23	6.0	67.6	2

Table 3.3: Benthic diatoms species richness and abundances of pollution-sensitive taxa along the uMsunduzi system during quarter 4 in March 2023. (Note SPI = pollution sensitivity index; % PTV = percentage pollution tolerant values).

Survey	Site	Number of species	SPI	PTV (%)	Deformed cells (%)
Quarter 4 March 2023	Upper Baynesspruit	33	6.2	60.9	2
	Mid Baynesspruit	28	4.7	82.7	2.75
	Lower Baynesspruit	33	3.7	82.5	2.75
	Refinery	25	11.6	42	0.5
	Alex Park	23	5.9	35	0
	Musson's Weir	32	7.7	36.3	0
	Darvill	29	7.7	35.5	0
	Grimthorpe	39	7.5	38	0
	Nkanyezini	38	6.5	28.1	1.25

3.4.4 Macroinvertebrates

During the 2022 survey, macroinvertebrate taxa did not differ significantly (Fig. 3.5). There was an even distribution of macroinvertebrate taxa within and between sampled sites, as shown in the NMDS plot (Fig. 3.5). The ANOSIM further supported that there were no significant differences in aquatic macroinvertebrate assemblages during the 2022 survey season (Global R = 0.1; P = 0.1). Furthermore, the SIMPER exhibited low dissimilarity percentages using the Bray-Curtis dissimilarity matrix for the 2022 season, except for the further downstream site, which was somewhat colonised by pollution-sensitive assemblages (Supplementary information Table S3.6). However, for the 2023 survey, macroinvertebrate assemblages were significantly different between sampled sites (Fig. 3.5). The different sites were separated from each other for the 2023 survey season, suggesting dissimilarity in the macroinvertebrate assemblages. The ANOSIM further confirmed the significant dissimilarity between groups for 2023 (Global R = 0.7; P = 0.001). Contrary to the 2022 survey season, the average dissimilarity percentages using the BrayCurtis dissimilarity matrix were high between groups for 2023, suggesting that new assemblages may have colonised the sampled sites because of improved conditions (Fig. 3.5).

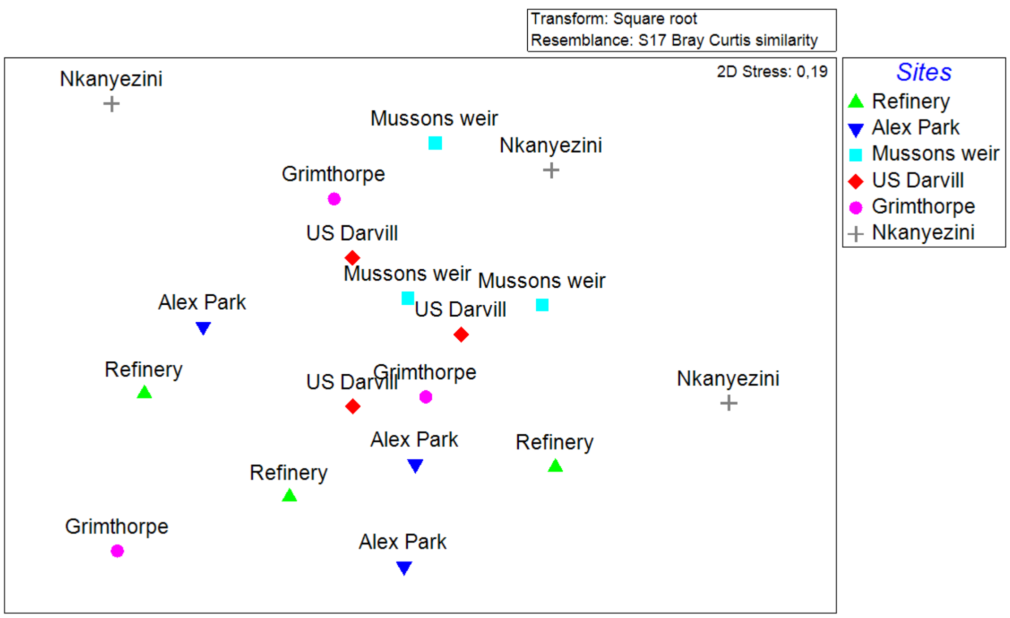

Figure 3.5: Non-metric multi-dimensional scaling (NMDS) plot of aquatic macroinvertebrates amongst sampling sites in the uMsunduzi River for 2022. The two-dimensional plot, with a stress level of 0.19, was derived from square root transformed macroinvertebrate abundances using Bray-Curtis similarity.

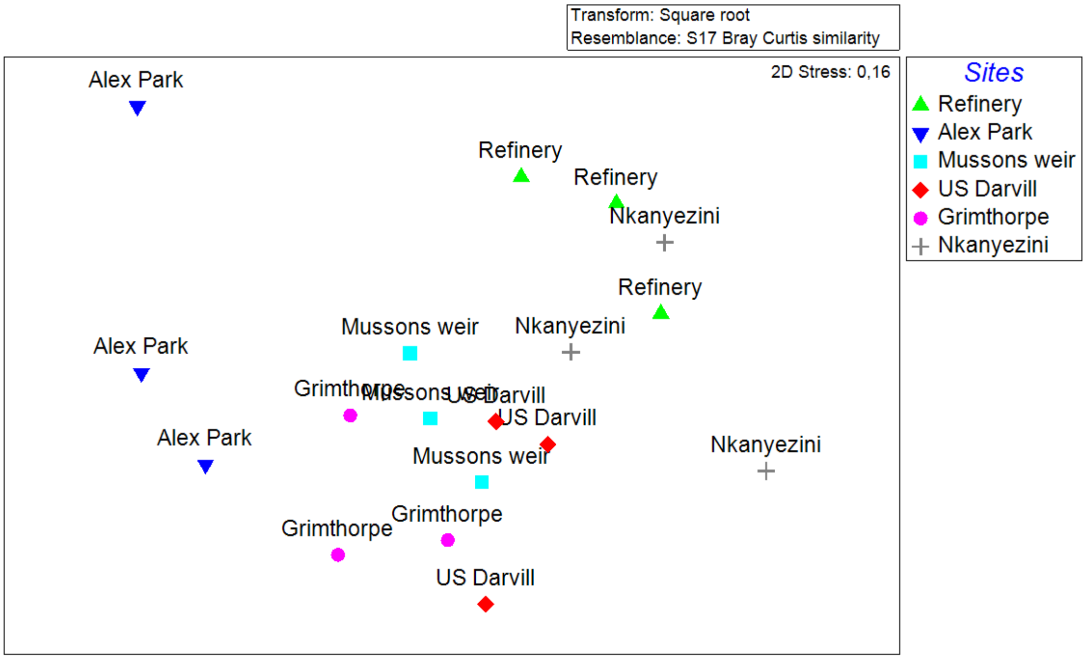

Figure 3.6: Non-metric multi-dimensional scaling (NMDS) plot of aquatic macroinvertebrate taxa sampled along the uMsunduzi River for 2023. The two-dimensional plot on 0.16 stress level was obtained from square root transformed macroinvertebrate data, using a Bray-Curtis similarity.

With the first sampling survey during the low flow season of quarter 2 (August 2022), all survey locations exhibited seriously modified ecological conditions, except for Inkanyezini (site FR3), which showed slightly improved river health (Table 3.4). The lowest SASS5 scores, ASPT scores and number of macroinvertebrate taxa were recorded at sites closest to the Baynesspruit tributary, which were Musson's Weir (site FC3) and Darvill wastewater treatment plant (site FR1) during quarter 2 (August 2023), and site FC2 in quarter 4 (March 2023) (Table 3.3).

During the quarter 4 (March 2023) survey, representing high flow season, river health was generally improved at several survey sites from seriously modified to poor condition, and at site FR3 from poor to fair condition (Table 3.4). This could be because of improvements in habitat conditions and water quality through dilution or flushing of contaminants during the high flow period. Conversely, two sites, namely FC2 and FC3, remained in seriously modified condition (Table 3.4).

At site FR2, the improved ecological health was related to an increase in the ASPT score rather than the SASS5 score, which suggests the presence of moderately sensitive taxa, namely *Chlorocyphidae* (Damselflies) and *Ecnomidae* (Caddisflies) (Table 3.4). Overall, there was a general trend of depressed SASS5 and ASPT scores in sampling sites located at the periphery of Pietermaritzburg city, and slightly improved scores were obtained from sites that are furthest upstream (site FC1) and downstream (site FR3), respectively (Table 3.4).

Table 3.4: The SASS scores, and the average contribution of each taxon to evaluate the recovery of water quality in the uMsunduzi River for 2022 and 2023. (SASS5 = South African Scoring System version 5; ASPT = average score per taxon).

Year	Site	SASS Score	ASPT	Number of taxa
2022	Alex Park	43	3.6	12
	US Darvill	40	4.0	10
	Grimthorpe	73	5.2	14
	Musson’s Weir	37	3.7	10
	Nkanyezini	113	6.0	19
	Refinery	69	5.0	14
2023	Alex Park	42	4.2	10
	US Darvill	84	5.6	15
	Grimthorpe	70	5.4	13
	Musson’s Weir	74	5.3	14
	Nkanyezini	121	5.8	21
	Refinery	110	5.8	19

3.5 Discussion

The uMsunduzi River is well known for its poor ecological health, owing to ongoing anthropogenic activities that prevent the self-remediation of the river to a pristine state (Gemmel and Schmidt 2013; Supplementary information Figs S3.2 -3.6). The real-time data were useful in detecting continuous water quality and periodic pollution events, and these can be linked to biomonitoring to evaluate the impacts of the water quality on aquatic biota (Burnett et al. 2020, 2024). Furthermore, real-time data can allow managers to address thresholds of potential concern, pinpoint pollution sources, and swiftly mitigate anthropogenic impacts to maintain desirable ecological conditions and water quality (Burnett et al. 2020, 2024). However, we had issues with probes, which constrained our study, as found in other studies (Burnett et al. 2020).

The physicochemical parameters monitored in the present study fluctuated amongst sites and between sampling seasons; however, they were comparable to previous monitoring surveys overall, except for a few outliers. The overall real-time monitoring results, although fragmented, depicted a uniform trend. Conductivity results were comparable to previous studies (Dickens and Graham 1998) on the uMsunduzi and Baynesspruit (GroundTruth 2021); however, pH levels were overall skewed towards the alkaline end (6.20 - 7.92), except for the mid-Baynesspruit (BS2) where acidic pH was occasionally recorded. The orthophosphate concentration was almost double that recorded in previous surveys after the spill event (GroundTruth 2019). However, concentrations at the two uMsunduzi sites were lower than those recorded previously during the monitoring survey after the spill event (GroundTruth 2019). The *E. coli* exceeded recommended thresholds (Note: 0 E. coli/ 100 ml for drinking; 1-10 MPN/100ml is low risk; 11-100MPN/ 100ml is medium risk; >100MPN/ 100ml is high risk) for all the survey quarters at the Baynesspruit and Darvill water chemistry monitoring sites, maintaining above 2000 MPN/100ml and more than 9200 MPN/100ml during quarter 4 of 2023 (March 2023). The water quality along the uMsunduzi River reflected the impacts of anthropogenic stressors driving the system's function. The maintained excessive *E. coli* levels at the Baynesspruit and Darvill suggest continuous inputs of influents dominated by faecal matter. Further, the occasional decline in pH, increased salinity and total dissolved solids, nitrates, ammonia and chemical oxygen demand indicate that toxic and organic effluents occasionally impact the Baynnesspruit. The Northdale residential area, the Willowton industrial complex and the subsistence farming at the Sobantu community are implicated in the pollution of the Baynesspruit tributary. Effluents from broken sewers, industrial spillage, and organic agricultural inputs significantly negatively affected the Baynesspruit tributary's water quality.

The diatom communities depicted that the uMsunduzi is still recovering from the poor ecological conditions following the toxic chemical spill, with few sites showing improved diatom species abundances. The sites further from the industrial and commercial activities showed improved diatom species richnesses. The FFS oil refinery site (FC1) and Inkanyezini (FR3) had the most diatom species among other sampling sites; however, some pollution-tolerant species were still prevalent, particularly *Gomphonema parvulum*, *Nitzchia palea*, *Sellaphora seminulum* and *Cocconeis placentula var. euglypta* suggesting impacts from agricultural inputs, and siltation. The Baynesspruit tributary was classified as seriously modified on all surveys, with Musson’s Weir (FC3) also categorised as seriously modified during quarter 3 (November 2022). This classification coincided with the high occurrence of pollution-tolerant diatom species in the samples collected from the Baynessprut and Musson’s Weir (FC3). During quarter 3 (November 2022), the Baynesspruit samples were dominated by *G. parvulum* and *S. seminulum*, species known to indicate heavy organic pollution. Moreover, at Musson’s Wier (FC3), *N. palea* was the dominant diatom species, indicating organic pollution for the site. The mid Baynesspruit sites (BS2) exhibited a significant number of deformed cells amongst all the sites and quarters (12.5%), indicating the impacts of occasional contamination with effluents containing heavy toxic chemicals. Across all survey sites, diatom communities were dominated by pollution-tolerant taxa, indicating severe organic pollution, high dissolved substances and poor ecological health in the uMsunduzi catchment, particularly the Baynesspruit tributary, which has turned into a eutrophic system.

Macroinvertebrate assemblages varied amongst sampling sites, with the furthest sites FFS refinery (FC1) and Inkanyezini (FR3), exhibiting improved conditions. The macroinvertebrate assemblages were not significantly different amongst survey locations during the 2022 monitoring year, and various macroinvertebrate taxa were shared amongst the survey sites. However, during the 2023 survey year, gradual segregation of taxa amongst the survey sites was observed, with the FFS refinery site (FC1), Inkanyezini (FR3) and Alex Park (FC2) having distinct macroinvertebrate assemblages. The significant difference in macroinvertebrate taxa indicates recovery, with pollution-sensitive species colonising the gradually improving water quality at Inkanyezini (FR3) and FFS refinery (FC1). The FFS refinery (FC1) and Inkanyezini (FR3) had the highest SASS5 scores and average score per taxon (ASPT), as well as the highest number of macroinvertebrate families for the 2023 survey year. The increase in ASPT for these sites indicated the contribution of pollution-sensitive taxa such as *Ecnomidae* (Caddisflies) and *Chlorocyphydae* (Damselflies), suggesting improved water quality. The other survey locations showed gradual improvement compared with the 2022 survey year, although they remained in poor ecological condition. On the contrary, Alex Park (FC2) remained in poor condition, dominated by pollution-tolerant assemblages. The fluctuating water quality at Musson's Wier (FC3) and Darvill (FR1) may occasionally be influenced by the inputs from their respective tributaries, the Dorpspruit and the Baynesspruit. The improvement in the water quality and associated macroinvertebrates was observed on the further upstream sites and downstream sites, while survey sites near the city and around industrial complexes showed deteriorating water quality. The Alex Park (FC2) survey location was directly below a hospital, with an impermeable wier containing refuse material from around the city, as well as sewage and industrial effluents, the high abundances of *Fistulifera saprophila*, a diatom species tolerant to organic pollution and a suite of pollution tolerant macroinvertebrates (*Ceratopogonidae*, *Hirudinae* and *Chironomidae*), indicated that the pollution is continuous at this site.

The further downstream site (FR3) was located in a rural community with fewer industrial activities but had sand mining evident. The sand mining interfered with the habitat structure, altering available niches. The presence of *S. seminulum* and *G. parvulum*, species tolerant to organic pollution and siltation, suggested that agricultural inputs and siltation from sand mining impacted this site. However, the high abundance of pollution-sensitive macroinvertebrates such as *Baetidae*, *Heptageniidae*, *Tricorythidae* and *Hydropsychidae* species indicated that the water quality had recovered somewhat at this site. The FFS refinery (FC1) was also located in a periurban community, with an oil refinery about 700 m off the river bank. The main stressors of this site were illegal refuse dumping and point source pollution (pers. obs.). The presence of *Fistulifera saprophila* may indicate occasional organic pollution from the oil refinery; however, the presence of pollution-sensitive macroinvertebrate taxa and high SASS5 scores and average scores per taxon indicated a gradual improvement in water quality at this site, although stressors limited full recovery to pristine conditions.

The findings from this study shine a light on the present state of water quality in the uMsunduzi River and are comparable to previous studies (Gemmel and Schmidt 2013; Agunbiade and Moodley 2016). The water quality of the uMsunduzi system is compromised by surrounding human activities such as wastewater discharges, as previously described in other studies (Dickens and Graham 1998, 2002; Gemmel and Schmidt 2013). The responses of taxa also indicate a trend towards declining water quality in the uMsunduzi River, with some sites gradually gaining pollution-sensitive species to indicate improvement.

3.6 Conclusions

The overall water quality of the uMsunduzi River in the present study was comparable to previous monitoring surveys, and various survey locations have shown gradual improvement since the chemical spill from the Willowton Group Ltd factory in 2019 and compared with previous studies. Various indices assessed along the uMsunduzi River and selected tributaries had a trend of poor water quality with potential for recovery. The sites away from industrial, commercial and municipal facilities were the ones where monitored indices showed some level of improvement in water quality. The Willowton Group Ltd factory mixed product chemical spill severely impacted the water quality of an already working urban river. Since then, parts of the uMsunduzi River free from the impacts of anthropogenic stressors have shown potential for rehabilitation; however, the full recovery to the pre-spill conditions at various sites is limited by the continued impacts of anthropogenic stressors. The preliminary real-time water quality results showed the potential for pinpointing pollution events and managing pollution sources for improved water quality should the challenges around water quality probes be resolved.

Monitoring the responses of various indices provides a holistic approach to monitoring water quality and devising viable strategies to rehabilitate a river after a chemical spill. This is particularly relevant in the present day and age to meet sustainable development goal 6 (SDG6), clean water and sanitation. Furthermore, the uMsunduzi River is a national treasure for the Dusi Canoe Marathon and contributes essential water resources for the eThekiwni and uMgungundlovu metropolitans.

It is recommended that existing legislation be enforced to manage and mitigate the intensity of anthropogenic impacts on the sites near the city to improve water quality, water provision, and ecosystem function. The legislative bylaws, such as the National Environmental Management Act (NEMA) number 10 of 2004, should be enforced, particularly section 9A, because it allows ministers to prohibit any activities threatening the well-being of the environment. The polluter pay principle should also be enforced so that industrial operations are carried out with minimal environmental damage and to curb polluters' impunity. The sewage infrastructure requires maintenance and upgrading to limit leakages into the river. Continuous monitoring of the water quality for the uMsunduzi River is recommended to ascertain pollution sources and to guide stakeholder engagements for future development, such as the Baynesspruit Conservancy and Catchment Action Plan.

3.7 Acknowledgments

We are grateful to the University of KwaZulu-Natal (ZA), the Willowton Group LTD (ZA) and the National Research Foundation (ZA, grant 98404) for funding. We thank postgrads and interns from the Downs lab for assisting with fieldwork. We thank the Ford Wildlife Foundation (ZA) for vehicle support. We thank GroundTruth water, wetlands and environmental engineering divisions and Umgeni Water for additional data.

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3.9 Supplementary information

Supplementary information Figure S3.1: Example of micro-invertebrate (SASS5) assessments and collection to evaluate water quality

Supplementary information Figure S3.2: Point source pollution from the outlet pipe upstream of the Alex Park (FC2) survey location

Supplementary information Figure S3.3: Treated effluent from Darvill Wastewater Treatment Plant flowing into the uMsunduzi mainstream, upstream of Grimthrope (FR2).

Supplementary information Figure S3.4: Real-time water quality probe at lower Baynesspruit (BS3), covered with debris washed downstream from surrounding urban land use.

Supplementary information Figure S3.5: The slimy floor from sewage and industrial pollution at lower Baynesspruit and uncollected or illegal dump refuse clogging the culvert.

Supplementary information Figure S3.6: High flows at Grimthope Bridge (FR2), hindering the collection of macroinvertebrates, diatoms and electronarcosis.

Supplementary information Table S3.1: Probe unique codes, deployment and replacementdates for broken and lost probes, as well as the inventory of active probes.

CID	UID 15/11/2022	UID 16/11/2022	UID 22/11/2022	UID 23/11/2022	UID 12/01/2023	UID 22/02/2023	UID 29/03/2023			UID 12/05/2023	UID 18/07/2023					Site	Date deployed	replacements	2nd replacements
1038	1				1	10	10		1	10						BS1	15/11/2022	03/2023	
1038				2			2			2						BS2	23/11/2022		
1038				4	4	9				9	18					BS3	23/11/2022	19/01/2023	23/08/2023
1038	3				3	5				5	6					FC3	15/11/2022	03/2023	18/07/2023
1038		5				7				7						FR3	16/11/2022	19/01/2023	
1038			6		6	8				8	17					FR1	22/11/2022	19/01/2023	23/08/2023
																Deployed (Green)			
																Lost(Gold)			
																Retrieved (Red)			

Supplementary information Table S3.2: Additional data from Umgeni Water routine surveys for the uMsunduzi River and Darvill Wastewater Treatment Plant.

Sampling point code	Description	Sample date	Result name	Result entry	Unit
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	% Weed cover	0	PERCENT
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	pH OS	7.6	NONE
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	Temperature OS	17.8	DEG_C
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	*E.coli*	48840	MPN_100ML
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	SS	29.6	MG_L
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	Turbidity	35.5	NTU
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	NO3	2.2	MG_N_P_L
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	COD	<20.0	MG_O2_L
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	Conductivity	25.2	MS_M
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	NH3	1.72	MG_N_P_L
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	SRP	7.91	UG_PL
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	TP	68.1	UG_PL
RMD014	Msunduzi u/s of Refuse Dump	3/27/2023	Al (AE)	765	UG_AL_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Cl2 (F) OS	<0.05	MG_CL2_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Cl2 (T) OS	<0.05	MG_CL2_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	NH3 (F)	0.0042	MG_N_P_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	% NH3	0.47	PERCENT
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	% Weed cover	0	PERCENT
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	pH OS	7	NONE
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Temperature OS	19.7	DEG_C
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	*E.coli*	61310	MPN_100ML
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	NO2	0.15	MG_N_P_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	NO3	2.28	MG_N_P_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	COD	<20.0	MG_O2_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Conductivity	25.8	MS_M
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	NH3	0.9	MG_N_P_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	SRP	10.9	UG_PL
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	TP	89.4	UG_PL
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Colour	8.2	MG_PT_CO_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	SS	25.6	MG_L
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Appearance	Slightly Turbid Yellow	NONE
RMD017	Msunduzi u/s of Darvill Maturation river	3/27/2023	Odour	Nil	NONE

Supplementary information Table S3.3: Diatom species and abundances for quarter 2 (August 2022) amongst all the survey locations.

Diatom species	FC1	FC2	FC3	FR1	FR2	FR3
Abnormal diatom valve or sum of deformities	12	2	0	2	4	1
Achnanthidium crassum (Hustedt) Potapova & Ponader	147	2	2	2	0	0
Achnanthidium minutissimum (Kützing) Czarnecki	0	3	0	1	0	0
Achnanthidium saprophilum (Kobayasi & Mayama) Round & Bukhtiyarova	23	0	0	0	0	1
Achnanthidium spp.	0	0	1	5	0	0
Amphora montana Krasske	3	0	0	0	1	0
Aulacoseira ambigua (Grunow) Simonsen	0	0	0	0	1	0
Aulacoseira granulata (Ehrenberg) Simonsen	0	0	0	0	1	0
Aulacoseira granulata var. angustissima (O.Müller) Simonsen	0	0	0	0	1	0
Cocconeis pediculus Ehrenberg	0	0	0	0	0	14
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	14	1	8	11	5	338
Craticula accomoda (Hustedt) Mann	0	0	0	0	2	0
Diadesmis confervacea Kützing	0	0	0	0	4	0
Diadesmis contenta (Grunow) D.G. Mann	0	0	0	1	0	0
Encyonema minutum (Hilse) D.G. Mann	15	0	0	0	0	0
Encyonopsis leei var. sinensis Metzeltin & Krammer	2	0	0	0	1	0
Eolimna minima(Grunow) Lange-Bertalot	0	1	0	0	0	1
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	1	5	0	1	1	3
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	45	213	0	17	0	0
Fragilaria biceps (Kützing) Lange-Bertalot	0	0	0	0	1	0
Fragilaria capucina Desmazieres	3	0	1	0	0	0
Fragilaria capucina var. vaucheriae (Kützing) Lange-Bertalot	0	0	0	0	0	8
Gomphonema parvulum (Kützing) Kützing	21	34	21	56	19	1
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	3	0	5	8	0	1
Lemnicola hungarica (Grunow) Round & Basson	0	0	0	0	1	0
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	48	1	0	0	0	0
Navicula antonii Lange-Bertalot	0	2	3	4	0	0
Navicula arvensis var. maior Lange-Bertalot	1	0	0	0	2	0
Navicula carissima Lange-Bertalot	2	0	0	0	0	0
Navicula cryptocephala Kützing	8	1	4	50	13	2
Navicula cryptotenella Lange-Bertalot	0	1	0	0	1	0
Navicula erifuga Lange-Bertalot	0	0	0	0	2	0
Navicula gregaria Donkin	24	4	6	29	12	5
Navicula libonensis Schoeman	0	0	0	0	1	0
Navicula notha Wallace	1	0	0	0	0	0
Navicula recens (Lange-Bertalot) Lange-Bertalot	0	0	1	0	0	0
Navicula rostellata Kützing	2	0	1	0	4	0

Navicula spp.	0	1	0	0	0	0
Navicula symmetrica Patrick	1	12	16	26	5	8
Navicula trivialis Lange-Bertalot	0	0	0	22	2	0
Navicula vandamii Schoeman & Archibald	1	0	0	0	1	0
Navicula veneta Kützing	0	2	4	0	4	0
Nitzschia amphibia Grunow	1	0	0	0	0	0
Nitzschia capitellata Hustedt	1	0	0	0	0	0
Nitzschia clausii Hantzsch	0	1	0	0	0	0
Nitzschia dissipata var. media (Hantzsch) Grunow	3	0	0	1	0	0
Nitzschia frustulum (Kützing) Grunow	1	0	0	1	0	0
Nitzschia linearis (Agardh) W.M.Smith	3	1	3	8	2	0
Nitzschia palea (Kützing) W.Smith	12	17	13	56	14	0
Nitzsschia spp.	2	10	5	12	8	0
Pinnularia acrospheria W. Smith	0	0	0	0	1	0
Pinnularia spp.	0	1	0	3	0	0
Pinnularia subbrevistriata Krammer	0	0	0	0	48	0
Planothidium delicatulum (Kützing) Round & Bukhtiyarova	0	0	0	0	0	1
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	2	0	0	0	0	1
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	0	0	2	0	0	0
Sellaphora pupula (Kützing) Mereschkowksy	1	0	0	5	18	0
Sellaphora seminulum (Grunow) D.G. Mann	8	84	3	73	18	14
Surirella angusta Kützing	1	0	1	3	2	0
Tryblionella hungarica (Grunow) D.G. Mann	0	3	0	5	4	2

Supplementary information Table S3.4: Diatom species and abundances for quarter 3 (November 2022) for selected sampling sites with data for this quarter.

Diatom species	**BS2**	**BS3**	**FC1**	**FC2**	**FC3**	**FR1**
Abnormal diatom valve or sum of deformities	14	9	0	4	1	2
Achnanthidium crassum (Hustedt) Potapova & Ponader	0	0	8	5	1	5
Achnanthidium saprophilum (Kobayasi & Mayama) Round & Bukhtiyarova	0	1	0	0	0	0
Achnanthidium spp.	22	0	8	3	3	0
Amphora montana Krasske	0	0	0	0	0	1
Caloneis spp.	0	0	0	1	0	0
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	15	1	1	2	1	0
Craticula molestiformis (Hustedt) Lange-Bertalot	0	0	0	1	0	0
Denticula sundayensis Archibald	0	0	0	1	0	0
Diadesmis confervacea Kützing	0	0	0	0	3	0
Encyonema krasskei (Krammer) Krammer	0	0	0	1	0	0
Encyonema minutum (Hilse) D.G. Mann	0	0	4	1	0	1
Encyonopsis leei var. sinensis Metzeltin & Krammer	0	0	0	1	0	0
Eolimna minima (Grunow) Lange-Bertalot	0	0	1	0	0	0
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	0	0	0	1	0	1
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0	0	0	0	1	0
Fragilaria biceps (Kützing) Lange-Bertalot	0	1	4	0	0	0
Fragilaria capucina Desmazieres	0	0	5	0	0	1
Gomphonema parvulum (Kützing) Kützing	29	165	18	6	17	27
Gomphonema pseudoaugur Lange-Bertalot	0	0	0	2	0	2
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	0	0	0	0	0	3

Gyrosigma scalproides (Rabenhorst) Cleve	0	0	0	0	0	1
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	0	0	0	1	0	0
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	0	0	0	0	1	0
Navicula antonii Lange-Bertalot	3	1	0	3	0	0
Navicula arvensis var. maior Lange-Bertalot	0	0	0	2	0	0
Navicula cryptocephala Kützing	2	3	2	3	0	2
Navicula cryptotenella Lange-Bertalot	0	0	0	1	0	2
Navicula erifuga Lange-Bertalot	0	0	0	0	1	0
Navicula germainii Wallace	0	0	0	0	0	1
Navicula gregaria Donkin	0	0	11	1	0	1
Navicula notha Wallace	0	0	0	1	0	0
Navicula riediana Lange-Bertalot & Rumrich	0	0	0	0	1	0
Navicula rostellata Kützing	4	1	0	2	2	0
Navicula spp.	4	0	0	5	0	2
Navicula symmetrica Patrick	2	1	4	25	6	4
Navicula veneta Kützing	5	0	1	3	1	0
Nitzschia amphibia Grunow	1	0	0	0	0	0
Nitzschia capitellata Hustedt	1	0	0	0	0	0
Nitzschia desertorum Hustedt	0	0	0	0	0	2
Nitzschia frustulum (Kützing) Grunow	3	1	0	2	2	0
Nitzschia linearis(Agardh) W.M. Smith	0	0	1	0	0	0
Nitzschia palea (Kützing) W.M. Smith	12	14	30	3	57	11
Nitzschia spp.	3	1	0	2	0	3
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	2	0	0	1	0	0

Pinnularia lundii Hustedt var. linearis Krammer	0	0	0	0	0	1
Pinnularia subbrevistriata Krammer	0	1	0	0	0	0
Placoneis spp.	0	0	0	0	0	1
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	2	1	0	1	1	0
Planothidium lanceolatum (Brébisson) Lange-Bertalot	0	0	0	1	0	0
Planothidium rostratum (Oestrup) Round & Bukhtiyarova	0	0	0	0	0	1
Psammothidium oblongellum (Oestrup) Van de Vijver	0	0	0	1	0	0
Sellaphora pupula (Kützing) Mereschkowksy	5	1	1	0	0	0
Sellaphora seminulum (Grunow) D.G. Mann	284	207	1	16	2	27
Tryblionella apiculata Gregory	1	0	0	0	0	0

Supplementary information Table S3.5: Diatom abundances and species richness for quarter 4 (March 2023) for the survey locations along the uMsunduzi River.

Diatom species	BS1	BS2	BS3	FC1	FC2	FC3	FR1	FR2	FR3
Abnormal diatom valve or sum of deformities	4	11	11	2	0	0	0	0	5
Achnanthes rupestris Krasske	1	0	0	0	0	0	0	0	0
Achnanthidium catenatum (Bily & Marvan) Lange-Bertalot	0	1	1	0	0	1	0	0	0
Achnanthidium crassum (Hustedt) Potapova & Ponader	2	0	0	54	1	6	4	2	2
Achnanthidium exiguum (Grunow) Czarnecki	0	0	1	0	0	0	0	0	1
Achnanthidium minutissimum (Kützing) Czarnecki	0	0	0	0	2	0	2	6	7
Achnanthidium spp.	0	0	3	9	5	0	0	2	2
Amphora montana Krasske	7	4	1	0	0	1	0	0	2
Amphora normanii Rabenhorst	0	0	0	0	1	0	0	0	0
Amphora pediculus (Kützing) Grunow	0	0	0	0	0	0	0	1	0
Amphora veneta Kützing	0	0	0	0	0	0	0	2	0
Aulacoseira ambigua (Grunow) Simonsen	0	0	0	0	0	1	2	0	0
Caloneis bacillum (Grunow) Cleve	0	2	0	0	0	0	0	0	0
Caloneis hyalina Hustedt	0	0	0	0	0	0	0	1	0
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	76	3	2	77	0	0	3	3	64
Craticula molestiformis (Hustedt) Lange-Bertalot	1	0	0	0	0	0	0	0	0
Cymbella kolbei Hustedt	0	0	0	0	0	0	2	0	0
Cymbella turgidula Grunow	0	0	0	1	0	1	0	3	0
Denticula sundayensis Archibald	0	0	0	0	0	0	0	1	1
Diadesmis confervacea Kützing	0	0	3	0	0	0	0	0	0
Discostella woltereckii (Hustedt) Houk & Klee	0	0	0	0	2	0	0	0	0
Encyonema minutum (Hilse) D.G. Mann	0	0	0	29	2	11	5	0	0
Encyonopsis leei var. sinensis Metzeltin & Krammer	0	0	0	1	1	0	0	0	0
Eolimna minima (Grunow) Lange-Bertalot	3	8	3	0	0	0	0	0	5
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	38	15	11	8	1	4	2	3	9
Fallacia monoculata (Hustedt) D.G. Mann	0	1	0	0	0	0	0	0	0
Fallacia tenera (Hustedt) Mann in Round	0	0	0	0	0	0	0	0	1
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	7	19	1	138	0	0	0	0	0

Fragilaria biceps (Kützing) Lange-Bertalot	0	0	0	3	0	0	0	0	0
Fragilaria capucina Desmazieres	1	0	1	16	0	11	11	8	10
Fragilaria capucina var. vaucheriae (Kützing) Lange-Bertalot	0	0	0	12	0	0	0	0	12
Fragilaria spp.	0	0	0	0	0	0	0	3	0
Geissleria decussis (Oestrup) Lange-Bertalot & Metzeltin	0	0	0	0	0	0	1	0	0
Gomphonema parvulum (Kützing) Kützing	44	44	33	8	7	65	31	21	16
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	6	3	5	4	1	7	7	3	3
Gomphonema spp.	0	0	0	2	0	1	1	3	14
Gyrosigma acuminatum (Kützing)Rabenhorst	0	0	0	0	0	0	0	0	3
Gyrosigma attenuatum (Kützing) Rabenhorst	0	0	0	0	0	2	0	1	0
Gyrosigma rautenbachiae Cholnoky	0	0	0	0	0	0	0	3	0
Hantzschia amphioxys (Ehrenberg) Grunow	0	0	1	0	1	0	0	0	0
Mayamaea atomus (Kützing) Lange-Bertalot	0	0	0	0	0	2	1	1	2
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	9	24	3	9	1	0	0	0	0
Navicula antonii Lange-Bertalot	1	10	3	0	2	2	0	0	1
Navicula arvensis var. maior Lange-Bertalot	7	2	9	0	0	2	3	0	1
Navicula cryptocephala Kützing	2	1	2	0	1	0	1	2	0
Navicula cryptotenella Lange-Bertalot	1	1	0	0	1	2	0	0	0
Navicula cryptotenelloides Lange-Bertalot	2	0	0	0	0	0	0	0	0
Navicula erifuga Lange-Bertalot	3	4	3	1	4	2	1	5	51
Navicula germainii Wallace	0	0	0	0	0	2	2	0	7
Navicula gregaria Donkin	1	1	0	0	1	1	0	4	0
Navicula reichardtiana Lange-Bertalot	0	1	0	0	0	0	0	0	0
Navicula rostellata Kützing	2	6	12	1	3	3	10	8	22
Navicula schroeteri Meister	0	0	0	1	0	0	0	0	0
Navicula sp.	0	0	0	2	0	0	1	3	2
Navicula symmetrica Patrick	22	14	7	10	19	177	52	48	24
Navicula tenelloides Hustedt	0	0	0	0	0	0	0	0	2
Navicula trivialis Lange-Bertalot	0	0	0	0	1	0	0	0	0
Navicula vandamii Schoeman & Archibald	1	0	0	1	0	0	2	1	0
Navicula veneta Kützing	5	0	4	0	0	2	0	1	0
Navicula vilaplanii (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater	1	0	1	0	0	0	0	0	0
Nitzschia amphibia Grunow	0	2	3	0	1	0	3	1	1

Nitzschia clausii Hantzsch	0	0	0	0	0	0	2	0	0
Nitzschia dissipata var. media (Hantzsch) Grunow	1	0	0	0	0	1	0	0	0
Nitzschia frustulum (Kützing) Grunow	0	0	2	0	0	1	0	2	1
Nitzschia intermedia Hantzsch	0	0	0	0	0	0	0	0	1
Nitzschia linearis(Agardh) W.M.Smith	0	0	1	0	0	0	0	1	0
Nitzschia palea (Kützing) W.Smith	11	6	79	1	25	66	25	38	64
Nitzschia spp.	9	3	5	7	17	15	16	5	26
Nitzschia terrestris (Petersen) Hustedt	0	0	0	0	0	1	0	0	0
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	0	0	1	0	0	0	0	0	0
Pinnularia acrospheria W. Smith	0	0	0	0	0	0	0	1	0
Pinnularia borealis Ehrenberg	0	0	0	0	0	1	0	1	0
Pinnularia gibba Ehrenberg	0	0	0	0	0	0	0	1	0
Pinnularia lundii var. linearis Krammer	0	0	0	0	0	1	0	0	0
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	3	2	2	0	0	0	0	0	0
Planothidium rostratum (Oestrup) Round & Bukhtiyarova	6	0	0	0	0	0	0	0	0
Planothidium spp.	0	0	0	0	0	0	1	0	0
Pleurosigma salinarum (Grunow) Cleve & Grunow	1	0	0	0	0	0	1	2	17
Pleurosira laevis (Ehrenberg) Compére	0	0	0	0	0	0	0	0	3
Reimeria sinuata (Gregory) Kociolek & Stoermer	0	1	0	0	0	0	0	0	0
Reimeria uniseriata Sala, Guerrero & Ferrario	0	1	0	0	0	0	0	0	1
Sellaphora pupula (Kützing) Mereschkowksy	0	0	3	0	0	0	0	1	1
Sellaphora seminulum (Grunow) D.G. Mann	125	221	193	5	0	2	7	6	15
Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli	0	0	0	0	0	5	0	0	1
Simonsenia delognei Lange-Bertalot	0	0	1	0	0	0	0	0	0
Stauroneis smithii Grunow	0	0	0	0	0	0	1	0	0
Staurosirella pinnata (Ehrenberg) Williams & Round	0	0	0	0	0	0	0	1	0
Tryblionella apiculata Gregory	0	0	0	0	0	1	0	0	0
Tryblionella debilis Arnott ex O'Meara	1	0	0	0	0	0	0	0	0
Tryblionella levidensis Wm. Smith	0	0	0	0	0	0	0	1	0

Supplementary information Table S3.6: Macroinvertebrate families and their abundances amongst the survey locations between 2022 and 2023.

Taxa	Aug-22 **FC1**	Aug-22 **FC2**	Aug-22 **FC3**	Aug-22 **FR1**	Aug-22 **FR2**	Aug-22 **FR3**	Mar-23 **FC1**	Mar-23 **FC2**	Mar-23 **FC3**	Mar-23 **FR1**	Mar-23 **FR2**	Mar-23 **FR3**
Porifera												
Coelenterata												
Turbellaria												
Oligochaeta	1	1		1	1		1		1	1	1	1
Leech	3	3		3	3		3	3	3	3	3	3
Amphipoda												
Potamonautidae		3					3	3	3			3
Atyidae	8					1						
Palaemonidae			3			3						
Hyracarina							8	8				
Notonemouridae												
Perlidae						8	12					
Baetidae 1spp		4				10		4				
Baetidae 2spp				6		8						
Baetidae >2spp	12				12		12		12	12	12	12
Caenidae	6	6		6	6		6		6	6	6	6
Ephemeridae												
Heptageniidae												13
Leptophlebiidae	9				9	12	9					9
Oligoneuridae						6						
Polymitarcyidae												
Prosopistomatidae												
Teloganodidae						9						
Tricorythidae							9					9
Calopterygidae												
Chlorocyphidae										10	10	10
Chlorolestidae									8			
Coenagrionidae	4	4		4	4		4	4	4	4	4	4
Lestidae												
Platycnemidae												
Protoneuridae												
Aeshnidae						4						
Corduliidae												
Gomphidae				6	6		6		6	6		6
Libellulidae	4	4			4			4			4	4
Pyralidae												
Belostomatidae											3	3
Corixidae	3	3							3			3
Gerridae			4			4	5	5				5
Hydrometridae												
Naucoridae					7		7		7	7	7	7
Nepidae			3			3				3		
Notonectidae												
Pleidae											4	4

Veliidae/Mesovel								5	5			
Corydalidae												
Sialidae												
Dipsuedopsidae												
Ecnomidae						5					8	
Hydropsychidae 1spp	4			4		8		4				
Hydropsychidae 2sp					6	6	6		6		6	6
Hydropsychidae >2spp										12		
Philopotamidae												
Polycentropodidae			4			4						
Psychomyiidae												
Lepidostomatidae												
Leptoceridae												
Hydroptilidae												
Calamoceratidae												
Pisuliidae												
Barbarochthonidae												
Glossosomatidae												
Hydrosalpingidae												
Petrothrincidae												
Sericostomatidae												
Dytiscidae							5			5		
Elmidae/Dryopidae									8			
Gyrinidae	5	5		5	5							
Haliplidae												
Helodidae												
Hydraenidae												
Hydrophilidae			5			5						
Limnichidae												
Psephenidae												
Athericidae												
Blepharoceridae												
Ceratopogonidae	5	5			5					5		
Chironomidae	2	2		2	2		2	2	2	2	2	2
Culicidae												
Dixidae												
Empididae						5						
Ephydridae						2						
Muscidae							1					
Psychodidae												
Simuliidae							5			5		
Syrphidae												
Tabanidae												5
Tipulidae												
Ancylidae							6					6

Bulininae												
Hydrobiidae												
Lymnaeidae												
Physidae	3	3		3	3					3		
Planorbinae												
Thiaridae												
Viviparidae												
Corbiculidae						3						
Sphaeriidae												
Unionidae												

CHAPTER 4

Pre, post and present ecological conditions in an environmentally and economically important river: the uMsunduzi River, Pietermaritzburg, South Africa

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Formatted for *Ecological Indicators*

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Running header: Ecological conditions in an important river

4.1 Abstract

We studied fish communities post a fish kill to understand the population dynamics and recovery of *Labeobarbus natalensis* as an ecological indicator species of the uMsunduzi River, KwaZulu-Natal, South Africa, from mid-2022 through 2023. We conducted quarterly fish community surveys at selected sites along the uMsunduzi River to assess fish species composition and abundance. We used fish translocation to improve population structures in the afflicted areas below the spill point using *L. natalensis* sourced from upstream healthy populations and understanding the movement and persistence of translocated *L. natalensis* by telemetry. We caught *L. natalensis* individuals upstream of the spill point and marked them with Biomark, full duplex pit tags, before translocation to downstream sites. However, no recaptures were recorded despite our efforts. We used the Fish Response Assessment Index (FRAI) to understand the river health of the uMsunduzi River. The results showed that fish communities are still recovering. The multivariate analysis showed conductivity, velocity depth and substrate as significant driving forces for the fish communities. The upstream sites significantly differed in fish species richness than the downstream sites. However, the size classes were comparable amongst all the species, except for the sharptooth catfish (*Clarias gariepinus*), which showed size classes above 500 mm. The FRAI also showed a trend towards poor ecological health for the uMsunduzi River, with fewer species than expected. The uMsunduzi sites varied between poor and seriously modified conditions; however, the downstream site (FR3) occasionally attained improved conditions and fish responses after the rainy seasons. The removal of barriers and limiting pollution inputs may allow for self-remediation and recovery of the uMsunduzi River; however, mitigating these impacts seems unlikely under present conditions.

Keywords: Fish communities; Biomonitoring; Fish kills; Telemetry; Pollution; Freshwater ecosystems

4.2 Introduction

Freshwater ecosystems are vital to maintaining human livelihoods and ensuring socio-economical sustainability (Rodriguez-Iturbe et al. 2009; Bergendahl et al. 2018; Thiem et al. 2022). These systems are crucial for water purification, transportation, water supply and conservation of aquatic organisms (Heathwaite 2010; Tickner et al. 2020; Vari et al. 2023). The stressors caused by natural processes and anthropogenic activities threaten freshwater ecosystems globally (Silva et al. 2017; Dudgeon et al. 2006; Tickner et al. 2020). The rapid growth in the human population drives changes in demographics, land use and industrial production (Heathwaite 2010; Xie et al. 2018; Danaher et al. 2022). The changing demographics and growth in population drive extensive agricultural activities and increase water demand, impacting freshwater ecosystems and associated biota as more water is appropriated for human use (Li et al. 2017; Xie et al. 2018; Grill et al. 2019). As the human population is projected to reach 9 billion by 2025, viable water management strategies are important to ensure clean water and sanitation as well as the protection of aquatic life (Dudgeon 2014; FWBON 2022).

Stressors on freshwater ecosystems are multifaceted with various impacts and are often a by-product of human activities (Chapagain et al. 2006; Tickner et al. 2020). Sources of pollution vary, and the stressor impact on the ecosystem differs (Dudgeon et al. 2006; Caballero and Navarro 2021; Piczak et al. 2023). Pollutants vary from non-chemical, such as thermal pollution from hydropower generation, to agrochemicals, pharmaceuticals, industrial effluents, and sewage inputs (Zhang et al. 2009; Mekonnen and Hoekstra 2018; Caballero and Navarro 2021; FWBON 2022).

Anthropogenic driver stressors are exacerbated by climatically driven factors, such as drought (Sabater et al. 2022). Drought reduces water in a freshwater ecosystem, raising water temperature and reducing the dilution effect of toxic chemicals when present; this severely compromises freshwater ecosystem well-being (Dlamini 2019; Dudgeon 2020; Jaureguiberry et al. 2022; Piczak et al. 2023).

The installation of instream physical barriers, such as dams, reservoirs, weirs and other water structures, limit the longitudinal movement of migratory fish (Pardini et al. 2017; O'Brien et al. 2019; GEO BON 2022; FWBON 2022). River connectivity and uninterrupted flows play a role in the lifecycle of migratory fish species, particularly catadromous fish (e.g., *Anguilla* spp.) and potamodromous fish (e.g., *Labeobarbus natalensis*) (Thronson and Quigg 2008; Burnett et al. 2018, 2021; Hanzen et al. 2020; 2021). The instream physical barriers fragment fish communities, reduce available biotopes and impact natural flows, which are essential in providing cues for reproduction (Alexander et al. 2012; O'Brien et al. 2019; Hanzen et al. 2021; Jaurenquiberry et al. 2022;). Habitat loss and fragmentation drive declines in populations of indigenous fish, making resources available for invasive species (Burnett et al. 2024). This imbalance impacts the overall ecological integrity of rivers as invasive species are more tolerant to water quality changes and niche decline (Ormerod et al. 2010; Strayer 2010; Evans et al. 2022; Burnett et al. 2024).

The tragedy of the commons, particularly unregulated fishing and water abstraction, drives unsustainable use of freshwater resources (Heathwaite 2010; Li et al. 2017; Xie et al. 2018; Danaher et al. 2022; Piczak et al. 2023). In such a predicament, monitoring techniques are required to understand the impact of stressors and guide the management of rivers to protect fish (Fisher et al. 2019; Wade et al. 2021; Evans et al. 2022; Burnett et al. 2024). Monitoring water quality often includes physicochemical measurements in situ and water samples for *Escherichia coli* and total coliforms (Li et al. 2010; Gemmel and Schmidt 2013; USEPA 2021). The physicochemical properties reflect the quality of water and associated biotopes; however, the sources of stressors and their impacts may be overlooked if biomonitoring is not considered (Ramesh et al. 2018; Lange et al. 2018; Burnett et al. 2020, 2024). Change in fish behaviour is known to be acute in response to environmental changes and relevant to river stressors (Heathwaite 2010; Burnett et al. 2020; Burnett et al. 2024). Fish behavioural responses provide a holistic approach to understanding the impacts of stressors on freshwater ecosystems (Wepener 2008; O'Brien et al. 2011, 2013a, 2016; Burnett et al. 2024). Indices used to assess the ecological integrity of freshwater ecosystems depend on data collected from aquatic fauna, flora, and surrounding environmental conditions (O'Brien et al. 2011; Agboola et al. 2019, 2020a; Evans et al. 2022).

The occurrence of fish kills has been rising, particularly because human activities devastate freshwater ecosystems globally (Stocks et al. 2022). The impact of anthropogenic stressors can be overlooked until fish mortalities are apparent as a line of evidence (La and Cooke 2014; Stocks et al. 2022). Therefore, monitoring biological responses allows for holistic mitigation of the anthropogenic effects and protection of aquatic fauna.

The uMsunduzi River, a historically pristine urban stream stretching 115 km through the KwaZulu-Natal Midlands has been subject to a plethora of anthropogenic impacts over the years (Gemmel and Schmidt 2013; Matongo et al. 2015). In addition to periodic inputs of sewage and toxic effluents, uMsunduzi River has been devastated by a severe mixed product spill from the Willowton Group Ltd factory (SAPA+ 2019). A valve was suspected to have burst in the fatty acid storage, bringing it down. The tank further dislodged caustic soda and edible oil holding tanks, leaving 240 tons of toxic effluent to flood the Baynesspruit (The Witness 2019). An estimated 1.6 million litres of the effluent reached the mainstream of the uMsunduzi River through the

Baynesspruit-uMsunduzi confluence above the Darvill Wastewater Treatment Plant and left a trail of destruction in the aquatic organisms of afflicted locations (The Witness 2019; Mdletshe 2019; DUCT 2019a,b).

The KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) is a long-lived, charismatic species that is often targeted by small-scale and recreational fishermen (Karssing et al. 2007; Impson et al. 2008). The niche specialisation at different life stages, long lifespan and potamodromous migratory behaviour of this species make it a suitable ecological indicator (Impson et al. 2008; Paxton et al. 2013; Burnett et al. 2021). Therefore, the recovery of the yellowfish population would greatly improve the ecological integrity of the uMsunduzi system. The KwaZulu-Natal yellowfish is presently listed as least concern in the International Union for Conservation of Nature (IUCN 2020). However, the sustained fragmentation of rivers and regulation of flows threatens the reproductive success of the species, and it may be in decline (Karssing et al. 2007; O'Brien et al. 2019). We predicted that fish communities have improved in the uMsunduzi River since the fish kill in 2019. This study aimed to monitor fish communities and the recovery of *L. natalensis* population as an ecological indicator species of the uMsunduzi River. Quarterly surveys were done to collect fish at designated sites, including in tributaries.

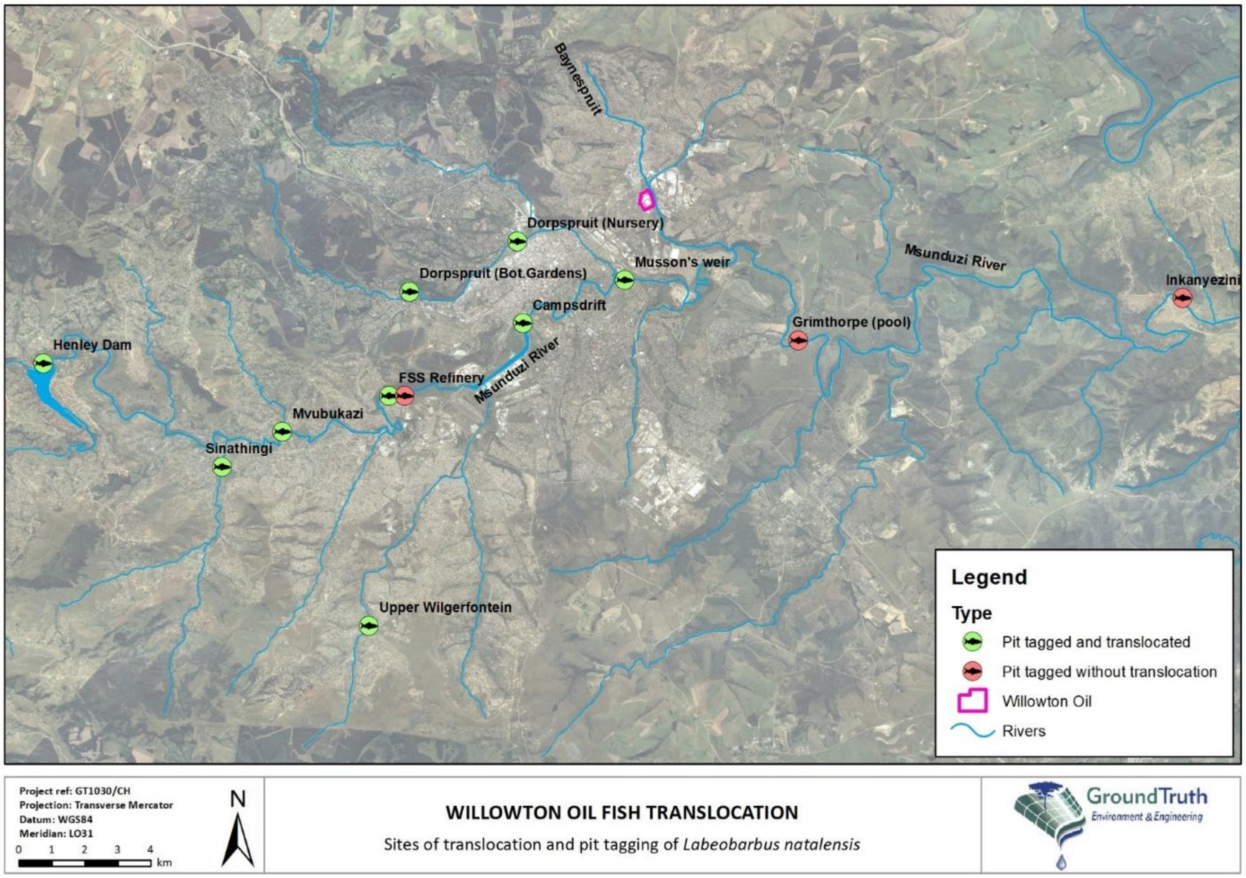

Figure 4.1: Fish collection and release sites along the tributaries and the main uMsunduzi system for translocated and PIT-tagged KwaZulu-Natal yellowfish individuals (See Supplementary Information Table S4.1). The main collection and release sites for fish were the FSS refinery fish capture site one, Alexandra Park (downstream of Campsdrift) fish capture site two, and Musson's Weir fish capture site three. Sampled areas in the region of the spill include Darvill Wastewater Treatment Plant fish release one, Grimthorpe (pool) fish release two and Inkanyezini fish release three.

4.3 Methods

4.4.1 Study area

The KwaZulu-Natal Province is the second-largest province, and in the eastern region of South Africa, covering 92,285 km^2 and characterised by remarkable variations in climate, topography, and geological attributes (Agboola et al. 2019). KwaZulu-Natal lies in the summer rainfall belt and ranges from low-lying coastal areas to the highest point of the Drakensberg mountains (3000 m asl) (Agboola et al. 2020a). The province is dominated by seasonal rainfall and weather conditions. Areas of the provinces located in the interior plateau experience a fluctuation in rainfall and temperatures whilst ocean climate maintains homeostatic climatic conditions along the province's coastal areas. KwaZulu-Natal is characterised by significant biodiversity, with some terrestrial and aquatic organisms endemic to parts of the province (Impson et al. 2008; Chakona et al. 2022), such as the KwaZulu-Natal yellowfish (Skelton 2000). KwaZulu-Natal has several socio-economically important rivers, such as the uMngeni River (Burnett et al. 2021). Furthermore, vast catchments are divided into water management areas to provide water resources to surrounding communities (Burnett et al. 2021; Evans et al. 2022).

The uMsunduzi River, an important tributary of the uMngeni River, jointly providing water to ~ 4 million people in the uMgungundlovu and eThekwini municipalities (Matongo et al. 2015), has been subject to the impacts of anthropogenic stressors over the years. The uMsunduzi River flows through the Msunduzi Municipal district with a population estimate of 618,536 and an area of 634 km^2 in the city of Pietermaritzburg. The uMsunduzi River has a catchment size of 875 km^2 and a tributary length of 115 km (Matongo et al. 2015). The uMsunduzi catchment is exposed to a variety of land use activities since it drains two-thirds of the metropolitan region, and the main river stem passes through agricultural, domestic, municipal, and industrial zones to its confluence with uMngeni River between Nagle Dam and Inanda Dam (Fig. 4.1, Namugize et al. 2018). The land use zones with associated activities along the uMsunduzi River provide a basic understanding of sources for anthropogenic stressors and their fate along the river's course to its confluence with the uMngeni River (Namugize et al. 2018). The uMsunduzi River receives runoff water from communal and municipal areas, chemical effluent from neighbouring industries, and effluent from sewers and wastewater treatment plants. Furthermore, the major wastewater treatment plant responsible for all the wastewater for the uMsunduzi district discharges final treated effluent into the uMsunduzi River. The Darvill Wastewater Treatment Plant receives wastewater from hospitals and domestic, commercial, and industrial sources, often with effluents that the facility is not designed to handle (Dickens and Graham 1998; Pole 2000; Gemmel and Schmidt 2013).

The mixed product spill from the Willowton Group Ltd factory further devasted an already stressed river with poor ecological health (Dickens and Graham 1998; Agunbiade and Moodley 2016; Gemmel and Schmidt 2013). The mixed product spill from the Willowton Group Ltd factory impacted the river, worsening the ecological integrity and functionality of the uMsunduzi system.

Sampling sites along the uMsunduzi River were strategically selected to encompass the land use activities and locations afflicted with catastrophic mixed product spill from the Willowton Group Ltd factory in Pietermaritzburg 2019 (DUCT 2019a,b). The sampled areas along the river for the present study comprised three sites upstream of the oil spill. These sites included the FSS refinery (Fish capture (FC) 1) (-29.633692, 30.339811); Alexandra Park site (FC2) (-29.608872, 30.380418); and Musson’s Weir site near the N3 (FC3) (-29.602114802614313, 30.404199586869773) (Fig. 4.1). Sampled areas in the region of the spill include Darvill Wastewater Treatment Plant (Fish release (FR) 1) (29.593554,30.438395); Grimthorpe Bridge (FR2) (-29.618502, 30.447223) and Inkanyezini (FR3) (-29.60699, 30.55220) (Fig. 4.1).

4.4.2 Fish community surveys

From mid-2022 through 2023, quarterly fish monitoring surveys were undertaken at selected sections along the uMsunduzi River during high and low flow seasons to assess the recovery of fish populations and rehabilitation after the 2019 acute pollution spill (Supplementary information Figs S4.1-S4.8). Quarterly monitoring was specifically important to better understand the uMsunduzi River ecosystem and capture the natural variation in fish species and the migration and spawning behaviour of *L. natalensis*.

In the initial stage of the surveys at each site along the uMsunduzi catchment, we established a transect of 50 m using a measuring tape, and then at 5 m intervals along the transect, we sampled for fish for at least 3 minutes, we then categorised the biotope sampled to account for point abundance sampling. We used an electro-fisher (SAMUS 725M electro-fisher, SAMUS Special electronics, Poland) with landing nets to capture fish in wadable river sections. In addition, where deep pools were present at the study site, we set gill nets (Multifilament nylon, 32 m long, 1,5 m deep, 75 and 118 mm stretched mesh sizes, Eigevis group of companies, Cape Town, South Africa) for the duration of the site survey, and we undertook seine netting (32 m, 18 mm mesh size Eigevis group of companies, Cape Town, South Africa). We transferred the captured fish to a holding container, a 750 L JoJo tank containing 500 L of water. We then identified the captured fish to species level, measured and recorded each individual's standard length (SL), fork length (FL), and total length (TL) using a measuring tape and body mass using a scale. We assessed the body condition of each fish caught before releasing it as soon as possible at the point of capture. The sampling duration at each site ranged between 2 - 3 h.

Furthermore, we collected associated habitat variables within every 5 m of the transect. The selected transect was representative of the niches in the section of the river. We determined the eco-hydraulic classification using the depth and water velocity collected, as well as sampled water quality and substrate type, to quantify available niches. The velocity and depth for each ecohydraulic class were quantified using a transparent velocity head rod (GroundTruth, Hilton, South Africa). The substrate type was categorised as either gravel, silt, mud, sand, cobbles, boulders or bedrock.

4.4.3 Fish translocation

We sourced *L. natalensis* individuals from healthy breeding local populations upstream of the spill point. Primarily adult *L. natalensis* specimens were collected from source (unimpacted) sites on the uMsunduzi over a 2–3-day period using a variety of fishing techniques, namely, electronarcosis, seine netting, fyke- netting and gill-netting as described earlier. We measured and recorded each individual's standard length (SL), fork length (FL), and total length (TL) using callipers and body mass using a scale. We assessed the body condition of each fish caught. Captured fish for translocation that met the minimum requirement for fish tagging of less than 2% body-mass to tag weight ratio were selected for passive integrated transponder (pit) tag fitment. We marked them with pit tags of unique codes for mark-recapture before translocation to downstream sites. We used full duplex (FDX) pit tags (12.5 mm x 2.03 mm, mass of 0.106 g, Biomark, USA). We placed each captured fish in an anaesthetic bath (25 L) using 0.1ml/l of clove oil and monitored signs of narcosis (Burnett et al. 2020, 2024). Once we observed the fish failing to maintain their upright position, we used the Biomark (USA) pit tag injector to administer the pit tag into the fish's abdominal cavity using a tag injector (Biomark, USA). The incision point was less than 2.5 mm, and to ensure the incision sealed, we applied a wound care gel (Aqua Vet, Veterinary hospital, Lydenburg, South Africa) as described in Burnett et al. (2020).

We attempted to capture fish of relatively large size (> 2 kg) to tag with external radio tags (3.6 g, Wireless Wildlife series IV tags, Potchefstroom, South Africa). However, despite our efforts, this size class was lacking from our surveyed localities, and non-suitable fish were captured (Chapter 5). Fish of reasonable size classes were sourced from upstream sites and Henley Dam, and translocated to downstream sites. However, the stress from the transport and the receiving water quality resulted in an unsuccessful tagging attempt (Chapter 5).

4.4.4 Fish Response Assessment Index (FRAI)

We used the Fish Response Assessment Index (FRAI) (Kleynhans 2007; Evans et al. 2022) to understand the river health of the uMsunduzi River. The FRAI is an evaluation of the impacts of environmental changes on fish communities and associated habitats to understand ecosystem health and function (Kleynhans 2007; Avenant 2010; Wepener et al. 2011). The environmental variables used in FRAI include habitat integrity and physicochemical parameters (Dickens and Graham 2002; Thirion 2007), linked to an existing database of the intolerance and preference ratings for a variety of southern African freshwater species (Kleynhans 1999, 2003; FBIS 2023).

The metrics that are assessed in FRAI are categorised as fish preferences and intolerances (Kleynhans and Louw 2007). The metric categories assessed in FRAI include biotope availability, flow modification, migration, cover, physicochemical indices and the impact of introduced species. Evaluating the response of fish species to environmental change is done through direct surveys or inferred from environmental change (Kleynhans 2007). In the present study, the assessment was conducted by direct fish and habitat sampling. The index was based on a combination of fish sample data and fish habitat data, and the responses of fish were evaluated based on the available knowledge of each species' ecological requirements (Kleynhans 2007). The FRAI results are represented as ecological categories (Kleynhans 2007). The FRAI results are automatic and adjusted scores. The automatic score is based solely on the differences in frequency of occurrence between expected and observed fish species at each specific site (Kleynhans 2007); however, it does not account for velocity-depth, cover, flow modification, physicochemical and sampling effort, so there is an adjusted score which can be manually altered to accommodate the variations in these factors. Manually adjusting the FRAI score allows the user to evaluate the sampling effort.

4.4.5 Data analyses

We summarised the data using descriptive statistics. The statistical analyses were carried out using R, version 4.2.3 (RStudio Team 2018) with added packages vegan (Oksanen et al. 2019), mvabund (Wang et al. 2012), and lme4 (Bates et al. 2014; Douglas et al. 2015). We conducted a redundancy analysis (RDA) comparing the relative abundance of fish for the site, habitat, and water quality. Furthermore, analyses of the fish communities with environmental variables monitored during the study were tested for multicollinearity and selected based on Pearson's correlation coefficient test (r < 0.50). We then performed an analysis of variance (ANOVA) for multiply variables to determine the relationship between fish communities and measured environmental variables.

4.4 Results

The overall present species richness and fish abundance in the uMsunduzi River were low. Furthermore, none of the tagged fish were recaught in subsequent sampling efforts. Of the thirteen expected fish species in the system (DWS 2014a; FBIS 2023), nine (69.2%) were not recorded at any of the sampling sites, including additional sites selected for sourcing fish for the reintroduction in the uMsunduze River tributaries and the upstream dam (Henley Dam) (Tables 4.1 and 4.2). Overall, 690 individual fish were recorded in the present study, and 186 *L. natalensis* translocated, making a total of 876 fish caught in 312 sampling efforts along the uMsunduzi system, including tributaries with recruiting fish stocks (Table 4.2, Fig. 4.2). Most of the fish caught were of small size classes (Fig. 4.3, Supplementary information Figure S4.3).

The three-key species throughout the study area were the KwaZulu-Natal yellowfish, Mozambique tilapia (*Oreochromis mossambicus*), and the sharptooth catfish (*Clarius gariepinus*), and to a lesser extent, the banded tilapia (*Tilapia sparmanii*) (Tables 4.1 and 4.2, Fig. 4.3). Mozambique tilapia were the most abundant fish species caught (Table 4.2). KwaZulu-Natal yellowfish were the second most abundant species across the study area, with numbers low in impacted sites (Table 4.2). Sharptooth catfish were found across the study area in impacted and unimpacted areas (Table 4.2) and all size classes. Class structure for Mozambique tilapia was predominantly in the 5 cm size class (Fig. 4.3). The KwaZulu-Natal yellowfish size class were from individuals < 350 mm size classes with predominately small individuals, indicating little recruitment into the impacted sites (Fig. 4.3).

The upstream sites were significantly different in fish species richness compared with the downstream sites ($p > 0.0010$, Table 4.2). However, the size classes were comparable amongst all the species ($p = 0.073$), except for the sharptooth catfish, which showed size classes above 500 mm ($p = 0.001$; Fig. 4.3).

The sharptooth catfish, banded tilapia and the Mozambique tilapia were associated with mud and undercut banks in pools and glides of low-flowing water, whereas the KwaZulu-Natal yellowfish juveniles were found in rapids of fast-flowing waters with boulders as dominant substrates and sub-adults found in deep pools. Fish surveys were conducted quarterly from June, August, and November 2022 and March, May, and September 2023. Overall, fish communities at fish collection sites had greater abundance and richness than downstream release sites (Table 4.2).

During the different sampling periods, August had the highest abundance of fish caught, with site FC3 contributing the most in abundance (Table 4.2). The sites near the city depicted low species richness, dominated mainly by the KwaZulu-Natal yellowfish. However, the downstream sites were slightly richer in species, particularly Musson’s Weir (FC3) and Inkanyezini (FR3), with all four fish species caught throughout the study occurring at these sites.

Table 4.1: Fish abundances in the uMsunduzi River of species that were caught during the 2022 and 2023 surveys. (Note: See Fig. 4.1 and Supplementary information Table S4.1 for site details).

Site	**Jun ‘22**	**Aug ‘22**	**Nov ‘22**	**Mar ‘23**	**Apr ‘23**	**Sep ‘23**	**Nov ‘23**	**Grand Total**
FC1	12	11	15	2	8	32		80
FC2	3	5	11	1		20		40
FC3	3	26	2	51			1	83
Total	**15**	**41**	**28**	**72**				**156**
FR1				19		11		30
FR2	3	17	11			11	10	52
FR3	6	70	28	22	42	12	5	185
Total	**4**	**2**	**12**	**46**				**64**
Grand total	**46**	**344**	**107**	**21**	**50**	**86**	**16**	**690**

Table 4.2: Fish relative abundance in the uMsunduzi River from 2017 to 2023 at selected sites (Fig. 4.1) to monitor fish community trends and recovery from periodic pollution. (Data from 2017-2020 sourced from Dlamini 2019, Burnett 2020 and unpublished data)

Site name	2017	2018	2020	2022	2023
US Darvill	-	17	4	-	11
Grimthorpe	-	41	6	17	32
Nkanyezini	64	25	4	104	97
Alex Park	-	14	-	39	20
Refinery	-	-	-	20	47
Musson’s Weir	-	-	-	34	47
Lower Baynesspruit	-	-	-	25	-

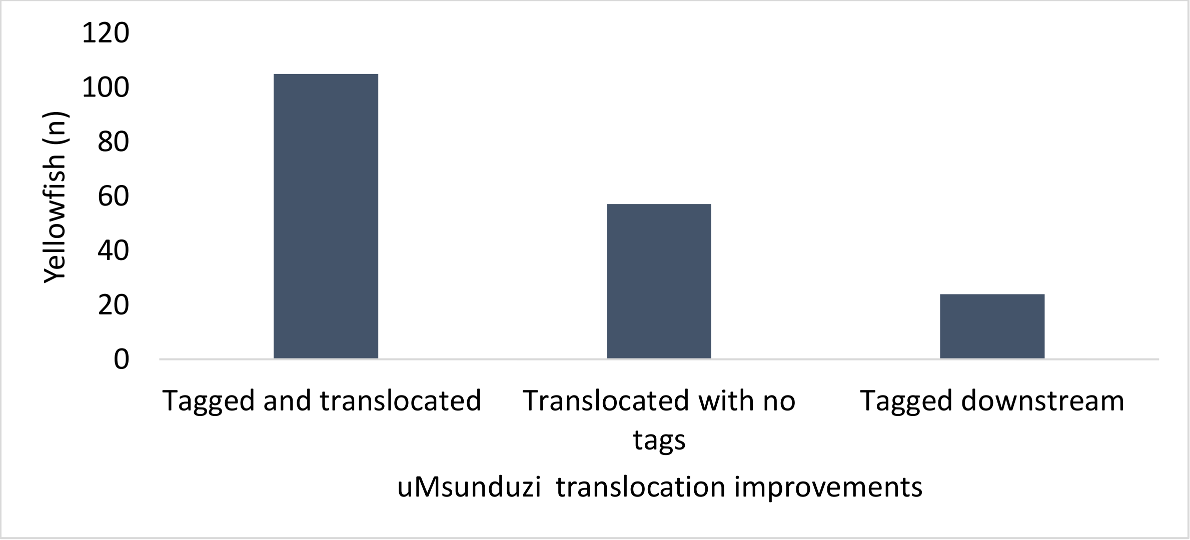

Figure 4.2: The KwaZulu-Natal yellowfish translocated from fish collection sites to re-inoculate impacted sites improve population dynamics and recovery of the uMsunduzi system post a fish kill event.

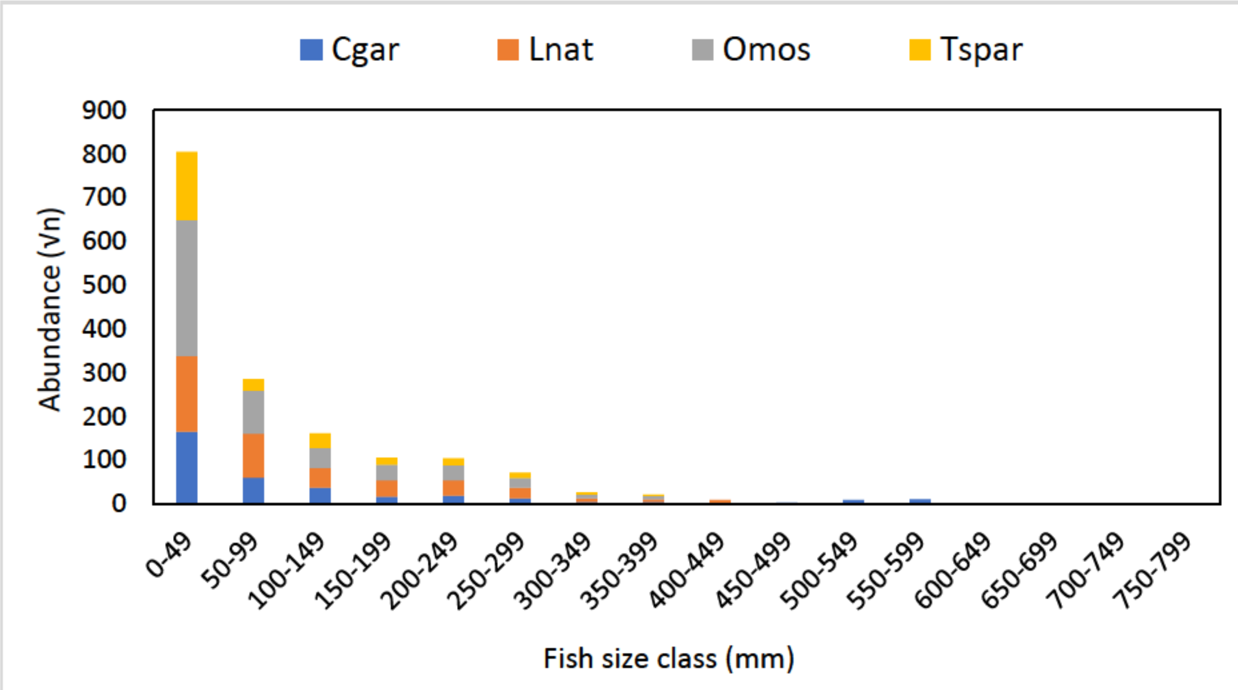

Figure 4.3: Size classes and abundance of the key fish species caught during various sampling seasons throughout the uMsunduzi system and associated tributaries. (Note Cgar = *Clarius gariepinus*, Lnat = *Labeobarbus natalensis*, Omos = *Oreochromis mossambicus*, Tspar = *Tilapia sparmanii*).

Ecological categories for the fish response assessment index based on fish responses to eco hydraulics, substrate type, physicochemical parameters, and impacts of alien fish species are shown in Table 4.3. The fish response assessment index (FRAI), using fish assemblages and adjusted FRAI scores, indicated a trend towards poor ecological conditions for uMsunduzi with FRAI Ecological Categories (ECs) ranging from moderately modified (C) to severely modified (D) (Table 4.4). There was much evidence of pollution and leaking sewerage (Supplementary information Figure S4.7). On occasional surveys, poor fish condition was observed. A yellowfish with a tail deformity was caught at one of the downstream impacted sites (Inkanyezini) during the

June 2022 survey (Supplementary information Figure S4.8). Moreover, during the August 2022 survey, a yellowfish with fungal infection was picked up by hand at the upstream collection site (Refinery). The poor fish condition may reflect the poor ecological conditions of the uMsunduzi River.

Table 4.3: Ecological categories for the Fish Response Assessment Index (FRAI) based on fish responses to eco hydraulics, substrate type, physicochemical parameters, and impacts of alien fish species (Source: Kleynhans 2007).

Ecological category	Name	Description	Acceptable/Unacceptable	Score
A	Natural	Unmodified	Acceptable	90-100
B	Good	Mostly natural with few modifications	Acceptable	80-89
C	Fair	Moderately modified	Acceptable	60-79
D	Poor	Largelt modified	Unacceptable	40-59
E	Seriously modified	Seriously modified	Unacceptable	20-39
F	Critically modified	Extremely modified	Unacceptable	0-19

Examples of the impact of anthropogenic stressors in the uMsunduzi system during the present study are highlighted. Fish source site one (FS1) (Fig. 4.1) was situated along an oil and natural gas company (FFS Refiners) located at least 700 m away from the riverbank. During the second quarter survey of 2022, fish with fungal infections were picked out by hand while swimming. The poor fish condition observed during sampling at the site may reflect the impacts of the industrial effluent from the surrounding oil and gas facility and refuse dump from the local community. Fish source site two (FS2) (Fig. 4.1) was in an urban area and located between a hospital and a park. Industrial activities in the urban area immediately upstream of the site affected the site's turbidity. During the present study, this site received sewage and industrial effluents that devastated aquatic populations and ecological health. Similarly, fish source site three (FS3) (Fig. 4.1), located in a residential area with a farm, experienced chronic waste refuse dumping and point source pollution during our study. Occasionally, fish infected with a disease that caused reddish blotches were observed here. The site also receives water from the Dorpsspruit tributary, which may influence water conditions and overall health.

Table 4.4: FRAI scores for the mainstream uMsunduzi river depicting quarters one, two and three, respectively, in 2022 and quarters four, one and two in 2023 along the uMsunduzi system to monitor ecological recovery.

2022						
Site	**June**		**August**		**November**	
	FRAI	**EC**	**FRAI**	**EC**	**FRAI**	**EC**
FC1	54.1	D	48.2	D	53.4	D
FC2	32.1	E	31.9	E	46.5	D
FC3	50.2	D	45.3	D	52.6	D
FR1	32.1	E	52.6	D	42.3	D
FR2	47.1	D	49.8	D	53.1	D
FR3	57.1	D	54.1	D	62.6	C
2023						
Site	**March**		**May**		**September**	
	FRAI	**EC**	**FRAI**	**EC**	**FRAI**	**EC**
FC1	39.1	E	48.1	D	56.1	D
FC2	42.5	D	46.9	D	50.1	D
FC3	49.2	D	47.1	D	43.2	D
FR1	45.3	D	43.2	D	52.1	D
FR2	48.5	D	53.1	D	42.8	D
FR3	56.9	D	67.2	C	70.2	C

Fish release site one (FR1) (Fig. 4.1) was associated with a wastewater treatment plant, dumping of refuse from nearby residential areas, extensive sugarcane farming and a turf field. Furthermore, the site received waters from the Baynesspruit tributary, which is known for its modified ecological state and potentially influenced the conditions of FR1. Fish release site two

(FR2) (Fig. 4.1) was particularly impacted by refuse dumping and point source pollution. It receives the treated effluent discharges downstream of the Darvill Wastewater Treatment Plant. Furthermore, occasional abstraction of water occurs at this site. Fish release site 3 (FR3) (Fig. 4.1) was in a relatively semi-pristine location with limited industrial activities. The prominent stressor at this site was the sand mining activity upstream of our sampling point. The removal of vegetation and sand increased the turbidity of the site and changed the hydrology and substrate resources. Overall, this site showed improved ecological conditions compared with other downstream sites since the mixed product spill from the Willowton Group Ltd factory. Redundancy analyses (RDA) of fish communities correlated to fish source and release sites (Fig 4.4), habitat characteristics (Fig 4.5), and in situ water quality (Fig. 4.6). The ANOVA test showed no significant results for substrate types, biotopes types, and water quality.

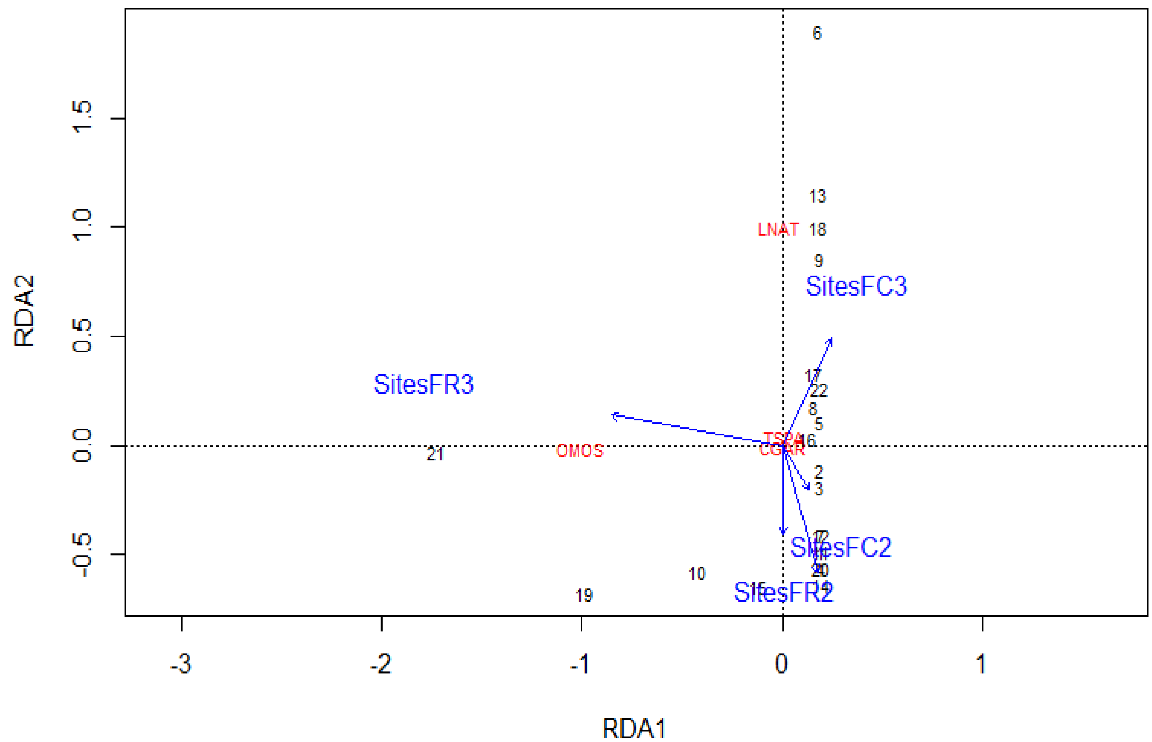

Figure 4.4: The redundancy analyses (RDA) of fish species and fish source and fish release sites in the uMsunduzi River to assess the influences of sites on fish community structures (RDA1 = 49.74; RDA2 = 8.55). (Note: Cgar = *Clarius gariepinus*, Lnat = *Labeobarbus natalensis*, Omos = *Oreochromis mossambicus*, Tspar = *Tilapia sparmanii*).

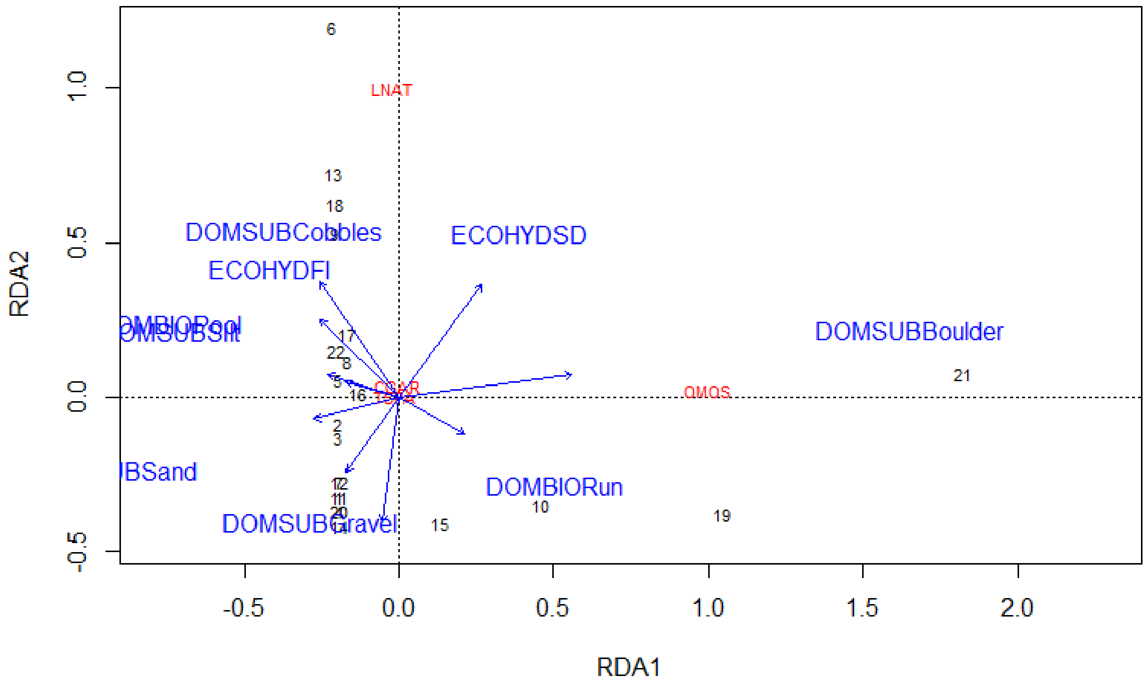

Figure 4.5: The redundancy analyses (RDA) of fish species and measure habitat in the uMsunduzi River to assess influences of ecohydraulic type (ECOHYD), dominant substrate type (DOMSUB) and dominant biotope type (DOMBIO) on fish community structures (RDA1 = 44.78; RDA2 = 21.17).

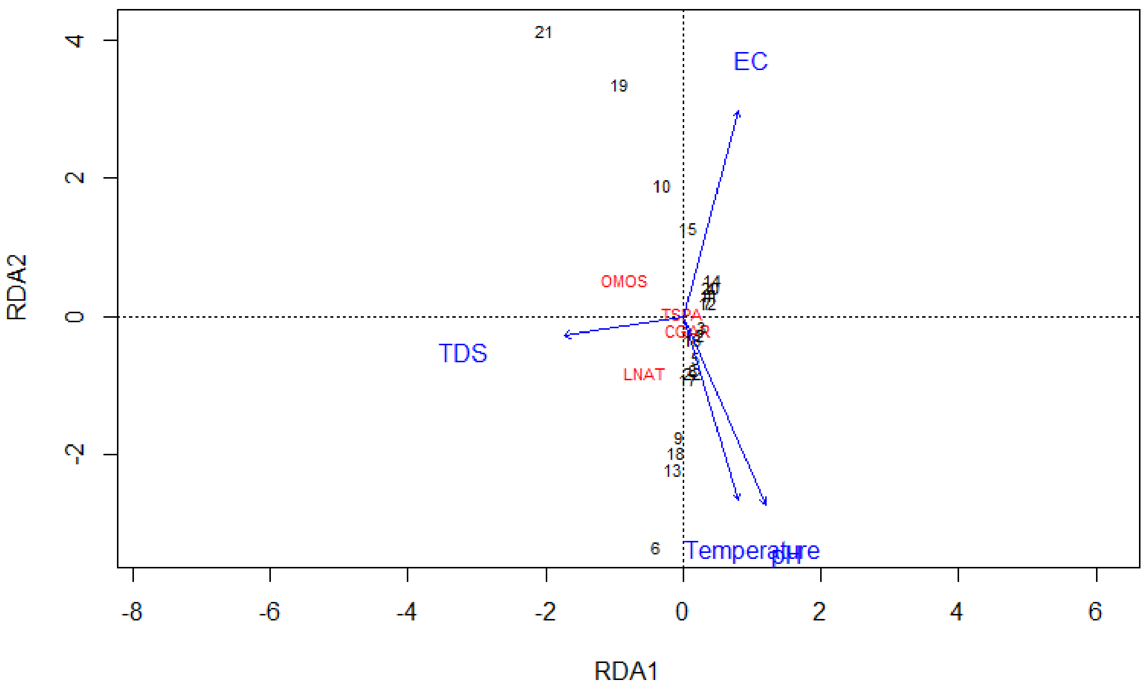

Figure 4.6: The redundancy analyses (RDA) of fish species and in situ water quality in the uMsunduzi River to assess influences of water quality on fish community structures (RDA1 = 25.671; RDA2 = 2.453). (Note: EC = electrical conductivity, TDS = total dissolved solids, Cgar = *Clarius gariepinus*, Lnat = *Labeobarbus natalensis*, Omos = *Oreochromis mossambicus*, Tspar = *Tilapia sparmanii*).

4.5 Discussion

The uMsunduzi River faces multiple anthropogenic threats from surrounding human activities that impact its ecological functions. Consequently, the uMsunduzi River has poor fish communities with low abundances, which are of concern not only in the pollution-impacted sites but also in the whole of the uMsunduzi system. Before the Willowton mixed product spill, the uMsunduzi system was already notorious for being chronically polluted, particularly from periodic spills over the years, such as the oil leak furnace in 2016, as well as sewage inputs from broken sewage systems and utility holes compounded by industrial effluent, solid waste and flow modification (Pole 2000; Gemmel and Schmidt 2013; Evans et al. 2022). The mixed product spill significantly impaired the aquatic organism structures in the uMsunduzi system. The relatively higher fish abundances in the upstream collection sites suggest that fish communities in the downstream impacted sites are still recovering. Three key species throughout the study area were the KwaZulu-Natal yellowfish, Mozambique tilapia, and the sharptooth catfish. To a lesser extent, the banded tilapia was present. However, none of the marked or tagged fish were caught in subsequent sampling along the uMsunduzi River.

The abundance of small-size classes in the key fish species in our study suggests that populations may still be recovering and fish stocks from tributaries colonising the mainstream. The anthropogenic impacts affecting the uMsunduzi River can also explain the slow rehabilitation and recovery to the pre-spill population structures. On occasional surveys, poor fish condition was observed. The poor fish condition may reflect the poor ecological conditions of the uMsunduzi River. The potential bioaccumulation of pollutants in sickly fish also presents a hazard to local fishermen who rely on such fish for food resources and income benefits (Viana et al. 2022). The lack of recaptures of tagged fish may indicate that there were high mortality rates, or alternatively, the population size downstream is greater than detected; however, a large sample size would be required.

The impacts of extensive agricultural practices, industrialisation and fragmentation threaten freshwater ecosystems globally (Zampatti et al. 2022). Locally, the KwaZulu-Natal Province enjoys a vast network of catchments that have been separated into water management areas to provide water across various metropolitan areas (Evans et al. 2022). Clean water flows from the headwaters of the uMsunduzi system; however, as the river passes through industrial, municipal, residential, urban, and agricultural lands through the KwaZulu-Natal Midlands, it is met with pollutants (Gemmel and Schmidt 2013). The poor biotope variability and ecological health associated with most sampling sites in the present study are concerning and highlight the impact of anthropogenic stressors in the uMsunduzi system.

The fish response assessment index (FRAI), using fish assemblages and adjusted FRAI scores, indicated poor ecological conditions for uMsunduzi with FRAI Ecological Categories (ECs) ranging from moderately modified (C) to largely/severely modified (D), complementing the findings from Dlamini (2019). The fish response to environmental conditions was poor, only four of the 13 species were caught during the present study. Some sites (Refinery (FC1), Alex Park (FC2), and Inkanyezini (FR3)) had a variety of available niches, from fast flowing rapids of cobble and boulders to glides of deep pools. Others (Musson's Weir (FC3), Darvill (FR1)) comprised of rapids and glides, with sand and cobble as the dominant substrates or (Grimthorpe (FR2)) were dominated by a bedrock as a substrate. However, fish responses did not vary markedly amongst survey locations, except for Inkanyezini, which occasionally showed improved FRAI scores. On the contrary, other sites (Alex Park (FC2), Darvill (FR1) and Refinery (FC1)) were occasionally depicted to be in a severe ecological state, showing seriously modified conditions. This showed that fish communities in the uMsunduzi River are in poor condition, and a holistic rehabilitation approach is required to curb the impacts of ongoing pollution and instream barriers.

Fish surveys were conducted quarterly from June, August, and November 2022 and March, May, and September 2023. Overall, fish communities at upstream fish collection sites had greater abundance and richness than downstream release sites. Mozambique tilapia were the most abundant, KwaZulu-Natal yellowfish were the second most abundant species with low numbers in impacted sites, and third was the sharptooth catfish found across the study area in both impacted and unimpacted areas and with a representation across all size classes. Class structures were predominantly from a size class, with the KwaZulu-Natal yellowfish size class being small individuals, indicating little recruitment into the impacted sites. Fish communities indicated that the recovery of fish populations in the uMsunduzi River was slow. The small size class for both KwaZulu-Natal yellowfish and Mozambique tilapia show that adult fish are lacking, and recovery has been slow to see the return of adult fish. The KwaZulu-Natal yellowfish, in particular, is considered a slow-growing fish species (Karssing 2007; Paxton et al. 2013), and recruitment of adults from up or downstream has not occurred. The abundance of fish upstream showed that the downstream impacted sites were still recovering to pre spill abundances. The flow regimes, biotope composition, river gradient and, to a lesser extent, physicochemical characteristics largely drove the fish communities. The impacts of sedimentation from sand mining, agricultural run-offs and river fragmentation drive the fish community composition in the uMsunduzi River, and the potential full recovery of populations of indigenous fish is hindered by fragmentation and regulated flows, limiting the necessary migratory trips to complete their life cycles (Coke 1964; Karssing 2008; Burnett et al. 2023, 2024).

The sites near the city had low species richness, dominated mainly by the KwaZulu-Natal yellowfish. However, the downstream sites were slightly richer in species, particularly Musson's Weir (FC3) and Inkanyezini (FR3). The fish species richness at Musson's Weir may be improved by the fish recruiting from the Dorpspruit tributary and moving downstream to colonise the mainstream uMsunduzi River through at Musson's Weir, particularly the banded tilapia as they have been shown to recruit in large schools along the Dorpspruit tributary at the National Botanical Gardens (N. Ngcobo unpublished data). The southern mouthbrooder (*Pseudocrenilabrus philander*) has also been caught in large abundance in the Dorpspruit (N. Ngcobo unpublished data); however, no individuals were caught in the mainstream uMsunduzi River in the present study. Furthermore, during the 2017 and 2018 surveys, the red-breasted tilapia (*Coptodon rendalli*) and the southern mouthbrooder (*Psuedocrenilabrus philander*) were caught at Inkanyezini prior the fish kill in August 2019 (Dlamini 2019). Although Inkanyezini has shown some recovery in fish species and abundance (even Anguillid species were caught at this site during 2022 surveys (N. Ngcobo unpublished data)), the red-breasted tilapia and the southern mouthbrooder have not returned yet, indicating that the river is still under recovery. The improved species richness at Inkanyezini was because of a suite of upstream tributaries recruiting fish stocks, such as the Impushini tributary. The fish stocks on the uMsunduzi tributaries were essential for fish translocation endeavours and repopulating the mainstream, particularly Sinathingi, Mvubukazi, Wilgerfontein and the Dorpspruit. These tributaries harbour healthier breeding populations of indigenous fish than the uMsunduzi River mainstem, with juveniles and sub-adults that could be PIT-tagged and translocated. The Natal mountain catfish (*Amphilius natalensis*) was caught amongst boulders of fast-flowing water at the Mvubukazi tributary during the preliminary presence/absence for the 2023 survey year (N. Ngcobo unpublished data). This suggests that the species may be imperilled by the impacts of fragmentation and ecological disturbances, limiting the species' occurrences in their distribution range.

The 2019 fish kill devasted indigenous fish populations in the uMsunduzi River, such that three years later, fish communities have not fully recovered, with populations dominated by small-size classes and some species having returned at some sites. Therefore, stakeholder engagement is encouraged to take collective decisions for further rehabilitation efforts, such as the collection of KwaZulu-Natal yellowfish from neighbouring catchments to translocate to the uMsunduzi River, the removal of redundant instream barriers to improve connectivity, managing flows to accommodate migratory fish species and limiting the anthropogenic impacts to allow self-remediation of the uMsunduzi River. However, population genetics are needed to make correct decisions around the translocation of KwaZulu-Natal yellowfish. The population of the uMsunduzi River can still be saved if correct mitigation is given. Stobie et al. (2018) indicated high diversity even to species level for KwaZulu-Natal yellowfish across its range, while Mashapa et al. (2023) showed high genetic diversity in Mozambique tilapia. Genetic consideration is needed and highlights the urgency to save sub-populations of fish exposed to severely polluted rivers that may jeopardise their survival. This has been the case with red-tailed barb (*Enteromius gurneyii*), which was not detected during our surveys, although several sites sampled had type specimens collected from them (O'Brien et al. 2017).

4.6 Conclusions

The fish communities' recovery in the uMsunduzi River is suppressed, primarily dominated by juveniles and sub-adult fish in low abundance. This indicates that there has been no significant recovery to pre-spill population structures even three years after the fish kill in 2019. The fish recruited from tributaries are important to repopulate the mainstream uMsunduzi; however, pollution and lack of connectivity limit these migrations and survival chances for the migrated fish. The tributaries of the uMsunduzi River depict potential for the river to recover to near pristine ecological conditions. Particularly important is the Mvubukazi tributary, where healthy sub-adults of the KwaZulu-Natal yellowfish were PIT-tagged, and a Natal mountain catfish was caught on a single occurrence for a presence/absence survey in 2023. The Willowton mixed chemical spill impacted an ecologically impaired river, such that the continued pollution and biological processes are hindering the recovery of fish communities and the river's ecological function to pre-spill conditions. Survey locations further away from the city centre, such as Inkanyezini (FR3) and the refinery sites (FC1) showed relatively healthy fish communities in terms of species richness and abundance, respectively. Moreover, Musson's Weir (FC3) and Grimthorpe (FR2) also showed recovery in species richness to some extent. The fragmented fish populations indicate that anthropogenic stressors drive fish distribution in the uMsunduzi River. Survey sites adjacent to residential areas, municipal areas, commercial zones and industrial complexes exhibited the poorest fish communities and responses of fish to environmental conditions, whereas sites further from the city and experiencing minimal impacts from anthropogenic stressors were improving in terms of water quality, habitat and fish communities. Therefore, fish are useful as ecological indicators, and the enforcement of legislation, such as the polluter pay principle and the National Environmental Management Act (NEMA) 10 of 2002, is recommended to curb the impunity of polluting industries. The obsolete historical instream barriers should be removed or modified, and non-redundant barriers should be fitted with fish ladders to better connectivity between downstream and upstream fish populations, including tributaries, to allow fish movement.

The low number of source fish to improve the populations may need to be sourced from the nearest similar sub-population. The study was constrained because of limited genetics knowledge, suggesting the KwaZulu-Natal complex be split into multiple species (Stobie et al. 2018). If other sub-populations are sourced, potential genetic issues may occur, as seen in the Elands River, South Africa, fish kill rehabilitation account (O'Brien et al. 2014). Continued monitoring of fish communities is recommended to guide future rehabilitation endeavours, such as the development of the Baynesspruit Conservancy and the Catchment Action Plan.

4.7 Acknowledgements

We are grateful to the University of KwaZulu-Natal (ZA), the Willowton Group LTD (ZA) and the National Research Foundation (ZA, grant 98404) for funding. We thank postgrads and interns from the Downs lab and School of Life Sciences for assisting in the field. We thank the Ford Wildlife Foundation (ZA) for vehicle support to carry out fieldwork for the present study.

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4.9 Supplementary information

Supplementary information Table S4.1. The geographical location (coordinates) of the sites sampled as presented in Fig. 4.1.

Site no.	Site name	Site abbreviation	Geographical coordinates X	Y
1	FSS Refinery	FC1	-29.633692	30.339811
2	Camps Drift	FC2	-29.613819	30.376365
3	Henley Dam	HD	-29,624771	30.245366
4	Musson's Weir	FC3	-29.602114	30.404199
5	Upper Wilgerfontein	UW	-29.696211	30.334310
6	Dorpspruit (Botanical Gardens)	DS1	-29.605243	30.345410
7	Mvubukazi	MV	-29.64339	30.310735
8	Sinathingi	SN	-29.652922	30.294289
9	Dorpspruit (Nursery)	DS2	-29.591490	30.374820
10	Mid Wilgerfontein	MW	-29.696219	30.334295

Supplementary information Figure S4.1: Boat cruise at Henley Dam to retrieve gill nets and fyke nets that were left overnight to find KwaZulu-Natal yellowfish (*L. natalensis*) for translocation and radio tagging.

Supplementary information Figure S4.2: Mid Baynesspruit (BS2), accessed through the Willowton Group Ltd factory, where the mixed chemical spill originated in August 2019.

Supplementary information Figure S4.3: A trailer with the 750L JoJo tank aerated with an oxygen tank for transporting fish from Henley Dam to re-inoculate downstream site on the uMsunduzi River and the survey team.

Supplementary information Figure S4.4: A catch of juvenile cichlid fish from a seine net effort at Musson's Weir (FC3).

Supplementary information Figure S4.5: KwaZulu-Natal yellowfish (*L. natalensis*) caught through a gillnet at Inkanyezini (FR3), to be PIT-tagged and released at the same location.

Supplementary information Figure S4.6: PIT tagged KwaZulu-Natal yellowfish (*L. natalensis*) Musson's Weir (FC3), and conditioned to be released at Inkanyezini (FR3).

Supplementary information Figure S4.7: Litter and debris trapped by the gill net deployed at Grimthorpe (FR2) because of anthropogenic refuse dumping.

Supplementary information Figure S4.8: A KwaZulu-Natal yellowfish (*L. natalensis)* with a deformed tail caught at Inkanyezini (FR3) during the June 2022 survey.

CHAPTER 5

Conditions of freshwater ecosystems need consideration in the application of telemetry techniques: Lessons from using telemetry to monitor fish in an urban river

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Formatted for the *Journal of Fish Biology*- short communication

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Running header: Fish telemetry use in a polluted South African urban river

5.1 Abstract

Fish telemetry is a tried and tested method for acquiring behavioural data on fish. Recent advances in telemetry in southern Africa have allowed data collection on fish remotely and in real-time. We used telemetry in an urban river, the uMsunduzi River, in Pietermaritzburg, South Africa, to understand fish responses to urban-related stressors. These included persistence in the impacted sites and their movement in the system in general. Several tagging efforts, including reintroductions made after an acute spill, to rehabilitate downstream impacted areas, were conducted using Passive Integrated Transponder (PIT) and radio-telemeter tagged fish to monitor movement. In 2018, nine suitable KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) were externally telemetry tagged on the uMsunduzi River in the Mpushini conservancy. However, a severe raw sewage discharge from the Darvill Wastewater Treatment plant jeopardised the study, killing all nine tagged fish during the fish kill event shortly after release. To exasperate attempts to use fish behaviour to monitor water quality, another acute spill from the Willowton Group Ltd factory decimated the population of KwaZulu-Natal yellowfish in 2019. Following these acute pollution events, an attempt was made to locate large KwaZulu-Natal yellowfish for telemeter tagging, but it was unsuccessful. Even three years after the 2019 fish kill, our efforts to capture suitable fish were unsuccessful. Fish communities were low in abundance and mainly consisted of juveniles and sub-adults with no suitable adults to radio-telemeter tag. An attempt to translocate fish and radio-telemeter tag them from Henley Dam on the uMsunduzi failed. Moreover, the PIT capture-mark-recapture component had no recaptures. The loss of suitable size classes for telemeter tagging is concerning as the KwaZulu-Natal yellowfish is a large, long-lived species, indicating chronic stress placed on the aquatic ecosystem by the urban environment. Intra-system translocation has not adequately helped recovery efforts, and a poor understanding of KwaZulu-Natal yellowfish genetics limits sourced fish localities. The technology for using fish behaviour to

monitor pollution events exists; however, the freshwater aquatic system has degraded to the point that innovative methods for monitoring the aquatic ecosystem cannot be implemented. This is also true for basic movement studies using telemetry techniques in a highly polluted urban river.

KEYWORDS: fish kill, acute spill; fish community recovery; translocation; rehabilitation

5.2 Introduction

Water is a finite resource that is pivotal to long-term sustainable development goals (Lynch et al. 2020). Freshwater ecosystems provide all the water resources available for human livelihoods and aquatic fauna (Lynch et al. 2016; Grill et al. 2019; He et al. 2021). The rapid expansion of the global human population, continued anthropogenic land use changes, industrialisation, and extensive agricultural practices to meet the rising food demand worsen the exploitation of freshwater resources and impact the ecological integrity of these systems globally (O'Brien et al. 2019; Du Plessis 2019; Thiem et al. 2022). Human livelihoods are intricately linked to freshwater resources; consequently, a large portion of all accessible water resources is appropriated for human use (Grill et al. 2019; Evans et al. 2022; Stocks et al. 2022). In addition, river ecosystems provide a plethora of ecosystem services such as transportation, remedial assimilation of waste, hydropower generation, food resources, income benefits, and water resources for agriculture, industry, and domestic use, especially for vulnerable communities (Thiem et al. 2022; Stocks et al. 2022).

Water demand has increased at twice the rate of the human population; as such, sustainable development goal 6 (SGD6) as water abstraction and pollution interfere with natural flow regimes and ecological responses, negatively impacting the sustainability of rivers (Gehrke et al. 1995; Tempelhoff 2009; Ngor et al. 2018; O'Brien et al. 2019; Du Plessis 2019). The poor implementation of the existing legislative framework, infrastructure failures, instream physical barriers lacking fish passage, and periodic pollution inputs of industrial effluents and sewage impact rivers to unacceptable ecological states (Tempelhoff 2009; O'Brien et al. 2018, 2019; Du Plessis 2019; Wade et al. 2020, 2021). The episodic spills of sewage, industrial effluent, and agricultural by-products limit the dissolved oxygen in the water, which is available for fish and often drives blooms of aquatic algae (Tempelhoff 2009; Kangur et al. 2016). Poor water quality is a health hazard to communities and aquatic life (Pole 2002; Adeyink et al. 2019; Besson et al. 2020). Riparian communities generally rely on river water for irrigating plants, fishing, and water for domestic use (Brand et al. 2009; Stocks et al. 2022).

International organisations and government institutions have set guidelines for acceptable indicator microorganisms in water intended for irrigation, recreation, and domestic use (Gemmel and Schmidt 2013). The guidelines of the World Health Organisation (WHO) adopted by the South African Department of Water and Sanitation suggest 0 *Escherichia coli*/100 ml for drinking water, 130 *E. coli*/100 ml for swimming, and up to 300 *E. coli*/ 100 ml is acceptable for intermediate contact with water (DWS 1996; Gemmel and Schmidt 2013). Various South African rivers exceed the specified *E. coli* guidelines and exhibit a decline in fish diversity, suggesting compromised ecological states (Dickens and Graham 1998; Pole 2002; GroundTruth 2021).

The persistence of poor water quality results in chronic stress for wild fishes (Chovanec et al. 2003; Chakona et al. 2020, 2022), with periodic spills inducing fish kills (Koehn 2021). Fish kill events can be traced back to as early as the 1500s and, in recent years, continue to devastate rivers globally, driven by human activities and unpredictable weather conditions (Doane et al. 1978; Boukamp 1980; La and Cooke 2011; Koehn 2021; Thiem et al. 2022). Anthropogenic stressors arise from different activities and can synergistically impact aquatic ecosystems (Doane et al. 1978; Dudgeon 2014, 2019; Du Plessis 2019). Therefore, fish kills require a holistic recovery approach that involves long-term monitoring of biological responses and collection of abiotic indices (Karr 1981, 1987; O'Brien 2013a,b; Thorstad et al. 2013; O'Brien et al. 2014; Matley et al. 2022; Stocks et al. 2022). Fish behavioural responses are acute and relevant to environmental disturbances, and as such, fish behaviour has been incorporated in various biomonitoring studies with the aid of telemetry (Beitinger 1990; Cooke et al. 2004, 2013; Burnett et al. 2024). Locations with a high impact of anthropogenic stressors can make for the use of fish behaviour to detect thresholds of potential concern for managers to mitigate (Burnett et al. 2020, 2024). Fish telemetry studies can provide important movement data related to their ecology biology (Cooke et al. 2013; Thorstad et al. 2013; Burnett et al. 2021), especially in urban environments where this information can assist in mitigating stressors impacting ecological health, fisheries, and river connectivity.

In South Africa, the KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) life history traits make it a suitable local ecological indicator as it is widespread, large-growing (ca. < 4 kg) and long-lived with some understanding of its behavioural ecology (Stobie et al. 2018; Burnett et al. 2020, 2021, 2024). The uMsunduzi River, KwaZulu-Natal, South Africa, is a working urban river with multiple stressors. This makes this an ideal case for using fish telemetry to study the response of fish to multiple stressors associated with an urban environment and improve how we manage it. In this study, we aimed to use fish telemetry to detect the movement response of fish to changing water quality variables and determine movements fish require for biological processes such as spawning or taking refuge in the urban uMsunduzi River. In addition, post a severe fish kill event, a second attempt was made to track fish, this time to determine the movement of relocated radiotelemetered tagged fish and pit-telemetry tagged fish and assess their persistence in impacted sites. We hypothesised that fish can be used to monitor movement response to multiple stressors, as suggested in the literature.

5.3 Methods

5.3.1 Study area

The uMsunduzi River flows through Pietermaritzburg, Msunduzi district, KwaZulu-Natal Province, South Africa (Chapter 4, Figure 4.1), with an area of 634 km^2 (Matongo et al. 2015; Namugize et al. 2018). The uMsunduzi River is an important tributary of the uMngeni River, a strategic water resource for uMgungundlovu and Durban, eThekwini, supporting more than four million residents (Matongo et al. 2015). The uMsunduzi River has a catchment size of 875 km^2 and a tributary length of 115 km. The uMsunduzi River's catchment has various land use zones along the water column as it drains two-thirds of the metropolitan region. The main river flows through agricultural, domestic, municipal, and industrial zones to its confluence with the uMngeni River between Nagle and Inanda Dams (Namugize et al. 2018). The land use activities along the uMsunduzi River provide a basic understanding of sources for anthropogenic stressors and their impacts along the river's course as it flows to join the uMngeni River. The uMsunduzi River receives runoffs from municipal operations, chemical effluents from industries, and sewage inputs from leaking sewers (Matongo et al. 2015; Namugize et al. 2018). The major wastewater treatment plant responsible for wastewater for the Msunduzi district, Darvill Wastewater Treatment, discharges effluent into the uMsunduzi River. This wastewater treatment plant receives wastewater from hospitals and domestic, commercial, and industrial sources.

Two important fish kill events happened over the course of this study. Firstly, in November 2018, where suspected untreated industrial and effluent waste was discharged into the uMsunduzi River following a power outage. Secondly, in August 2019, a chemical spill from the Willowton Group Ltd factory of 240 tons of crude oil, fatty acids, and caustic acid effluent flooded the Baynesspruit, a tributary of the uMsunduzi River, when a valve burst and dislodged storage tanks at an edible oils and margarine processing factory (SABC, 2019). Approximately 1.6 million litres of the effluent entered the main stem of the uMsunduzi River via the Baynesspruit-uMsunduzi confluence and negatively impacted aquatic life in the 82 km stretch of the river up to Inanda Dam (Environmental Justice Atlas, 2020). The spill left a trail of destruction, with at least 15 tons of dead fish collected and livestock deaths reported by local farmers (eNCA 2019; Mail and Guardian 2019; Supplementary information Figure S5.1).

A rehabilitation program was initiated in 2021 to reintroduce KwaZulu-Natal yellowfish, and radio-telemeter and PIT-tagged fish (Chapter 4) were used to determine reach scale movements across impacted and unimpacted sites. The radio-telemeter tagging of KwaZulu-Natal yellowfish was attempted. An added telemetry component of the rehabilitation was the use of water quality probes (Chapter 4) that communicate and transfer recorded environmental data through a remote network to a central data management system. The real-time data allowed for monitoring of minor pollution events that may go undetected as part of routine sampling. We used six submersible water probes (Wireless Wildlife, Potchefstroom, South Africa) that were deployed at five locations across the study area (Chapter 4). To detect the transmission from these probes, two base stations (Wireless Wildlife, Potchefstroom, South Africa) were installed to detect two of the five real-time water quality probes (Wireless Wildlife, Potchefstroom, South Africa) along the uMsunduzi River. A base station was installed at Newton School (Chapter 4) along the uMsunduzi River to detect the probe at the site (FC3) Musson's Weir near the N3. A relay station was installed at the Willowton Group Ltd factory to detect the water quality probe at mid-Baynesspruit. Due to technical issues with the real-time water quality probes, the relay station was replaced with a base station on the 24th of February, 2023.

5.3.2 Fish collection, tagging and monitoring

As part of a preliminary study to determine where fish were moving in the system and to set up an adequate network of receivers to track fish in real-time and remotely, as per Burnett et al. (2020; 2021, 2024). In November, unbeknown to the 2018 fish kill event, gillnets with a stretched mesh size of 75 mm, 92 mm, 118 mm, 120 mm and 150 mm were deployed around 06h00 and monitored at the Mpushini Bridge (-29630066, 30.476383) to capture KwaZulu-Natal yellowfish. Once a fish entered the gillnet, it was removed as quickly as possible to avoid further entanglement and placed in a holding tank filled with river water before radio-telemeter tagging took place. Once a sufficient number of fish were caught, the gillnets were removed to avoid unnecessary bi-catch.

In efforts to aid rehabilitation and monitor recovery from the 2019 fish kill event in the uMsunduzi River, we sourced local KwaZulu-Natal yellowfish specimens from upstream of the impacted site, fitted them with passive integrated transponder (PIT) tags, and translocated them to re-introduction sites as impacted by the spill. When KwaZulu-Natal yellowfish of substantial size were sourced from healthy upstream populations, external radio-telemeter tags were administered through a surgical procedure (Thorstad et al., 2013; Burnett et al., 2020) and translocated into downstream sites, with the premise that adults will move into and repopulate these areas. The efforts were directed towards a post-spill biomonitoring programme to track the natural recovery of the KwaZulu-Natal yellowfish population on the upper uMsunduzi River and provide a comprehensive indication of the habitat conditions from which the behaviour of fish could be assessed. Various collection techniques were used, for example, running seine nets, glass eel nets, sets of gill nets with large mesh sizes (75 mm to 150 mm stretched mesh), electro-shocker (SAMUS 725M Electro-fisher, SAMUS Special Electronics, Poland), fishing rods, and keep nets, to maximise the efforts and probability of finding fish suitable for radio telemeter tagging to track movements. On unsuccessful attempts to source suitable fish for tagging from selected upstream sites, in August 2021, fish from the upstream impoundment (Henley Dam, Chapter 4 Figure 4.1) were caught by deploying sets of gill nets and fyke nets overnight. We deployed sets of gill nets (mesh size 73 mm:75 mm, 118 mm:118 mm and a set of 125 mm:150 mm) and three fyke nets.

Fish caught in the nets were retrieved the following morning (06h00) to be measured, weighed, and identified to species. To enhance the probability of finding more fish suitable for telemeter tagging, at least three efforts of seine nets were run. The seine net was pulled using an inflatable boat with a trawling motor powered by a deep-cycle truck battery, and two people would hold the ends of the seine net by the bank. In addition, local fishermen were asked to notify us when they had caught a large enough fish with the intention to respond immediately and pay for the fish if suitable for tagging; a keep net was given to aid in this process and keep the fish in good condition for tagging. We transported the KwaZulu-Natal yellowfish of suitable sizes caught in Henley Dam using a 750 L horizontal JoJo water tank mounted on a trailer. The water in the tank was from the river and aerated with oxygen through an oxygen tank mounted on the trailer to provide dissolved oxygen during the transportation period. We transported fish to Musson's Weir (FS3) on the uMsunduzi River the same day as capture and radio-telemeter tagged. All fish radio-telemeter tagged in the study from November 2018 and August 2021 were tagged with external radio-telemeter tags. We measured and assessed the KwaZulu-Natal yellowfish collected for radio telemeter tagging for relevant parameters such as body mass, length, and body condition. To ensure minimal interference with locomotion, the body mass to tag ratio of less than 2% of body mass was accounted for, as suggested by (Jepsen et al. 2002; Hanzen et al. 2020; Burnett et al. 2024). We used radio-telemeter tags attached externally to fish following the protocol described by Bridger and Booth (2003) and Burnett et al. (2020) to monitor the movement of adult KwaZulu-Natal yellowfish in the uMsunduzi River. We transferred the KwaZulu-Natal yellowfish to an anaesthetic bath of 0.4ml/l of clove oil. We observed the fish for signs of narcosis, such as limited fin movement and involuntary upside-down swimming with only the gill movement before the tagging process. When fish were under full anaesthesia, external radio-telemeter tags (Wireless Wildlife, Potchefstroom, South Africa, mass 16 g) were surgically attached using sterile hollow needles to thread the tag attachment wire (diameter 0.508 mm) through the muscular tissue below the dorsal fin and tighten the wire from the other side to secure the tag. We administered wound care gel (Aqua Vet, veterinary hospital, Lydenburg, South Africa) and Terramycin©, an antibiotic that contains oxytetracycline, to the attachment area on the fish to minimise infection and promote swift wound recovery. We transferred each radio-telemeter tagged fish to a recovery container (25 L) with river water that was pumped consistently into the container and allowed to spill over using a 12V battery-powered bilge pump. Upon recovery, telemeter-tagged fish were released into the river to be tracked manually using a radio receiver and antennae. A manual tracking setup used for the present study included Yagi antennae and wide-range scanning radio receivers (DJ-X10; ALINCO INC.,Osaka, Japan). The antennae maximised the reception of radio signals from the radio-telemeter tagged fish to determine presence, movement trends and habit preferences over the study period. The radio-telemeter tagged fish were tracked daily after release and then weekly. Location data were obtained, and fish were manually tracked based on Burnett et al. (2020). This phenomenon laid the base for assumptions that if no movement was recorded from a radiotelemeter tagged fish for a prolonged period, they were assumed to have expelled the tag or died.

As part of the rehabilitation plan for PIT-tag fish, we sourced KwaZulu-Natal yellowfish of desired body mass to tag ratio from areas with surviving populations in the uMsunduzi River and its tributaries. Monthly fish collection surveys at sites along the uMsunduzi River were initiated in the early mornings (06h00) when three sets of gill nets (multifilament nylon, 32m long, Eigenvis group of companies, Cape Town, South Africa; mesh size 73 mm:75 mm, 118 mm:118 mm and a set of 125 mm:150 mm) were deployed. The gill nets were left for at least 3 h whilst suitable pools to run the seine net were identified. The seine net was run for three efforts before retrieving the sets of gill nets. Fish caught in the nets were identified, measured, weighed, and tagged with pit tags. We processed KwaZulu-Natal yellowfish of more than 100mm for PIT tagging as described earlier and in Chapter 4.

Fish translocation improvements were intended to aid the recovery of the uMsunduzi River sites below the chemical spill point. The translocated indigenous fish were individually marked with pit tags. The pit tags used in the study were full duplex (FDX) pit tags (12.5 mm x 2.03 mm, 0.106 g) (Biomark, USA). We placed captured fish in an anaesthetic bath using 0.1ml/l of clove oil and monitored signs of narcosis (Burnett et al. 2020, 2024). Once the captured fish could not maintain their upright position, we used a pit tag injector (Biomark, USA) to inject the tag into the fish's abdominal cavity. We applied wound care gel to ensure the incision sealed. After being pit tagged, the fish were kept in a bucket with flowing river water to recover before being released into the water. We used a bilge pump powered by a small 12V battery to pump river water into the recovery bucket. The scannable pit tags on the translocated fish were used to initiate a capture-mark-recapture to monitor population dynamics and the persistence of translocated fish in the river. Fish captured on subsequent surveys post translocation were scanned with the pit tag reader (Biomark, USA) to detect any recaptures from previously pit-tagged and translocated KwaZulu-Natal yellowfish. We undertook monthly surveys along the river using the netting techniques mentioned earlier to catch fish and scan them for pit tags. If fish were not telemeter tagged, we then PIT- tagged them (Supplementary information Figures S5.2 - S5.4).

5.3.3 Data analyses

We used descriptive statistics to summarise the morphological characteristics, body mass of fish caught and released, and subsequent tracking or recapture. Statistical tests were run on IBM SPSS (version 27.0.1, 2022), and the means were reported with standard deviations (± SD). We used analysis of variance to compare the body mass of fish caught at the different sites along the uMsunduzi River. Benferroni and Turkey posthoc tests were performed to compare size classes.

5.4 Results

To monitor the movement of fish and their response to water quality changes as per Burnett et al. (2020, 2024), suitable fish were captured and radio-telemeter tagged in November – December 2018. Here, nine fish were radio-telemeter tagged on the Mpushini Bridge downstream of the city of Pietermaritzburg. The mean body mass ($\pm$ SD) of KwaZulu-Natal yellowfish caught in 2018 was 1472 $\pm$ g, while their mean standard length was 400.56 $\pm$ 230 mm. All were tagged and released as per Table 5.1. Following the start of the fish tracking process, two major fish kills occurred, one within days of radio-telemeter tagging all nine fish and another eight months later in August 2019.

Table 5.1: KwaZulu-Natal yellowfish that were tagged with external radio tags and translocated in 2018 to track their behavioural responses along the uMsunduzi River before the major fish kill in 2019.

Tag code	SL	Weight	Tag date
142:198	390	1300	20/11/2018
142:300	400	1500	20/11/2018
142:250	420	1650	20/11/2018
142:550	380	1260	20/11/2018
142:600	395	1280	06/12/2018
142:650	390	1200	06/12/2018
142:450	450	1850	06/12/2018
142:348	400	1970	06/12/2018
142:499	380	1240	06/12/2018

Table 5.2: Comparison of class sizes for KwaZulu-Natal yellowfish caught for telemetry and translocation at Henley Dam and Inkanyezini (FR3) in 2021 - 2023.

Site	SL (mm)	Body mass (kg)
Henley Dam	400	1.425
Henley Dam	380	1.425
Henley Dam	443	2
Henley Dam	370	1.295
Henley Dam	360	1.31
Henley Dam	410	1.66
Henley Dam	365	1.26
Henley Dam	400	1.7
Henley Dam	350	1.02
Henley Dam	415	1.87
Henley Dam	410	1.69
Henley Dam	420	1.89
Henley Dam	350	1.06
Henley Dam	420	1.56
Henley Dam	130	0.1
Henley Dam	135	0.75

Henley Dam	185	0.88
FR3	160	0.1
FR3	160	0.11
FR3	180	0.125
FR3	150	0.085
FR3	135	0.056
FR3	105	0.07
FR3	154	0.09
FR3	270	0.37
FR3	170	0.065
FR3	180	0.086
FR3	210	0.52
FR3	230	0.74
FR3	230	0.6
FR3	230	0.14
FR3	300	0.305
FR3	230	0.19
FR3	277	0.295
FR3	160	0.073
FR3	350	0.48

The mean body mass (± SD) of KwaZulu-Natal yellowfish caught in 2021-2023 at Henley Dam for telemetry and translocation was 1190 ± 9050 g (n = 17), while their mean standard length was 349.59 ± 155 mm (Table 5.2**)**. The mean body mass (± SD) of KwaZulu-Natal yellowfish caught in 2021-2023 at Inkanyezini (FR3) for telemetry and translocation was 237 ± 180 g (n = 19) while their mean standard length was 204.26 ± 65 mm (Table 5.2). These were released at the same location as it is a fish release site and were not detected again. Sub-adult KwaZulu-Natal yellowfish with nuptial tubercles were caught at the refinery site (FC1) along with males that released sperms during quarter 3 of 2023 (September 2023), showing breeding potential.

The mean body mass (± SD) of KwaZulu-Natal yellowfish caught in 2021-2023 for PITtagging was 382.36 ± 183 g (n = 186), while their mean standard length was 183.29 ± 123.91

mm (Supplementary information Table S5.1**)**. So far, we have translocated 186 KwaZulu-Natal yellowfish over the three years since the chemical spill in the uMsunduzi River. Of these, 105 (56.5%) were fitted with PIT tags, 57 (30.6%) were translocated without PIT tags, and a further 24 (12.9%) fish were PIT-tagged in an impacted downstream site (FR3) Inkanyezini. So far, no recaptures of the PIT-tagged fish have been obtained despite the regular sampling.

Of the expected 13 fish species from the uMsunduzi River, only four species were caught (Chapter 4 Table 4.1). The fish communities depicted a general trend towards small-size classes of the key fish species caught throughout the survey (Chapter 4). Moreover, the KwaZulu-Natal Yellowfish at Henley Dam depicted larger size classes, whereas the further downstream site Inkanyezini (FR3) depicted a range of size classes, mostly with juvenile and sub-adult fish (Table 5.3). The lack of large fish posed a challenge for external telemeter fish tracking and monitoring. Some of the fish caught after the chemical spill in the uMsunduzi River showed poor condition or deformities. For example, at the refinery (FC1, Chapter 4 Figure 4.1) sampling site during quarter two of 2022, a KwaZulu-Natal yellowfish with a fungal infection was caught by hand while swimming weakly. Furthermore, a yellowfish with a deformed tail was caught during the June 2022 survey at Inkanyezini (FR3, Chapter 4 Figure 4.1).

5.5 Discussion

The mixed product chemical spill interrupted the onset of the tracking when all the tagged KwaZulu-Natal yellowfish were killed during the spill, and no suitable adult KwaZulu-Natal yellowfish fish for tagging have been caught along the river below the chemical spill since then. The real-time data for the present study is fragmented (Chapter 3), although the long-term trends in water quality would have been useful in detecting pulses of periodic pollution events that might be overlooked. Regarding aquatic fauna, real-time water quality monitoring provides the context of the living conditions of aquatic organisms on the Baynesspruit and uMsunduzi Rivers.

The fish communities were dominated by small-size classes recruiting from tributaries to repopulate the mainstream; however, anthropogenic stressors impacted the health of fish, such that the body condition of fish along the uMsunduzi River was generally poor, with fungal infections and deformities. The lack of adult KwaZulu-Natal yellowfish is concerning and suggests that KwaZulu-Natal yellowfish populations in the uMsunduzi system have not fully recovered, even three years after the fish kill. The KwaZulu-Natal yellowfish spawn at different times of the year but are mostly recorded to start breeding from spring through to autumn (Skelton 2001; Burnett et al. 2021). This species is potamodromous, with adults migrating upstream to find suitable spawning niches (Write and Coke 1975a; Karssing 2008). It is likely that when the August 2019 fish kill occurred, KwaZulu-Natal yellowfish were still in their refugia habitat of large instream pools characteristic of the reach from the Baynespruit confluence downstream to Nkanyenzini village. Spawning female adults range from a minimum body mass of 600 g to above 2 kg and > 100 mm total length (Impson et al. 2008). The males of KwaZulu-Natal yellowfish generally reach breeding age as yearlings at 125 mm total length, whereas females can only produce eggs after two years of age (Write and Coke 1975a, 1975b; Cooke et al. 2001; Impson et al. 2008). The tributaries of the uMsunduzi River serve as valuable nurseries for fish that then populate the mainstream uMsunduzi. However, instream physical barriers and continued pollution from the dysfunctional sewage network and toxic industrial effluents (Ngcobo et al. in review) continue to impede the recovery of the uMsunduzi River to pre-spill conditions and telemetry application for monitoring fish presence and movement. The increased frequency of occurrence of fish kills is less than ideal and can inflate fish telemetry costs that are already difficult to implement locally (Burnett et al. 2021, 2024).

Before the major mixed chemical product spill, suitable adult KwaZulu-Natal yellowfish could be sourced from the uMsunduzi River, tagged with external radio tags, and tracked for a certain period along the uMsunduzi River (Umngeni Report 2019). However, following the 2018 and consequential August 2019 spill, KwaZulu-Natal yellowfish of small-size classes are only present. The KwaZulu-Natal yellowfishes of suitable size classes for external radio telemetry were lacking, with sub-adults suitable for PIT tags being mostly sourced from surrounding tributaries. We tagged and translocated a substantial number of the KwaZulu-Natal yellowfish, and no recaptures were made despite extensive sampling efforts on subsequent surveys following translocation. A suite of healthy sub-adult KwaZulu-Natal yellowfish was sourced from tributaries for translocation, and sub-adult KwaZulu-Natal yellowfish with nuptial tubercles were caught at the refinery site (FC1) along with males that released sperms during quarter 3 of 2023 (September 2023). The nuptial tubercles indicate that the KwaZulu-Natal yellowfish are breeding. This suggests that breeding individuals repopulate the mainstream uMsunduzi; however, the impacts of chemical and physical barriers limit the completion of life cycles and fragment populations, creating a time lag in the recovery process. The majority of the PIT-tagged sub-adults were sourced from these tributaries. However, the lack of connectivity and the impacts of chronic pollution limit interaction between downstream and upstream populations in the uMsunduzi River, such that there have been no recaptures from the PIT-tagged and translocated KwaZulu-Natal yellowfish from 2022 through to 2023.

The lack of suitable adults for external telemetry and sustained small-size classes suggests slow recovery and the impact of biological processes because of acute and chronic pollution from human activities. The slow recovery is particularly evident because, before the spill, adult KwaZulu-Natal yellow fish suitable for external radio-telemetry were tagged and tracked along the uMsunduzi and uMngeni Rivers (Umgeni Water Report 2019).

The uMsunduzi River has not attained the pre-spill fish community abundance three years after the spill. No adult fish were caught downstream of the spill that could be fitted with external radio-telemeter tags, suggesting that even three years post the spill, anthropogenic stressors were limiting the recovery and return to healthy indigenous fish populations and the application of telemetry to monitor and manage the river. The water quality probes were clogged with disposable nappies (pers. obs.) and other waste debris that washed downstream, causing water damage to the data transmitting cord and data loss. The poor fish communities, malfunction, and loss of real-time water quality probes suggest that the uMsunduzi River has a lot of ecological challenges and recovery to pre-spill conditions will be relatively slow.

5.6 Conclusions

The impacts of pollution on the biological processes in the uMsunduzi system drive fish community structures and impede the recovery of the river to pre-spill conditions. The life history traits of the KwaZulu-Natal yellowfish have allowed successful monitoring of the recovery of the uMsunduzi River following the 2019 fish kill event. The poor fish communities and compromised ecological integrity of the river can be linked to the impacts of anthropogenic stressors. The instream physical barriers prevent intra-system migrations, whereas habitat and flow modification limit available niches required by fish at different life stages of their development, and the continued pollution further stresses the fish communities at chronic levels. The fish population, dominated by small size classes of juveniles and sub-adults, showed recruitment in adjacent tributaries such as Wilgerfontein, Sinathingi, Mvubukazi and the Dorpspruit to repopulate the mainstream uMsunduzi River. The application of telemetry in the uMsunduzi River was not without challenges, probe malfunction and loss, as well as the lack of adult KwaZulu-Natal yellowfish for external radio tagging, were the main challenges. As a highly mobile fish, obsolete instream barriers must be removed, and functional barriers must be fitted with fish ladders to improve connectivity across the urban riverscape. The technical challenges with water quality probes and the lack of suitable fish for radio-telemeter tagging suggest that the conditions of an urban river can be challenging when applying telemetry, and fish populations in the uMsunduzi River have not recovered significantly since the fish kill event in August 2019. The illegal dumping of physical material, such as disposable diapers, created further challenges by covering the probes, promoting dysfunctionality and loss. The governing local authorities and parastatal organisations delegated with water provision and protection of natural resources must direct efforts to clean up the uMsunduzi River, enforce the existing legislature to improve the ecological conditions of the river so that technological innovations can be deployed to improve the knowledge of indigenous fish. relevant

5.7 Acknowledgements

We are grateful to the University of KwaZulu-Natal (ZA), the Willowton Group (ZA) and the National Research Foundation (ZA, grant 98404) for funding. We thank various postgrads and interns in the Downs lab and School of Life Sciences for their assistance in the field. We thank the Ford Wildlife Foundation (ZA) for vehicle support.

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5.9 Supplementary information

Supplementary information Figure S5.1: Newspaper articles highlighting pollution events in the uMsunduzi River in Pietermaritzburg, South Africa.

Supplementary information Figure S5.2: Scanning the KwaZulu-Natal yellowfish (*L. natalensis*) with a PIT tag reader at Musson's Weir (FC3), subsequent to translocation of fish.

Supplementary information Figure S5.3: Sub-adult of the *KwaZulu-Natal y*ellowfish caught at the refinery (FR1) by electronarcosis.

Supplementary information Figure S5.4: A KwaZulu-Natal yellowfish with nuptial tubercles (breeding marks) caught at the refinery (FC1) during quarter 3 (September 2023).

Supplementary information Figure S5.5: A KwaZulu-Natal yellowfish caught at Musson's Weir (FC3), PIT-tagged and translocated.

Supplementary *i*nformation Table S5.1: PIT-tagged KwaZulu-Natal yellowfish (*L. natalensis*) without translocation from the survey locations downstream of the spill point.

Unique PIT tag code	Species	SL (mm)	From site	Date
17393	*L. natalensis*	160	FR3	08/2022
17433	*L. natalensis*	160	FR3	08/2022
17399	*L. natalensis*	180	FR3	08/2022
17422	*L. natalensis*	230	FR2	11/2022
16729	*L. natalensis*	150	FR3	03/2023
16989	*L. natalensis*	135	FR3	03/2023
16763	*L. natalensis*	105	FR3	03/2023
16767	*L. natalensis*	154	FR3	03/2023
17538	*L. natalensis*	270	FR3	05/2023
17566	*L. natalensis*	170	FC1	05/2023
17510	*L. natalensis*	192	FC1	05/2023
17497	*L. natalensis*	174	FC1	05/2023
17543	*L. natalensis*	150	FC1	05/2023
17534	*L. natalensis*	170	FR3	09/2023
17493	*L. natalensis*	180	FR3	09/2023
17523	*L. natalensis*	210	FR3	09/2023
17490	*L. natalensis*	230	FR3	09/2023
17491	*L. natalensis*	230	FR3	09/2023
17535	*L. natalensis*	230	FR3	11/2023
17532	*L. natalensis*	300	FR3	11/2023
17518	*L. natalensis*	230	FR3	11/2023
17489	*L. natalensis*	277	FR3	11/2023
17560	*L. natalensis*	160	FR3	11/2023

Supplementary information Table S5.2: PIT-tagged and translocated KwaZulu-Natal yellowfish (*L. natalensis*) along the uMsunduzi catchment, including tributaries for translocation improvements

PIT tag code	Species	SL (mm)	From site	To site	Date
17548	*L. natalensis*	350	FC1	FR1	08/2022
17452	*L. natalensis*	210	FC1	FR1	08/2022
17397	*L. natalensis*	170	FC1	FR1	08/2022
17388	*L. natalensis*	200	FC1	FR1	08/2022
17313	*L. natalensis*	100	FC1	FR1	08/2022
17395	*L. natalensis*	130	FC1	FR1	08/2022
17441	*L. natalensis*	90	FC1	FR1	08/2022
17348	*L. natalensis*	110	FC1	FR1	08/2022
17449	*L. natalensis*	260	FC1	FR2	08/2022
17400	*L. natalensis*	165	FC2	FR2	08/2022
17474	*L. natalensis*	130	FC2	FR2	08/2022
17445	*L. natalensis*	275	FC2	FR2	08/2022
17446	*L. natalensis*	300	FC2	FR2	08/2022
17413	*L. natalensis*	400	Henley Dam	FR2	08/2022
17442	*L. natalensis*	380	Henley Dam	FR2	08/2022
17431	*L. natalensis*	443	Henley Dam	FR2	08/2022
17480	*L. natalensis*	370	Henley Dam	FR2	08/2022
17423	*L. natalensis*	360	Henley Dam	FR2	08/2022
17391	*L. natalensis*	410	Henley Dam	FR2	08/2022
17463	*L. natalensis*	365	Henley Dam	FR2	08/2022
17477	*L. natalensis*	400	Henley Dam	FR2	08/2022
17461	*L. natalensis*	350	Henley Dam	FR2	08/2022
17473	*L. natalensis*	415	Henley Dam	FR2	08/2022
17451	*L. natalensis*	410	Henley Dam	FR2	08/2022
17437	*L. natalensis*	420	Henley Dam	FR2	08/2022
17418	*L. natalensis*	350	Henley Dam	FR2	08/2022
17447	*L. natalensis*	420	Henley Dam	FR2	08/2022
17484	*L. natalensis*	130	Henley Dam	FR2	08/2022
17426	*L. natalensis*	135	Henley Dam	FR2	08/2022
17401	*L. natalensis*	185	Henley Dam	FR2	10/2022
16413	*L. natalensis*	270	FC3	FR3	10/2022
16444	*L. natalensis*	235	FC3	FR3	11/2022
17483	*L. natalensis*	100	Wilgerfontein	FR2	11/2022
17455	*L. natalensis*	105	Wilgerfontein	FR2	11/2022
17419	*L. natalensis*	117	Wilgerfontein	FR2	11/2022
17392	*L. natalensis*	120	Wilgerfontein	FR2	11/2022
17436	*L. natalensis*	115	Wilgerfontein	FR2	11/8/2022
17410	*L. natalensis*	125	Wilgerfontein	FR2	11/2022
17424	*L. natalensis*	115	Wilgerfontein	FR2	11/2022

17390	*L. natalensis*	130	Wilgerfontein	FR2	11/2022
17456	*L. natalensis*	105	Wilgerfontein	FR2	11/2022
17482	*L. natalensis*	135	Wilgerfontein	FR2	11/2022
17398	*L. natalensis*	112	Wilgerfontein	FR2	11/2022
17405	*L. natalensis*	123	Wilgerfontein	FR2	11/2022
17406	*L. natalensis*	110	Wilgerfontein	FR2	11/2022
17403	*L. natalensis*	172	Dorpspruit	FR2	11/2022
17488	*L. natalensis*	185	Dorpspruit	FR2	11/2022
17434	*L. natalensis*	115	Dorpspruit	FR2	11/2022
17416	*L. natalensis*	125	Dorpspruit	FR2	11/2022
17415	*L. natalensis*	121	Dorpspruit	FR2	11/2022
17466	*L. natalensis*	110	Dorpspruit	FR2	11/2022
17481	*L. natalensis*	150	Dorpspruit	FR2	11/2022
17428	*L. natalensis*	122	Dorpspruit	FR2	11/2022
17438	*L. natalensis*	100	Dorpspruit	FR2	01/2023
17425	*L. natalensis*	140	Mvubukazi	FR2	01/2023
17469	*L. natalensis*	120	Mvubukazi	FR2	01/2023
17471	*L. natalensis*	150	Mvubukazi	FR2	01/2023
17421	*L. natalensis*	160	Mvubukazi	FR2	01/2023
17394	*L. natalensis*	145	Mvubukazi	FR2	01/2023
17486	*L. natalensis*	150	Mvubukazi	FR2	01/2023
17402	*L. natalensis*	142	Mvubukazi	FR2	01/2023
17415	*L. natalensis*	110	Mvubukazi	FR2	01/2023
17457	*L. natalensis*	205	Mvubukazi	FR2	01/2023
17462	*L. natalensis*	120	Mvubukazi	FR2	01/2023
17407	*L. natalensis*	130	Mvubukazi	FR2	01/2023
17489	*L. natalensis*	110	Mvubukazi	FR2	01/2023
16735	*L. natalensis*	150	Sinathingi	FR2	01/2023
16736	*L. natalensis*	120	Sinathingi	FR2	01/2023
16747	*L. natalensis*	180	Dorpspruit	FR2	01/2023
16735	*L. natalensis*	150	Wilgerfontein	FR2	01/2023
16736	*L. natalensis*	120	Wilgerfontein	FR3	03/2023
16774	*L. natalensis*	215	FC3	FR3	05/2023
17540	*L. natalensis*	110	FC1	FR1	05/2023
17530	*L. natalensis*	100	FC1	FR1	05/2023
17517	*L. natalensis*	130	FC1	FR1	05/2023
17542	*L. natalensis*	120	FC1	FR1	05/2023
17544	*L. natalensis*	115	FC1	FR1	05/2023
17531	*L. natalensis*	110	FC1	FR1	05/2023
17519	*L. natalensis*	120	FC1	FR1	05/2023
17554	*L. natalensis*	200	FC3	FR3	05/2023
17520	*L. natalensis*	200	FC3	FR3	05/2023
17523	*L. natalensis*	220	FC1	FR1	09/2023

17531	*L. natalensis*	175	FC1	FR1	09/2023
17574	*L. natalensis*	150	FC1	FR1	09/2023
17547	*L. natalensis*	165	FC1	FR1	09/2023
17559	*L. natalensis*	205	FC1	FR1	09/2023
17568	*L. natalensis*	156	FC1	FR1	09/2023
17498	*L. natalensis*	140	FC1	FR1	09/2023
17503	*L. natalensis*	185	FC1	FR1	09/2023
17524	*L. natalensis*	200	FC1	FR1	09/2023
17583	*L. natalensis*	160	FC1	FR1	09/2023
17576	*L. natalensis*	180	FC1	FR1	09/2023
17509	*L. natalensis*	210	FC1	FR1	09/2023
17576	*L. natalensis*	180	FC1	FR1	09/2023
17509	*L. natalensis*	210	FC1	FR1	09/2023
17507	*L. natalensis*	145	FC1	FR1	09/2023
17567	*L. natalensis*	170	FC1	FR1	09/2023
17573	*L. natalensis*	280	FC1	FR1	11/2023
17492	*L. natalensis*	270	FC1	FR1	11/2023
17495	*L. natalensis*	190	FC1	FR1	11/2023
17588	*L. natalensis*	150	FC1	FR1	11/2023
17546	*L. natalensis*	180	FC1	FR1	11/2023

Supplementary information Table S5.3: KwaZulu-Natal yellowfish (*L. natalensis*) translocated without PIT tags, to improve population structures and recovery along the downstream sites that were impacted by the fish kill in August 2019

Species	**SL (mm)**	**From site**	**To site**	**Date**
L. natalensis	95	Wilgerfontein	FR2	11/8/2022
L. natalensis	85	Wilgerfontein	FR2	11/8/2022
L. natalensis	90	Wilgerfontein	FR2	11/8/2022
L. natalensis	66	Wilgerfontein	FR2	11/8/2022
L. natalensis	50	Wilgerfontein	FR2	11/8/2022
L. natalensis	98	Dorpspruit	FR2	11/9/2022
L. natalensis	84	Dorpspruit	FR2	11/9/2022
L. natalensis	95	Dorpspruit	FR2	11/9/2022
L. natalensis	120	Dorpspruit	FR2	11/9/2022
L. natalensis	45	FC3	FR3	8/2022
L. natalensis	42	FC3	FR3	8/2022
L. natalensis	43	FC3	FR3	8/2022
L. natalensis	42	FC3	FR3	8/2022
L. natalensis	39	FC3	FR3	8/2022
L. natalensis	40	FC3	FR3	8/2022
L. natalensis	44	FC3	FR3	8/2022
L. natalensis	43	FC3	FR3	8/2022
L. natalensis	55	FC3	FR3	8/2022
L. natalensis	45	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	50	FC3	FR3	8/2022
L. natalensis	80	Mvubukazi	FR2	01/2023
L. natalensis	80	Mvubukazi	FR2	01/2023
L. natalensis	115	Mvubukazi	FR2	01/2023
L. natalensis	95	Mvubukazi	FR2	01/2023
L. natalensis	65	Mvubukazi	FR2	01/2023
L. natalensis	80	FC1	FR1	05/2023
L. natalensis	50	FC3	FR3	05/2023
L. natalensis	160	FC1	FR1	09/2023

L. natalensis	145	FC1	FR1	09/2023
L. natalensis	110	FC1	FR1	09/2023
L. natalensis	144	FC1	FR1	09/2023
L. natalensis	130	FC1	FR1	09/2023
L. natalensis	145	FC1	FR1	09/2023
L. natalensis	130	FC1	FR1	09/2023
L. natalensis	165	FC1	FR1	09/2023
L. natalensis	150	FC1	FR1	09/2023
L. natalensis	140	FC1	FR1	09/2023
L. natalensis	135	FC1	FR1	09/2023
L. natalensis	110	FC1	FR1	09/2023

Supplementary information Table S5.4: KwaZulu-Natal Yellowfish (*L. natalensis*) tagged from the downstream impacted sites (FR2) and (FR3) without translocation

Unique PIT tag code	Species	SL (mm)	From site	Date
17393	*L. natalensis*	160	FR3	08/2022
17433	*L. natalensis*	160	FR3	08/2022
17399	*L. natalensis*	180	FR3	08/2022
17422	*L. natalensis*	230	FR2	11/2022
16729	*L. natalensis*	150	FR3	03/2023
16989	*L. natalensis*	135	FR3	03/2023
16763	*L. natalensis*	105	FR3	03/2023
16767	*L. natalensis*	154	FR3	03/2023
17538	*L. natalensis*	270	FR3	05/2023
17566	*L. natalensis*	170	FC1	05/2023
17510	*L. natalensis*	192	FC1	05/2023
17497	*L. natalensis*	174	FC1	05/2023
17543	*L. natalensis*	150	FC1	05/2023
17534	*L. natalensis*	170	FR3	09/2023
17493	*L. natalensis*	180	FR3	09/2023
17523	*L. natalensis*	210	FR3	09/2023
17490	*L. natalensis*	230	FR3	09/2023
17491	*L. natalensis*	230	FR3	09/2023
17535	*L. natalensis*	230	FR3	11/2023
17532	*L. natalensis*	300	FR3	11/2023
17518	*L. natalensis*	230	FR3	11/2023
17489	*L. natalensis*	277	FR3	11/2023
17560	*L. natalensis*	160	FR3	11/2023

Supplementary information Table S5.5: Translocated KwaZulu-Natal yellowfish (*L. natalensis*), sourced from upstream fish source sites, including tributaries with breeding populations to re-inoculated downstream sites impacted by the fish kill in August 2019

PIT tag code	Species	SL (mm)	From site	To site	Date
17548	*L. natalensis*	350	FC1	FR1	08/2022
17452	*L. natalensis*	210	FC1	FR1	08/2022
17397	*L. natalensis*	170	FC1	FR1	08/2022
17388	*L. natalensis*	200	FC1	FR1	08/2022
17313	*L. natalensis*	100	FC1	FR1	08/2022
17395	*L. natalensis*	130	FC1	FR1	08/2022
17441	*L. natalensis*	90	FC1	FR1	08/2022
17348	*L. natalensis*	110	FC1	FR1	08/2022
17449	*L. natalensis*	260	FC1	FR2	08/2022
17400	*L. natalensis*	165	FC2	FR2	08/2022
17474	*L. natalensis*	130	FC2	FR2	08/2022
7445	*L. natalensis*	275	FC2	FR2	08/2022
17446	*L. natalensis*	300	FC2	FR2	08/2022
17413	*L. natalensis*	400	Henley Dam	FR2	08/2022
17442	*L. natalensis*	380	Henley Dam	FR2	08/2022
17431	*L. natalensis*	443	Henley Dam	FR2	08/2022
917480	*L. natalensis*	370	Henley Dam	FR2	08/2022
17423	*L. natalensis*	360	Henley Dam	FR2	08/2022
17391	*L. natalensis*	410	Henley Dam	FR2	08/2022
17463	*L. natalensis*	365	Henley Dam	FR2	08/2022
17477	*L. natalensis*	400	Henley Dam	FR2	08/2022
17461	*L. natalensis*	350	Henley Dam	FR2	08/2022
17473	*L. natalensis*	415	Henley Dam	FR2	08/2022
17451	*L. natalensis*	410	Henley Dam	FR2	08/2022
17437	*L. natalensis*	420	Henley Dam	FR2	08/2022
17418	*L. natalensis*	350	Henley Dam	FR2	08/2022
17447	*L. natalensis*	420	Henley Dam	FR2	08/2022
17484	*L. natalensis*	130	Henley Dam	FR2	08/2022
17426	*L. natalensis*	135	Henley Dam	FR2	08/2022
17401	*L. natalensis*	185	Henley Dam	FR2	10/2022
16413	*L. natalensis*	270	FC3	FR3	10/2022
16444	*L. natalensis*	235	FC3	FR3	11/2022
17483	*L. natalensis*	100	Wilgerfontein	FR2	11/2022
17455	*L. natalensis*	105	Wilgerfontein	FR2	11/2022
17419	*L. natalensis*	117	Wilgerfontein	FR2	11/2022
17392	*L. natalensis*	120	Wilgerfontein	FR2	11/2022
17436	*L. natalensis*	115	Wilgerfontein	FR2	11/8/2022
17410	*L. natalensis*	125	Wilgerfontein	FR2	11/2022

17424	*L. natalensis*	115	Wilgerfontein	FR2	11/2022
17390	*L. natalensis*	130	Wilgerfontein	FR2	11/2022
17456	*L. natalensis*	105	Wilgerfontein	FR2	11/2022
17482	*L. natalensis*	135	Wilgerfontein	FR2	11/2022
17398	*L. natalensis*	112	Wilgerfontein	FR2	11/2022
17405	*L. natalensis*	123	Wilgerfontein	FR2	11/2022
17406	*L. natalensis*	110	Wilgerfontein	FR2	11/2022
17403	*L. natalensis*	172	Dorpspruit	FR2	11/2022
17488	*L. natalensis*	185	Dorpspruit	FR2	11/2022
17434	*L. natalensis*	115	Dorpspruit	FR2	11/2022
17416	*L. natalensis*	125	Dorpspruit	FR2	11/2022
174150	*L. natalensis*	121	Dorpspruit	FR2	11/2022
17466	*L. natalensis*	110	Dorpspruit	FR2	11/2022
17481	*L. natalensis*	150	Dorpspruit	FR2	11/2022
17428	*L. natalensis*	122	Dorpspruit	FR2	11/2022
17438	*L. natalensis*	100	Dorpspruit	FR2	01/2023
17425	*L. natalensis*	140	Mvubukazi	FR2	01/2023
17469	*L. natalensis*	120	Mvubukazi	FR2	01/2023
17471	*L. natalensis*	150	Mvubukazi	FR2	01/2023
17421	*L. natalensis*	160	Mvubukazi	FR2	01/2023
17394	*L. natalensis*	145	Mvubukazi	FR2	01/2023
17486	*L. natalensis*	150	Mvubukazi	FR2	01/2023
17402	*L. natalensis*	142	Mvubukazi	FR2	01/2023
17415	*L. natalensis*	110	Mvubukazi	FR2	01/2023
17457	*L. natalensis*	205	Mvubukazi	FR2	01/2023
17462	*L. natalensis*	120	Mvubukazi	FR2	01/2023
17407	*L. natalensis*	130	Mvubukazi	FR2	01/2023
17489	*L. natalensis*	110	Mvubukazi	FR2	01/2023
16735	*L. natalensis*	150	Sinathingi	FR2	01/2023
16736	*L. natalensis*	120	Sinathingi	FR2	01/2023
16747	*L. natalensis*	180	Dorpspruit	FR2	01/2023
16735	*L. natalensis*	150	Wilgerfontein	FR2	01/2023
16736	*L. natalensis*	120	Wilgerfontein	FR3	03/2023
16774	*L. natalensis*	215	FC3	FR3	05/2023
17540	*L. natalensis*	110	FC1	FR1	05/2023
89175	*L. natalensis*	100	FC1	FR1	05/2023
17517	*L. natalensis*	130	FC1	FR1	05/2023
17542	*L. natalensis*	120	FC1	FR1	05/2023
17544	*L. natalensis*	115	FC1	FR1	05/2023
17531	*L. natalensis*	110	FC1	FR1	05/2023
17519	*L. natalensis*	120	FC1	FR1	05/2023
17554	*L. natalensis*	200	FC3	FR3	05/2023
17520	*L. natalensis*	200	FC3	FR3	05/2023

17523	*L. natalensis*	220	FC1	FR1	09/2023
3891	*L. natalensis*	175	FC1	FR1	09/2023
917574	*L. natalensis*	150	FC1	FR1	09/2023
17547	*L. natalensis*	165	FC1	FR1	09/2023
17559	*L. natalensis*	205	FC1	FR1	09/2023
17568	*L. natalensis*	156	FC1	FR1	09/2023
17498	*L. natalensis*	140	FC1	FR1	09/2023
17503	*L. natalensis*	185	FC1	FR1	09/2023
17524	*L. natalensis*	200	FC1	FR1	09/2023
17583	*L. natalensis*	160	FC1	FR1	09/2023
17576	*L. natalensis*	180	FC1	FR1	09/2023
17509	*L. natalensis*	210	FC1	FR1	09/2023
17576	*L. natalensis*	180	FC1	FR1	09/2023
17509	*L. natalensis*	210	FC1	FR1	09/2023
17507	*L. natalensis*	145	FC1	FR1	09/2023
17567	*L. natalensis*	170	FC1	FR1	09/2023
17573	*L. natalensis*	280	FC1	FR1	11/2023
17492	*L. natalensis*	270	FC1	FR1	11/2023
17495	L. natalensis	190	FC1	FR1	11/2023
17588	L. natalensis	150	FC1	FR1	11/2023
17546	L. natalensis	180	FC1	FR1	11/2023

Supplementary information Table S5.6: KwaZulu-Natal yellowfish (*Labeobarbus natalensis*) translocated without PIT tags, sourced from sites upstream of the spill point and tributaries to improve population structures on the downstream sites impacted by the fish kill

Species	SL (mm)	From site	To site	Date
L. natalensis	95	Wilgerfontein	FR2	08/2022
L. natalensis	85	Wilgerfontein	FR2	08/2022
L. natalensis	90	Wilgerfontein	FR2	08/2022
L. natalensis	66	Wilgerfontein	FR2	08/2022
L. natalensis	50	Wilgerfontein	FR2	08/2022
L. natalensis	98	Dorpspruit	FR2	09/2022
L. natalensis	84	Dorpspruit	FR2	09/2022
L. natalensis	95	Dorpspruit	FR2	09/2022
L. natalensis	120	Dorpspruit	FR2	09/2022
L. natalensis	45	FC3	FR3	08/2022
L. natalensis	42	FC3	FR3	08/2022
L. natalensis	43	FC3	FR3	08/2022
L. natalensis	42	FC3	FR3	08/2022
L. natalensis	39	FC3	FR3	08/2022
L. natalensis	40	FC3	FR3	08/2022
L. natalensis	44	FC3	FR3	08/2022
L. natalensis	43	FC3	FR3	08/2022
L. natalensis	55	FC3	FR3	08/2022
L. natalensis	45	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022

L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	50	FC3	FR3	08/2022
L. natalensis	80	Mvubukazi	FR2	01/2023
L. natalensis	80	Mvubukazi	FR2	01/2023
L. natalensis	115	Mvubukazi	FR2	01/2023
L. natalensis	95	Mvubukazi	FR2	01/2023
L. natalensis	65	Mvubukazi	FR2	01/2023
L. natalensis	80	FC1	FR1	05/2023
L. natalensis	50	FC3	FR3	05/2023
L. natalensis	160	FC1	FR1	09/2023
L. natalensis	145	FC1	FR1	09/2023
L. natalensis	110	FC1	FR1	09/2023
L. natalensis	144	FC1	FR1	09/2023
L. natalensis	130	FC1	FR1	09/2023
L. natalensis	145	FC1	FR1	09/2023
L. natalensis	130	FC1	FR1	09/2023

L. natalensis	165	FC1	FR1	09/2023
L. natalensis	150	FC1	FR1	09/2023
L. natalensis	140	FC1	FR1	09/2023
L. natalensis	135	FC1	FR1	09/2023
L. natalensis	110	FC1	FR1	09/2023

CHAPTER 6

Conclusions and recommendations

6.1 Background

Most available clean water for human use comes from freshwater ecosystems, particularly inland rivers (Grill et al. 2019; Zampatti et al. 2022). These ecosystems provide us with a plethora of ecosystem services such as water purification, power generation, food supply, and water for domestic, agricultural, and industrial uses (Holmlund and Hammer 1999; Yeakley et al. 2016; Agboola et al. 2020a). The natural resources and the ecosystem services derived from aquatic ecosystems are especially important in developing countries, which host vulnerable communities that are susceptible to poverty (Steyn et al. 2019; Evans et al. 2022). The uMsunduzi River, as a major tributary of the uMngeni River, provides water to two large metropolitans in KwaZulu-Natal province, namely the uMgungundlovu and eThekwini municipalities, making them socioeconomically valuable South Africa (Matongo et al. 2015; Namugize et al. 2018; Dlamini 2019). Protecting these valuable freshwater resources from the impacts of stressors is important to improve ecological health and ensure long-term access and use of the resources. Unfortunately, the impacts of anthropogenic activities have put these systems among some of the deteriorated riverine ecosystems in South Africa (Tempelhoff 2009; Gemmel and Schmidt 2013). The ecological function of an aquatic ecosystem can be evaluated through the traditional biotic indices and biological indices such as diatoms, macro-invertebrates and fish, which have often been used to monitor freshwater ecosystems owing to their acute sensitivity to environmental changes, mobility, relatively long lifespan in the case if fish (Wright and Coke 1975a,b; Karr 1981; O'Brien et al. 2019; Evans et al. 2022; Chapter 4). The present study evaluated the water quality and integrity of biological communities of the uMsunduzi River in KwaZulu-Natal, South Africa. We gathered literature on the science of fish kills. We assessed water quality in situ and water samples were also collected for analyses. Furthermore, diatoms and macro-invertebrates were used to correlate ecological categories to water quality. We further assessed fish community structures upstream and downstream of the spill point and tributaries. We pit tagged the local KwaZulu-Natal yellowfish *Labeobarbus natalensis*, to understand movement and persistence as it is an indicator species.

6.2 Findings

The intensity and occurrence of fish kill events have increased owing to industrialisation, developments and lack of environmental awareness. The fish kills are most frequent around the cities, impacting aquatic ecosystems and immediate beneficiaries of ecosystem services such as fishing and water collection (Chapter 2). Water quality monitoring is therefore necessary to understand aquatic ecosystem health and manage the impact of stressors on the physicochemical parameters and associated biota (Chapter 3). The inclusion of biota in monitoring provides a holistic approach to understanding stressor impacts on aquatic organisms. Furthermore, this has allowed for baseline data to use aquatic organisms as biological indicators (Chapter 3). The inclusion of diatoms, macroinvertebrates, and fish allows for early detection of stressors and can be mitigated before the fish kill events (Chapter 3). Water quality results show recovery at some sites; however, certain sites remain poor because of immediate stressors. Diatoms and macro-invertebrates depicted recovery from 2022 to 2023, with pollution-sensitive taxa becoming more abundant.

The first hypothesis for this study was that fish communities in the uMsunduzi River would act as ecological indicators, with behavioural responses relevant to environmental changes such as flow, biotope composition, and water quality (Chapter 4). This hypothesis was accepted as the results in Chapter 4 indicated that the fish communities are driven by water quality and habitat characteristics. The Fish Response Assessment Index (FRAI) showed that the uMsunduzi River can be classified in a poor ecological category and some slow recovery in fish abundance during the 2023 survey year (Chapter 4). Multivariate analyses indicated that the anthropogenic impacts responsible for shifts in community structure, amongst other things, were velocity-depth classes, substrate type, and fluctuation in physicochemical properties.

The second hypothesis of this study was that populations of *L. natalensis* along the uMsunduzi River also act as an ecological indicator, responding to environmental changes, namely changes in habitat and water quality caused by human activities around the river (Chapter 4). This hypothesis was accepted as the results of the study showed how *L. natalensis* populations in the uMsunduzi River progressively diminished, both in abundance and structure, down the catchment gradient as the compounded impact of water quality and quantity changes in the system increased (Chapters 3 and 4); however, the further downstream site at Inkanyezini (FR3) has shown some improvement in species richness and abundance since the spill (Ngcobo et al. in review; Chapter 4). The absence of juvenile and smaller adult *L. natalensis* illustrated the impacts of pollution and related biological responses are not suited for fish population recovery, as they impair the required biotopes and alter flows. The third hypothesis of the present study was that the telemetry techniques can be applied in the uMsunduzi River to monitor water quality and the behavioural responses and population dynamics of *L. natalensis* to ascertain the ecological recovery and promote rehabilitation to pre spill conditions (Chapter 5). This hypothesis was rejected, as it became apparent that the ecological conditions of a river need consideration before telemetry techniques can be employed to monitor the ecological integrity. The lessons were learned from attempts to monitor water quality and *L. natalensis* population dynamics in the uMsunduzi River (Chapter 5). The lack of recaptures from the PIT-tagged fish may suggest that the individuals are not surviving and recolonising the impacted areas; contrary, the fish might be moving out of the sampling locations during high flows of rainy seasons, or the fish population is greater than presently detected. However, extensive sampling efforts have been made after PIT tagging and translocation of yellowfish. Moreover, the lack of adult *L. natalensis* for attachment external radio transmitters indicates that telemetry techniques presently cannot be applied to monitor fish behavioural response in the uMsunduzi and water quality in the uMsunduzi system (uMngeni Report 2019; Chapter 5). A suite of challenges was experienced with water quality probes in the Baynesspruit tributary and the mainstream uMsunduzi, such as theft, chronic pollution, and refuse dumping, resulting in probes being lost, dysfunctional, and covered in debris (Chapter 4). The submerging of water quality probes by cumulative debris interferes with signal transmission, limiting remote data transfer to the network of receivers.

This study illustrated how the numerous barriers in the uMsunduzi River system have many negative effects on the systems as they alter natural flow regimes, impact sedimentation processes, and alter water quality. The high demand for water resources for human well-being and sustainable development is the main reason for South Africa’s uneven distribution of water resources. In addition to the demand, the existing legislative framework lacks implementation, and increased fish kills threaten indigenous fish populations and potential fisheries that provide income benefits to local subsistence fishermen to provide for their families. The poor fish communities and impaired *L. natalensis* populations in the uMsunduzi River are of great concern, particularly after the fish kill in August 2019.

6.3 Recommendations

It is recommended that the forces noted to drive fish communities and the ecological health of the uMsunduzi River be improved. Locations characterised by modified flows, biotope structures, and poor water quality have often been the sample points near the city, and the impunity of polluting industries and broken sewage pipes affect the ecological health of such locations. Efforts to mitigate these issues would likely result in improved ecological health and, subsequently, the health of fish communities as well as populations of indigenous indicator species.

Specific recommendations are as follows:

- Rehabilitation efforts to improve riparian and instream vegetation to prevent erosion and habitat fragmentation.
- Planting indigenous vegetation and floating wetlands to clean up the water and improve niche availability for aquatic organisms.
- Get the relevant authorities and stakeholders in water and sanitation to take action.
- Fish passages need to be constructed in all functional barriers to allow migratory fish such as *L. natalensis* and *Anguillid* spp. to migrate between upstream and downstream areas according to seasonal conditions.
- The removal of redundant instream physical barriers, and improvement of pollution barriers and human-made barriers would improve flow regimes, allow the movement of *L. natalensis* within the river, improve connectivity between downstream and upstream populations of *L. natalensis* and possibly encourage breeding to improve fisheries along the uMsunduzi River.
- The effects of fragmentation on the genetic resilience of the *L. natalensis* must be investigated to ascertain the detrimental effects of river fragmentation on fish populations and

inform possible translocation of fish from other catchments to re-inoculate the *L. natalensis* populations in the uMsunduzi River in an attempt to improve population dynamics of the species as a valuable indicator and target fish for anglers.

- The use of telemetry techniques to monitor the ecological water quality and flow requirements of *L. natalensis*, behavioural responses, and population recovery is necessary to inform water resource managers on when to address thresholds of potential concern to maintain a health system, with near pre-spill abundances and species richness in the local fish populations. However, until habitat, connectivity and water quality issues have been resolved, telemetry will not be applicable to the uMsunduzi system.

6.4 Conservation implications

The present study's findings can be of great use in guiding management and conservation plans in the uMsunduzi catchment, providing an evidence-based management approach. The continual monitoring and historical data allow for analysis of trends in biological responses and abiotic indices over time, which can directly inform necessary management measures to maintain desired ecological conditions (e.g., the incorporation of fish ladders to allow fish migration), and to minimize anthropogenic effects on the ecological functioning of aquatic ecosystems, with the improvement water security. Moreover, the results from the present study can inform municipalities about the ill effects of pollution on our aquatic ecosystems and the importance of implementing the existing legislature to curb the illegal dumping of toxic byproducts from the adjacent industrial complexes, maintenance of the ageing and broken sewage networks and the improvement of fisheries to promoted rehabilitation of the uMsunduzi River to historically pristine conditions.

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