

**Source to Sea conservation of the fish communities of the greater
Incomati River Catchment, southern Africa**

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South Africa is a water-scarce country, with its water resources under constant strain due to ongoing resource use and development. With high levels of poverty, inequality and unemployment, many people in South Africa rely on natural river systems to meet their daily water and natural product requirements. The National Water Act of South Africa made provision for this by legislating “*fresh*” or “*clean*” water required to meet domestic use as a basic human need. But, continued population growth, land transformations, intensified agriculture and industrial diversification coupled with climate change and unsuccessful law enforcement are threatening and degrading the ecosystems and the biodiversity that depends on them. Degradation of freshwater systems at the current rate may cause two-thirds of the human population to face severe water shortage and water stress as soon as 2030 in the region and losses of associated ecosystem services.

Freshwater fishes and their populations and community structures are indicators of key ecological attributes of complex ecosystems. A lot is known about fishes and this information is used to understand, monitor and manage ecosystems. Changes in ecosystem function and associated condition drives changes in fish communities that is characterised and used to determine the amount of degradation that specific ecosystem has undergone. Multiple Lines of Evidence (LoEs) can be implemented to evaluate the present condition of biotic communities in important catchments, characterise drivers of changes in communities and evaluate the threat of multiple stressors affecting biotic communities and the ecosystems they live in.

The study aimed to characterise and evaluate the effects of multiple anthropogenic stressors affecting the rivers of the Incomati River Catchment from source to sea, using fish communities and migratory species as ecological indicators of the rivers through multiple lines of evidence.

The greater Incomati River Catchment is a semi-arid environment located in the south-eastern parts of South Africa, northern parts of Eswatini and southern parts of Mozambique. The greater Incomati River Catchment is identified as an ecologically important Water Management Area (WMA) in South Africa due to the high number of endemic fish species and conservational fish protected areas. The greater Incomati River Catchment is home to 70 known expected species throughout the catchment. The greater Incomati River Catchment is also one of the most stressed ecosystems in South Africa, partly due to land use transformations, agriculture and urbanisation. Currently, many of these environmental stressors are driving the fish communities negatively that could lead to the degradation of the

ecosystem and the provided ecosystem services. Fifty of the expected 70 fish species has been collected throughout the catchment, along with four invasive fish species. Using multiple lines of evidence and exciting biotic indices, the current state of the fish communities in the greater Incomati River Catchment is in a moderately-modified state with a low to moderate relative risk assessment score.

Various anthropogenic activities in the catchment are leading to environmental drivers such as barrier formation, sedimentation, flow alteration, water quality alterations and habitat modification to occur. Water quality modifications and alterations in substrate diversity contributed significantly to affect the fish communities and the ecological and social services throughout the greater Incomati River Catchment. The risk assessment of the different risk regions supports the effects of these anthropogenic activities and identified areas of concern due to adverse ecological effects that occur or will occur due to the exposure of one or more stressors. Management considerations are needed to address flow alterations and limited water quality and habitat stressors in the Uanetze and Sabie River sub-catchments, considerable water quality, flow alterations, habitat changes and alien invasive species in the Crocodile River sub-catchment, the extreme effect of barriers and altered flows with alien invasive species affecting fish communities in the Komati River sub-catchment and lastly, invasive species, overexploitation and agricultural water abstraction in the Incomati River sub-catchment.

Additionally, anguillid eels were used as an ecological indicator species to the effects of anthropogenic stressors in the greater Incomati River Catchment. A 40.2% decline in anguillid eel populations indicate the drastic effects of environmental dysconnectivity and overexploitation. A Relative Risk Assessment indicate the risk associated to the different life cycle stages of anguillid eels as well as the different anthropogenic stressors that affects the populations at each stage.

This study successfully characterized and evaluated the effects of multiple anthropogenic stressors on the rivers of the greater Incomati River Catchment, from source to sea. By utilizing fish communities and migratory species as ecological indicators, I have provided valuable insights into the health and resilience of these aquatic ecosystems. Through multiple lines of evidence, the findings highlight the extent of human-induced pressures and their implications for riverine biodiversity and ecosystem functioning.

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“A river has many curves, but it always reaches the ocean.”

- Donald L. Hicks

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DECLARATION

I, Annelize Van der Merwe, declare that the thesis hereby submitted by me for the Degree of Doctor of Philosophy at the University of Mpumalanga, is my own original research work. The thesis was carried out under the supervision of Prof. GC. O'Brien. Neither this thesis, nor any part of it has been previously submitted by me for any degree or examination to another University. I also affirm that the information and material used from other sources are fully recognized and acknowledged.

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LIST OF ABBREVIATIONS

AVEG	Aquatic vegetation
BN	Bayesian Network
CMA	Catchment Management Agencies
DO	Dissolved oxygen
DWS	Department of Water and Sanitation
EC	Electrical conductivity
ERYCA	Elands River Yellowfish Conservation Area
EWR	Ecological Water Requirements
FAII	Fish Assemblage Integrity Index
FEPA	Freshwater Ecosystem Priority Areas
FRAI	Fish Response Assessment Index
HF	High Flow season
IBI	Index of Biotic Integrity
IIMA	Interim IncoMaputo Agreement
IUA	Integrated Units of Analysis
IUCMA	Incomati-Usuthu Catchment Management Agency
IUCN	International Union for Conservation of Nature
IWRM	Integrated Water Resources Management
KNP	Kruger National Park
LF	Low Flow season
LoE	Lines of Evidence
MTPA	Mpumalanga Park and Tourism Agency
MVEG	Marginal vegetation
NEM:BA	National Environmental Management Biodiversity Act
NEMA	National Environmental Management Act
NFEPA	National Freshwater Ecosystem Priority Area
NWA	National Water Act
PCA	Principle Component Analyses
PES	Present Ecological State
RDA	Redundancy Analysis ordination technique
RDM	Resource-Directed Measures
RQOs	Resource Quality Objectives

RRM-BN	Relative Risk Model through the incorporation of Bayesian Networks
SDGs	Sustainable Developmental Goals
SUBS	Substrate diversity
TDS	Total Dissolve Salts
TEMP	Water Temperature
TVHR	Transparent Velocity Head Rod
UN	United Nations
WIO	Western Indian Ocean
WMA	Water Management Area
WWTW	Waste Water Treatment Works

Chapter 1

General introduction

South Africa is a water-scarce country, with its water resources under constant strain due to ongoing resource use and development (Schneider *et al.*, 2014). Continued population growth, land transformations, intensified agriculture and industrial diversification coupled with climate change and unsuccessful law enforcement are threatening and degrading ecosystems and the biodiversity that depends on them (Fouchy *et al.*, 2018). The consistent overuse and anthropogenic pressure on freshwater resources have caused the quality of southern African river systems regionally to reach its lowest level of functionality (Karr 1999; Saunders *et al.*, 2002; du Plessis 2019). Despite the multiple advantages human communities gain from freshwater ecosystems in the form of ecosystem services, and the ecological value of fishes, these ecosystems are constantly overused and abused (Apostolaki *et al.*, 2020). Freshwater fish species represent part of the world's greatest diversity and provide numerous services to especially vulnerable human communities (Dudgeon 2010; van der Sleen and Albert 2022). At the current rate of degradation of freshwater systems may cause two-thirds of the human population to face severe water shortage and water stress as soon as 2030 in the region, as well as losses of associated ecosystem services (Arthington *et al.*, 2023; Thieme *et al.*, 2024).

With high levels of poverty, inequality and unemployment, many people in southern Africa rely on natural river systems to meet their daily water and natural product requirements (Nkondo *et al.*, 2012). “Clean” water, as a basic human need that requires coordinated efforts from governmental entities, to consider its long-term sustainability (Ali 2023). In South Africa, the National Water Act (36 of 1998) makes provision for daily water requirements by legislating a basic human need, as well as an ecological reserve flow for all the major river systems in the country (RSA 1998; Nkondo *et al.*, 2012). In South Africa regionally progressive legislation includes the concept of the “Ecological Reserve” emphasising the efficiency of water management and ensures its sustainable use by citizens while protecting ecosystems (RSA 1998; Bourblanc 2015). This legislation is progressive in the region and being implemented leading to alignment opportunities with neighbouring countries, especially countries in southern Africa who share river catchments with South Africa (ORASECOM 2012; LIMCOM 2013). Transboundary governmental entities within

the Incomati River Catchment, excluding South Africa, include Botswana (Department of Water Affairs, Water Utilities Corporation and Dept. & National Parks of Wildlife), Zimbabwe (Ministry of Environment, Water and Climate, Zimbabwe National Water Authority, Ministry of Parks and Wildlife Management - ZIMPARKS) and Mozambique (Ministry of Public Works, Housing & Water, ARA Sul and Ministry Coordination of Environmental Affairs). These transboundary governmental entities has a Tripartite Interim (IncoMaputo) Agreement signed in 2002 (TIA 2002). The agreement aims to ensure equitable and sustainable use of shared water resources, protect water quality, and prevent significant harm to downstream users (TIA 2002). While each country has its own laws, acts and regulations regarding Environmental Management and Water Resource Protection, the catchment must work together to ensure parallel management and protection of this shared water resource.

National water laws and regulations in countries like South Africa, Mozambique, and Eswatini not only govern the allocation and protection of water resources but also play a crucial role in the conservation of freshwater ecosystems and biodiversity. By protecting and managing the freshwater biodiversity of a system, it will ensure that the ecosystem stays stable and resilient (Elmqvist *et al.*, 2003). The Biedouw River Restoration Project demonstrated how river rehabilitation, alien species removal (fish and plant) and long-term monitoring have recolonized and restored river functionality to allow for the re-establishment of the endangered Clanwilliam Sandfish (*Labeo seeberi* Gilchrist & Thompson, 1911) (Freshwater Research Centre (FRC) 2022). Additionally, the Thames River Restoration Project in the United Kingdom demonstrated how a “*biologically dead*” river system (Thames River) is now considered as one of the cleanest metropolitan rivers in the world (Attrill and Thomas 1996; Zoological Society of London (ZSL) 2015). Massive wastewater treatment improvements, habitat restoration, strict environmental regulations and continuous monitoring, improved the condition of the river system to such a degree that it allowed the re-establishment of over 125 fish species (Attrill and Thomas 1996; Zoological Society of London (ZSL) 2015). Case studies like these (Zoological Society of London (ZSL) 2015; Kingsford, Bino, and Finlayson 2021; Freshwater Research Centre (FRC) 2022) now serves as a model for freshwater conservation and species recovery. Conserving fish species not only preserves biodiversity but also provides a valuable tool for monitoring ecosystem health, as fish act as sensitive indicators of environmental change (Karr and Dionne 1991; Inwati *et al.*, 2025).

Freshwater fishes and their populations and community structures are indicators of key ecological attributes of complex ecosystems (Kumar 2020; Sajina *et al.*, 2021). Fish longevity allows long-term exposure and response to environmental variability but are limited to their biological requirements and or tolerances (Fausch *et al.*, 1990). A lot is known about fishes and this information is used to understand, monitor and manage ecosystems. The high mobility rates of fish allow colonisation of a wide range of habitats; and ease of identification allows ease of application to observed changes in fish community composition either through taxonomic compositions, life history traits or functional traits that inform us about ecosystem functionality (Skelton 2001; Kumar 2020; Sajina *et al.*, 2021). Changes in ecosystem function and associated condition drives changes in fish communities that is characterised and used to determine the amount of degradation that specific ecosystem has undergone (Ormerod *et al.*, 2010; Zhao *et al.*, 2016). The study of fish communities requires knowledge on fish species specific habitat preferences, as well as environmental drivers e.g., velocity, depth, substrate cover and roughness, and the determination of species distribution and abundance within a community (Lamouroux *et al.*, 1999; Muñoz-Mas *et al.*, 2019). Apart from the ecological importance fish communities provide the aquatic ecosystems, indicator migratory species also provide a linking function between ecosystems (Burnett *et al.*, 2018). South Africa is ranked 94th out of 180 assessed countries in environmental performance due to the poor management and conservation efforts of its rivers in particular (Environmental Performance Index 2020). There are also examples of the need to address and manage the vulnerable ecosystems for fish and fisheries towards a sustainable future for them and the human livelihoods who depend on them in southern Africa (Jackson *et al.*, 2016; Gammage and Jarre 2021). A trade-off now exists between the use and maintenance of a healthy and diverse freshwater ecosystem and the use and utilisation of the rivers by human communities.

Unregulated environmental variability in rivers drive fish community structures and or the population structure of indicator migratory species in various ways namely: changes in diversity, abundance and ratios of juveniles, sub-adults and adult individuals, and changes in the ratio of different species (Grossman *et al.*, 2010; Oberholster *et al.*, 2013; Erös *et al.*, 2014). The physical and or biological attributes of rivers vary between locations and over time. This drives natural variability of fish communities, when the natural variability is understood the abnormal changes in fish communities can be used to identify environmental variability that is unnatural and potentially destructive.

The greater¹ Incomati River is passing through South Africa (Mpumalanga), eSwatini and Lower Mozambique. This important river sustains >4 million people by providing ecosystem services, and other services for socio-economic development throughout the region (Anderson 2005; Sherwill *et al.*, 2007; Schreiner *et al.*, 2011). With all the rivers originating within the mountains of South Africa, South Africa has International legal obligations to ensure that sufficient quality and quantity of water flows downstream to countries like Eswatini and Mozambique. (Zaag and Carmo 2003; Hallowes *et al.*, 2010; Zikhali-Nyoni 2021). These obligations are governed by various international acts and agreements intended to promote equitable and sustainable use of transboundary water resources (Hallowes *et al.*, 2010; Zikhali-Nyoni 2021). South Africa must therefore manage its water resources not only for domestic purposes, but also in a sustainable way that respects the rights and developmental needs of its transboundary countries, while encouraging collaboration and preventing possible confrontations over shared water.

The Incomati River Catchment is a highly diverse catchment, supporting an estimated 70 fish species of which the majority are ecologically important, and many have conservation importance (Scott *et al.*, 2006). The greater Incomati River Catchment is divided into five main sub-catchments that share the water resources, but each with its own anthropogenic stressors. The main river sub-catchments within the Incomati include the Uanetze-, Sabie-, Crocodile-, Komati- and Incomati Rivers. The Uanetze River sub-catchment (including the Massintonto River sub-catchment) is a seasonal river catchment with minimal sometimes no-flows during the winter low flow period but usually maintains pools that species use for refugia (Tedesco *et al.*, 2008). The Sabie River sub-catchment one of the catchment's largest perennial rivers and although in the 1960's was the most severely impacted of the catchment, today it is one of the most pristine rivers in the greater Incomati River Catchment due to its relative high biodiversity, which has recovered from only a few tolerant species in the 1960s. In 1989 the Corumana Dam was built on the Sabie River in Mozambique below the Kruger National Park (Isaacman and Isaacman 2013). This permanent barrier limits any further migrations of fishes into the Sabie River from the rest of the greater Incomati River Catchment. The Crocodile River has been the key ecosystem for fish communities and other aquatic animals in the catchment with a few partial barriers (O'Brien *et al.*, 2014b; O'Brien *et al.*, 2019). The Crocodile River also has the highest representation of fishes that occur in the

¹ To minimize confusion - the word "greater" is inserted when referring to the whole catchment (South Africa & Mozambique) while when referring to the Incomati River Catchment (sub-catchment only within Mozambique) it is referred to the Incomati River Catchment.

greater Incomati River Catchment (Skelton 2001). The Komati River is the most developed and used river system in the catchment with a high abundance of large dams, excessive water abstraction and water quality contamination from the mining industry in its upper reaches (Kleynhans *et al.*, 2015). The greater Incomati River is the lower section within Mozambique below the confluences where the other main tributaries flows into a large (>1500km²) floodplain with major socio-ecological importance (Roux *et al.*, 2013). The Incomati Estuary is linked to the Incomati Floodplain and the western Indian Ocean. In the lower reaches of the Incomati River, many endemic fishes and other aquatic animals occur making this a particularly important part of the whole catchment (Ashton 2007; Roux *et al.*, 2013).

Problem statement and Justification

Unregulated environmental drivers can alter fish community composition in multiple ways, including shifting the balance between major trophic groups (herbivores, omnivores, predators), changing species ratios, or disrupting the interactions among species (Gulland 1987; Broadway *et al.*, 2015; Tesfay Gebrekiros 2016). Breaking these linkages between biotic communities and the ecosystem services they provide could lead to serious ecological and social consequences.

The transboundary Incomati River Catchment is one of the most ecologically important systems in southern Africa, yet it is under increasing pressure from development, water abstraction, land-use changes, pollution, and climate-driven stressors. Preserving the ecological integrity of this catchment is therefore urgent, not only to maintain biodiversity but also to safeguard the ecosystem services that support human livelihoods. South Africa has initiated multi-partner projects to identify areas of conservation and biodiversity concern, classifying them into National Freshwater Ecosystem Priority Areas (NFEPA) (Dudgeon *et al.*, 2006; Driver *et al.*, 2011). These NFEPA aim to ensure that rivers, wetlands, and estuaries remain in as natural a condition as possible to conserve ecosystems and water resources for both human and ecological use (Driver *et al.*, 2011).

The majority of the greater Incomati River Catchment within South Africa is classified as FEPA (Driver *et al.*, 2011), motivated by the high number of endemic species of conservation concern (Skelton *et al.*, 1995; Scott *et al.*, 2006) and protected areas such as Kruger National Park (KNP). Of the estimated 70 fish species in the Incomati Catchment, 13 (18%) are listed as threatened on the IUCN Red List (IUCN 2021b), while many others are data deficient. Despite these protections, many areas now fall within developed zones where

enforcement of environmental protection is weak, reducing the effectiveness of current legislation (O'Brien *et al.*, 2014b). Furthermore, existing water resource protection and fisheries management programs do not adequately account for river connectivity or migratory species, which are crucial for maintaining ecosystem function (Fausch *et al.*, 2002; Silva *et al.*, 2018). Species such as anguillid eels are both ecologically and economically important but remain understudied and undervalued, highlighting a critical gap for sustainable water resource management.

The central question arises: Is there enough being done to conserve these species and their habitats? Can protected areas like KNP maintain healthy fish populations while allowing other parts of the basin to support heavier development? Achieving this would provide a sustainable regional balance, where conservation efforts in priority areas complement responsible resource use across the catchment.

Rivers within the greater Incomati Catchment are overused, and information regarding their management, wellbeing, and sustainability is limited. Since environmental drivers directly influence freshwater ecosystems, it is imperative to study and predict how current and future stressors will affect fish communities (Lee *et al.*, 2023). Understanding which species occur naturally, which are present now, and how communities differ across the catchment will allow managers to evaluate river health, prioritize conservation efforts, and anticipate future impacts.

Good management practices and regular monitoring are essential to ensure the resilience of fish communities and to mitigate environmental impacts within the Incomati River Catchment. Conservation efforts must integrate knowledge of how environmental drivers shape species composition and biogeography along the river system (Pool *et al.*, 2010; Mueller *et al.*, 2020), supporting a regional strategy that balances ecosystem protection in key areas like KNP with sustainable use in the wider catchment.

Study Aim and Objectives

This study aims to contribute to the conservation and sustainable management of the rivers and their fishes in the transboundary, Incomati River Catchment. Towards this it is aimed to improve understanding of the fishes of the catchment, and changes in fish communities between the past, prior to anthropogenic activities, and today. This study will also elucidate the source to sea dynamics of the fish in the catchment and their migratory requirements. Afterwards, the study will evaluate the risk of multiple anthropogenic stressors affecting the

fishes of the Incomati River Catchment, to contribute to the sustainable management of the Incomati River Catchment and its fishes.

To achieve the aims of the study, six key objectives for the study, each addressed in a chapter of the study, have been established including:

- Review the knowledge of fish communities in the greater Incomati River Catchment, southern Africa along with their wellbeing associated to the different conditions of the rivers,
- Evaluate the compositions, wellbeing and integrity of fish communities and key environmental drivers through the implementations of established biological index Lines of Evidence,
- Evaluate the Relative Risk of multiple established anthropogenic stressors affecting the fish communities in the rivers of the greater Incomati River Catchment,
- Characterise anthropogenic stressors affecting the wellbeing of anguillid eels in the greater Incomati River Catchment,
- Undertake a regional scale ecological risk assessment of multiple stressors affecting anguillid eel populations in the greater Incomati River Catchment, and
- Provide recommendations for sustainable management and conservation.

Thesis Structure

This thesis format includes seven chapters of which four are data chapters for publication. The thesis is structured into a series of chapters designed to be published separately. By adopting a manuscript format for chapters there are un-avoided overlapping duplication of introductions and methodologies in the chapters. The chapters of the thesis include:

- *Chapter 1: General introduction:* This chapter provides the general introduction, justification, motivations, research questions, hypotheses, aim and objectives of the study and thesis structure.
- *Chapter 2: Review of the fish of the greater Incomati River Catchment:* This chapter focuses on the fish of the Incomati River Catchment, their rivers and past and present stressors affecting the condition of the rivers and the fishes. A broad section of the literature review provides a background summary on the importance of the regional Incomati Catchment Rivers', the laws protecting them, and the different approaches used to manage, monitor and conserve them. This chapter then considers the fish of the catchment, species, distributions, biology and ecology and their use as ecological

indicators towards the conservation and management of the rivers. The review includes information on the historical wellbeing, conservation status and current threats of stressors to fish communities in the catchment.

- *Chapter 3: A holistic catchment scale assessment of fish communities and what is driving communities for catchment scale management and conservation consideration.* This data chapter includes an evaluation of the wellbeing of fish communities using multiple Lines of Evidence (LoE) to determine the condition of communities and environmental drivers affecting communities in the greater Incomati River Catchment, southern Africa. Multiple LoEs including the Fish Response Assessment Index (FRAI) and Multivariate statistics (Redundancy Analysis) were used to assess the fish communities in the catchment while providing insight into what anthropogenic stressors affect fish communities. The multivariate analysis was used to validate the FRAI results and to provide insight into the main environmental drivers of change affecting fish communities in the catchment. The findings include the identification of stressors affecting fish community composition, and the ecological wellbeing of river using fish as ecological indicators. Fish community linkages and the importance of migrations are considered in this evaluation so that sustainable fish community management can be considered from source to sea. This included the evaluation of the main river systems, barriers, and or condition of the main tributaries. This chapter will be structured for the submission to the peer-reviewed journal named *Journal of Fish Biology*.
- *Chapter 4: An ecological risk assessment of multiple anthropogenic stressors affecting the fish communities of the greater Incomati River Catchment, southern Africa:* This data chapter details the relative ecological risk assessment of multiple flow and non-flow, physical and biological stressors, affecting fish communities in the greater Incomati River Catchment. Subsistence and ecological ecosystem service endpoints were selected to represent ecosystem services in the catchment from a fish perspective and to determine the effect of multiple environmental drivers related to anthropogenic sources and stressors on fish related selected endpoints. Hypothetical scenarios were established and evaluated to identify and test management options. The Relative Risk assessment allows for the determination of the relative risk or impact associated for fish communities for that specific endpoint. Endpoints represent specific ecosystem services provided by fish species and thus the risk towards those

fish and ecosystem services. The Incomati River Catchment, important to many human and biotic communities, requires the proper management strategies to preserve the ecological integrity of the catchment. Each river system unique to its own stressors and communities, plays its role in making the Incomati River Catchment the most ecologically important catchment in southern Africa. This chapter will be structured for the submission to the peer-reviewed journal named *Integrated Environmental Assessment and Management* (IEAM).

- *Chapter 5: A regional scale Risk Assessment of the greater Incomati River Catchment, southern Africa, and the associated drivers affecting anguillid eels using the Relative Risk Model:* This is the final data chapter and have been prepared to demonstrate the risk of natural and anthropogenic stressors to the different life stages of anguillid eels throughout the greater Incomati River Catchment, to inform ecological and social endpoints on the relative risk involve for anguillid eels. The anguillid eels within the greater Incomati River Catchment are under threat due to multiple environmental drivers that act in synergy to cause effect on their population numbers. The distribution of anguillid eels has reduced with 40.2% throughout the greater Incomati River Catchment from 1960 to 2024. Four scenarios, of which three were hypothetical, were evaluated to assess the risk when management and monitoring strategies are aimed to prioritise different detrimental stressors. This study demonstrates the probable effects that environmental drivers have on the anguillid eel populations and the ecological wellbeing within the Wester Indian Ocean. Anthropogenic stressors like barriers, overfishing (exportation) and habitat destructions are greatly influencing the wellbeing of anguillid eels within the catchment with future scenarios prediction the outcomes if current management processes and strategies are not attended to. This chapter will be structured for the submission to the peer-reviewed journal named *Integrated Environmental Assessment and Management* (IEAM).
- *Chapter 6: Provided recommendations for sustainable management and conservation:* This is a final discussion and conclusion chapter where findings of all data chapters will be discussed and recommendations for the different outcomes will be provided to aid management discissions. Reference is made back to the main aim to provide an in-depth evaluation of the anthropogenic stressors affecting fish

communities and indicator species within the greater Incomati River Catchment.

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Mpumalanga. It represents a significant contribution to both the management and scientific understanding of the fish communities in the Incomati River Catchment, offering novel insights through its approach, findings, and outcomes. The research demonstrates a systematic, reliable methodology, and addresses key challenges relevant to sustainable resource use and environmental governance. All necessary ethical clearances and country permits were obtained to ensure responsible and compliant conduct throughout the study area. The ethical clearance number was obtained from the University of Mpumalanga (UMP/O'Brien/2/2023), provincial research permits were obtained from Mpumalanga Park and Tourism (MPB 5789) which allowed sampling and collection within Mpumalanga, South Africa. Sampling within transboundary countries (Mozambique and Eswatini) was allowed following the approval and allocation of an invitation letter and sampling letter. All local community chiefs and tribe leaders were informed of our whereabouts and permission letters were presented to them before sampling commenced.

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Chapter 2

Review of the greater Incomati River Catchment

This review of the greater Incomati River Catchment, its rivers, threats and or stressors affecting river health or wellbeing and the fish of the greater Incomati River Catchment, their biology and ecology and the health of wellbeing of fish communities is provide as a foundation of knowledge for this thesis. This section includes an overview of rivers and their governance, fish as ecological indicators of rivers globally and regionally, the greater Incomati River Catchment, threats and or stressors affecting the wellbeing of rivers in the catchment, knowledge of fish populations and communities and their use as ecological indicators in the catchment. Finally, this review considers available knowledge and or efforts towards the conservation and management of fish in the Incomati River Catchment.

Rivers and their governance of the Incomati Catchment

Freshwater ecosystems, including rivers, lakes, groundwater or wetland systems all support a variety of biodiversity and human communities (Apostolaki *et al.*, 2020). These systems not only provide biodiversity (supporting ecosystem services) and provisioning ecosystem services that human communities depend on, they have important regulatory and cultural services which all benefit from (Apostolaki *et al.*, 2020). Freshwater aquatic ecosystems provide renewable resources and ecosystem services but are susceptible to multiple anthropogenic stressors (Costanza *et al.*, 1997; O'Brien *et al.*, 2019). Ecosystem services of freshwater ecosystems include the provisioning services of resources (food, water), environmental regulation services (water purification or waste assimilation, erosion processing and flood mitigation), cultural services including recreation and ecotourism, and ecological processes or supporting services including biodiversity (Apostolaki *et al.*, 2020). These services are provided through complex habitats, temperature- and flow regimes, food web function, and migratory pathways (Costanza *et al.*, 1997; Holmlund and Hammer 1999; Fischlin *et al.*, 2007). The livelihoods of human communities depend on the services provided by freshwater ecosystem, that they derive directly from the quality and quantity of available freshwater resources (Brown and King 2013; Apostolaki *et al.*, 2020). Despite the multiple advantages human communities gain from freshwater ecosystems, these ecosystems are constantly used and abused (Skelton *et al.*, 1995; Apostolaki *et al.*, 2020). The consistent

overuse and anthropogenic pressure on freshwater bodies have caused the quality of southern African river systems to reach its lowest level of functionality over the past ten years (Ashton 2010; Woodborne *et al.*, 2012; du Plessis 2019).

Southern Africa is a water-scarce region and receives an average annual precipitation of 464mm (59% of the global average), with its water resources under constant strain because of national development (Schneider *et al.*, 2014). Since 1960, the population has grown fourfold resulting in increased resource demands that alter land use through agriculture and infrastructure expansion (Fouchy *et al.*, 2018). These impacts accumulate in aquatic ecosystems due to drainage, physicochemical pollution from urban developments and waste, altered flow regimes and disturbance (Fouchy *et al.*, 2018; O'Brien *et al.*, 2019). Degradation of freshwater systems at the current rate may cause two-thirds of the human population to face severe water shortage and water stress as soon as 2030 (Karr 1999; Saunders *et al.*, 2002). It is imperative that ecological functionality be preserved for sustainability and to conserve ecosystem services, (Ward and Stanford, 1995; Silva *et al.*, 2018; O'Brien *et al.*, 2019).

With high levels of poverty, inequality and unemployment, many people in southern Africa rely on natural river systems to meet their daily water requirements (RSA, Mozambique, Eswatini)(Swatuk 2008; Hallowes *et al.*, 2010; Kit 2011; Kyei and Chitiga-Mabugu 2021). In South Africa, the National Water Act (36 of 1998) makes provision for daily water requirements by legislating a basic human need, as well as an ecological reserve flow for all the major river systems in the country (RSA 1998; Nkondo *et al.*, 2012). In addition to the NWA, progressive legislation in South Africa including the National Environmental Management Act (NEMA) no 107 of 1998 and the National Environmental Management: Biodiversity Act (NEM:BA) no 10 of 2004 have added requirements for species conservation through the establishment of Freshwater Ecosystem Priority Areas (FEPA) (Driver *et al.*, 2011). This legislation is progressive in the region and being implemented leading to alignment opportunities with neighbouring countries, especially countries in southern Africa who share river catchments with South Africa (ORASECOM 2012; LIMCOM 2013). In South Africa the FEPA are established to ensure rivers, wetland areas and estuaries are managed and protected to remain in as natural and good condition as possible to conserve ecosystems and water resources for human use (Driver *et al.*, 2005). Unfortunately, these protected areas are now in developed areas where protection is poor and additional environmental protection is not required or enforced. With all of these systems and

governmental legislations in place, South Africa's current water resources protection and fisheries management programmes does not incorporate or consider river connectivity management and migratory species (Fausch *et al.*, 2002; Silva *et al.*, 2018). The use of migratory fishes for ecological and economic benefits in southern Africa are understudied and undervalued and need to be considered for the sustainable vision for water resources (Silva *et al.*, 2018; Agboola *et al.*, 2020).

In South Africa the National Water Resource Strategy incorporates an Integrated Water Resources Management (IWRM) process for the strategic development and management of water, land and related resources (DWS 2014). Two complementary strategies in South Africa are proposed to maximise economic and social welfare without compromise to ecosystems (King and Pienaar 2011). The first, Resource-Directed Measures (RDM) are to protect water resources through targets and goals for the preferred condition of water resources in freshwater systems (King and Pienaar 2011; Dickens *et al.*, 2020). The second, is source-directed control that specifies criteria for controlling the water resource use and effects on aquatic ecosystems (King and Pienaar 2011; Rowlston *et al.*, 2011; Dickens *et al.*, 2020).

Other southern African countries like Eswatini and Mozambique have also developed legal and institutional frameworks to protect water resources and ensure sustainable use. These frameworks reflect the growing commitment to the Integrated Water Resources Management (IWRM), environmental protection and equitable water allocation (LIMCOM 2003; Wilkinson *et al.*, 2015). Eswatini has foundational legislations that governs the management and conservation of water resources through 1^{stly}) the Water Act No. 7 of 2003 that makes provision for the management and conservation of water resources (Government of Eswatini 2003), 2^{ndly}) Water Pollution Control Regulation of 2010 that is complemented to the Environmental Management Act that mandate the maintenance of water quality (Government of Eswatini 2010) and 3^{rdly}) the Environmental Management Act 2002 and Eswatini Environment Authority (EEA) which is empowered to oversee environmental protection through environmental scrutiny (Government of Eswatini 2002). Mozambique also have their own fundamental laws to ensure water resource protection and management namely, 1^{stly}) Water Law No 16/91 of 1991 that employ a river basin approach to regulate use, conservation and management of water resources (Government of Mozambique 1991), 2^{ndly}) the National Water Policy (1995) and Water Tariff Policy (1998) these policies underscores the decentralization of water management to provincial and basin-level entities to satisfy the

basic human needs of rural communities as well as ensuring environmental protection and efficient water use (Government of Mozambique 1995, 1998), and 3^{rdly}) by supporting Decrees and Institutional Reforms that enacted a series of decrees and ministerial instruments (E.g., ARA-SUL & FIPAG which stands for *Fundo de Investimento e Património do Abastecimento de Água*, which translates to Water Supply Investment and Asset Fund) (ARA-SUL 2020; FIPAG 2022).

Conjointly, these transboundary countries are committed to the sustainable management of the shared Incomati River Catchment through the Tripartite Interim Agreement (2002) (TIA 2002; Hallowes *et al.*, 2010). This agreement, through equitable and sustainable water use, coordinated planning, data sharing, and joint decision-making, promotes integrated water resources management and the protection of the aquatic ecosystems of the greater Incomati River Catchment. This agreement is further strengthened through the establishment of the INMACOM (Incomati & Maputo Watercourse Commission) in 2022 which formalises a long-term cooperation to manage this water resource in a sustainable manner (Government of Mozambique 2022).

In 2012 the United Nations (UN) developed the Sustainable Developmental Goals (SDGs): a set of universal goals to meet the environmental, political and economic challenges faced by the world (Dickens *et al.*, 2019; 2020). The Brundtland Commission defines sustainable development as a “*development that meets the needs of the present generation, without compromising the needs for future generations*” (Imperatives 1987 Ch 2- pg 1). The 2030 Agenda for Sustainable Development compiled 17 SDGs in the hope that these could be achieved by 2030 (Dickens *et al.*, 2019). Global SDGs contribute to the management of water resources on multiple spatial scales (Dickens *et al.*, 2019). Each country must contextualise and implement the different goals and targets for its own purpose at a country level (Assembly 2015). Specific goals and target descriptions in the 2030 Agenda will help lead to a sustainable future (Dickens *et al.*, 2019). Southern African countries (South Africa, Namibia, Botswana, Zimbabwe, Mozambique and Eswatini) aim to achieve a balance between the use and protection of its natural resources and achieve the SDGs by 2030 (Dickens *et al.*, 2019; Lynch *et al.*, 2019; United Nations 2023). For example, SDG 6 emphasizes the availability of safe drinking water and well-managed sanitation for all. Water Resource Management in southern Africa is progressive, yet still adaptable to meet the need of legislation and ensure protected, sustainable resources (King and Pienaar 2011; O’Brien *et al.*, 2019). Projections on the rate of progress on this SDG suggest the need for double the

time allocated as 129 countries (including southern African countries) are not on track to achieve sustainable managed water resources in 2030 (Silva *et al.*, 2018; Lynch *et al.*, 2020).

Coordination of water, land, and resources across shared or transboundary river catchments can be obtained through the management and conservation of freshwater ecosystems (Arthington *et al.*, 2023; Kuehne *et al.*, 2023; Thieme *et al.*, 2024). If the correct monitoring and management strategies are implemented and linked, freshwater ecosystems can be protected in a manner that would ensure continuity of ecosystem services and the ecosystem and associated processes that are depended on (Ashton 2010; Boulton *et al.*, 2016; Bănăduc *et al.*, 2022). Management of ecosystems can be facilitated through adaptive management approaches that take into consideration the change in responses of ecosystems and organisms (Ashton 2010).

Fish as ecological indicators of river health

It is common practice for management agencies globally to use river biota (invertebrates and fish) as ecological indicators to assess the ecological state and health of freshwater ecosystems (Roux *et al.*, 1999; Arthington *et al.*, 2010; Wepener *et al.*, 2011; Agboola, Downs, and O'Brien, 2019). Aquatic macroinvertebrates are the most used indicators/organisms to assess river health and ecological condition (Kleynhans 2007). Their fast generation time, environmental sensitivity ranges, amount of species compositions and ease of sampling makes them valuable indicators for all the assess (Kleynhans 2007; Kleynhans and Louw 2007). Although, macroinvertebrates are a well-recognised indicator biota, freshwater fish species can provide a longer timeframe of accumulated stressors in the environment. Freshwater fish species represent part of the world's greatest diversity (Kuehne *et al.*, 2023). Fishes are successfully used as ecological indicators due to their relatively long lifespan, broad habitat requirements, mobility, wide range of trophic levels within the ecosystem and social importance to human communities (Sajina *et al.*, 2021; Pinna *et al.*, 2023). Fish and fish community composition makes good ecology indicators of long-term effects on river systems (Sajina *et al.*, 2021; Pinna *et al.*, 2023). Fish communities have been shown to respond predictably to almost all known anthropogenic disturbances (Poikane *et al.*, 2017; Sajina *et al.*, 2021; Pinna *et al.*, 2023). In particular, the responses of fish communities in a freshwater ecosystem can reflect the amount of degradation that specific ecosystems has undergone (Das and Chakrabarty 2007; Zhao *et al.*, 2016). Different levels of biological organisation (community, population, species, life stages) elucidate degrees of responses to a

particular stressor (O'Brien *et al.*, 2014; Burnett *et al.*, 2018; Murphy *et al.*, 2018)(Figure 1). Lower organisational levels demonstrate a more rapid stressor response and provide a cause-and-effect understanding (Valesini *et al.*, 2017) and higher levels demonstrate delayed stressor responses and provide knowledge of broader ecological implications (Valesini *et al.*, 2017)(Figure 1). Changes in fish community composition either through taxonomic compositions, life history traits or functional traits can all affect the ecosystem functionality (Zhao *et al.*, 2016). For these reasons, ecosystem functioning is largely dependent on the responses of freshwater fish populations and community compositions (Das and Chakrabarty 2007, Zhao *et al.*, 2016).

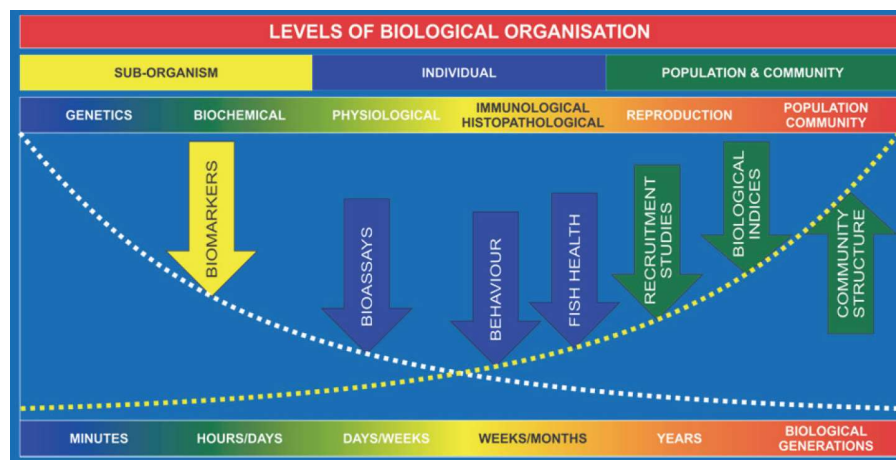


Figure 1: Different levels of Biological Organisation and the degree of information one can gain. Adapted from O'Brien *et al.*, 2014.

Since the quality and quantity of freshwater ecosystems can be influenced by environmental drivers it is important to study and predict the effects that different environmental drivers can have on fish communities (Wagner *et al.*, 2020; Lee *et al.*, 2023). Knowledge of the fishes expected to occur within a given reach of river and what a species' biology and ecology is or environmental preferences, and how communities differ will allow for the evaluation of the state of fish communities and the rivers in that they live in. Fish are good ecological indicators providing a variable degree of sensitivity as individual species are more/less tolerant of certain environmental factors and specific stressors.

In southern Africa there are more than 300 freshwater fish species of which 70 are endangered (Darwall *et al.*, 2009; Skelton 2024). These species are particularly influenced by eutrophication, bank erosion, channelisation, pollution and sand mining. A trade-off now

exists between the use and maintenance of a healthy and diverse freshwater ecosystem and the use and utilisation of the rivers by human communities. According to the Environmental Performance Index of 2020, South Africa is ranked 94th in the world (out of 180 countries) due to the poor management and conservation efforts of its river systems (Environmental Performance Index 2020).

Unregulated environmental drivers can cause change to a fish community composition in various ways namely, changing the balance between major elements (herbivores, omnivores, predators), changing the ratio of different species or changing the way the different species are linked to one another (Gulland 1987).

The greater Incomati River Catchment

The greater Incomati River Catchment includes the Incomati River and four main tributaries originating in South Africa namely: the Uanetze-, Sabie-, Crocodile- and Komati Rivers in eastern southern Africa, with the rivers flowing in an easterly direction into the Indian Ocean at Macaneta in Mozambique. The transboundary greater Incomati River Catchment occurs in a semi-arid part of southern Africa and includes approximately 46 750 km² of which 28 681 km² is located in South Africa (61.3%), 33% in Mozambique and 6% in Eswatini (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2024). The average discharge at the estuary of the Indian Ocean is 100 – 200 m³s⁻¹ (O'Brien *et al.*, 2024). The greater Incomati River Catchment experiences high seasonal flow variability with high flows during the wet season (November to March) and low flow during the dry season (April to October) (Roux *et al.*, 2016). The river's flow regime is categorised as torrential with a mean annual precipitation of 740 mm.a⁻¹ (mm per annum). Main river sub-catchments within the greater Incomati River Catchment include the Uanetze, Sabie, Crocodile, Komati and Incomati Rivers. These main rivers are exposed to different land use and water-use changes from as early as the 1950's (Hallowes *et al.*, 2010; O'Brien *et al.*, 2024).

The Uanetze River Catchment (along with the Massintonto River Catchment) is a seasonal river catchment with minimal flows during the low flow period (O'Brien *et al.*, 2024). The Uanetze Catchment within South Africa's side originates within the Kruger National Park, a protected area with minimal anthropogenic disturbances. The tributaries of the Uanetze Catchment are known to have around 26 freshwater fish species within the catchment, that generally utilises the catchments rivers as refuse areas after flooding events or spawning grounds (Scott *et al.*, 2006; Skelton 2024).

The Sabie River is currently classified as one of the most pristine rivers in the greater Incomati River Catchment due to high biodiversity and re-establishment of migratory pathways and species that have preserved ecological functionality in this system (Kleynhans 1986; Roux *et al.*, 2016; O'Brien *et al.*, 2018a; O'Brien *et al.*, 2018b). The Sabie River has 46 known freshwater fish species (Scott *et al.*, 2006; Roux *et al.*, 2016; South *et al.*, 2025). Surprisingly in the 1960s the Sabie River was considered the most polluted river of the Kruger National Park, considering its condition compared to the larger Limpopo, Shingwedzi, Letaba, Olifants and Crocodile Rivers that flow through the park (Pienaar 1968; 1978). The Sabie River has recovered from being a “*barren wasteland for fish*” (Pienaar 1978), to one of southern Africa’s most valuable conservation refugia for fish (Riddell *et al.*, 2022; South *et al.*, 2025). The fish recovery is largely attributed to river connectivity and the recruitment of fish into the Sabie River from the Crocodile, Komati and lower Incomati Rivers (NDW 2011; O'Brien *et al.*, 2018a; 2018b; Burnett *et al.*, 2021). Today, the Sabie River is disconnected from the rest of the Incomati Catchment through the construction of the Corumana Dam built in 1989 (NDW 2011). This system is under threat from multiple stressors including water quality, quantity and habitat alterations, alien species and disturbance to wildlife impacts (O'Brien *et al.*, 2024), from: forestry, agriculture, domestic water-abstraction, waste water treatment works, sand mining, historical mining and light industries. (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2024). In the lower reaches of the Sabie River, Corumana Dam restricts fish migrations, particularly for important catadromous migratory species such as anguillid eels and other threatened and or endangered fishes (NDW 2011; Fouchy *et al.*, 2018; O'Brien *et al.*, 2018a).

The Crocodile River system, has approximately 49 freshwater fish species, while some new species are proposed to occur and are being described (Mazungula and Chakona 2021). The Crocodile River is ecologically one of the most important and biodiverse river systems in the greater Incomati River Catchment today (Scott *et al.*, 2006; O'Brien *et al.*, 2024). The main threats on this river is domestic water use abstraction (South Africa and Mozambique), industrial and mining activity, forestation and irrigation (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2024). Additional threats include multiple weirs and dams blocking the connectivity of the system in the upper reaches (O'Brien *et al.*, 2019) as well as disturbance to wildlife through illegal fishing/netting and sand mining (Soko and Gyedu-Ababio 2015; McLoughlin *et al.*, 2021; O'Brien *et al.*, 2024). The Crocodile River is the most important and biologically diverse today due to the relative connectivity to the Indian Ocean that allows for anguillid

eels to migrate upwards (Hanzen *et al.*, 2022; Dlamini *et al.*, 2024) as well as the presence of critically endangered species (Eg., *Chiloglanis bifurcus*, *Kneria spp*) (IUCN 2021a).

The Komati River system is the dominant tributary of the greater Incomati River Catchment in South Africa and is well studied in South Africa because of its historical importance and more recent development interest (Dlamini 2021; Chawiya 2023; Dlamini *et al.*, 2023). The Komati is often synonymised with the greater Incomati River causing confusion but only refers to the southernmost large tributary of the Incomati River Catchment in South Africa (Nkomo and van der Zaag 2004). There are 43 freshwater fish species and is considered to be the most stressed river system in the catchment due to extraction for irrigation, power stations, mining and industrial water use, afforestation, and domestic water usage (in both South Africa and Eswatini) (Kleynhans *et al.*, 2015a; Saraiva Okello *et al.*, 2015). While the water quality conditions are deteriorating (O'Brien *et al.*, 2019), physical barriers in this river system and its tributaries affect the aquatic ecosystems and the pathways of important migratory fish (Kleynhans *et al.*, 2015a).

The Incomati River Catchment, the lower Incomati River section within Mozambique, splits into multiple branches where it converges again before the river reaches the estuary. This region supports a diverse array of critically important species within its delta zones, wetlands and estuary (WIOSAP 2020). The main economic activities within the lower Incomati River Catchment include commercial farming, fishing and sugarcane production. The Incomati River Catchment is home to freshwater and estuary fish species that utilises the vast array of habitats (Taucale 2007). Major dams within the country like Corumana Dam plays a crucial role in flood control, water storage and irrigation and domestic water supply while an additional dam, Moamba-Major Dam, is under construction to be built on the main stem of the Incomati River (De Boer and Droogers 2016). The Moamba-Major Dam will support irrigation, domestic water supply to Maputo and produce hydropower (De Boer and Droogers 2016).

Known Threats and challenges in the Incomati Catchment

The greater Incomati River Catchment is identified as an ecologically important Water Management Area (WMA) in South Africa due to the high number of endemic fish species and conservational fish protected areas (Driver *et al.*, 2011; Desai *et al.*, 2019; O'Brien *et al.*, 2024). The greater Incomati River Catchment is also one of the most stressed ecosystems in

South Africa, partly due to land use transformations, agriculture and urbanisation (Soko and Gyedu-Ababio 2015; Jackson *et al.*, 2016; O'Brien *et al.*, 2024).

Growing agriculture, forestry and urbanisation over the past two decades, were identified as some of the main drivers of increased water scarcity in the greater Incomati River Catchment (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2019; O'Brien *et al.*, 2024). Unprotected areas (excluding KNP) in the greater Incomati River Catchment are extensively transformed into plantations/woodlots, sugarcane croplands and urban/industrial areas. These transformations continue to cause change to the climate, weather patterns, energy and water transportation, alter species distribution, and reduce viable habitats (Sleeter *et al.*, 2018; Riddell *et al.*, 2022).

Flow alteration and connectivity threats

Rivers in their natural state flow undisturbed from source to sea without any physical barriers (dams, weirs, etc.), water quality, flow and or habitat stressors (Vannote *et al.*, 1980; Hadeed and Thomson 2006). Free-flowing rivers are rare and more at risk than their terrestrial counterparts as they concentrate the impacts of land use and degradation (Dudgeon *et al.*, 2006; Hadeed and Thomson 2006; Moorhouse and Macdonald 2015). Changes in the flow regime caused by for example water abstractions, barriers and channelisation will disrupt available habitats, species distribution, hydrology and nutrient exchange (Bunn and Arthington 2002; Poff *et al.*, 2007; Zeiringer 2018). In turn these changes will affect the fish communities through changes in their distribution, life histories and movement patterns (Bunn and Arthington 2002; O'Brien *et al.*, 2019).

The nature of trophic interactions and life cycles in aquatic ecosystems rely on the connectivity of various habitats (Silva *et al.*, 2018; O'Brien *et al.*, 2019). Physical barriers decrease ecological connectivity of river systems by impeding flows, nutrient transfer, causing sediment build-up and preventing biota from migrating up or downstream (Zarfl *et al.*, 2015). River infrastructure for hydropower, water supply, irrigation, and recreation provide immediate benefits to people but hinder ecosystem services and negatively affect their wellbeing over time (Aylward *et al.*, 2005; MEA 2005). Hydrological connectivity is important for the downstream movement of nutrients and the multidimensional dispersal of organisms (Pringle 2010; Branco *et al.*, 2014). Terrestrial connectivity to aquatic ecosystems is equally important for nutrient sources as some, species through different life-stages, depend on both habitats (Jungwirth *et al.*, 2003). Dams negatively affect available habitat,

migratory cues, reproduction and the feeding of many freshwater fish species (Brink *et al.*, 2018).

South Africa is ranked 8th among the countries with the highest number of dams per land area (Asmal *et al.*, 2000; O'Brien *et al.*, 2019). The largest protected area in South Africa, the Kruger National Park (KNP), extends across five major rivers, with their origins upstream of the park. All these rivers systems have dams or weirs, and additional planned construction will result in a conservation area that hosts barriers but inhibit the function and biodiversity of aquatic ecosystems (Nel *et al.*, 2007; O'Brien *et al.*, 2018b). The rivers of the KNP have been referred to islands of rivers amongst large dams (O'Brien *et al.*, 2018a). Unfortunately, very little protection has been afforded to river connectivity maintenance and migrations in South Africa (O'Brien *et al.*, 2019). Water storage and flow regulation for agriculture and other resource use activities have led to 610 formal dams and 1430 gauging weirs to be constructed that act a partial or complete barriers to fish migration on river ecosystems in South Africa (O'Brien *et al.*, 2019).

Flow regime modifications throughout the greater Incomati River Catchment is based on land cover and usages that cause change to the flow from the natural condition (DWS 2014; O'Brien *et al.*, 2024). Flow modifications in the greater Incomati River Catchment are due to weir and dam constructions, water abstraction for irrigation and channelisation (O'Brien *et al.*, 2019; O'Brien *et al.*, 2024) (Figure 2). Increasing agricultural growth and irrigation demands have led to the establishment of 12 major dams and more than 200 informal farm dams within the greater Incomati River Catchment (Jackson *et al.*, 2016; O'Brien *et al.*, 2019) (Figure 3). Among these are gauging weirs constructed to regulate flow, water withdrawal and control flooding (Silva *et al.*, 2018; O'Brien *et al.*, 2024). The dams and gauging weirs are anthropogenic barriers that hinder the migration of fish (Branco *et al.*, 2017; Silva *et al.*, 2018; O'Brien *et al.*, 2019). The Incomati Water Management Area (WMA) has more than 10 fish passages constructed on these barriers to improve connectivity and migration of fish but none are in a functional state (Bok *et al.*, 2007; O'Brien *et al.*, 2019; O'Brien *et al.*, 2024).

Indicator species have been used to infer the effects of high diversity on endangered and threatened species to provide a potential cause of low migratory management (Driver *et al.*, 2012; O'Brien *et al.*, 2019). Barriers affect the migration of catadromous species, like anguillid eel, upstream in search of suitable habitats for the completion of their life cycle and potamodromous species, like yellowfish, to feeding or spawning grounds (Branco *et al.*,

2017; O'Brien *et al.*, 2019). Apart from blocking the migration of fish, barriers (any physical anthropogenic structure) actively change the river flow through changes to the duration, rate, frequency, and timing of water availability (Jumani *et al.*, 2019). The advantages that barriers provide to human communities (water provisioning and storage, hydropower) are outweighed by the disadvantages (Rivers-Moore *et al.*, 2011; Jumani *et al.*, 2019).

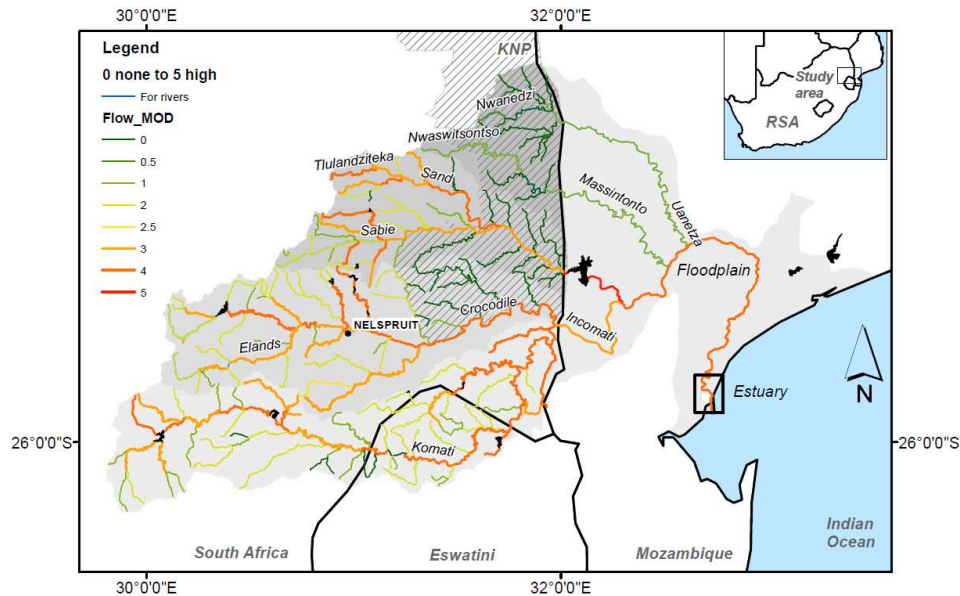


Figure 2: Flow regime modification scores of the rivers in the greater Incomati River Catchment (Data from DWS 2014).

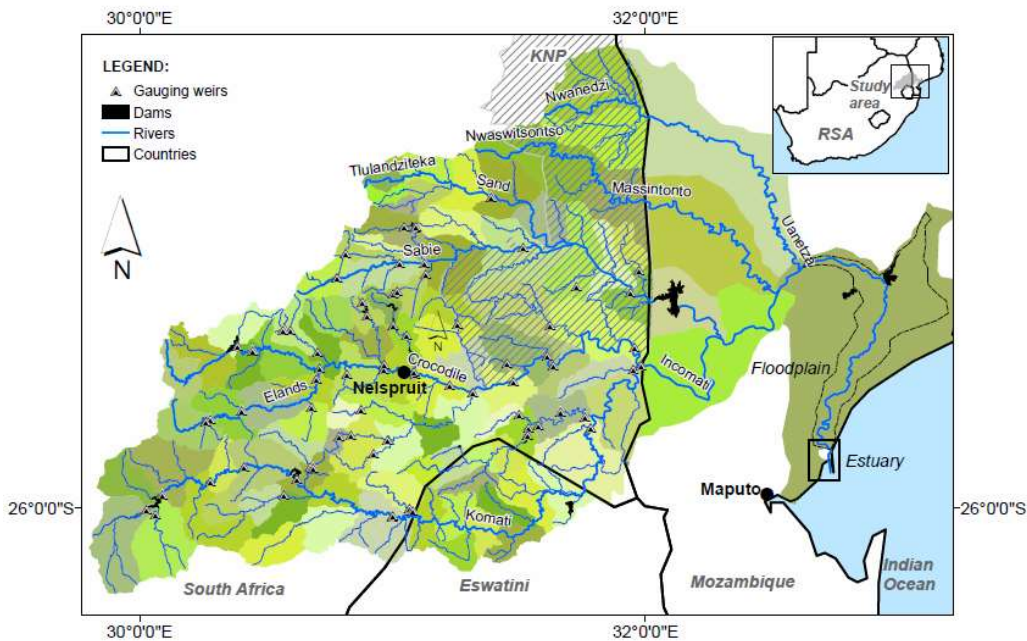


Figure 3: Gauging weirs and large dams within the greater Incomati River Catchment. Different colours represent the different sub-quaternary catchments (Data from IUCMA website).

Water quality threats

Water pollution is a critical environmental problem throughout South Africa that will further intensify if no proper management is implemented (Singkran 2017). The degradation of water resources can be attributed to numerous causes for example natural: weathering of soils and rock, high and low flow fluctuations, or anthropogenically: domestic wastewater discharge, agricultural drainage and dam constructions (de Necker *et al.*, 2020).

To evaluate the impact of anthropogenic water use it is important to understand how fish communities respond to the changes in water quality parameters (Schinegger *et al.*, 2016). The metabolic rate of fish is proportional to the water temperature, that means when the temperature increases the fish metabolic rate will increase and more energy and food will be required by the fish (Svobodová *et al.*, 1993; Volkoff and Rønnestad 2020). The most common and threatening water quality threat are chemical pollutants (nutrient pollution, heavy metal contaminants) as they are intensified through the process of bioaccumulation (Kotze *et al.*, 1999; Coetzee *et al.*, 2002).

Water quality threats in the greater Incomati River Catchment are based on anthropogenic usage and land cover changes that will likely alter the physio-chemical conditions away from the reference conditions in all rivers (DWS 2014; O'Brien *et al.*, 2024). In the greater Incomati River Catchment, the major water quality threats that influence the rivers condition are from irrigation, agricultural run-off and cultivation, unmanaged wastewater works and mining activities (Das and Chakrabarty 2007; Deksissa *et al.*, 2003; Roux *et al.*, 2013; O'Brien *et al.*, 2024). Major water quality threats and alterations occur throughout the catchment with minimal alterations in the KNP area (Figure 4).

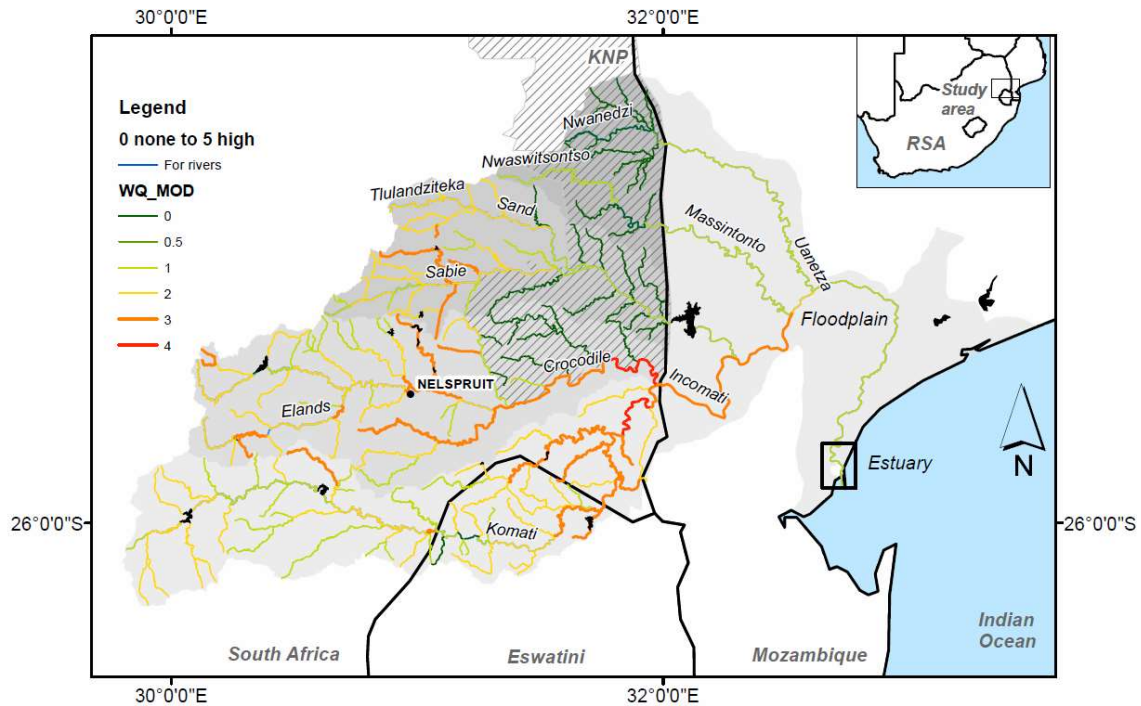


Figure 4: Water quality modification scores of the rivers within the greater Incomati River Catchment (Data from DWS 2014).

Habitat threats

Habitat alterations are one of the main causes of biodiversity loss (Tóth *et al.*, 2019; Christian 2023). Habitat availability is vital for species survival as it is used to escape predation and competition (Niebuhr *et al.*, 2015). Habitats are mainly threatened due to agriculture, intensive farming, riparian vegetation removal, and instream sand mining (Carpenter *et al.*, 2011). Habitat availability and flow are strongly related to one another as changes in either of them will impact the other (Freeman *et al.*, 2001; Spurgeon *et al.*, 2019).

Instream habitat (runs, rapids, riffles, pools) modifications are based on the frequency of flow alterations, physical modification and sediment changes that occur (DWS 2014). Instream habitats in the greater Incomati River Catchment are influenced by the presence of barriers, sedimentation and sand mining, erosion from neglected riparian zones and invasive species (e.g. carp, crustacea and mullusca) (Roux *et al.*, 2013; Molnar *et al.*, 2015; O'Brien *et al.*, 2024). In the greater Incomati River Catchment major instream habitat modifications occur outside the KNP as unprotected areas in the catchment are extensively transformed into plantations/woodlots, sugarcane and macadamia monocultures and urban/industrial areas (Figure 5).

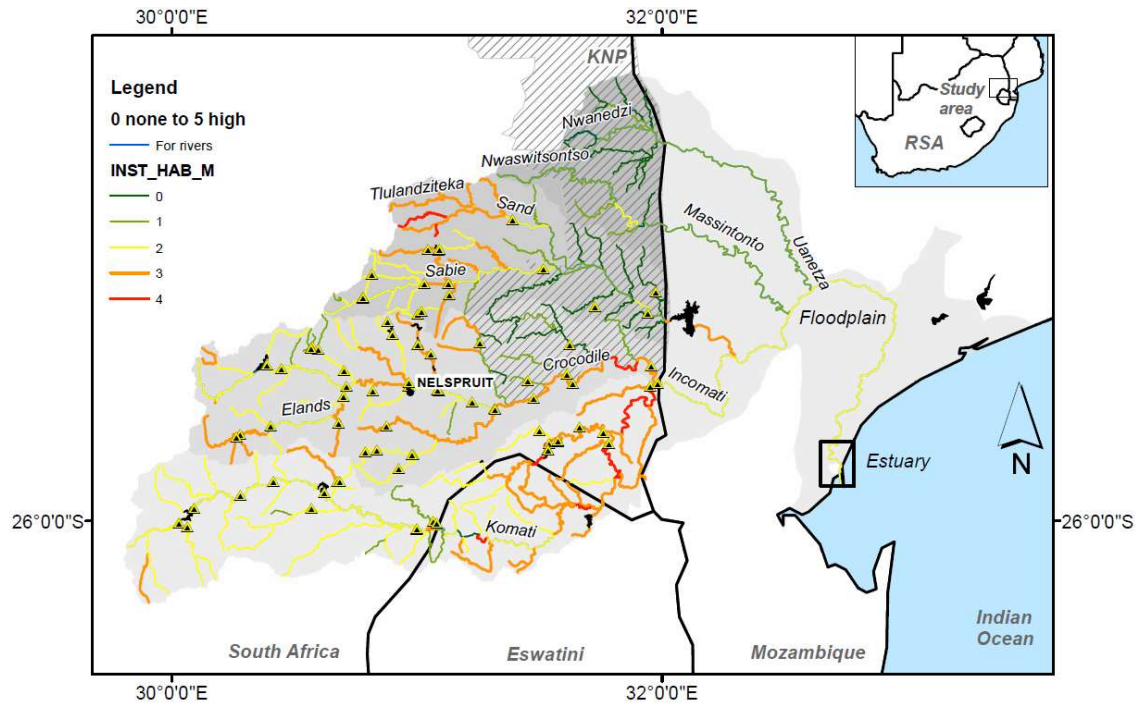


Figure 5: Instream habitat modification scores and barrier indications of the rivers in the greater Incomati River Catchment (Data from DWS 2014).

Other threats

Other major threats within the greater Incomati River Catchment include Disturbance to wildlife, overexploitation, alien invasive species, pesticides and endocrine disruptors, to only name a few (Gaynor *et al.*, 2018; O'Brien *et al.*, 2024). Disturbance to wildlife and overexploitation work hand-in-hand and refers to a reduction in a specifically targeted species that will result in the loss of species-specific regulating and provisioning services (Rosser and Mainka 2002; Gaynor *et al.*, 2018). The overexploitation of fish species may disrupt the fish community composition in turn causing a bottom-up effect in the ecosystem (Rosser and Mainka 2002). Alien invasive species can cause change to a functional ecosystem (MacRae and Jackson 2001). Invasive species are disruptive as they are more abundant and have more aggressive behaviour compared to native species (Cucherousset and Olden 2011). Some invasive species can also interbreed with native species that reduces the genetic uniqueness of native species. South African rivers are already threatened by 21 alien invasive freshwater fish species (Weyl *et al.*, 2020). Six of these species occur throughout the greater Incomati River Catchment of which the Largemouth Bass (*Micropterus salmoides*) and Common Carp (*Cyprinus carpio*) being the most abundant (Weyl *et al.*, 2020). Alien invasive species are

destructive through predatory and competitive actions (Cucherousset and Olden 2011). Collectively these actions can change the trophic dynamics and destabilise ecosystem functioning (Cucherousset and Olden 2011; Weyl *et al.*, 2020). Lastly, the relies of pesticides and endocrine disruptors from agricultural activities or wastewater treatment plants can cause severe ecological, physiological, and biochemical impacts on aquatic environments. Once present in rivers systems, these contaminants can alter water quality, degrade habitat integrity, and threaten biodiversity (Aneck-Hahn *et al.*, 2009).

Known knowledge on the fish communities of the greater Incomati River Catchment

The Incomati fish fauna demonstrate a Zambezian origin (Stankiewicz and de Wit 2006) characterised by high diversity and regional endemism, including species with restricted ranges due to incomplete connectivity and preferred temperature ranges throughout the catchment (Scott *et al.*, 2006; Skelton 2024). The greater Incomati River Catchment supports an estimated 70 species of freshwater fishes (Table 1; Appendix Data 1) (Darwall *et al.*, 2011; O'Brien *et al.*, 2024; Skelton 2024). In addition to a high diversity of species, there are many fishes that have high conservation value that occur in the catchment. This includes one critically endangered species *Chiloglanis bifurcus*, four endangered fishes *Chetia brevis*, *Serranochromis meridianus*, *Silhouettea sibayi*, *Kneria sp. nov. 'South Africa'*, three vulnerable (*Chiloglanis emarginatus*, *Oreochromis mossambicus*, *Enteromius gurneyi*), five near threatened (*Anguilla bicolor*, *Anguilla mossambica*, *Anguilla bengalensis*, *Enteromius brevipinnis*, *Labeobarbus nelspruitensis*) and the rest least concern or insufficient data (IUCN 2021)(Table 1).

Table 1: Fish species expected (taxonomic authority) to occur in the greater Incomati River Catchment including their IUCN conservation status (Scott et al., 2006; Department of Water and Sanitation 2014).

Species name	Common name	IUCN classification class
<i>Amphilius natalensis</i> (Boulenger, 1917)	Natal mountain catfish	Least concern
<i>Amphilius uranoscopus</i> (Pfeffer, 1889)	Stargazer (mountain catfish)	Least concern
<i>Anguilla labiata</i> (Peters, 1852)	African mottled eel	Near threatened
<i>Anguilla bicolor</i> (McClelland, 1844)	Shortfin eel	Near threatened
<i>Anguilla marmorata</i> (Quoy & Gaimard, 1824)	Giant mottled eel	Least concern
<i>Anguilla mossambica</i> (Peters, 1852)	Longfin eel	Near threatened
<i>Awaous aeneofuscus</i> (Bleeker, 1852)	Freshwater goby	Least concern
<i>Brycinus imberi</i> (Peters, 1852)	Imberi	Least concern
<i>Chelon dumerili</i> (Valenciennes, 1836)	Grooved mullet	Data deficient
<i>Chetia brevis</i> (Trewavas, 1935)	Orangefringed largemouth	Endangered
<i>Chiloglanis anoterus</i> (Boulenger, 1911)	Pennant tail suckermouth	Least concern
<i>Chiloglanis bifurcus</i> (Bailey & Holland, 1994)	Incomati suckermouth	Critically endangered
<i>Chiloglanis emarginatus</i> (Peters, 1868)	Pongolo suckermouth	Vulnerable
<i>Chiloglanis paratus</i> (Crass, 1960)	Sawfin suckermouth	Least concern
<i>Chiloglanis pretoriae</i> (Skelton, 1986)	Shortspine suckermouth	Least concern
<i>Chiloglanis swierstrai</i> (Boulenger, 1905)	Lowveld suckermouth	Least concern
<i>Clarias gariepinus</i> (Burchell, 1822)	Sharptooth catfish	Least concern
<i>Coptodon rendalli</i> (Boulenger, 1897)	Redbreasted tilapia	Least concern

<i>Crenimugil seheli</i> (Fabricus, 1775)	Bluespot mullet	Least concern
<i>Ctenopoma multispine</i> (Peters, 1844)	Climbing perch	Least concern
<i>Eleotris fusca</i> (Forster, 1801)	Dusky sleeper	Least concern
<i>Engraulicypris brevianalis</i> (du Toit, 1982)	River sardine	Least concern
<i>Enteromius afrohamiltoni</i> (Crass, 1960)	Hamilton's barb	Least concern
<i>Enteromius annectens</i> (Gilchrist & Thompson, 1917)	Broadstriped barb	Least concern
<i>Enteromius anoplus</i> (Peters, 1852)	Chubbyhead barb	Least concern
<i>Enteromius brevipinnis</i> (Peters, 1868)	Shortfin barb	Near threatened
<i>Enteromius crocodilensis</i> (Fowler, 1930)	Rosefin barb	Least concern
<i>Enteromius eutaenia</i> (Boulenger, 1904)	Orangefin barb	Least concern
<i>Enteromius gurneyi</i> (Günther, 1868)	Redtail barb	Vulnerable
<i>Enteromius paludinosus</i> (Peters, 1852)	Straightfin barb	Least concern
<i>Enteromius radiatus</i> (Pfeffer, 1896)	Beira barb	Least concern
<i>Enteromius toppini</i> (Boulenger, 1916)	Toppini	Least concern
<i>Enteromius trimaculatus</i> (Steindachner, 1864)	Threespot barb	Least concern
<i>Enteromius unitaeniatus</i> (Weber, 1897)	Longbeard barb	Least concern
<i>Enteromius viviparus</i> (Weber, 1897)	Bowstripe barb	Least concern
<i>Glossogobius callidus</i> (Smith, 1959)	River goby	Least concern
<i>Glossogobius giuris</i> (Hamilton, 1822)	Tank goby	Least concern
<i>Hemigrammopetersius barnardi</i> (Poll, 1967)	Barnard's robber	Least concern
<i>Hippichthys cyanopilos</i> (Bleeker, 1854)	Blue-spotted pipefish	Least concern

<i>Hippichthys heptagonus</i> (Bleeker, 1849)	Belly pipefish	Least concern
<i>Hydrocynus vittatus</i> (Castelnau, 1861)	Tigerfish	Least concern
<i>Kneria</i> sp. nov. (unknown)	Kneria	Endangered
<i>Labeo congoro</i> (Pellegrin, 1926)	Purple labeo	Least concern
<i>Labeo cylindricus</i> (Peters, 1852)	Redeye labeo	Least concern
<i>Labeo molybdinus</i> (Peters, 1852)	Leaden labeo	Least concern
<i>Labeo rosae</i> (Castelnau, 1861)	Rednose labeo	Least concern
<i>Labeo ruddi</i> (Boulenger, 1907)	Silver labeo	Data deficient
<i>Labeobarbus marequensis</i> (Steindachner, 1897)	Largescale yellowfish	Least concern
<i>Labeobarbus nelspruitensis</i> (Gilchrist & Thompson, 1911)	Incomati chiselmouth	Near threatened
<i>Labeobarbus polylepis</i> (Boulenger, 1907)	Smallscale yellowfish	Least concern
<i>Marcusenius pongolensis</i> (Roberts, 1969)	Bulldog	Least concern
<i>Micralestes acutidens</i> (Peters, 1852)	Silver robber	Least concern
<i>Micropanchax johnstoni</i> (Günther, 1894)	Johnston's topminnow	Least concern
<i>Microphis brachyurus</i> (Kaup, 1856)	Opossum pipefish	Least concern
<i>Microphis fluviatilis</i> (Kaup, 1856)	Freshwater pipefish	Data deficient
<i>Oligolepis acutipennis</i> (Valenciennes, 1837)	Sharptail goby	Least concern
<i>Opsaridium peringueyi</i> (Gilchrist & Thompson, 1913)	Southern barred minnow	Least concern
<i>Oreochromis mossambicus</i> (Peters, 1852)	Mozambique tilapia	Vulnerable
<i>Oreochromis placidus</i> (Trewavas, 1933)	Black tilapia	Least concern
<i>Petrocephalus wesselsi</i> (Peters, 1852)	Southern churchill	Least concern

<i>Pseudocrenilabrus philander</i> (Weber, 1897)	Southern mouthbrooder	Least concern
<i>Redigobius dewaali</i> (Peters, 1852)	Checked goby	Least concern
<i>Schilbe intermedius</i> (Rüppell, 1832)	Silver catfish	Least concern
<i>Serranochromis meridianus</i> (Regan, 1921)	Lowveld largemouth	Endangered
<i>Silhouettea sibayi</i> (Barnard, 1935)	Sibayi goby	Endangered
<i>Synodontis zambezensis</i> (Pappenheim, 1911)	Brown squeaker	Least concern
<i>Terapon jarbua</i> (Fabricius, 1775)	Crescent grunter	Least concern
<i>Tilapia sparrmanii</i> (Smith, 1840)	Banded tilapia	Least concern
<i>Valamugil buchanani</i> (Bleeker, 1853)	Bluetail mullet	Least concern

Various studies have been conducted within the greater Incomati River Catchment regarding the distribution or condition of fish species (Kleynhans 1986; James 1992; Kleynhans *et al.*, 1992; Rivers-Moore *et al.*, 2003; O'Brien *et al.*, 2014; Kleynhans, *et al.*, 2015; O'Brien *et al.*, 2018c) although knowledge regarding the specific health of individuals, populations and communities and species stressor-response relationships are low. Current knowledge on species distribution, condition and fish community stressors are used as reference for current or future studies to allow for the evaluation of the whole representation of what is occurring in the greater Incomati River Catchment (Table 2).

Table 2: Summary table of main studies/reports conducted within the greater Incomati River Catchment relating to the condition or health of fish communities and their stressors-responses (Resourced from Google Scholar and Scopus).

Citation	River System	Summary/Main Findings
Kleynhans <i>et al.</i> , 1992	Elands and Crocodile River	In September 1989 an effluent chemical spill occurred in the Elands River below the SAPPI Ngodwana Mill. Fish mortalities occurred up to 40km below the mill where 14 fish species were documented to have been affected. During the 1990 survey evaluations it was evident that recolonisation was taking place although at a very slow pace. The recovery of the critically endangered, <i>Chiloglanis bifurcus</i> , was of concern and re-introduction interventions were suggested. Lower sections of the river reach took between 6-11 months to show signs of recovery although the abundance of species has not yet recovered to pre-spill levels.
O'Brien <i>et al.</i> , 2014	Elands-, Houtbosloop-, Crocodile-, Ngodwana Rivers.	This study evaluated the past efforts implemented to conserve critically endangered, <i>Chiloglanis bifurcus</i> , and genetically unique populations of <i>Labeobarbus polylepis</i> . The study discussed that the water-use activities in the area were continuing to increase although the impacts on the fish communities were offset by previously implemented conservation efforts. In conclusion, the Elands River and associated tributaries were considered to be in a healthy state.
Kaiser-Reichel <i>et al.</i> , 2025	Seasonal rivers within the catchment	As seasonal rivers play a crucial role in shaping aquatic ecosystems, the study

		evaluated the diversity of fish communities within the seasonal rivers of Kruger National Park, Incomati River Catchment. This study provide a clearer understanding of their community structure and support a better understanding and management of theses systems.
Burnett <i>et al.</i> , 2018	Crocodile River	The study evaluated the spatial movement of <i>Labeobarbus marequensis</i> using radio transmitters. The study concluded that Yellowfish preferred to use deep runs and fast glides along with the use of submerged boulders as cover. Tagged individuals had reduced activity responses to rapid water discharge change. Unmanaged anthropogenic water resource use could affect the movement of yellowfish and their associated habitat availability.
Erasmus <i>et al.</i> , 2023	Incomati River	The study determined the trace element concentrations and bioaccumulation patterns in muscle and liver tissue of <i>Anguilla mossambica</i> and <i>A. marmorata</i> , as well as the chronic health risk to community consumers in the lower Incomati region. The study concluded that bioaccumulation occurred and elements like As, Cr, and Ni are found to pose human health risk through carcinogenic effects.
Soko and Gyedu-Ababio 2015	Crocodile River	The study evaluated the present ecological state of the river using fish community structure and their response to changes in the water quality. The study concluded that the water constituents increase as the river moves downstream. Anthropogenic activities deteriorate the water quality condition along with the distribution of fish species along the Crocodile River. Agricultural activities in the upper reaches with industrial, domestic and agriculture in the middle and lower reaches where the main sources of pollution in the catchment. The FRAI assessment of the river had the majority of the river reaches in a class-C category.
Kleynhans 1999	Crocodile River	The report developed a fish assemblage index. With this framework, fish are categories according to their intolerance levels, specialisations, habitat preferences and flow requirements. The index aimed to

		measure the biological integrity of the river based on the attributes of the fish assemblages. In the Crocodile River a general decrease in the relative FAII score per fish habitat segment was observed in a downstream direction, while the relative FAII per segment remained consistently high in the Elands River. Ecological classes ranged from A to C.
Rivers-Moore <i>et al.</i> , 2003 Linking source: Rivers-Moore and Jewitt 2004	Sabie River	The study observed that the principle abiotic driver of fish species distribution patterns in the Sabie River is determine by the water temperature and secondly the flow volume of the river. Specific sites within the Sabie River had unique fish species compositions and diversity increased as the river moves downstream. The study concluded that fish species can be used as biological indicators of annual thermal changes.
Jewitt <i>et al.</i> , no date	Sabie River	The results for the study indicated that an uncontrolled flow regime, including the removal of major flood events could have dramatic effects on the fish populations in the Sabie River. The study stimulated hypothetical flow manipulations through models. The dominate fish species of the Sabie River is found to be highly sensitive to the removal of flooding events. The removal of flooding events reduce the rate of sediment removal that affects fish populations.
Roux <i>et al.</i> , 2016	Komati River and tributaries	The study conducted an Instream Ecostatus Rating assessment of sites throughout the Komati River and associated tributaries. The overall assessment was derived from the fish assemblage (FRAI) and macro-invertebrate assemblage (MIRAI) assessments. The overall catchment Ecological Category Rating for the Komati River catchment were consistent a Class C rating. Specific sites illustrated higher or lower ecological ratings.
Roux <i>et al.</i> , 2013	Crocodile River and tributaries	The study conducted an Instream Ecostatus Rating assessment of sites throughout the Crocodile River and associated tributaries. The overall assessment was derived from the fish assemblage (FRAI) and macro-invertebrate assemblage (MIRAI) assessments. The overall catchment Ecological Category Rating for the

			Crocodile River catchment were consistent a Class C rating. Specific sites illustrated higher or lower ecological ratings.
Roux <i>et al.</i> , 2016	Sabie River and tributaries		The study conducted an Instream Ecostatus Rating assessment of sites throughout the Sabie River and associated tributaries. The overall assessment was derived from the fish assemblage (FRAI) and macro-invertebrate assemblage (MIRAI) assessments. The overall catchment Ecological Category Rating for the Sabie River catchment were consistent a Class B rating. Specific sites illustrated higher or lower ecological ratings.
O'Brien <i>et al.</i> , 2024	Incomati Catchment	River	This study performed a risk assessment. The risk assessment was completed using available and newly collected social and ecological evidence, in collaboration with multiple partners from the Incomati Basin over a three-year period. The study have aligned existing information, enhanced understanding of the basin's hydrology, and generated new data to address key knowledge gaps. In this study, it was identified and described resource users, the nature of their resource use, and the threats posed by their combined activities to the ecological and social attributes of the rivers within the basin. The study identified physical and chemical stressors, as well as the impacts of river connectivity loss and disturbances caused by alien invasive fauna and flora. The study characterised the aquatic biodiversity of the basin and examined how both ecosystems and the communities that depend on them are, or may be, affected by multiple stressors. Additionally, the study developed a framework that provides stakeholders with environmental flow (e-flow) and associated non-flow driver information to achieve a sustainable balance between the use and protection of water resources. This framework enables stakeholders to identify stressors, evaluate their impacts, and address the excessive use or degradation of water resources. Finally, the framework facilitates collaboration among regulators and water managers, enabling shared stewardship of

water resources across the basin. It is adaptable and can be applied iteratively, incorporating machine-learning capabilities from ongoing monitoring to continuously refine projections and improve future applications.

Conservation efforts and management

In comparison to the developed part of the world, in southern Africa human and financial resources are limited (Slinger *et al.*, 2010), this results in relatively limited conservation and management activities. Today, for large parts of the greater Incomati River Catchment has one water resource use regulator and or conservation manager who is individually responsible for aquatic ecosystem conservation and management. This includes for example one aquatic conservation manager in South Africa (Mpumalanga Parks and Tourism Agency) who is responsible for multiple sub-catchments in Mpumalanga in South Africa, while there are no dedicated freshwater conservationists in Eswatini and Mozambique. There are terrestrial conservationists in all three countries associated with protected areas (Kruger and Sabie Conservation Area in Mozambique). The Incomati River Catchment is jointly managed through a network of transboundary and national institutions that coordinate the sustainable use, regulation, and protection of shared water resources across Mozambique, South Africa, and Eswatini (Table 3).

Table 3: The Transboundary managers and regulators of the greater Incomati River Catchment.

Transboundary / Regional Regulators					
Incomati Maputo Watercourse Commission	&	INMACOM	Tripartite river basin commission for Mozambique, Eswatini and South Africa which provides institutional oversight for cooperation in managing, conserving, and regulating water resources.	(Velpuri <i>et al.</i> , 2023)	
Southern African Development Community		SADC	Regional policy / protocol context and convening role for shared watercourse institution		
National Regulators					
Department of In Water and Africa Sanitation (DWS)		South	National regulator for water allocation and transboundary responsibilities	(Department of Water and Sanitation (South Africa) 2008)	

<i>Direcção Nacional de Águas</i> / National Directorate of Water (DNA) and regional water administration <i>Administração Regional de Águas</i> — ARA Sul	In Mozambique	National regulator and regional water administration covering southern basins.	(National Directorate of Water (DNA) n.d.)
Ministry of Natural Resources & Energy (MNRE), Department of Water Affairs	In Eswatini	National water authority / policy lead for shared basins; implements national side of tripartite agreements	(Ministry of Natural Resources & Energy, Department of Water Affairs 2003)
Catchment / sub-catchment Authorities			
Inkomati-Usuthu Catchment Management Agency (IUCMA)	In South Africa	South African catchment management agency operating inside the Inkomati-Usuthu Water Management Area (manages water resources on the South African side)	(Inkomati-Usuthu Catchment Management Agency (IUCMA) 2020)
Komati Basin Water Authority (KOBWA)	South Africa & Eswatini	Carries operational/cooperative functions between the two states	(Komati Basin Water Authority (KOBWA). 2022)
Administration Regional de Águas — ARA-Sul	Mozambique	The Mozambican regional agency responsible for river basins in southern Mozambique.	(De Boer and Droogers 2016)
Local user Technical Bodies that Play regulatory/operational roles			
Irrigation districts, Water User Associations, river operations committees and dam operators	E.g., Crocodile River operations committees, local reservoir operators	Manage allocation/operations on the ground and feed into basin decision-making.	(Pollard <i>et al.</i> , 2023)

In the region, water resource use regulators/managers have resources, and their mandates include sustainability considerations. Conservationists in the region generally refer to freshwater conservation of formal water resource use regulators and managers, assuming that their sustainability mandate will meet conservation requirements. Apart from these regulatory/management efforts there are limited direct conservation activities in the region.

Efforts to ensure fish population perseverance are vital to aquatic ecosystem service provision (Lynch *et al.*, 2016; Silva *et al.*, 2018). Fishes of ecological importance, often referred to as flagship or umbrella species, are those that benefit other co-occurring species by filling key roles in aquatic ecosystems (Skelton 2000; Kalinkat *et al.*, 2017). Species with complex reproductive cycles, system-specific endemism, environmental preference, and/or human use and preference are good examples (Skelton 2000; Kalinkat *et al.*, 2017). Focus on fishes of ecological importance for conservation efforts allow for the protection of whole ecosystems and ensure ecosystem service continuation.

A case study by O'Brien *et al.*, (2014) illustrated that a well-managed and detailed conservation plan on system-specific endemic species had a suitable degree of success years after an accidental paper mill effluent spill in the Elands River, greater Incomati River Catchment (Kleynhans *et al.*, 1992; O'Brien *et al.*, 2014). In 1989, large amounts of effluents high in salts and chemicals were accidentally spilled into the Elands River (Kleynhans *et al.*, 1992). This system is well-known for its high percentage of endemic species like *Labeobarbus polylepis* and critically endangered *Chiloglanis bifurcus* and *Kneria spp.* Conservation and management efforts were formally initiated in 1992 (James 1992) by monitoring the population structures of these fish combined with the relocation and introduction of *C. bifurcus* (± 100) into the Ngodwana River (a tributary of the Elands). Additionally, management allowed the establishment of a fish sanctuary, the Elands River Yellowfish Conservation Area (ERYCA), for the protection of the genetically unique *L. polylepis* (O'Brien *et al.*, 2014). Population abundances of all species stabilised and are increasing after conservation efforts were put in place (O'Brien *et al.*, 2014). The Elands River Catchment was further declared a fish sanctuary within the National Freshwater Ecosystem Priority Area (NFEPA) program (Nel *et al.*, 2011)(Figure 6).

National Freshwater Ecosystem Priority Areas are river regions identified through a systematic biodiversity planning and collaborations based on a range of criteria (Nel *et al.*, 2011). The FEPA is aimed to meet national biodiversity goals for freshwater ecosystems as well as to develop a basis for enabling effective implementation of protection measurements. Updated Ecological Reserve Determination data, sub-national Present Ecological State (PES) assessments, EcoStatus data and River Health Data is used to guide discussions at expert review workshops and subsequently update the river condition. River condition was used to inform the selection of rivers as FEPAs and the subsequent FEPA map categories. For example, only river ecosystems in good condition (A or B ecological category) were chosen

as FEPAs because these rivers provide the best examples of representative examples of South Africa's freshwater ecosystems and associated biodiversity (Kleynhans and Louw 2007; Nel *et al.*, 2011). Unfortunately, the majority of these protected areas are now in developed areas where protection is poor and additional environmental protection is not required or enforced.

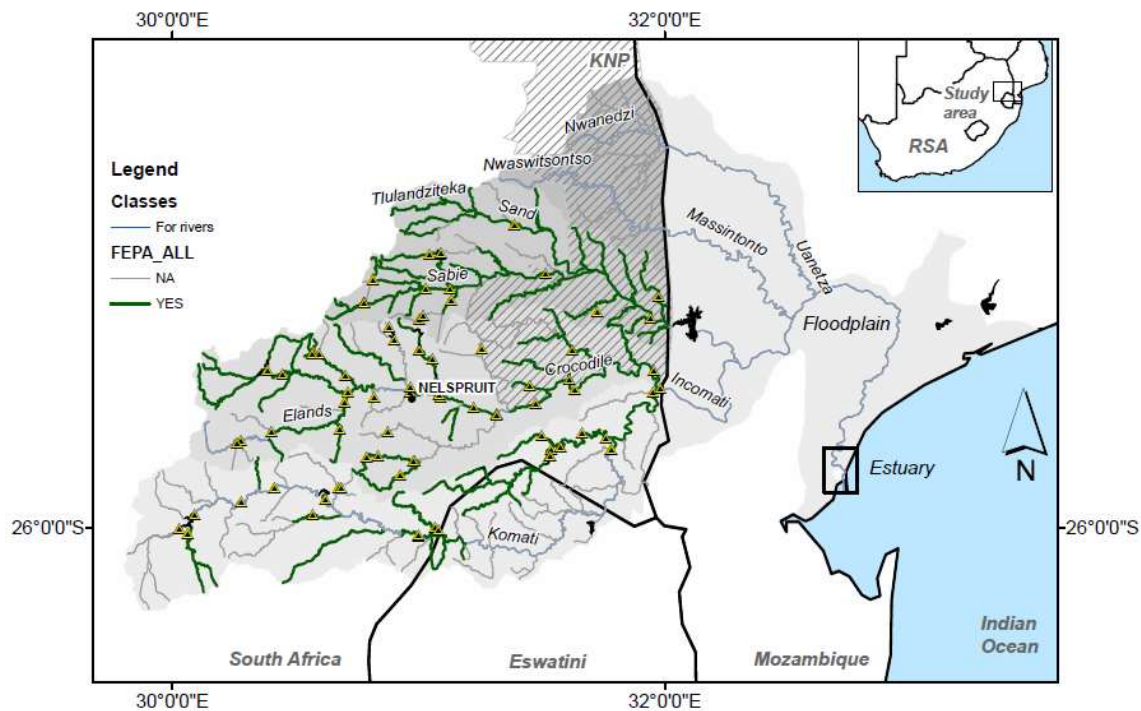


Figure 6: Rivers in the greater Incomati River Catchment that is categorised as a Freshwater Ecosystem Priority Area (FEPA)(Data from DWS 2014).

Other examples are demonstrated by Skelton (2000) with three case studies on how conservation of freshwater fishes in southern Africa has grown and developed in the 20th century. These examples emphasise that knowledge of the species in question are important before conservation management strategies are put in place. They further inform on how researchers and conservation managers can work together to devise integrative conservation plans (Cambray *et al.*, 1997; Skelton 2000).

The literature highlights the need for freshwater fish conservation, that is essential to human welfare (Holmlund and Hammer 1999; Skelton 2000). These case studies are examples of how anthropogenic disturbance and stressors can be remediated through effective conservation strategies and knowledge of the species in question (O'Brien *et al.*, 2014).

South African Water Resource Management legislation regulates the catchment, and includes the implementation of the Resource Directed Measures (RDM) of the NWA (108 of 1998) (Basson and Rossouw 2003; Brown and Woodhouse 2004; Colvin *et al.*, 2008; de Lange *et al.*, 2010; DWS 2016, 2019; Wilkinson *et al.*, 2015; Denby *et al.*, 2017). The Incomati-Usuthu Catchment Management Agency (IUCMA), established in 2006, was the first of many Catchment Management Agencies (CMA) in South Africa (Denby *et al.*, 2017). It is of socio-economic importance as it support >2.5 million South Africans and >1 million Mozambicans (Anderson 2005; Schreiner *et al.*, 2011; Sherwill *et al.*, 2007). The Department of Water and Sanitation (DWS) and Incomati-Usuthu Catchment Management Agency must implement the NWA of South Africa (Act 108 of 1998) and Resource Quality Objectives for each river reach as deficits exist in water allocation between the sub-catchments, and ecological functionality needs to be preserved (Roux *et al.*, 2010; Chibwe *et al.*, 2012; South Africa Department of Water Affairs 2013; Rogers and Luton 2016; Denby *et al.*, 2017).

Research Quality Objectives (RQO) are defined for all prioritised resource units for every Integrated Units of Analysis (IUA) in terms of the water quality and quantity, and Habitat and Biotic assessments (Department of Water and Sanitation 2016). The Integrated Unit of Analysis (IUA) are classified based on the water resource class classification (Class I/Class II/Class III) in terms of its extent of permissible utilisation and protection. The RQO specifies an ecological category (Target Ecological Category) to a water resource based on the ecological condition of that water resource in terms of deviation of its biophysical components from a historical condition (Department of Water and Sanitation 2016)(Figure 7). The outcome goals of set target RQOs is for management and conservation efforts in each IUA to be orientated to preserve that IUA in that ecological category to ensure continuation of ecological functionality.

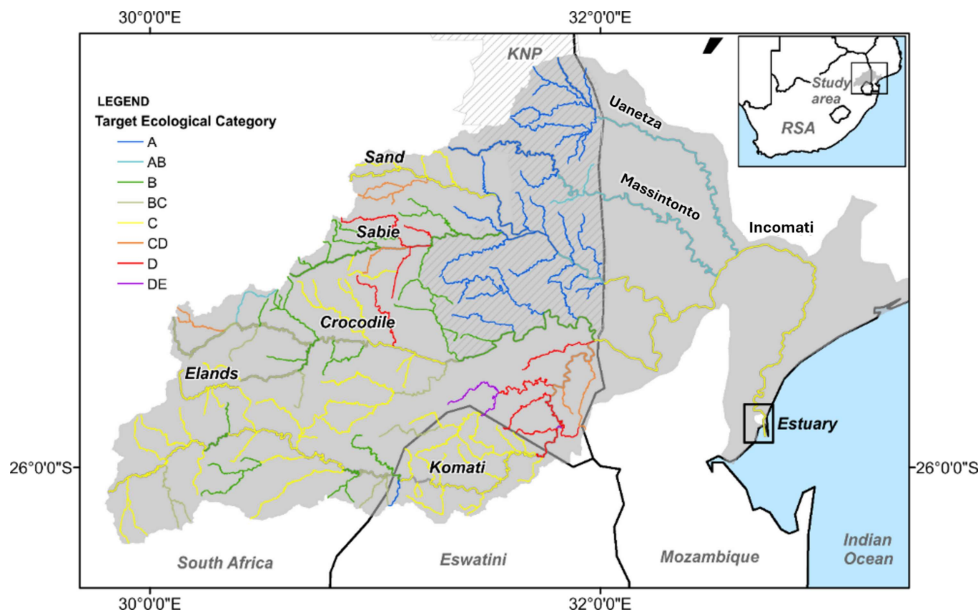


Figure 7: The quarter sub-catchments within the greater Incomati River Catchment and the associated target ecological category as set out by the Resource Quality Objectives of the greater Incomati Catchment (Department of Water and Sanitation 2016). A - Unmodified/natural; B - Largely natural; C - Moderately modified; D - Largely modified; E - Seriously modified and F - Critically / Extremely modified.

Fish as ecological indicators for ecosystem protection

The health of an ecosystem is based on its ability to sustain a biotic community (Karr 2020). Fish are considered to be good ecological indicators, with many species being intolerant to certain environmental factors and specific stressors (Kwak and Peterson 2007). The use of fish community response to assess/monitor the ecological wellbeing allows for the evaluation of direct/indirect influences on fish communities and in turn the ecosystem in which they live (Ansara-Ross *et al.*, 2008, Wepener *et al.*, 2011).

Multiple Lines of Evidence (LoEs) that incorporate aquatic animals as indicator organisms are used to inform management agencies on the impacts environmental drivers have on the health of an aquatic ecosystems (Fausch *et al.*, 1990; Ansara-Ross *et al.*, 2008).

Biotic Lines of evidence include to Fish Response Assessment Index (FRAI). The South African Department of Water and Sanitation (DWS) started developing a National Aquatic Ecosystem Monitoring Program in 1995 with the purpose of determining the integrity and/or health of an aquatic ecosystem using biota (Roux 1997). Adapted from the Index of Biotic Integrity (IBI) (USA) (Karr 1981) which initially resulted in the Fish Assemblage Integrity Index (FAII) (Kleynhans 1999), the Fish Response Assessment Index (FRAI) is the current biotic index used for monitoring South African rivers (Kleynhans and

Louw 2007). The FRAI assessment is based on the tolerance, intolerance and preferences of the reference fish communities in question and the response of the reference species to environmental determinants or drivers (Kleynhans 2007)(Appendix Data 2). The tolerance, intolerance and preference levels of the reference fish community are categorised into metric groups that related to the environmental determinants or drivers (Kleynhans 1999; Dickens and Graham 2002; Kleynhans 2007; Levin *et al.*, 2019). Changes in the environmental conditions relates to fish stress and forms the basis of an ecological response assessment. The output of the assessment is the present ecological state (Ecological Category) of the fish communities based on the A-F classification category (Kleynhans 2007) (Table 4). The FRAI assessment doesn't essentially follow the same procedure as the initial FAI assessment. As the FRAI assessment is essentially improved to incorporate strong habitat-based cause-and-effect conditions. Keep in mind that the FRAI is still a rapid assessment with informed opinion based impact ratings and user training, moderation and justification is recommended (Kleynhans 2007).

Table 4: Fish Response Assessment Index scoring system based on Ecological assessment output (Kleynhans 2007).

Ecological Categories	Description	Acceptable/ Unacceptable state	Score (%)
A	Unmodified, natural state	Acceptable	90-100
B	Largely natural with few modifications	Acceptable	80-89
C	Moderately modified	Acceptable	60-79
D	Largely modified	Unacceptable	40-59
E	Seriously modified	Unacceptable	20-39
F	Critically modified	Unacceptable	0-19

Statistical Lines of Evidence include using different statistical methods. A multivariate statistical approach is used to evaluate the composition of fish communities and predicts the drivers of change, summarises and provides the environmental condition of communities and identifies important drivers of a community that can be used to identify and evaluate impacts (O'Brien *et al.*, 2009, Wepener *et al.*, 2011). Multivariate statistical approaches provide a more rational approach to assess the patterns of association between fish communities and the

different environmental drivers (Van den Brink *et al.*, 1998). Combined with Monte Carlo permutation tests, multivariate statistical analysis can test the differences in the fish community and quantify drivers of change (Van den Brink *et al.*, 2003).

In recent times (1980's to 2000), Bayesian Network assessments have been established and incorporated into scientific assessments as new statistical line of evidence. Since, major developments in the field of ecological risk assessments were completed by Stephenson (2000); Claassen *et al.*, (2001); O'Brien and Wepener (2012) and Govender *et al.*, (2021). The "South African Ecological Risk Assessment Guidelines" promote risk assessments as a process that evaluates the likelihood that adverse ecological effects occur or will occur due to the exposure of one or more stressors and endpoint (Claassen *et al.*, 2001). Modern ecological risk assessment approaches incorporate the use of Bayesian Network (BN) Probability modelling approaches (Govender *et al.*, 2021). In South Africa these BN approaches have been valuable to the Integrated Water Resource Management (IWRM) of South Africa and now are receiving broad attention in South Africa and the region (Castelletti and Soncini-Sessa 2007; Barton *et al.*, 2008; O'Brien *et al.*, 2019; O'Brien *et al.*, 2021). The incorporation of BNs in ecological risk assessments to evaluate the probable effect of multiple stressors has been formalised globally and applied in South Africa (O'Brien *et al.*, 2019; O'Brien *et al.*, 2021). This approach incorporates the use of the Relative Risk Model established by Landis and Wiegers (1997), and applied by O'Brien *et al.*, (2019); Vezi *et al.*, (2019); Agboola *et al.*, (2020); Wade *et al.*, (2020); O'Brien *et al.*, (2021); and Govender *et al.*, (2021). These case studies demonstrate how available evidence describes, sources resulting in stressors, affect multiple receptors in the content of the social and ecological attributes or endpoints. The specific endpoint in the risk assessment represents the relative risk or impact associated for fish communities for that endpoint. The approach has been applied to evaluate the risk of multiple stressors on regional scales (O'Brien *et al.*, 2019; Agboola *et al.*, 2020; O'Brien *et al.*, 2021) local scales (Vezi *et al.*, 2019; Wade *et al.*, 2020), to multiple water resource types including rivers, floodplains/wetlands and estuaries (Vezi *et al.*, 2019; O'Brien *et al.*, 2021) and to evaluate the effect of multiple water quality, quantity, habitat and other stressors in the region and abroad.

Combining the use of different indices and using Multiple LoE allows for the evaluation of the wellbeing of ecosystems with a greater level of certainty.

Summary

The greater Incomati River Catchment is an ecologically important Water Management Area in South Africa (O'Brien *et al.*, 2019). This is attributed to the high biodiversity and number of endemic species, including the high prevalence of Freshwater Ecosystem Priority Areas (FEPA). An estimated 70 fishes with a Zambezian origin occur in the region (Russell 2011). And there is a high level of endemism of fishes that are vulnerable and have been responding to important environmental stressors like barriers and connectivity restrictions, flow and sediment alterations, water quality stressors, overfishing and immigrating alien invasive species. These environmental stressors have shaped and determined the fish community compositions throughout the catchment.

Past studies throughout the catchment allows us to evaluate the condition of the river sections based on the different environmental stressors they experience. The IUCMA vision is to maintain sufficient, equitable and quality water resources for all users throughout the catchment by determining and monitoring the health and condition of its rivers (Ecological Category). The target Ecological Category of the different river sections differ based on the Integrated Unit of Analyses and Management Class. Past monitoring studies classified the Ecstatus of the main river systems between a Class B to Class C with large sections and tributaries currently out of these ranges (Kleynhans *et al.*, 2015; Roux *et al.*, 2013, 2016). Past studies has also evaluated the new distribution range extensions of critically endanger species (O'Brien *et al.*, 2014), Tigerfish and Large scale Yellowfish behavioural response (Burnett, 2013; O'Brien *et al.*, 2018a), catadromous anguillid eel species movement and barrier conquering (Hanzen *et al.*, 2019; O'Brien *et al.*, 2019), general fish community compositions and environmental driver influences (Jewitt *et al.*, n.d.; Rivers-Moore and Jewitt 2004; Soko and Gyedu-Ababio 2015) and much more. With the extent of environmental monitoring and species-specific studies completed within the greater Incomati River Catchment, there still seems to be a need for specific environmental stressors management and protection. For example, very little protection has been afforded to river connectivity maintenance and migrations (O'Brien *et al.*, 2019).

Fish are important biotic indicator species that help us monitor and evaluate the effects of anthropogenically caused stressors. Providing resources to all southern African human communities the economic and ecological value and importance of fishes are of top priority. Since the quality and quantity of freshwater ecosystems are influenced by environmental stressors it is important to study and anticipate the effects that different environmental stressors have on fish communities. Knowledge of the fishes expected to occur within a river

reach, what species presently occur in the rivers and their population conditions, and how communities differ will allow us to evaluate the state of fish communities and the rivers in which they live.

Efforts to conserve freshwater ecosystems continuously requires a better understanding of how the different environmental drivers influence the composition of fish communities and shape the biography of different species along this river system. Good management practises and regular monitoring events are needed to ensure the wellbeing of fish communities and the reduction of environmental impacts in the greater Incomati River Catchment.

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Chapter 3

A holistic catchment scale assessment of fish communities and what is driving communities for catchment scale management and conservation consideration.

Freshwater ecosystems are threatened worldwide and face multiple stressors. Degradation of freshwater systems at the current rate may cause two-thirds of the human population to face severe water shortage and water stress as soon as 2030 in the region and losses of associated ecosystem services. The greater Incomati River Catchment is one of the most ecologically important water resources in South Africa due to the high diversity and endemism of fish species. Land use development is increasingly affecting habitat, flow regimes and water quality of the rivers in the catchment. In addition, invasive species and migration barriers contribute to the reduction of the wellbeing of fish communities. Multiple Lines of Evidence were used to assess the status and composition of fish communities associated with different environmental drivers in the catchment. A total of 50 out of the expected 70 fish species were collected with critically endangered species like the Orange-fringed Largemouth (*Chetia brevis*) and Kneria (*Kneria* cf. *Crocodile*) being absent throughout the study area. The greater Incomati River Catchment is in a moderately altered yet stable state with degraded fish communities and ecosystems observed at multiple sites. The rivers in the catchment are dynamic, and each has its own collection of stressors that require unique management consideration. Flow reduction and habitat modification were identified as the main drivers of change throughout the catchment. Monitoring of anthropogenic stressors is important to protect the valuable integrity of the greater Incomati River Catchment and its fish communities. The study has demonstrated that the use of multiple Lines of Evidence can inform stakeholders about the different environmental drivers that affect freshwater ecosystems.

Keywords: Redundancy Analyses, Fish Response Assessment Index, Environmental drivers

Introduction

Human population growth, land transformations, intensified agriculture and industrial diversification coupled with climate change and limited sustainability policies and legislation, and or inability to implement policies and legislation, is having a major impact on the biodiversity and ecosystem wellbeing worldwide (Allan *et al.*, 2005; Aukema *et al.*, 2017; Dudgeon *et al.*, 2006; Portner *et al.*, 2023). Degrading ecosystems and biodiversity loss, hinders the functionality and ability of the ecosystem to provide ecosystem services to human communities (Holmlund and Hammer 1999; Daily 2000; Hooper *et al.*, 2005; Hussein 2021). Freshwater fish species represent part of the world's greatest diversity and provide numerous resources and ecosystem services to human communities (Dudgeon *et al.*, 2006; Dudgeon 2010). Fish from lakes and rivers supply human communities with a valuable, self-sustainable food source, allows for water purification, nutrient cycling and recreational activities (Rodríguez *et al.*, 2006; Embke *et al.*, 2020). Despite the multiple advantages human communities gain from freshwater ecosystems and the ecological value of fishes, these freshwater ecosystems are constantly used, overused, and deteriorated (Skelton *et al.*, 1995; Dudgeon *et al.*, 2006).

Consistent overuse and anthropogenic pressure on freshwater bodies have caused the quality of southern Africa river systems to reach its lowest level of functionality over the past ten years (Oberholster and Ashton 2009; Woodborne *et al.*, 2012; Dabrowski *et al.*, 2013). According to the Environmental Performance Index (2020), South Africa is ranked 94th in the world (out of 180 countries) due to the poor management and conservation efforts of its river systems (Environmental Performance Index 2020). Changes in fish community composition either through taxonomic compositions, life history traits or functional traits can all affect the ecosystem functionality (Zhao *et al.*, 2016). For these reasons, ecosystem functioning is largely dependent on the responses of freshwater fish as it reflects the amount of degradation that specific ecosystem has undergone (Das and Chakrabarty 2007; Zhao *et al.*, 2016). Fish species are used and highlighted as ecological indicators due to their relatively long lifespan, broad habitat requirements, mobility, wide range of trophic levels within the ecosystem and social importance to human communities (Wichert and Rapport 1998; Sajina *et al.*, 2021). Various Lines of Evidence (LoE) such as monitoring tools (FRAI, RDA) are incorporated and used to inform management agencies on the impact's environmental drivers (pollution, habitat change, flow alteration, overutilisations) have on the health and functionality of freshwater ecosystems (Fausch *et al.*, 1990; Ansara-Ross *et al.*, 2008). Resource Quality

Objectives (RQOs), as a requirement within the National Water Act (1998), specifies an ecological category (Target Ecological Category) to a water resource based on the ecological condition of that water resource in terms of deviation of its biophysical components from a historical condition (Department of Water and Sanitation 2016). The Fish Response Assessment Index (FRAI) has been established as a commonly used index of the condition of attributes/ecological condition of fish communities in South Africa (Kleynhans 2007). The purpose of this assessment is to measure the biological integrity from the attributes of a river based on the fish assemblages of the river (Kleynhans 2007; Kleynhans *et al.*, 2008). The FRAI assessment is one of the components used to assess if the water resource are within an acceptable ecological condition/category for ecosystem services to be provided (Kleynhans 2007).

A multivariate statistical approach (e.g., Redundancy Analysis ordination technique (RDA)) is further used to evaluate the composition of fish communities and drivers of change. It predicts, summarises and provides the environmental condition of communities and identifies important drivers of a community that can be used to identify and evaluate impacts (O'Brien *et al.*, 2009; Wepener *et al.*, 2011). The use of multiple LoEs allows the evaluation of wellbeing of ecosystems to provide a greater level of certainty as individual indices alone are not robust. If the correct monitoring and management strategies are implemented and linked, freshwater ecosystems can be protected in a manner that would ensure continuity of ecosystem services and the ecosystem and associated processes upon which human communities depend on (Ashton 2010).

The greater Incomati River Catchment is a semi-arid, transboundary catchment located in the south-eastern parts of South Africa, northern parts of Eswatini and southern parts of Mozambique (Kleynhans *et al.*, 2015). Main river sub-catchments within the greater Incomati include the Uanetze-, Sabie-, Crocodile- Komati- and Incomati sub-catchment Rivers. These main sub-catchments are exposed to different land use and water-use changes from as early as the 1950s (Hallowes *et al.*, 2010; O'Brien *et al.*, 2024). The Uanetze River sub-catchment (along with the Massintonto River Catchment) is a seasonal river catchment with minimal flows during the low flow period. The Uanetze River sub-catchment within South Africa's side is completely within the Kruger National Park, a protected area with minimal anthropogenic disturbances. The Sabie River sub-catchment, through its re-establishment from the Crocodile- and Komati Rivers are currently classified as one of the most pristine southern Africa ecosystems due to the high species diversity it maintains (Kleynhans 1986;

O'Brien *et al.*, 2018A; O'Brien *et al.*, 2018B). The Crocodile River sub-catchment is an ecologically important and biologically diverse river system with at least 49 fish species that occur frequently throughout the river (Roux *et al.*, 2013). The Komati River Catchment is the most stressed river system in the catchment with multiple barriers and water quality related stressors. The greater Incomati River Catchment, the lower section within Mozambique, splits into multiple branches where it converges again before the river reaches the estuary. The Incomati River sub-catchment flows through Mozambique when all three major river sub-catchments have converged, forms a floodplain and then flow into the Indian Ocean at a discharge of 100-200m³s⁻¹.

Growing agriculture, forestry and urbanisation over the past two decades, were identified as some of the main drivers of increased water scarcity in the catchment (Saraiva Okello *et al.*, 2015; Fouchy *et al.*, 2018). Unprotected areas (excluding KNP) in the greater Incomati River Catchment are extensively transformed into plantations/woodlots, sugarcane croplands and urban/industrial areas (van Eekelen *et al.*, 2015). The increasing agricultural growth and irrigation demand has led to the establishment of 12 major dams and more than 200 informal farm dams (Jackson *et al.*, 2016; O'Brien *et al.*, 2019). These barrier formations continue to cause change to the climate, weather patterns, energy and water transportation, alter species distribution, and reduce viable habitats (Sleeter *et al.*, 2018). Among these are gauging weirs constructed to regulate flow, water withdrawal and control flooding (Silva *et al.*, 2018; O'Brien *et al.*, 2019). The dams and gauging weirs are anthropogenic barriers that hinder the migration of fish and transport of sediment (Branco *et al.*, 2017; Silva *et al.*, 2018; O'Brien *et al.*, 2019). The Incomati Water Management Area (WMA) has more than 10 fish passages constructed on these barriers to improve connectivity and migration of fish, but none are in a functional state (Bok *et al.*, 2007; O'Brien *et al.*, 2019).

This study aims to contribute to the conservation and management of the rivers in the Incomati River Catchment by determining the state of fish communities and environmental drivers affecting the fish communities while providing management considerations. The multiple LoEs used in this study included the Fish Response Assessment Index (FRAI) (Kleynhans 2007) and Multivariate statistics (Redundancy Analysis) as these assessments are used to provide insight into what the main drivers of change were for the catchment.

Materials and Methods

Ethics

All necessary ethical clearances and country permits were obtained to ensure responsible and compliant conduct throughout the study area. The ethical clearance number was obtained from the University of Mpumalanga (UMP/O'Brien/2/2023), for sampling in the Kruger National Park, the project was registered with South Africa National Parks (SS840). provincial research permits were obtained from Mpumalanga Park and Tourism (MPB 5789) which allowed sampling and collection within Mpumalanga, South Africa. Sampling within transboundary countries (Mozambique and Eswatini) was allowed following the approval and allocation of an invitation letter and sampling letter. All local community chiefs and tribe leaders were informed of our whereabouts and permission letters were presented to them before sampling commenced.

Study area

Fifty-three sites were selected throughout the greater Incomati River Catchment to represent the spatial variability and complexity of the system (Figure 8; Table 5). The sites were selected based on historical data availability and included sites on the mainstem rivers and tributaries of the Uanetze, Sabie, Crocodile, Komati and Incomati sub-catchments. Sampling sites were in South Africa, Eswatini, and Mozambique and included sites selected in protected areas (Kruger National Park). The sampling sites ranged from highveld plateaus with small, shallow streams with rocky substrates to large deep, slow flowing sites that's dominated by sandy substrates. The majority of the river systems sampled are perennial river systems while some tributaries and the mainstem of the Uanetze River Catchment are seasonal. The catchment is dominated by natural- (waterfalls) and artificial barriers (weirs or dams) that hinder the movement of fish species throughout the catchment and between regions. The local communities also differ throughout the catchment that allows different regions of the river to be utilised differently from an ecosystem service' point of view.

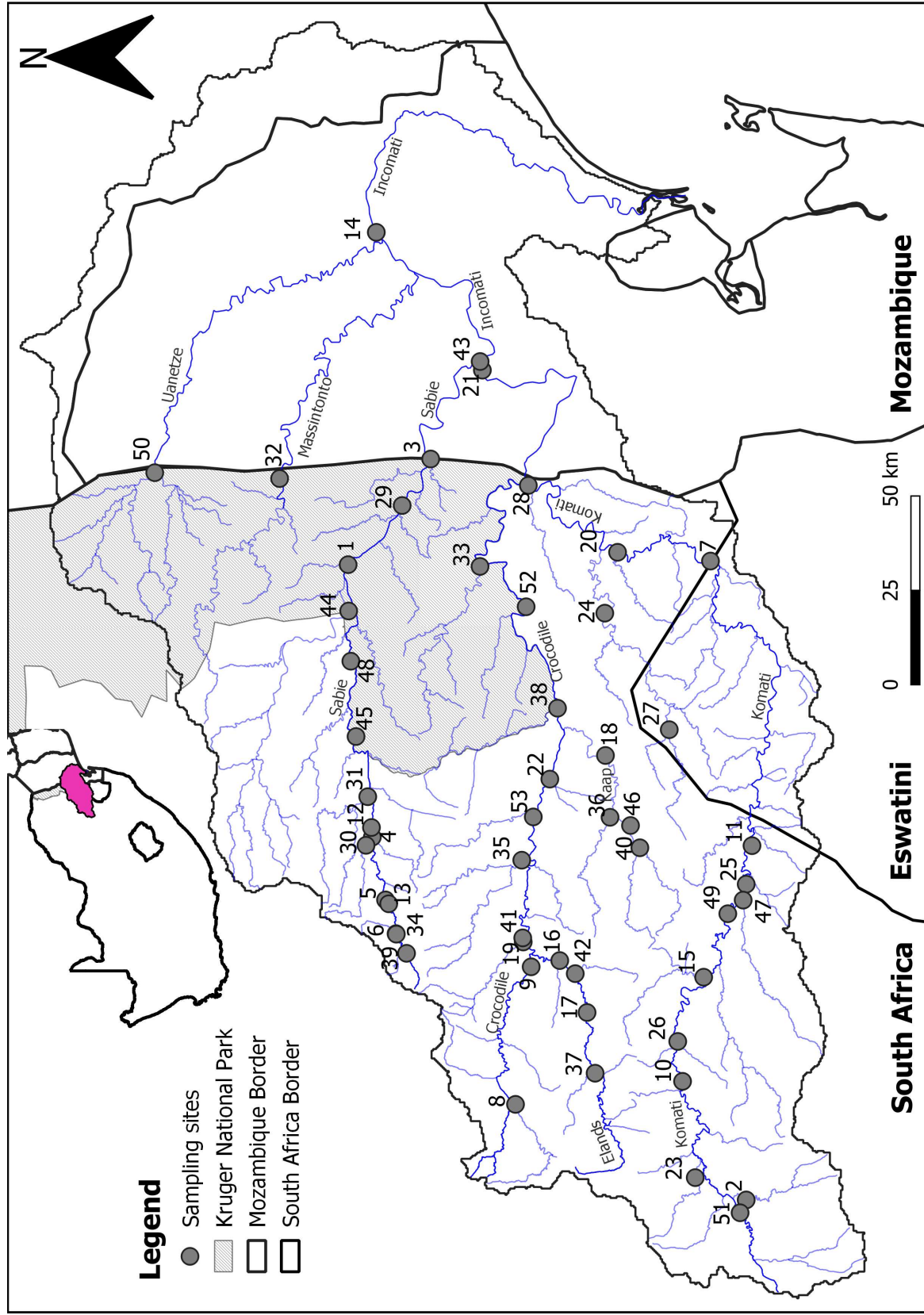


Table 5: Detailed sampling locations of the sites sampled in the greater Incomati River Catchment with corresponding map nr, Site codes and GPS locations.

Map Nr	Sub Catchment	River system	Site Name	Site code	Latitude	Longitude
1	Sabie	Sabie	ANTHOCLYSTER	X33A-ANT	-24.96583	31.75008
2	Komati	Boesmanspruit	BOESMANSPRUIT	X11B-BOE	-26.023406	30.061365
3	Sabie	Sabie	BORDER	X33D-BOR	-25.18518	32.03094
4	Sabie	Sabie	BRAND	X31B-BRA	-25.030106	31.025696
5	Sabie	Sabie	BRUG1	X31B-BRU	-25.06522	30.85893
6	Sabie	Sabie	CASTLE	X31A-CAS	-25.093152	30.769191
7	Komati	Komati	DOWN_ESWATINI	X13J-DOW	-25.928516	31.759672
8	Crocodile	Crocodile	EWR_C2	X21B-EWR_C2	-25.410503	30.31546
9	Crocodile	Crocodile	EWR_C3	X21E-EWR_C3	-25.452111	30.681083
10	Komati	Komati	EWR_K1	X11F-EWR_K1	-25.854333	30.376639
11	Komati	Komati	EWR_K2	X12K-EWR_K2	-26.038806	31.003139
12	Sabie	Sabie	EWR_S	X31D-EWR_S	-25.027778	31.051389
13	Sabie	Sabie	EWR_S1	X31D-EWR_S1	-25.073611	30.848611
14	Incomati	Incomati	FLOODPLAIN	FLOOD	-25.040656	32.633429
15	Komati	Gladdespruit	GLADDESPRUIT	X11K-GLA	-25.91042	30.653459
16	Crocodile	Elands	GOEDGENOEG	X21K-GOE	-25.528051	30.697699
17	Crocodile	Elands	HEMLOCK	X21J-HEM	-25.600597	30.559655
18	Crocodile	Kaap	HONEYBIRD	X23H-HON	-25.64947	31.24286
19	Crocodile	Houtbosloop	HOUTBOSLOOP	X22B-HOU	-25.431013	30.747095
20	Komati	Komati	IFRO4	X13J-IFR	-25.68168	31.78295
21	Incomati	Incomati	INCOMATI	X13L-INC	-25.322458	32.267084
22	Crocodile	Crocodile	KANYAMAZANE	X22K-KAN	-25.501687	31.18005
23	Komati	Komati	KLEIN_KOMATI	X11D-KLE	-25.888018	30.12071
24	Komati	Lomati	KLEINDORINGKOP	X14H-KLE	-25.647721	31.621155
25	Komati	Komati	KOMAT	X12H-KOM	-26.02341	30.90073

26	Komati	Komati	LEKKE	X11H-LEK	-25.8415123	30.482707
27	Komati	Lomati	LOMATI	X14B-LOM	-25.818972	31.311388
28	Komati	Komati	LOW_KOMATI	X13K-LOW	-25.444556	31.960754
29	Sabie	Sabie	LOW_SABIE	X33B-LOW	-25.109384	31.907326
30	Sabie	Mac Mac	MACMAC	X31C-MAC	-25.01333	31.00405
31	Sabie	Marite	MARITE	X31G-MAR	-25.01795	31.13328
32	Uanetze	Massintonto	MASSINTONTO_SA	X40D-MAS	-24.78377	31.978932
33	Crocodile	Mbyamiti	MBYAMITI	X24H-MBY	-25.31621	31.745376
34	Sabie	Sabie	MERRY_PEBBLES	X31A-MER	-25.093815	30.76792
35	Crocodile	Nels	NELS	X22F-NEL	-25.427128	30.964531
36	Crocodile	Noordkaap	NOORDKAAP	X23B-NOO	-25.661645	31.078546
37	Crocodile	Elands	NSININI	X21G-NSI	-25.621988	30.398245
38	Crocodile	Nzikasi	NZIKASI	X24C-NZI	-25.522125	31.368514
39	Sabie	Sabie	OLIFS	X31A-OLI	-25.120955	30.716955
40	Crocodile	Queen	QUEEN	X23E-QUE	-25.740912	30.997523
41	Crocodile	Crocodile	RIVULETS	X22B-RIV	-25.429708	30.757919
42	Crocodile	Elands	RONDEWAL	X21K-RON	-25.569221	30.66293
43	Sabie	Sabie	SABIE_MOZ	X33D-SAB	-25.316521	32.290041
44	Sabie	Sand	SAND_EXETER	X32G-SAN	-24.967222	31.626944
45	Sabie	Sabie	SKURUKWANE	X31K-SKU	-24.986846	31.293247
46	Crocodile	Suidkaap	SUIDKAAP	X23F-SUI	-25.716048	31.056677
47	Komati	Teespruit	TEESPRUIT	X12F-TEE	-26.015345	30.858366
48	Sabie	Sabie	TINGA	X31M-TIN	-24.97305	31.49346
49	Komati	Komati	TJAKASTAD	X12G-TJA	-25.97453	30.82221
50	Uanetze	Uanetze	UANETZE_SA	X40A-UAN	-24.452061	31.994505
51	Komati	Vaalwaterspruit	VAALWATERSPRUIT	X11A-VAA	-26.007191	30.027323
52	Crocodile	Crocodile	VANGRAAN	X24F-VAN	-25.43872	31.638135
53	Crocodile	White River	WHITERIVER	X22H-WHI	-25.457741	31.079465

Fish sampling

Fish communities were evaluated at the different sampling sites in the greater Incomati River Catchment during five sampling events undertaken from May 2020 to October 2023 within South Africa, Eswatini and Mozambique. Limited sampling sites were sampled throughout Mozambique and Eswatini due to assess restrictions, sampling safety in areas and the availability of historical data. Although this poses as a limitation to the study, some data needed to be included to validate the study area as the Greater Incomati Catchment and not only the Inkomati Catchment. Fish communities were sampled using various active sampling techniques including a SAMUS (Bonar *et al.*, 2015) backpack- and generator driven electro-fishing and angling techniques, and the use of 10ft cast nets, and small (6m x 1.2m in 6mm mesh), medium (25m x 1.8m in 12mm mesh) and large (45m x 2.5m in 20mm mesh) seine nets. Selected passive sampling techniques including fine mesh (5mm) double wing, single trap fykes, and standard 15mm mesh double trap fykes with one connecting wing, based on habitat availability (Oliver *et al.*, 2015). The sampling approach included an evaluation of available pool, glide, rapid, riffle and backwater habitats available at each reach considered in the study, during each survey the appropriate sampling method was used so that all habitats and potential species could be evaluated (Smith *et al.*, 2014). Expected fish were initially considered for each site using DWS (2014), Scott *et al.*, (2006) and Skelton (2021,2024). All fish collected were identified to species and the length (SL) and condition of each individual was measured (Skelton 2024). Fish species, individual sizes and abundances were documented per biotope along with the associated habitat characteristics. The habitat variability considerations included three depth (mm) and velocity (m/s) measurements per biotope, using the transparent velocity head rod (Le Coz *et al.*, 2024). For each biotope sampled the substrate distributions including percentage silt, mud, gravel, cobble, boulders and bedrock were determined using the substrate classification of Wadeson and Rowntree (1998) and Rowntree *et al.*, (2000). Habitat cover characteristics including the availability of and condition of plant roots, undercut banks and substrates for cover, adequate water column or depth for cover and marginal, aquatic and overhanging vegetation for cover and other features providing cover such as bridges adapted from Fouché (2009) and Kleynhans (2007). *In situ* water quality attributes were monitored using a calibrated HANNA HI98198 Dissolved Oxygen meter and a EUTECH PCD 650 water quality meter. This included pH, electrical conductivity ($\mu\text{S}/\text{cm}$), temperature ($^{\circ}\text{C}$), total dissolve salts (ppm) and dissolved oxygen concentrations (mg/L).

The ecological wellbeing of the fish communities

The use of multiple lines of evidence like the Fish Response Assessment Index (FRAI) and multivariate statistical methods is used to consider the effect of environmental drivers on fish populations and communities.

1. Fish Response Assessment Index

The present ecological state of the fish communities in the greater Incomati River Catchment was determined through the Fish Response Assessment Index (FRAI) (Kleynhans, 2007). The eight-step process of the FRAI methodology was used to obtain modelled and adjusted FRAI scores (FRAI I), score each metric, and assess which altered driver component contributed to the ecological state obtained at each site. The output of the assessment is the present ecological state of the fish communities based on the A-F category, where “A” represents an ‘unmodified natural’ system and “F” a ‘critically/extremely modified’ system (Kleynhans 2007) (Table 6).

Table 6: Fish Response Assessment Index scoring system based on assessment output (Kleynhans 2007).

Ecological Categories	Description	Acceptable/ Unacceptable	Score (%)
A	Unmodified, natural state	Acceptable	90-100
B	Largely natural with few modifications	Acceptable	80-89
C	Moderately modified	Acceptable	60-79
D	Largely modified	Unacceptable	40-59
E	Seriously modified	Unacceptable	20-39
	Critically modified	Unacceptable	0-19

2. Statistics

In this study direct or constrained multivariate statistical analyses were undertaken to determine the potential for significant differences in fish communities, and environmental drivers that can explain significant differences in fish community structures (Akhi *et al.*, 2020; Das *et al.*, 2025). The statistical analyses involved overlaying captured variance of the

explanatory environmental variables onto fish sample and taxa ordination diagrams. The linear response mode used to achieve this is a redundancy analysis (RDA), a derivative of principle component analyses (PCA) using the Canoco version 4.5 software package (Ter Braak 1994; Leps and Smilauer 2003). Because abundance data were available, the data were transformed using a Log X+2 - transformation (Van den Brink *et al.*, 2003).

Results

During the five surveys from May 2020 to October 2023, 484 sampling efforts were carried out with an average of eight efforts per site representing different biotopes at each site. The habitats ranged from shallow rocky streams to deep channels with variable substrate types and velocities. Habitat availability ranged from undercut banks to large areas with aquatic vegetations and overhanging structures. Sites on the main rivers were sampled once during the high flow season and once during the low flow survey.

The fish sampling resulted in the collection of 6684 individuals from the 53 sites. Fifty of the 70 expected different freshwater fish species, and four alien invasive species were study (Table 7). *Kneria sp.*, *Crocodile* and *Chetia brevis* individuals were collected during a different study within the same study area, however not included due to not being one of the study's sites.

The Uanetze River sub-catchment had to lowest species richness with only eight species of the expected 27 species collected (Table 7)(Darwall *et al.*, 2009; Skelton 2024). The Sabie River sub-catchment had the highest freshwater fish species richness present (36 fish species collected of the 45 expected species)(Darwall *et al.*, 2009; Skelton 2024) followed by the Crocodile River sub-catchment (35 fish species collected of the expected 45)(Darwall *et al.*, 2009; Skelton 2024) (Table 7). From the Komati River sub-catchment, 32 species were collected from the expected 48 species (Darwall *et al.*, 2009; Skelton 2024), and 21 species were collected from the Incomati River sub-catchment for the expected 44 species (Darwall *et al.*, 2009; Skelton 2024) (Table 7). The invasive species collected were *Gambusia affinis* (Baird & Girard 1853) (3 sites, n=12), *Micropterus salmoides* (Lacepede 1802) (5 sites, n=9), *Serranochromis robustus* (Gunther 1864) (1 site, n=1) and *Xiphophorus helleri* Heckel 1848 (2 sites, n=5). *Oreochromis mossambicus* and *O. niloticus* hybrids were also collected within the floodplain area sites (1 site, n= 21) (Mashaphu *et al.*, 2024). Thirty-nine anguillid eel individuals (*Anguilla bengalensis*, *Anguilla mossambica* and *Anguilla marmorata*) were collected but were expected at all of the sites, during the study (O'Brien *et al.*, 2019; Hanzen *et al.*, 2022). *Micralestes acutidens* was collected in the highest abundance throughout the greater Incomati River Catchment. *Oreochromis mossambicus* (IUCN category Vulnerable), *Anguilla bengalensis labiata*, *Anguilla mossambica* (IUCN category Near Threatened), *Serranochromis meridianus* (IUCN category Endangered) and *Chiloglanis bifurcus* (IUCN category Critically Endangered), were the only species with an IUCN category above least concerned that were sampled during the survey.

1. Fish Response Assessment Index (FRAI)

The FRAI scores are representative of the tolerance, intolerance and preferences of the reference fish communities in question at each site and the response of the reference species to environmental determinants or drivers (Kleynhans 2007). The assessment incorporates changes in the environmental conditions related to fish stress and forms the basis of an ecological response assessment.

The highest Fish Response Assessment Index score was observed at the X21J-HEM site on the Elands River, Crocodile sub-catchment during the 2020 survey (Figure 8-Map Nr 17, FRAI score 80%, Class BC)(Table 7). The lowest Fish Response Assessment Index score was observed at the X23B-NOO site on the Noordkaap River, Crocodile sub-catchment during the 2022 survey (Figure 8-Map Nr 36, FRAI score 52.2%, Class D)(Table 7).

In the Uanetze River sub-catchment, the FRAI assessment for all sites ranged from 68.2% – 73.4%, all categorised in a Class-C category (Table 7). In the Sabie River sub-catchment, the FRAI assessment for the sites ranged from 67.4% - 77.4%, all categorised in a Class-C category (Table 7). In the Crocodile River sub-catchment, the FRAI assessment for the sites ranged from 52.2% - 80%, the majority of the sites categorised in a Class-C category while the highest site assessment was categorised in a Class-B/C assessment and the lowest assessment categorised in a Class-D assessment (Table 7). In the Komati River sub-catchment, the FRAI assessment for the sites ranged from 64% - 77.9%, all categorised in a Class-C category (Table 7). In the Incomati River sub-catchment, the FRAI assessment for the sites ranged from 64.7% - 70.6%, all categorised in a Class-C category (Table 7).

Table 7: The abundance, species richness and ecological condition (EC) (Kleynhans, 2007) of each site for the timeframe it was sampled.

Ma p Nr	River	SQ code	Year	Site name	Site code	Abundanc e	Richnes s	FRA I Score	EC
1	SABIE	X33A	2020(JUN)	ANTHOCLYSTER	X33A-ANT	156	12	77.4	C
1	SABIE	X33A	2021(MAY)	ANTHOCLYSTER	X33A-ANT	260	14	77.4	C
2	KOMATI	X11B	2023(JUN)	BOESMANSPRUIT	X11B-BOE	3	1	70.3	C
3	SABIE	X33D	2020(JUN)	BORDER	X33D-BOR	36	10	67.4	C
4	SABIE	X31B	2020(JUL)	BRAND	X31B-BRA	88	10	75.1	C
5	SABIE	X31B	2020(JUN)	BRUG1	X31B-BRU	146	3	75.1	C
6	SABIE	X31A	2020(JUN)	CASTLE	X31A-CAS	39	3	75.5	C
7	KOMATI	X13J	2023(JUN)	DOWN_ESWATINI	X13J-DOW	35	5	65	C
8	CROCODILE	X21B	2023(JUN)	EWR_C2	X21B-EWR_C2	23	4	72.3	C
9	CROCODILE	X21E	2022(JUN)	EWR_C3	X21E-EWR_C3	16	5	70.8	C
10	KOMATI	X11F	2020(JUN)	EWR_K1	X11F-EWR_K1	36	5	72	C
10	KOMATI	X11F	2021(AUG)	EWR_K1	X11F-EWR_K1	48	5	71.9	C
11	KOMATI	X12K	2020(JUN)	EWR_K2	X12K-EWR_K2	17	3	67.2	C
12	SABIE	X31D	2022(AUG)	EWR_S	X31D-EWR_S	40	7	74.4	C
13	SABIE	X31D	2023(JUN)	EWR_S1	X31D-EWR_S1	46	3	72.8	C
14	INCOMATI		2022(JUN)	FLOODPLAIN	FLOOD	249	20	70.6	C
15	KOMATI	X11K	2022(NOV)	GLADDESPRUIT	X11K-GLA	2	2	66.9	C
15	KOMATI	X11K	2023(JUN)	GLADDESPRUIT	X11K-GLA	7	3	66.8	C
16	ELANDS	X21K	2020(APR)	GOEDGENOEG	X21K-GOE	116	9	74.9	C
16	ELANDS	X21K	2021(MAY)	GOEDGENOEG	X21K-GOE	47	7	75.2	C
17	ELANDS	X21J	2020(MAY)	HEMLOCK	X21J-HEM	129	8	80	BC
17	ELANDS	X21J	2021(MAY)	HEMLOCK	X21J-HEM	227	8	71.7	C

18	CROCODILE	X23H	)	2022(NOV)	HONEYBIRD	X23H-HON	43	7	59.1	CD
19	CROCODILE	X22B		2023(JUN)	HOUTBOSLOOP	X22B-HOU	27	5	67.7	C
20	KOMATI	X13J	)	2020(MAY)	IFRO4	X13J-IFR	42	10	64	C
21	KOMATI	X13L	)	2022(MAY)	INCOMATI	X13L-INC	27	6	64.7	C
22	CROCODILE	X22K		2020(JUL)	KANYAMAZANE	X22K-KAN	41	10	72.8	C
22	CROCODILE	X22K		2021(JUN)	KANYAMAZANE	X22K-KAN	90	15	74.8	C
22	CROCODILE	X22K		2022(NOV)	KANYAMAZANE	X22K-KAN	263	11	74.8	C
23	KOMATI	X11D		2023(JUN)	KLEIN_KOMATI	X11D-KLE	5	2	66.1	C
24	KOMATI	X14H		2022(NOV)	KLEINDORINGKOP	X14H-KLE	12	5	67.5	C
25	KOMATI	X12H	)	2020(MAY)	KOMAT	X12H-KOM	28	4	70.9	C
26	KOMATI	X11H	)	2020(MAY)	LEKKE	X11H-LEK	68	5	69.7	C
26	KOMATI	X11H		2021(MRT)	LEKKE	X11H-LEK	47	5	72	C
27	KOMATI	X14B		2023(JUN)	LOMATI	X14B-LOM	31	12	67.6	C
28	KOMATI	X13K		2023(JUN)	LOW_KOMATI	X13K-LOW	10	2	68.1	C
29	SABIE	X33B		2022(AUG)	LOW_SABIE	X33B-LOW	160	10	67.6	C
30	SABIE	X31C		2020(APR)	MACMAC	X31C-MAC	44	6	68.6	C
30	SABIE	X31C		2021(JUN)	MACMAC	X31C-MAC	172	8	68.6	C
31	SABIE	X31G		2023(JUN)	MARITE	X31G-MAR	3	2	67.5	C
32	UANETZE	X40D		2021(MRT)	MASSINTONTO_SA	X40D-MAS	167	7	69.8	C
32	UANETZE	X40D		2023(JUN)	MASSINTONTO_SA	X40D-MAS	160	4	68.2	C
33	CROCODILE	X24H		2021(MRT)	MBYAMITI	X24H-MBY	212	6	71.5	C
34	SABIE	X31A		2022(NOV)	MERRY_PEBBLES	X31A-MER	15	1	72.4	C
35	CROCODILE	X22F		2022(NOV)	NELS	X22F-NEL	19	4	67.5	C
36	CROCODILE	X23B		2022(NOV)	NOORDKAAP	X23B-NOO	9	3	52.2	D
37	CROCODILE	X21G		2022(NOV)	NSININI	X21G-NSI	92	5	69.2	C

38	CROCODILE	X24C	2020(MRT)	NZIKASI	X24C-NZI	84	7	71.4	C
38	CROCODILE	X24C	2021(MRT)	NZIKASI	X24C-NZI	213	13	70.4	C
39	SABIE	X31A	2020(JUN)	OLIFS	X31A-OLI	38	5	74	C
40	CROCODILE	X23E	2022(NOV)	QUEEN	X23E-QUE	39	4	61.1	CD
41	CROCODILE	X22B	2020(APR)	RIVULETS	X22B-RIV	614	9	73.7	C
41	CROCODILE	X22B	2023(JUN)	RIVULETS	X22B-RIV	59	4	72.5	C
42	CROCODILE	X21K	2020(JUN)	RONDEWAL	X21K-RON	56	9	78.1	BC
42	CROCODILE	X21K	2021(JUN)	RONDEWAL	X21K-RON	25	7	74.5	C
42	CROCODILE	X21K	2022(NOV)	RONDEWAL	X21K-RON	119	7	71	C
43	SABIE	X33D	2022(JUN)	SABIE_Moz	X33D-SAB	39	8	69	C
44	SABIE	X32G	2023(MRT)	SAND_EXETER	X32G-SAN	260	20	70.8	C
45	SABIE	X31K	2020(JUL)	SKURUKWANE	X31K-SKU	68	8	74.3	C
45	SABIE	X31K	2021(JUN)	SKURUKWANE	X31K-SKU	93	9	75.3	C
45	SABIE	X31K	2022(AUG)	SKURUKWANE	X31K-SKU	302	19	75.2	C
46	CROCODILE	X23F	2022(NOV)	SUIDKAAP	X23F-SUI	34	8	57.2	D
47	TEESPRUIT	X12F	2023(JUN)	TEESPRUIT	X12F-TEE	10	5	68.7	C
48	SABIE	X31M	2020(JUL)	TINGA	X31M-TIN	144	11	75.1	C
49	KOMATI	X12G	2020(MAY)	TJAKASTAD	X12G-TJA	180	11	76.4	C
49	KOMATI	X12G	2021(MRT)	TJAKASTAD	X12G-TJA	96	13	77.9	C
50	UANETZE	X40A	2023(JUN)	UANETZE_SA	X40A-UAN	63	2	73.4	C
51	VAALWATERSPRUIT T	X11A	2023(JUN)	VAALWATERSPRUIT T	X11A-VAA	8	1	68.7	C
52	CROCODILE	X24F	2020(MAY)	VANGRAAN	X24F-VAN	234	17	68.8	C
52	CROCODILE	X24F	2021(APR)	VANGRAAN	X24F-VAN	101	15	69.6	C
52	CROCODILE	X24F	2022(AUG)	VANGRAAN	X24F-VAN	62	8	68.7	C
52	CROCODILE	X24F	2023(JUN)	VANGRAAN	X24F-VAN	221	10	70.2	C
53	CROCODILE	X22H	2023(JUN)	WHITERIVER	X22H-WHI	32	7	69.9	C

2. Environmental Stressors Assessment

(Note: For the Redundancy analyses all graph results are presented in the Appendix Data 3, Figure 9 is a representation within the chapter on how the redundancy analyses results look like while Table 8 summarises the results of the redundancy analyses in a more readable manner).

Inter river catchment comparisons for all sampling efforts and fish communities were overall significantly different from one another ($F = 9.165$; $p = 0.001$) (Figure 9). A total of 70.2% of the total variation was presented in this analysis with 45.1% explained on axis 1 and 25.1% explained on axis 2.

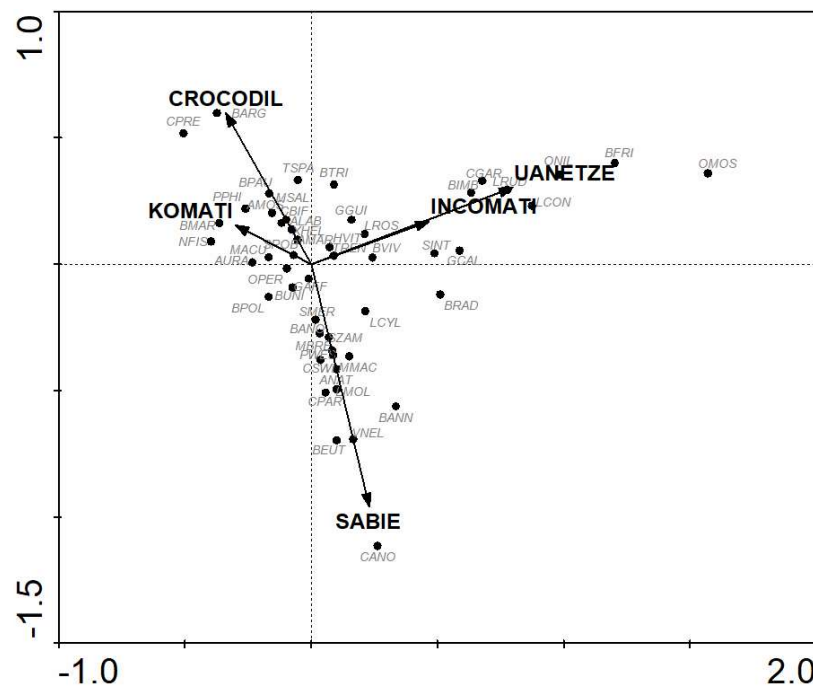


Figure 9: Redundancy analysis biplot based on the fish communities among the different main river catchments for all the sampling efforts. A total of 70.2% of the total variation were presented in this analysis with 45.1% explained on axis 1 and 25.1% explained on axis 2.

The Intra-River sub-catchment analyses for each of the regions in the main river catchments for all sampling efforts and fish communities were evaluated (Appendix - Figure 30; Table 8). The sites within the Uanetze River sub-catchment differ significantly from one another ($F = 6.578$; $p = 0.001$) with 93.4% of the variation explained on axis 1 and 6.6% explained on axis 2 (Figure 30A). From the sampling sites in the Uanetze River Sub-catchment, all sites sampled contributed significantly to the assessment and had significant differences in the fish community per site (MASSINTO, UANETZE). The MASSINTO site contributed the most to the analyses ($F = 8.560$; $p = 0.001$). The sampling sites on the

Massintonto River (MASSINTO) was more associated with species like *Enteromius radiatus* (BRUD) while the Uanetze sites didn't illustrate species with strong associations. (Appendix - Figure 30A).

The sites within the Sabie River sub-catchment differ significantly from one another ($F = 3.533$; $p = 0.001$) with 30.1% of the variation explained on axis 1 and 19.7% explained on axis 2 (Appendix - Figure 30B; Table 8). From the sampling sites in the Sabie River sub-catchment, 10 out of the 15 sampling sites had a significant effect on the overall sub-catchment assessment (MACMAC, BRUG, SKURUKWA, SAND, LOW_SABI, ANTHOCLY, BRAND, CASTLE, EWR_S1, TINGA). The MACMAC site contributed the most to the analyses ($F = 6.10$; $p = 0.001$). The association of fish species toward specific sites are difficult to illustrate due to the high number and clustering of sampling sites and number of fish species observed (Appendix - Figure 30B; Table 8).

The sites within the Crocodile River sub-catchment differ significantly from one another ($F = 3.572$; $p = 0.001$) with 37.7% of the variation explained on axis 1 and 17.5% explained on axis 2 (Appendix - Figure 30C; Table 8). From the sampling sites in the Crocodile River sub-catchment, eight out of the 15 sites sampled contributed significantly to the assessment and had significant differences in the fish community per site (HEMLOCK, GOEDGENO, KANYAMAZ, VAN_GRAA, MBYIAMIT, RONDEWAL, NSIKASI, RIVULETS). The HEMLOCK site contributed the most to the analyses ($F = 7.280$; $p = 0.001$). The association of fish species toward specific sites are difficult to illustrate due to the high number and clustering of sampling sites and number of fish species observed (Appendix - Figure 30C; Table 8).

The sites within the Komati River sub-catchment differ significantly from one another ($F = 1.728$; $p = 0.002$) with 36.0% of the variation explained on axis 1 and 22.1% explained on axis 2 (Appendix - Figure 30D; Table 8). From the sampling sites in the Komati River sub-catchment, four out of the 15 sites sampled contributed significantly to the assessment and had significant differences in the fish community per site (TJAKASTA, KOMAT, EWR_K1, LEKKER). The TJAKASTA site contributed the most to the analyses ($F = 4.76$; $p = 0.002$). The association of fish species toward specific sites are difficult to illustrate due to the high number and clustering of sampling sites and number of fish species observed (Appendix - Figure 30D). It can be observed that the TJAKASTA site is breaking away from the rest of the Komati River sub-catchment sites with a high association of *Labeobarbus marequensis* (BMAR) at this site (Appendix - Figure 30D; Table 8).

The sites within the Incomati River sub-catchment differ significantly from one another ($F = 3.752$; $p = 0.001$) with 73.5% of the variation explained on axis 1 and 18.6% explained on axis 2 (Appendix - Figure 30E; Table 8). From the sampling sites in the Incomati River sub-catchment, the Floodplain site (FLOODPLA) contributed the most to the analyses ($F = 7.480$; $p = 0.001$). The Floodplain site was more associated with slow flowing preferable species (CSWI, BRAD, ONIL and GCAL), while the other sites (SABIE_MOZ and INCOMATI) was more associated with fish species of variable preferences and sizes (large to small growing species) (Figure 30E; Table 8).

The study sampled different fish communities from 2020 to 2023. The overall sampling year evaluation contributed significantly to the assessment ($F = 4.244$, $p = 0.001$) with 70.8% explained on axis 1 and 19.7% explained on axis 2 (Appendix - Figure 31A; Table 8). The 2023 sampling year contributed the most to the assessment ($F = 7.700$, $p = 0.001$).

The Uanetze River sub-catchment had a significant difference in the fish communities between sampling years ($F = 2.729$, $p = 0.037$) with 100% explained on axis 1 and 0% explained on axis 2 (Appendix - Figure 31B; Table 8). The 2021 sampling year did not significantly contribute to the assessment ($F = 2.730$, $p = 0.051$) although the association of collected fish species with the different sampling years are moderately distributed towards the 2021 sampling year (Appendix - Figure 31B; Table 8).

The Sabie River sub-catchment had a significant difference in the fish communities between sampling years ($F = 1.969$, $p = 0.002$) with 57.9% explained on axis 1 and 24.3% explained on axis 2 (Appendix - Figure 31C; Table 8). The 2021 sampling year contributed the most to the assessment ($F = 2.580$, $p = 0.005$), while the 2020 sampling year did not significantly contributed to the assessment ($F = 1.430$, $p = 0.118$). The association of collected fish species with the different sampling years are moderately distributed throughout the assessment (Appendix - Figure 31C; Table 8).

The Crocodile River sub-catchment had a significant difference in the fish communities between sampling years ($F = 4.246$, $p = 0.001$) with 72% explained on axis 1 and 15.3% explained on axis 2 (Appendix - Figure 31D; Table 8). The 2023 sampling year contributed the most to the assessment ($F = 6.240$, $p = 0.001$). Some sampled fish species had high associations with the 2020 sampling year, e.g., the anguillid eels while the remaining species are distributed throughout (Appendix - Figure 31D; Table 8).

The Komati River sub-catchment had a significant difference in the fish communities between sampling years ($F = 4.840$, $p = 0.001$) with 91.2% explained on axis 1 and 5.1% explained on axis 2 (Appendix - Figure 31E; Table 8). The 2020 sampling year contributed the most to the assessment ($F = 10.380$, $p = 0.002$), while the 2021 sampling year did not significantly contribute to the assessment ($F = 0.860$, $p = 0.476$). The 2022 sampling year has a moderate association with *Micralestes acutidens* (MACU) and the 2023 sampling year had a moderate association with 'no fish' collected (NFIS) (Appendix - Figure 31E; Table 8).

Seasonality assessment was completed against high flow and low flow season of sampling events throughout the greater Incomati River Catchment. The low flow season (LF) was considered from March to August and the high flow season (HF) was from September to February. The overall seasonality assessment of the fish communities in the greater Incomati River Catchment was a significant contributor to the availability of fish in the system ($F = 1.647$, $p = 0.043$) (Appendix - Figure 32A; Table 8) with 100% explained on axis 1 and 0% explained on axis 2. The low flow (LF) variable contributed the most to the assessment ($F = 1.650$, $p = 0.041$). The collected fish species are distributed throughout the assessment with no clear association towards and seasonality.

The seasonality assessment of the fish communities in the Sabie River sub-catchment was not significant contributor to the availability of fish in the system ($F = 1.365$, $p = 0.119$) with 100% explained on axis 1 and 0% explained on axis 2 (Appendix - Figure 32B; Table 8).

The seasonality assessment of the fish communities in the Crocodile River sub-catchment was a significant contributor to the availability of fish in the system ($F = 4.181$, $p = 0.001$) with 100% explained on axis 1 and 0% explained on axis 2 (Appendix - Figure 32C; Table 8). The low flow (LF) variable contributed the most to the assessment ($F = 4.180$, $p = 0.001$). The collected fish species are distributed throughout the assessment with no clear association towards any seasonality.

The seasonality assessment of the fish communities in the Komati River sub-catchment was a significant contributor to the availability of fish in the system ($F = 4.840$, $p = 0.001$) with 91.2% explained on axis 1 and 5.1% explained on axis 2 (Appendix - Figure 32D; Table 8). The low flow (LF) variable did not significantly contribute to the assessment ($F = 0.720$, $p = 0.625$). The collected fish species are distributed throughout the assessment with no clear association towards seasonality.

The Velocity-Depth relationship for all the sampling sites combined were a significant ($F = 2.545$, $p = 0.001$) driver of fish communities in the greater Incomati River Catchment. A total variation of 59.0% was explained on axis 1 and 16.6% explained on axis 2 (Appendix - Figure 33A; Table 8). The sub-catchment evaluations of the Velocity-Depth relationship for each of the sub-catchments revealed that the Uanetze- and Incomati sub-catchments had no significant Velocity-Depth relationships for all sites sampled in these sub-catchments ($F = 1.008$, $p = 0.332$ and $F = 0.716$, $p = 0.608$)(Appendix - Figure 33B and Appendix - Figure 33F; Table 8).

The Velocity-Depth relationship for the sites sampled in the Sabie River sub-catchment had an overall significant relationship and effected the observed fish communities ($F = 1.562$, $p = 0.016$) with 58.2% explained on axis 1 and 16.7% explained on axis 2 (Appendix - Figure 33C; Table 8). Only one velocity measurement (VEL2) significantly contributed to the assessment ($F = 5.480$, $p = 0.001$). The Velocity relationship that had a significant effect on the fish communities (VEL2) had no specific association with any species. The majority of the species had a moderate weak association towards both Velocity-Depth relationship and are distributed towards the centre of the relationship (Appendix - Figure 33C; Table 8).

The Velocity-Depth relationship for the sites sampled in the Crocodile River sub-catchment had a significant relationship and effected the observed fish communities ($F = 1.682$, $p = 0.003$) with 40.7% explained on axis 1 and 26.1% explained on axis 2 (Appendix - Figure 33D; Table 8). The Velocity relationship had high association with rheophilic species (AURA, CPRE) and the Depth relationship had associations with more pool-loving or slow-shallow preferable species (BEUT, OPER, MACU).

The Velocity-Depth relationship for the sites sampled in the Komati River sub-catchment had an overall significant relationship and effected the observed fish communities ($F = 2.125$, $p = 0.002$) with 73.9% explained on axis 1 and 12.3% explained on axis 2 (Appendix - Figure 33E; Table 8). Only one velocity measurement (VEL2) significantly contributed to the assessment ($F = 8.350$, $p = 0.001$). The Velocity relationship that had a significant effect on the fish communities (VEL2) had a specific association with rheophilic *Chiloglanis* species (CANO, CSWI). The majority of the species had a moderate weak association towards both Velocity-Depth relationship and are distributed towards the centre of the relationship (Appendix - Figure 33E; Table 8).

The Water quality assessment determined the relationship between *in situ* variables and fish community composition. Water Quality was a significant ($F = 3.381, p = 0.001$) driver of fish communities in the greater Incomati River Catchment with 42.3% explained on axis 1 and 28.8% explained on axis 2 (Appendix - Figure 34A; Table 8). Electrical conductivity (EC) ($F = 5.180, p = 0.001$) and water temperature (TEMP) ($F = 4.520, p = 0.001$) accounted for the greatest variation in the analyses. The pH and Dissolved oxygen (DO) did not significantly contribute to the analyses. The collected fish species are distributed throughout the association analyses with *Oreochromis mossambicus* (OMOS) showing a high association with electrical conductivity.

Water Quality was not a significant ($F = 1.753, p = 0.109$) driver of fish communities in the Uanetze River sub-catchment with 77.1% explained on axis 1 and 22.9% explained on axis 2 (Appendix - Figure 34B; Table 8).

Water Quality was a significant ($F = 3.256, p = 0.001$) driver of fish communities in the Sabie River sub-catchment with 43.8% explained on axis 1 and 23% explained on axis 2 (Appendix - Figure 34C; Table 8). Water temperature (TEMP) ($F = 6.640, p = 0.001$) accounted for the greatest variation in the analyses, while all remaining *in situ* variable also significantly contributed to the analyses except electrical Conductivity (EC) that did not significantly contribute to the analyses ($F = 1.300, p = 0.164$). The collected fish species are distributed throughout the association assessment with no clear associations (Appendix - Figure 34C).

Water Quality was a significant ($F = 5.578, p = 0.001$) driver of fish communities in the Crocodile River sub-catchment with 47.6% explained on axis 1 and 27.2% explained on axis 2 (Appendix - Figure 34D; Table 8). Dissolved oxygen (DO) ($F = 5.150, p = 0.001$) accounted for the greatest variation in the analyses, while all remaining *in situ* variable also significantly contributed to the analyses. The collected fish species are distributed throughout the association assessment with a few species like *Chiloglanis pretoriae* (CPRE) and *Amphilius uranoscopus* (AURA) associating with Dissolved oxygen and *Chiloglanis bifurcus* (CBIF) and invasive *Gambusia affinis* was associated with pH (Appendix - Figure 34D; Table 8).

Water Quality was a significant ($F = 2.100, p = 0.007$) driver of fish communities in the Komati River sub-catchment with 59.6% explained on axis 1 and 26.9% explained on axis 2 (Appendix - Figure 34E; Table 8). Total Dissolve Salts (TDS) ($F = 4.660, p = 0.003$) accounted for the greatest variation in the analyses, while Dissolved oxygen (DO) and

Electrical Conductivity (EC) did not significantly contribute to the assessment. The collected fish species are distributed throughout the association assessment with a few species associating with water temperature and *Glossogobius giuris* (GGIU) showing a high association with TDS (Appendix - Figure 34E; Table 8).

Water Quality was a significant ($F = 1.688, p = 0.031$) driver of fish communities in the Incomati River sub-catchment with 79.1% explained on axis 1 and 20.9% explained on axis 2 (Appendix - Figure 34F; Table 8). Water temperature (TEMP) ($F = 2.040, p = 0.027$) accounted for the greatest variation in the analyses. The pH, Dissolved oxygen (DO), Total Dissolve Salt (TDS) and Electrical Conductivity (EC) did not significantly contribute to the assessment. The collected fish species are distributed throughout the association assessment with *Labeo rosae* (LROS) showing a high association with water temperature (Appendix - Figure 34F; Table 8).

The Substrate diversity assessment was determined by evaluating the different types of silt, mud, sand, gravel, cobble, boulders, bedrock and the fish community compositions at these substrate units. The Substrate diversity was a significant ($F = 3.281, p = 0.001$) driver of fish communities in the greater Incomati River Catchment with 52.0% explained on axis 1 and 15.8% explained on axis 2 (Appendix - Figure 35A; Table 8). Sand ($F = 8.890, p = 0.001$), Mud ($F = 5.260, p = 0.001$), Boulders ($F = 3.010, p = 0.001$), Cobble ($F = 2.790, p = 0.001$), and Silt ($F = 2.240, p = 0.028$) substrates accounted for the greatest variability in the analyses. Bedrock, Gravel and Other substrate did not significantly contribute to the analyses. The collected species are distributed throughout the association analyses with specific species like *Chiloglanis pretoriae* (CPRE), *Amphilius uranoscopus* (AURA), *Enteromius crocodalensis* (BARG) and *Labeobarbus polylepis* (BPOL) have a high association with Cobble and Boulder substrates (Appendix - Figure 35A; Table 8).

The Substrate diversity was not a significant ($F = 0.991, p = 0.507$) driver of fish communities in the Uanetze River sub-catchment with 51.2% explained on axis 1 and 30.9% explained on axis 2 (Appendix - Figure 35B). Individually, Bedrock ($F = 2.540, p = 0.044$) had a significant effect on the fish communities in the Uanetze River sub-catchment (Appendix - Figure 35B; Table 8).

The Substrate diversity was a significant ($F = 1.925, p = 0.001$) driver of fish communities in the Sabie River sub-catchment with 41.1% explained on axis 1 and 25.4% explained on axis 2 (Appendix - Figure 35C; Table 8). Sand ($F = 4.680, p = 0.001$), Boulders

($F = 2.300$, $p = 0.006$) and Gravel ($F = 2.000$, $p = 0.022$) substrates accounted for the greatest variability in the analyses, while the other substrate types did not significantly contribute to the analyses. The collected species are distributed throughout the association analyses (Appendix - Figure 35C; Table 8).

The Substrate diversity was a significant ($F = 2.191$, $p = 0.005$) driver of fish communities in the Crocodile River sub-catchment with 45.2% explained on axis 1 and 25.2% explained on axis 2 (Appendix - Figure 35D; Table 8). Sand ($F = 6.090$, $p = 0.001$), Boulders ($F = 2.780$, $p = 0.004$) and Silt ($F = 2.730$, $p = 0.046$) substrates accounted for the greatest variability in the analyses, while the other substrate types did not significantly contribute. The collected species are distributed throughout the association analyses with a few specific species that has a high association towards Sand and Silt (Appendix - Figure 35D; Table 8).

The Substrate diversity was a significant ($F = 2.013$, $p = 0.004$) driver of fish communities in the Komati River sub-catchment with 61.6% explained on axis 1 and 15.4% explained on axis 2 (Appendix - Figure 35E; Table 8). Boulders ($F = 4.710$, $p = 0.001$) and Cobbles ($F = 4.020$, $p = 0.003$) substrates accounted for the greatest variability in the analyses, while the other substrate types did not significantly contribute. The collected species are distributed throughout the association analyses (Appendix - Figure 35E; Table 8).

The Substrate diversity was not a significant ($F = 0.991$, $p = 0.507$) driver of fish communities in the Incomati River sub-catchment with 56.7% explained on axis 1 and 23.1% explained on axis 2 (Appendix - Figure 35F; Table 8). Individually, mud ($F = 4.070$, $p = 0.001$) had a significant effect on the fish communities in the Incomati River sub-catchment (Appendix - Figure 35F; Table 8).

The Cover features assessment was determined by evaluating the different types of cover features such as undercut bank, roots, vegetation, substrate variability, depth in relation to the fish community. The Cover features was a significant ($F = 3.261$, $p = 0.001$) driver of fish communities in the greater Incomati River Catchment with 46.6% explained on axis 1 and 21.5% explained on axis 2 (Appendix - Figure 36A; Table 8). All Cover features significantly contributed to the analyses with Substrate diversity (SUBS) ($F = 7.170$, $p = 0.001$) accounting for the greatest variability in the analyses. The collected fish species are distributed throughout the association analyses with specific species having high associations to some cover features (Appendix - Figure 36A; Table 8).

The Cover features was not a significant ($F = 2.378$, $p = 0.688$) driver of fish communities in the Uanetze River sub-catchment with 58.8% explained on axis 1 and 23.9% explained on axis 2 (Appendix - Figure 36B; Table 8).

The Cover features was a significant ($F = 1.617$, $p = 0.002$) driver of fish communities in the Sabie River sub-catchment with 34.9% explained on axis 1 and 23.1% explained on axis 2 (Appendix - Figure 36C; Table 8). Substrate diversity (SUBS) ($F = 3.290$, $p = 0.001$) and Roots ($F = 2.440$, $p = 0.007$) accounted for the greatest variability in the analyses, while other cover features did not significantly contribute to the analyses. The collected fish species are distributed throughout the association analyses (Appendix - Figure 36C; Table 8).

The Cover features was a significant ($F = 2.673$, $p = 0.001$) driver of fish communities in the Crocodile River sub-catchment with 41.1% explained on axis 1 and 31% explained on axis 2 (Appendix - Figure 36D; Table 8). Aquatic vegetation (AVEG) ($F = 4.800$, $p = 0.001$) accounted for the greatest variability in the analyses, while other cover features like Roots, Substrate diversity (SUBS), Depth and Marginal vegetation (MVEG) all significantly contributed to the analyses. The collected fish species are distributed throughout the association analyses (Appendix - Figure 36D; Table 8).

The Cover features was a significant ($F = 3.477$, $p = 0.001$) driver of fish communities in the Komati River sub-catchment with 57.8% explained on axis 1 and 16.6% explained on axis 2 (Appendix - Figure 36E; Table 8). Substrate diversity (SUBS) ($F = 6.490$, $p = 0.001$) accounted for the greatest variability in the analyses, while other cover features like Other (OTHER), Roots, Marginal vegetation (MVEG) and Depth all significantly contributed to the analyses. The collected fish species are distributed throughout the association analyses (Appendix - Figure 36E; Table 8).

The Cover features was not a significant ($F = 1.324$, $p = 0.293$) driver of fish communities in the Incomati River sub-catchment with 40.7% explained on axis 1 and 23.4% explained on axis 2 (Appendix - Figure 36F; Table 8).

Table 8: Results for the different sub-catchments (rows) and the different Redundancy Analyses assessment (columns) of the fish communities in the greater Incomati River Catchment. The *F*-value and significance (*p*-value) is indicated.

	Intra-river	Sampling Year	Seasonality	Velocity-Depth	Water Quality	Substrate Diversity	Cover Features
Uanetze River sub-catchment	F: 6.578 <i>p</i> : 0.001	F: 2.729 <i>p</i> : 0.037	Insufficient Data	Non-significant F: 1.008 <i>p</i> : 0.332	Non-significant F: 1.753 <i>p</i> : 0.109	Non-significant F: 0.991 <i>p</i> : 0.507	Non-significant F: 2.378 <i>p</i> : 0.688
Sabie River sub-catchment	F: 3.533 <i>p</i> : 0.001	F: 1.969 <i>p</i> : 0.002	Non-significant F: 1.365 <i>p</i> : 0.119	F: 1.562 <i>p</i> : 0.016	F: 3.256 <i>p</i> : 0.001	F: 1.925 <i>p</i> : 0.001	F: 1.617 <i>p</i> : 0.002
Crocodile River sub-catchment	F: 3.572 <i>p</i> : 0.001	F: 4.246 <i>p</i> : 0.001	F: 4.181 <i>p</i> : 0.001	F: 1.682 <i>p</i> : 0.003	F: 5.150 <i>p</i> : 0.001	F: 2.191 <i>p</i> : 0.005	F: 2.673 <i>p</i> : 0.001
Komati River sub-catchment	F: 1.728 <i>p</i> : 0.002	F: 4.840 <i>p</i> : 0.001	F: 4.840 <i>p</i> : 0.001	F: 2.125 <i>p</i> : 0.002	F: 2.100 <i>p</i> : 0.007	F: 2.013 <i>p</i> : 0.004	F: 3.477 <i>p</i> : 0.001
Incomati River sub-catchment	F: 3.752 <i>p</i> : 0.001	Insufficient Data	Insufficient Data	Non-significant F: 0.716 <i>p</i> : 0.608	F: 2.040 <i>p</i> : 0.027	Non-significant F: 0.991 <i>p</i> : 0.507	Non-significant F: 1.324 <i>p</i> : 0.293

Discussion

The fish communities of the greater Incomati River Catchment in South Africa vary considerably. A total of 71% of the expected fish species (50 out of 70) were collected in the study between 2020 and 2023 in the greater Incomati River Catchment. From the different sub-catchments, a total of 29% (8 out of 27 expected) from the Uanetze River sub-catchment 83% (36 out of 43 expected) from the Sabie River sub-catchment, 77% (35 out of 45 expected) from the Crocodile River sub-catchment, 66% (32 out of 48 expected) were collected from the Komati River sub-catchment, and 52% (23 out of 44 expected) from the Incomati River sub-catchment. Unfortunately, no communities considered in the study are in a natural or pristine condition with most occurring in a moderately modified condition (Kleynhans and Louw, 2007). Although more than 70% of expected fish species were collected, most of these fish communities have a decreasing trend and are moderately affected by multiple stressors in the catchment with limited consideration of sustainability and or conservation (Hoguane *et al.*, 2010; O'Brien *et al.*, 2024). The absence of many of the ecologically important fishes from this study that have local and or international conservation status is concerning (*Chetia brevis*; *Kneria spp.*). Although, other studies have observed these species at different sites (SAIAB). The abundances and relative collection difficulty is still of concern. A concerning four alien invasive species were also collected throughout the study area (*Gambusia affinis*, *Micropterus salmoides*, *Serranochromis robustus*, *Xiphophorus helleri*). Additional invasive species like the common carp (*Cyprinus carpio*) and Silver carp (*Hypophthalmichthys molitrix*) were not collected throughout the study duration although it is known to be present in South Africa and Mozambique.

1. Fish Community Assessments per sub-catchment

The FRAI scores representing the wellbeing of the communities between the different sampling years includes small changes in the ecological state with some ecological decrease at specific sites within the greater Incomati River Catchment (Table 7). The overall ecological wellbeing of the river systems occurs in a moderately modified state (Kleynhans and Louw 2007). The moderately modified state reflects a loss or change to the habitat structure and moderate change to the fish communities, but ecosystem functioning is intact (Kleynhans and Louw 2007). Previous Ecstatus evaluation studies (Roux *et al.*, 2013, 2016; Kleynhans *et al.*, 2015) completed by Mpumalanga Park and Tourism Agency for the Inkomati Catchment Management Agency summarised that all the sites evaluated in the Komati- and Crocodile

River sub-catchments, were overall rated into moderately modified categories, while the Sabie River sub-catchment was rated into a largely modified category. Similar findings were observed in the current study at sites with overlapping locations.

The highest ecological score were observed at the X21J-HEM site during the 2020 sampling event where it was classified into a largely natural condition (80%, Class BC). The condition of this site declined to a moderately modified condition (Class C) during a re-evaluation in 2021 (Table 7). Roux *et al.*, (2013) also evaluated the site and classified it into a moderately modified state (Class C). Improvement in the ecological condition of the site was observed during the 2020 sampling event, while a decline in the ecological category in 2021 was attributed to habitat removal/alterations and water quality deterioration. The lowest ecological score was observed at the X23B-NOO site on the Noordkaap River during the 2022 sampling event where it was classified into a largely modified condition (52.2%, Class D). This classification indicates a large change or loss of natural habitat, biota and basic ecosystem function (Kleynhans and Louw, 2007). The site had low species abundance and richness, with varying stressors occurring. During the Roux *et al.*, (2013) evaluation, this site was classified it into a moderately modified category (Class C) and possible combined degradation occurred between sampling events. Along with the Noordkaap, the Suidkaap (FRAI score - 57.2%), Queens (FRAI score - 61.1%) and Kaap River (FRAI score - 59.1%) systems are important tributaries of the Crocodile River that provides refuge and breeding grounds for smaller and younger fish. However, while these river needs to provide these important functions, they are currently in a moderately to largely modified state. The main stressors affecting the fish communities in these tributary river systems include habitat alteration, water quality degradation (from WWTW) and pollution (Roux *et al.*, 2013).

From available Resource Quality Objectives, the majority of the sites throughout the greater Incomati River Catchment were below the recommended Ecological category for that reach (Department of Water and Sanitation 2016). Concerns should be raised in the Sabie- and Crocodile River sub-catchments as the majority of the required fish ecological classes should be between an Unmodified A-class and a Largely modified B-Class (Department of Water and Sanitation 2016). From this study, sites are classified within a Moderately modified C-Class. This indicates that the river's ecological conditions fail to align with the set environmental and management goals. Failure to address environmental issues that could have let to this reduction can have cascading effects, undermining ecological integrity and human health.

Areas of concern of these FRAI evaluations per site include the lack of robustness/sensitivity of the assessment. For example, some re-evaluated sites had the same ecological outcome score and ranking even though species abundances and richness were different (lower) compared to the initial evaluation (Table 7). Note that miss-use of assessment could be possible and ecological FRAI scores should not be used as the only evaluation of the environment. The FRAI assessment is a rapid assessment method that does not address the population status of any species (Kleynhans *et al.*, 2007).

2. Redundancy analyses evaluation per environmental stressor

The fish communities differed significantly between the different sub-catchments within the greater Incomati River Catchment.

The sampling sites (Intra-river assessment) all differ significantly from one another within each sub-catchment (Appendix - Figure 30; Table 8). Sampling sites all had different habitat variability, water quality alteration or flow alterations due to the altitude, climate, geomorphic composition, rain fall, historic connectivity and more. These environmental conditions allowed for different species to be expected in various areas, however, different anthropogenic stressors also determine if these expected species are still observed in the different areas presently (DWS 2014).

Within the Uanetze River sub-catchment all sites differed significantly with unique species richness and species associations at each site (Appendix - Figure 30A; Table 8). Rivers in this sub-catchment are seasonal and located within the Kruger National Park. Seasonal rivers, especially in KNP are important to provide refuge for smaller fish to escape predators and floods (Henning *et al.*, 2007; Kaiser-Reichel *et al.*, 2024). These seasonal rivers are subjected to an intensified degree of anthropogenic stressors due to the short duration of water flow availability (Datry *et al.*, 2014; Magand *et al.*, 2020).

Most of the sites within the Sabie River sub-catchment differed significantly from one another. Sampled sites in the Sabie ranged from high-mountainous areas with high velocities and substrate diversity to low land sites dominated by sandy substrates and deep-slow flows (Appendix - Figure 30B; Table 8). Sites within the upper Sabie River had high association and abundances to rheophilic species like *Amphilius* and *Chiloglanis* species while the lower Sabie River sites had unique species like *Serranochromis meridianis* (SMER), *Opsaridium peringueyi* (OPER) and *Synodontis zambezensis* (SZAM). The Sabie River is currently considered to be the most pristine river within the greater Incomati River Catchment due to

the high species richness and uniqueness (Riddell *et al.*, 2019). The Sabie River has re-established its species composition from its connection with the Crocodile- and Komati River sub-catchments (Riddell *et al.*, 2019). The Sabie River is also one of the major sub-catchments that had no anguillid eel species present as large dams like Corumana dam on the border of South Africa prevents the migration of anguillid eels into South Africa throughout the Sabie River sub-catchment.

The Crocodile River sub-catchment, eight of the sampled 15 sites differed significantly. The association of fish species towards specific sites are difficult to illustrate due to the high number and clustering of sampling sites and number of fish species observed (Appendix - Figure 30C; Table 8). These eight sites that significantly differed from all the other sites had high abundances of *Micralestes acutidens* (MACU), *Labeobarbus marequensis* (BMAR) and various *Enteromius* species (BVIV, BARG, BPAU). These species have high preferences or requirements for available flow, water quality and regional migration as offered by these sites (Kleynhans and Louw 2007). Three of the significantly different sampling sites were from the Elands River. The Elands River has a high number of endemic fish species, unique habitats, and exclusive environmental drivers (Kleynhans *et al.*, 1992; Ferreira 2006). The Elands River is of high importance to the greater Incomati River Catchment as two critically endangered fish species occur in this catchment (O'Brien *et al.*, 2014). Management in the Elands River allowed for the establishment of a fish sanctuary, the Elands River Yellowfish Conservation Area (ERYCA) and a declared fish sanctuary within the National Freshwater Ecosystem Priority Areas (NFEPA) program (Driver *et al.*, 2011; O'Brien *et al.*, 2014). Two of these significantly different sites in the Crocodile River sub-catchment were also seasonal tributaries (Mbyiamiti and Nsikasi rivers). These rivers are located within the Kruger National Park, away from major anthropogenic stressors. Seasonal rivers like these are very important to provide refuge for protection, recruitment and migration (Kaiser-Reichel *et al.*, 2024). However, these rivers are understudied and little is known about their fish community structure. Kaiser-Reichel *et al.*, (2024) highlighted the importance of these seasonal rivers from a conservation and ecosystem services point of view along with the understanding of better management strategies to protect and manage these seasonal rivers. The Crocodile River sub-catchment has a large number of dams within the catchment primary for irrigation purposes that also affects the flow (Velocity-depth) regime within the catchment (Roux *et al.*, 2013). Long distant migratory anguillid eels species have been collected throughout the Crocodile- and Elands River systems that indicates some connectivity between river reaches.

Four of the 15 sites in the Komati River sub-catchment differed significantly from one another (Appendix - Figure 30D; Table 8). These sites are all located close to one another in the mid-upper reaches of the river and is associated with species like *Labeobarbus polylepis* (BPOL) and *Chiloglanis pretoria* (CPRE). These are cold-water loving species that require fast-flowing habitats and variety of substrates (Kleynhans *et al.*, 2007). These sites had similar habitat descriptions and characteristics. The Komati River sub-catchment, has the most amount of anthropogenic weirs and dams that prevents the migration of species between river reaches (Kleynhans *et al.*, 2015), as observed anguillid eel species are restricted to the lower reaches of the system.

In the Incomati River sub-catchment, two of the three sites were significant and had high associations with alien invasive species (ONIL and GAFF)(Figure 30E). Alien invasive species like *O. niloticus* interbreeds with *O. mossambicus* species reducing the overall species genetic pool (Mashaphu *et al.*, 2024; Mojekwu and Hoareau, 2024). *Oreochromis mossambicus* species are tolerant to changing saline conditions (King and Sardella 2017) which makes them well-adapted for floodplain/estuarine environments.

The yearly sampling and seasonality of sampling differed significantly between the different sub-catchments (Appendix - Figure 31; Appendix - Figure 32; Table 8). Rainy-high flow seasons play a crucial role in facilitating fish movement and spawning events (Winemiller and Jepsen 1998; Poff and Zimmerman 2010). During these journeys many fish species take advantage of connected water bodies to travel between habitats and river reaches (Lucas and Baras 2008). These movement events of fishes allow for easier sampling and more positive collection events of species. Yearly sampling also differed due to availability and access to sample.

Environmental stressors like weir or dam construction, water extraction and land development could affect the velocity and depth within river systems (Thomas and Bovee 1993; Bunn and Arthington 2002). The Velocity-Depth environmental stressor had a significant effect on the distribution of fish species throughout the greater Incomati River Catchment (Appendix - Figure 33A; Table 8). The Incomati and Uanetze River sub-catchments had no significant Velocity-Depth relationship. Sites within these sub-catchments were either too little for the analyses or Velocity-Depth related environmental stressors did not affect the species distribution (Appendix - Figure 33B and F; Table 8). For the Crocodile-, Sabie- and Komati River sub-catchments, most of the fish species had a moderate to high association with the Velocity environmental driver (Appendix - Figure 33C, D, E; Table 8).

The Komati River sub-catchment is the most ecologically stressed due to their high water demands (dams and weirs for ESKOM and agriculture) and number of impoundments (Kleynhans *et al.*, 2015). Velocity in general affect fish communities more than depth, although the impact depends on the species (Shiau *et al.*, 2020). Velocity fluctuations could exhaust fish, especially fingerlings when they are still young (Enders *et al.*, 2003; Pavlov and Savvaitova 2008). Many species also depend on higher flows for feeding, spawning and shelter while low flows could affect oxygenation in the river (Enders *et al.*, 2003; Pavlov and Savvaitova 2008). Environmental cues for fish migration and breeding are also affected with altered flow regimes (Poff *et al.*, 1997). Although not the main contributor to this studies analyses but still significant, depth variability also affects fish communities through limited habitat availability and increased water temperature (Poff and Zimmerman 2010). The study analyses demonstrated that more pool-loving species were associated with the Depth variable while rheophilic species were associated with the Velocity variable throughout the greater Incomati River Catchment.

Water Quality stressors significantly affected the fish communities within the greater Incomati River Catchment with Electrical conductivity and Temperature having the largest effect on the assessment (Appendix - Figure 34; Table 8). Within each sub-catchment, various water quality parameters affected the fish communities. The Sabie- and Incomati River sub-catchments were mostly affected by high water temperature alterations, the Crocodile River sub-catchment were affected by low Dissolved oxygen alterations, the Komati River sub-catchment were affected by high Total Dissolve Salts (TDS), and the Uanetze River sub-catchment was not significantly affected by any water quality parameters (Appendix - Figure 34B; Table 8). Agriculture and Industrial discharge, sedimentation, damming activities, mining activities, nutrient overload and chemical contamination are all the primary cause for water quality alterations throughout the greater Incomati River Catchment (Svobodová *et al.*, 1993; Soko and Gyedu-Ababio 2015; Namugize *et al.*, 2018). The Sabie River sub-catchment is predominantly affected by commercial plantations, smaller towns with poor management, sewerage work and sawmills (Roux *et al.*, 2016). These upstream activities affects the water quality that in turn causes observed water quality issues within the Kruger National Park (Roux *et al.*, 2016). Environmental health issues due to poor management and non-operating development (sewage and domestic waste) needs to be addressed to preserve the condition of the most pristine river sub-catchment in the greater Incomati River Catchment (O'Brien *et al.*, 2018B; Riddell *et al.*, 2019). The Incomati River sub-catchment is affected by rural

communities relying on the water resources for all needs, intense floodplain agriculture as well as already altered water quality received from South Africa (Taucale 2007). The Crocodile River sub-catchment is mainly affected by dam development, agricultural activities, sedimentation, eutrophication and intensive coal mining (Roux *et al.*, 2013). These activities could lead to reduced oxygen levels as excessive sedimentation and eutrophication conditions can cause hypoxic conditions within the system (Baxa *et al.*, 2021). The high association of *Chiloglanis* and *Amphilius* species with high Dissolved oxygen indicate their intolerance levels and preference conditions that these species need in the system (Kleynhans 2007)(Appendix - Figure 34D; Table 8). The Komati River sub-catchment is dominated by high agricultural fields that results in high loads of pesticide, fertilisers and salts within the system (Kleynhans *et al.*, 2015). Supported by this study, high Total Dissolve Salts had a significant effect on the fish communities with species like *Glossogobius giuris*, a high salt tolerant species and 'no-fish' efforts having a high association with TDS (Appendix - Figure 34E; Table 8). Water quality parameters did not significantly affect the fish communities within the Uanetze River sub-catchment (Appendix - Figure 34B; Table 8). This could be due to limited number of sampling sites within the sub-catchment or fish communities are not affected by water quality parameters (*Oreochromis mossambicus*). Although not significant in this study, water quality parameters in seasonal rivers are affected through chemical and nutrient mass imbalances or fluctuating temperatures (Day *et al.*, 2020).

Substrate diversity (silt, mud, sand, gravel, cobble, boulders, bedrock) and Cover features (undercutbank, roots, vegetation, substrate variability, depth) are important material providing habitat, breeding grounds and foraging areas for fish (Allouche 2002). Substrates like gravel, coarse sand, and cobbles are important structures used by rheophilic species like yellowfish during spawning to lay their eggs on, while Cichlid species prefer sandy substrates to make nest for their eggs (Allouche 2002; Kleynhans 2007). Throughout the greater Incomati River Catchment, the Substrate and Cover features had a significant effect on the fish communities (Appendix - Figure 35; Appendix - Figure 36; Table 8). Substrate diversity in the Sabie River sub-catchment was significantly influenced by Sand, Boulder and gravel (Appendix - Figure 35C; Table 8), while the Cover features were influenced by Substrate diversity and roots as habitat (Appendix - Figure 36C; Table 8). Species associated to these substrates include *Chiloglanis* (CPRE, CANO), *Amphiliids* and *Labeobarbus polylepis* (BPOL) species in the upper reaches of the catchment while species like *Chiloglanis swierstrai* (CSWI) were associated to sand substrates in the lower reaches on the catchment

(Appendix - Figure 35C; Table 8). These species, along with other Sabie River specific species (*Labeobarbus nelspruitensis*, VNEL) also had a high association with Substrate diversity as a Cover feature (Appendix - Figure 36C; Table 8). These coarse substrates along with the diversity of different substrates are important to species in the upper reaches of the Sabie River for refuge, feeding and breeding (Kleynhans and Louw 2007; Roux *et al.*, 2016). Sabie River sub-catchment specific species refer to species like *Labeobarbus nelspruitensis* (VNEL), *Chiloglanis anoterus* (VNEL), *Enteromius annectens* (BANN), *Schilbe intermedius* (SINT) and *Synodontis zambezensis* (SZAM) (Skelton *et al.*, 1995; Roux *et al.*, 2016). These species needs their specific preferred substrates and cover features for their survival and continuation of the population and were currently only collected in the Sabie River sub-catchment (Kleynhans 1986). The Crocodile River sub-catchment was influenced by Sand, Boulders and Silt Substrate diversities along with Aquatic vegetation as Cover features (Appendix - Figure 35D; Appendix - Figure 36D; Table 8). Large growing species like anguillid eels (AMOS, AMAR, ABEN), *Hydrocynus vittatus* (HVIT) and Labeo spp (LMOL, LCYL) were associated with Silt, Boulder and Sand Substrates while species like *Glossogobius giuris* (GGUI) were associated with Aquatic vegetation. *Glossogobius guiris* uses vegetation for feeding and hide from large predators (Weliange and Amarasinghe 2007). Large growing species utilises deep areas usually associated by sand, silt and some boulder substrates. What is more important for them is to freely move around in the habitat and be able to ambush prey from vegetated (anguillid eels) or open areas. Substrate diversity and Cover features in the Komati River sub-catchment was significantly influenced by Boulders, Cobbles and Substrate diversity (Appendix - Figure 35E; Appendix - Figure 36E; Table 8). Throughout the Komati River sub-catchment boulders and cobbles where the dominate substrate type. Species like *Tilapia sparrmanii* (TSPA), *Labeobarbus polylepis* (BPOL), *Clarias gariepinus* (CGAR) and *Enteromius spp* were all associated with the boulders and cobbles substrates while the cover feature associations are orientated towards the majority of the cover features (Appendix - Figure 35E; Appendix - Figure 36E; Table 8). The Komati River sub-catchment is characterized by lots of riffle areas and fast flows in the upper reaches. Substrate preferable species were associated with sites and used boulders and cobbles within the riffle areas to feed or escape fast flowing areas for protection from predators. The Substrate diversity and Cover features did not significantly affect the fish communities within the Uanetze- and Incomati River sub-catchments (Appendix - Figure 35B and F; Appendix - Figure 36B and F; Table 8). This could be due to limited sampling

sites within these sub-catchments. Additionally, the Uanetze sub-catchment due to its seasonality, could have seasonal specific cover features available to the fish, E.g., aquatic vegetation is only available during high flow seasons. This constant change in cover features could drive fish to relay on different structures for protection (Sanful *et al.*, 2021).

Conclusion

This study provides an evaluation of the current ecological wellbeing of sites along the greater Incomati River Catchment. It demonstrates the possible effects that environmental drivers have on the fish community composition and the ecological wellbeing of the different river systems. A total of 50 out of the expected 70 fish species were collected with critically endangered species like *Chetia brevis* and *Kneria spp* being absent from this study (Skelton 2001). From a combined present-absent point of view this is not bad, however the per region reach present-absent evaluations were much lower.

Fish communities in the greater Incomati River Catchment are in a moderately stable but altered state with changes in many of the environmental stressors leading to the reduction in fish community compositions. From available Resource Quality Objectives, the majority (>60%) of the sites throughout the greater Incomati River Catchment are below the recommended Ecological category for that reach (Department of Water and Sanitation 2016). When river reaches don't meet the set-out required RQOs, it indicates that the environmental conditions (water quality, flow, habitat) fail to align to the environmental and management goals. These goals support the rivers to provide ecosystem health, human needs and regulatory compliance.

The Incomati River sub-catchment was not significantly affected by velocity-depth requirements nor substrate and cover diversity. What is important is that water quality alterations had a significant effect on the analyses of the Incomati sub-catchment, but more importantly that this sub-catchment is an important linkage between marine and inland riverine environments for anguillid eels. Organised targeting of anguillid eels (O'Brien *et al.*, 2024) should be addressed to conserve these Vulnerable species and allow them the opportunity to migrate inland towards South Africa. The Incomati sub-catchment also had a lot of invasive species and management should be aimed to eradicate them and prevent their spread to new habitats or inbreeding with *O. mossambicus*. The Uanetze River sub-catchment, a seasonal river catchment within the Kruger National Park. Fish communities within the catchment was not significantly affected by velocity-depth requirements, water quality alteration or substrate and cover diversity. Seasonal rivers had different ecological processes and timing of these processes that allows them to be so unique. Although not significant, possibly due to a lack in sampling numbers, environmental stressors within the Uanetze River sub-catchment should not be ignored. This seasonal river provide habitat, refuge and breeding grounds for fish species within KNP. The Sabie-, Crocodile- and Komati

River sub-catchments were all significantly affected by velocity-depth requirements, water quality alteration and substrate-cover diversity. What makes each sub-catchment unique is the effect of these environmental stressors on the fish communities. The Crocodile River sub-catchment is important for its relative connectivity to the Incomati River sub-catchment that allow long distant migrators to inhabit the catchment. The Crocodile River sub-catchment also has the important Elands River Yellowfish Conservation Area (ERYCA) and a declared fish sanctuary within the National Freshwater Ecosystem Priority Areas (NFEPA) program (Driver *et al.*, 2011; O'Brien *et al.*, 2014). It is important to manage the environmental stressors within the Crocodile River sub-catchment to ensure the conservation of these important and biologically unique species. The Sabie River sub-catchment, partially protected within the KNP, is home to many specialised species with a high species richness. Habitat and flow specific species occur within the Sabie River and stressors related to these requirements should be monitored and managed to ensure the continuation of the populations. The Komati River sub-catchment has some connectivity to allow anguillid eels in the lower reaches but high densities of dams and weirs in the middle and upper reaches of the river prevents further penetration. The Komati River sub-catchment also had high abundances of invasive species that possibly escape or populate in channels or stocked off-channel farm dams.

Each sub-catchment is unique to its own environmental drivers that require precise orientated monitoring and management. Monitoring of anthropogenic stressors is important to protect the valuable integrity of the greater Incomati River Catchment and its fish communities. Sites with high sedimentation loads, barrier construction and water abstraction for irrigation needs management and monitoring prioritisation to minimise the chances of reduced flows and substrate availability in the catchment. A recommendation is to start evaluating the purposes of barriers at sites and remove it if the purpose is no longer served. Failure to resolve noncompliance (RQOs) can have far-reaching consequences, jeopardizing both ecological integrity and human welfare. Addressing this will result in multiple positive outcomes as native migratory species will have new habitats, sedimentation will move out (exposing habitats) and water quality parameter will improve (Winter 1990; Hill *et al.*, 1994; Duvail *et al.*, 2017).

The study has demonstrated that the use of multiple Lines of Evidence can inform stakeholders about the different environmental drivers that effect freshwater ecosystems. These methods can be used globally on large catchments or river systems to inform

governmental bodies about the different environmental drivers causing threat to that fish community. Early detection or response to these environmental drivers will not only aid the fish communities' wellbeing but will ensure that the ecosystem services provided by these fish communities are still in place to benefit from.

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Chapter 4

An ecological risk assessment of multiple anthropogenic stressors affecting the fish communities in the Incomati River Catchment, southern Africa

Freshwater ecosystems worldwide are increasingly threatened by anthropogenic activities, leading to habitat degradation and biodiversity loss. This study presents a regional-scale ecological risk assessment of multiple human-induced stressors impacting fish communities in the greater Incomati River Catchment, Southern Africa. By analysing key environmental stressors, including flow alteration, habitat modification, water abstraction, invasive species and pollution, their cumulative impact on fish communities and ecosystem function were addressed. Findings indicate that continued environmental stressors may significantly alter fish community structures, with potential cascading effects on ecosystem services. For the ecological fish wellbeing, the risk regions (sub-catchments) within the greater Incomati River Catchment are all in a low to moderate-risk distribution signifying that ecosystem functioning is on the threshold for possible failure. For the subsistence fish wellbeing, the risk regions (sub-catchments) within the greater Incomati River Catchment are all in a zero to moderate risk distribution, signifying possible failure of the ecosystem in some regions to provide for the demands of human needs. The Komati River sub-catchment is the most stressed region within the greater Incomati River Catchment. The study underscores the necessity of sustainable water management strategies and conservation interventions to mitigate risks and maintain ecological integrity. Ensuring connectivity and resilience within freshwater systems is critical to preserving biodiversity and securing long-term water resources for both ecological and human needs. This assessment provides a foundation for future research and policy development aimed at sustainable river catchment management in the region.

Keywords: Bayesian Network Assessment, Drivers of change, Fisheries

Introduction

Globally water resources are under threat due to growing anthropogenic land transformation, water usage and environmental neglect (Dudgeon *et al.*, 2006; Fischlin *et al.*, 2007; Thieme *et al.*, 2024). Ecosystems are under strain to keep up with the constant demand to supply human communities with services vital to their survival and comfort. Degradation of freshwater systems at the current rate may cause two-thirds of the human population to face severe water shortages and water stress as soon as 2030 (Karr 1999; Saunders *et al.*, 2002; Kuehne *et al.*, 2023). It is imperative that ecological functionality be preserved to conserve ecosystem services, and one of the best ways to achieve this is to ensure that ecosystems remain connected and maintained (Ward and Stanford 1995; Silva *et al.*, 2018; O'Brien *et al.*, 2019).

The National Water Act (No. 36 of 1998) (NWA) of South Africa makes provision for basic human water resources needs, as well as an ecological reserve flow for all the major river systems in the country (RSA 1998; Nkondo *et al.*, 2012). The NWA and the South African National Water Resources Strategy aim to adopt an equitable balance between the use and protection of ecosystems through the Integrated Water Resource Management (IWRM) framework (DWA 2004; van Koppen and Schreiner 2014). Through process and implementation, the IWRM aims to establish and maintain the integrity and condition of water resources in South Africa (DWA 2004). Relative to South Africa, water resource management in Mozambique and Eswatini are limited, limiting management in the southern Africa region.

The greater Incomati River Catchment is a semi-arid transboundary catchment located in the south-eastern region of South Africa, northern parts of Eswatini and southern parts of Mozambique. The water resources of the greater Incomati River Catchment, are socio-ecologically important and support the livelihoods of >2.5 million South Africans and >1 million Mozambicans (Anderson 2005; Schreiner *et al.*, 2011). The main rivers (Uanetze, Sabie, Crocodile, Komati and Incomati) descend from Highveld plateaus and flow east to the coastal plains of Mozambique. These main rivers contribute 94% of the total discharge in the catchment. The water resources of the greater Incomati River Catchment are being used excessively, and their condition is still deteriorating (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2019). Growing agriculture, forestry and urbanisation, are the main drivers of increased water scarcity and intensive land cover transformations are accelerating the degradation of the catchments aquatic and terrestrial ecosystems (Saraiva Okello *et al.*, 2015; O'Brien *et al.*,

2019, 2024). The greater Incomati River Catchment is a biodiversity hotspot for the region with many intact ecosystems, primarily in the Kruger National Park (Riddell *et al.*, 2019). The greater Incomati River Catchment supports an estimated 70 fish species of which several have conservation importance (Darwall *et al.*, 2011; Kleynhans *et al.*, 2015). Despite the presence of several protected and conservation areas, fish communities in the greater Incomati River Catchment remain largely understudied (Rahel and Magnuson 1983; Jackson *et al.*, 2001; O'Brien *et al.*, 2024). This knowledge gap is particularly concerning because multiple environmental and anthropogenic drivers—such as flow alterations, pollution, habitat degradation, and land-use changes can alter ecosystem structure and function. Without comprehensive data on fish populations, it is difficult to assess the full impact of these stressors or to implement effective conservation and management strategies (Nelson 2005). Multiple stressors can act simultaneously to alter ecosystem functioning and to understand their interactive effects, relative risk assessments can be used as part of adaptive management strategies (Govender *et al.*, 2021).

The “*Ecological Risk Assessment Guidelines*” promote risk assessments as a process that evaluates the likelihood that adverse ecological effects occur or will occur due to the exposure of one or more stressors to an endpoint (Claassen *et al.*, 2001). Ecological risk assessment approaches have been established in South Africa from the early 2000s and include major developments by the Claassen *et al.*, (2001), O'Brien and Wepener, (2012) and Govender *et al.*, O'Brien (2021). Modern ecological risk assessment approaches incorporate the use of Bayesian Network (BN) Probability modelling approaches (Govender *et al.*, 2021). In South Africa these BN approaches are receiving broad attention as they have been considered to be valuable to the IWRM of South Africa (Castelletti and Soncini-Sessa 2007; Barton *et al.*, 2008; O'Brien *et al.*, 2019; O'Brien *et al.*, 2021). The incorporation of BNs in ecological risk assessments to evaluate the probable effect of multiple stressors has been formalised globally and applied in South Africa (O'Brien *et al.*, 2019; 2021). Ecological risk assessments demonstrate how available evidence describes sources resulting in stressors, affect multiple receptors in the content of the social and ecological attributes or endpoints (Govender *et al.*, 2021). The specific endpoint in a risk assessment represents the relative risk or impact associated with a particular objective (Landis and Wiegiers 2005). Multiple ecosystem response components and multiple lines of evidence (LoE) have been included in risk assessments to evaluate the effect of multiple water quality, quantity, habitat and other stressors on the biotic community (Vezi *et al.*, 2019; O'Brien *et al.*, 2021).

Freshwater fish provide a holistic view of complex systems as they integrate functionality across trophic levels (Whitfield and Elliott 2002). Their taxonomy and ecologies have been well described and the ease of identification allow fish to be used as ecological indicators (Fausch *et al.*, 1990). Fish communities have been shown to respond predictably to almost all known anthropogenic disturbances (Poikane *et al.*, 2017; Pinna *et al.*, 2023). In particular, the responses of fish communities in a freshwater ecosystem can reflect the amount of degradation that specific ecosystems has undergone (Das and Chakrabarty 2007, Zhao *et al.*, 2016).

The aim of this study is to use a Relative Risk Model incorporating Bayesian Networks (RRM-BN), to evaluate the effects multiple stressors have on fish communities of the greater Incomati River Catchment as indicators of ecological wellbeing and subsistence fish as an indicator of the human livelihood component of the catchment. This study incorporates source-stressor and stressor-receptor relationships to contribute to the determination of a sustainable balance between water resources. Risk assessment developments that provide an understanding of the socio-ecological consequences of multiple stressors for water resources and support evidence-based trade-off decisions will make a significant contribution to the sustainable management of this water resource (O'Brien *et al.*, 2019).

Materials and Methods

Study area and data collection

The greater Incomati River Catchment is located in the south-eastern part of South Africa and occupies portions of Eswatini, Mozambique and South Africa. Thirty-nine sites were selected throughout the greater Incomati River Catchment (Table 9; Figure 10). The sites were selected based on the availability of historical data (previous studies conducted at the same site), and included sites on the mainstem rivers and tributaries, of the Uanetze-, Sabie-, Crocodile, Komati- and Incomati River sub-catchments. Limited sampling sites were sampled throughout Mozambique and Eswatini due to access restrictions, sampling safety in areas and the availability of historical data. Although this poses as a limitation to the study, some data needed to be included to validate the study area as the Greater Incomati Catchment and not only the Inkomati Catchment. The sampling sites selected had various available habitat characteristics and structures utilised by fish species.

Biological and environmental data collection occurred during the low flow and high flow seasons of May 2020 to October 2023. All necessary ethical clearances and country permits were obtained to ensure responsible and compliant conduct throughout the study area. The ethical clearance number was obtained from the University of Mpumalanga (UMP/O'Brien/2/2023), provincial research permits were obtained from Mpumalanga Park and Tourism (MPB 5789) which allowed sampling and collection within Mpumalanga, South Africa. Sampling within transboundary countries (Mozambique and Eswatini) was allowed following the approval and allocation of an invitation letter and sampling letter. All local community chiefs and tribe leaders were informed of our whereabouts and permission letters were presented to them before sampling commenced.

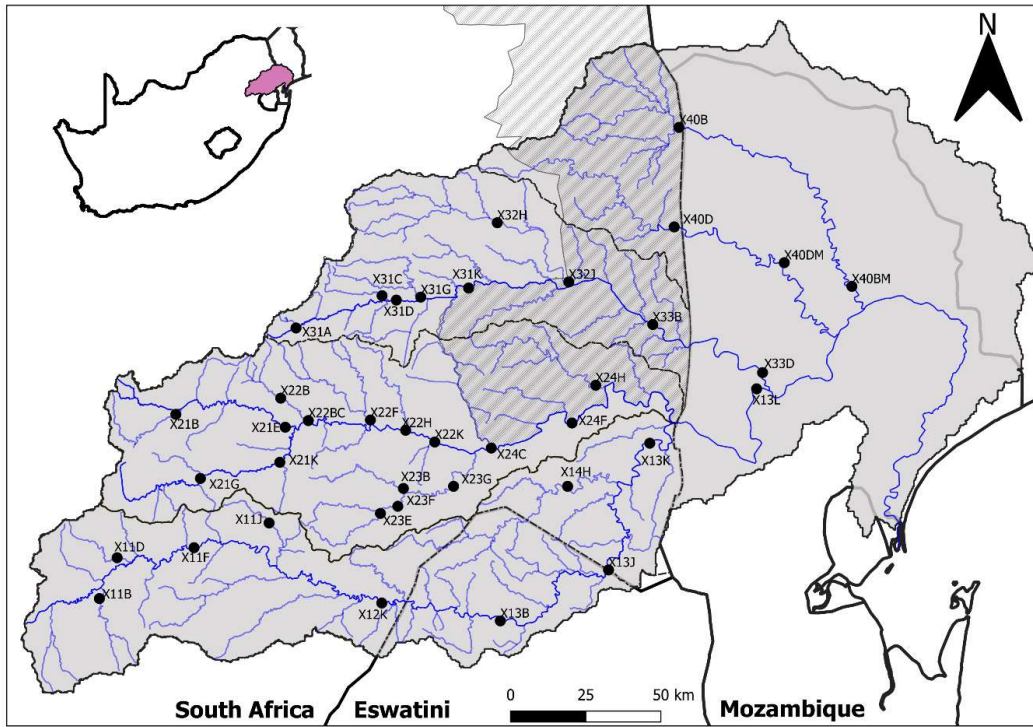


Table 9: The sampling locations (code names) per river system sampled between 2020 and 2023.

SITE CODE (NAME)	RIVER	DESCRIPTION	LATITUDE	LONGITUDE
X40B Uanetze	Uanetze	Uanetze KNP seasonal	-24.451542	31.994502
X40BM Uanetze	Uanetze	Uanetze in Mozambique	-24.982093	32.571741
X40D Massintonto	Massintonto	Massintonto KNP seasonal	-24.78377	31.978932
X40DM Massintonto	Massintonto	Massintonto in Mozambique	-24.903239	32.346172
X31A Sabie	Sabie	Sabie River at Merry pebbles	-25.121	30.717
X31C Mac-Mac	Mac-Mac	MacMac/Mac Mac/4	-25.01333	31.00405
X31D Sabie	Sabie	2Sabie at EWR-S	-25.02792	31.05166
X31G Marite	Marite	Marite/Marite/5	-25.01795	31.13328
X31K Sabie	Sabie	Sabie/KNP/3	-24.98759	31.29287
X32H Sand	Sand	Sand River at Exeter	-24.769709	31.388545
X32J Sand	Sand	Sand at EWR-S8	-24.96742	31.62734
X33B Sabie	Sabie	Sabie River at Lower Sabie	-25.109384	31.907326
X33D Sabie	Sabie	Sabie River in Mozambique	-25.269875	32.273776
X21B Crocodile	Crocodile	Crocodile at EWR-C2	-25.40925	30.31592
X21E Crocodile	Crocodile	Crocodile at EWR-C3	-25.45211	30.68108
X21G Elands	Elands	Elands River at Nsinini	-25.623031	30.397948
X21K Elands	Elands	Elands River at Roodewal	-25.569221	30.66293
X22B Houtbosloop	Houtbosloop	Houtbosloop	-25.35516	30.66591
X22BC Crocodile	Crocodile	Rivulets	-25.430069	30.75796

X22F Nels	Nels	Nels at UMP	-25.428142	30.965178
X22H White River	White River	Lower White River	-25.462396	31.082854
X22K Crocodile	Crocodile	Crocodile at Kanyamazane	-25.501687	31.18005
X23B Noordkaap	Noordkaap	Noordkaap	-25.656522	31.075219
X23E Queens	Queens	Queens	-25.73986	30.998596
X23F Suidkaap	Suidkaap	Suidkaap	-25.716048	31.056677
X23G Kaap	Kaap	Kaap River at Honeybird	-25.649406	31.24264
X24C Nsikazi	Nsikazi	Nsikazi	-25.521647	31.368499
X24F Crocodile	Crocodile	Crocodile at Van Graan	-25.438058	31.637947
X24H Mbyamiti	Mbyamiti	Mbyamiti	-25.312103	31.716999
X11B Boesmanspruit	Boesmanspruit	Boesmanspruit	-26.024106	30.060851
X11D Klien Komati	Klien Komati	Klein Komati	-25.887745	30.120044
X11F Komati	Komati	Komati at EWR-K1	-25.85421	30.37664
X11J Gladdespruit	Gladdespruit	Gladdespruit	-25.77172	30.62717
X12K Komati	Komati	Komati at EWR-K2	-26.03881	31.00314
X13B Komati	Komati	Komati River at Silingani	-26.09839	31.39875
X13J Komati	Komati	Komati weir below Eswatini	-25.928497	31.759773
X13K Komati	Komati	Lower Komati River	-25.505497	31.897398
X14H Lomati	Lomati	Lomati River Kleindoringkop	-25.64945	31.6232
X13L Incomati	Incomati	Incomati in Mozambique	-25.324015	32.253866

Fish community and ecosystem evaluation

Fish Community sampling: Standard instream biotopes within each site were effectively sampled using a range of sampling methods (see Methods section in Chapter 3). Biotopes were selected according to the habitat flow (fast, medium, slow), depth (deep, medium, shallow), substrate type (boulders, cobble, sand, gravel, bedrock) and habitat cover features (overhanging/aquatic/marginal vegetation, undercut bank, roots or substrate diversity) (Rowntree *et al.*, 2000). Fish were identified on site to species level (Scott *et al.*, 2006; Skelton 2024), standard length were recorded, any deformities were noted and fish were returned unharmed back to the river.

Ecosystem Evaluation: Habitat characteristics and associated environmental variables were collected for each effort/biotope at each site. Multiple metrics were used to describe/represent habitat characteristics including depth and velocity ranges per biotope/effort, percentage cover of substrate types and a rating system used to evaluate the availability of and suitability of cover characteristics (Koizumi and Matsumiya 1997; Vallès and Oxenford 2015). Three depth and velocity measurements were collected within each biotope. A transparent velocity head rod (TVHR, GroundTruth) was used to determine the depth and flow velocities along with the discharge (Le Coz *et al.*, 2024). A calibrated EUTECH PCD 650 water quality meter was used to document *in situ* water quality measurements. This included the pH, electrical conductivity, temperature, and dissolved oxygen concentrations. A sub-surface water sample was collected at each site and analysed at the University of North-West (South Africa) for metal and salt analyses.

Regional ecological risk assessment approach

The methodology for the regional scale ecological risk assessment includes the well-established Relative Risk Model (RRM) from the Regional-scale Ecological Risk Assessment framework established by Wiegiers *et al.*, (1998) and optimised for application in Africa by O'Brien and Wepener (2012); Vezi *et al.*, (2019) and O'Brien *et al.*, (2021). The RRM approach incorporates the use of both Bayesian Networks (BNs) and Monte Carlo randomisation procedures that conform to current best international practice (Van Den Brink *et al.*, 2016). This regional scale ecological risk assessment approach is carried out on a regional spatial scale and allows for the consideration of multiple sources of multiple stressors affecting multiple endpoints, with the inclusion of local ecosystem dynamics and the

characteristics of the landscape that may affect the risk estimate. The 10 steps of the RRM are (Chen and Landis 2005):

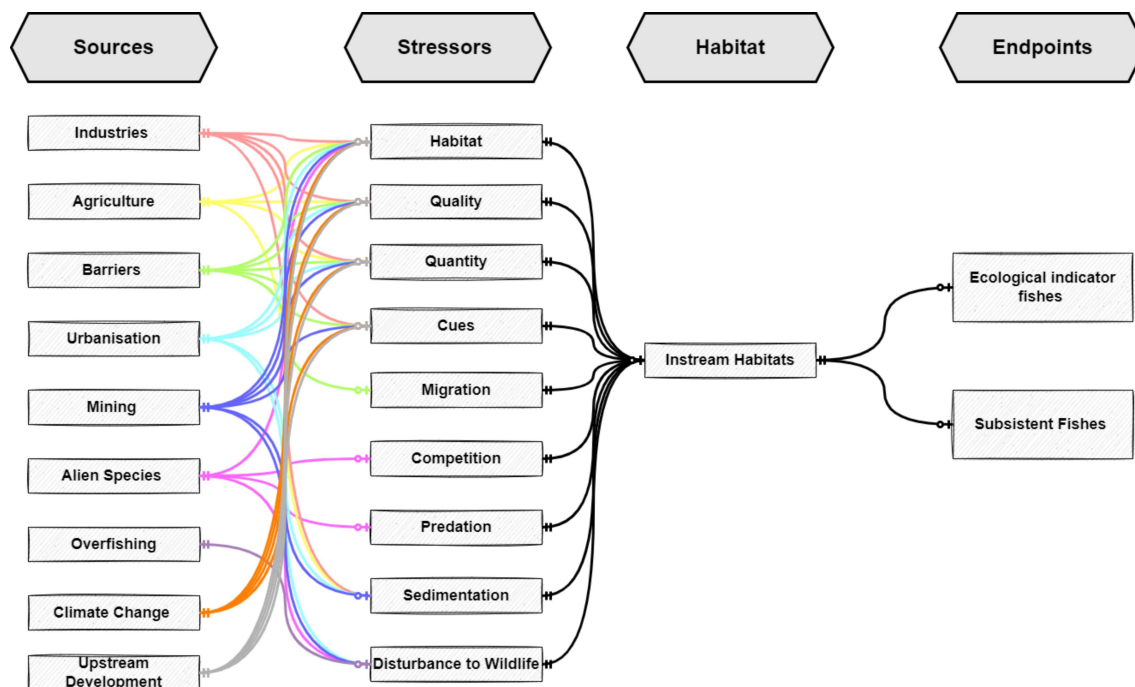
List the important management goals for the region.

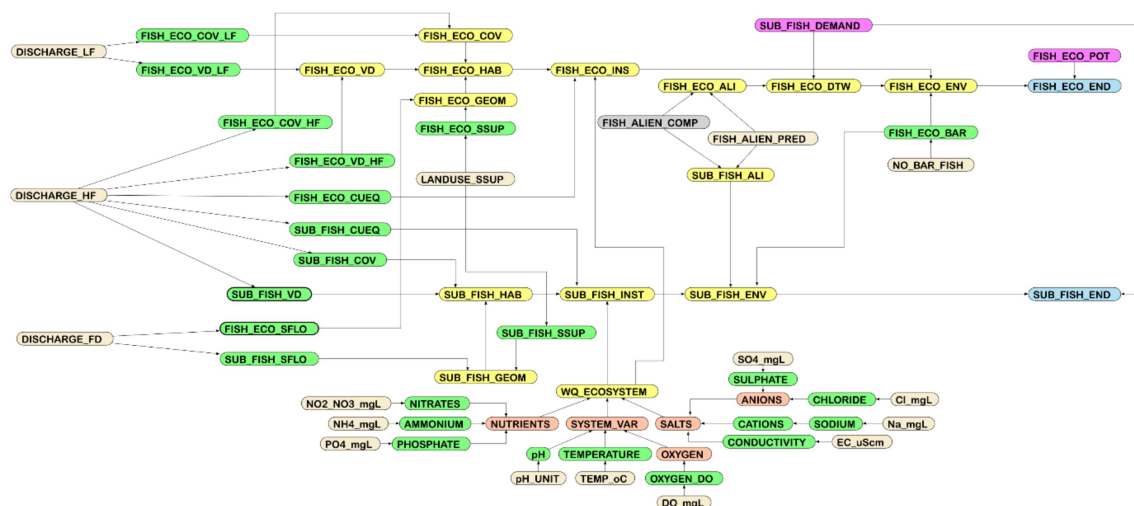
1. Generate a map on which the potential sources and habitats relevant to the established management goals are indicated.
2. Demarcate the map into risk regions based on a combination of the management goals, sources, and habitats.
3. Construct conceptual models from available information that represent probable risk pathways between sources, stressors and receptors in a range of habitats to the ecological and/or social endpoints selected for the study.
4. Decide on a ranking scheme to calculate the relative risk to the assessment endpoints. Ranking schemes are used to represent the state of variables, with unique measures and units to be comparable as non-dimensional ranks and combined in BN-RRMs. The outputs from these first five steps will be used to generate a holistic risk assessment framework for the study using Microsoft Excel and the freeware NeticaTM BN software by Norsys Software (Version 6.09 – <http://www.norsys.com>) .
5. Calculate the relative risks (Zero, Low, Moderate, High).
6. Evaluate uncertainty and sensitivity analysis of the relative rankings. All aspects of uncertainty associated with the entire application of the RRM-BN approach for the study including objectives and endpoint selection for the assessment, availability and use of evidence, expert solicitations, and model uncertainty for example, will be addressed.
7. Generate testable hypotheses for future field and laboratory investigations to reduce uncertainties and to confirm the risk rankings. The preliminary risk assessment will result in a range of hypotheses that need to be tested to reduce the uncertainty associated with preliminary assessments. The hypotheses will include a list of research questions that will guide future field surveys and adaptive management activities.
8. Test the hypotheses that were generated in Step 7.
9. Communicate the results in a fashion that effectively portrays the relative risk and uncertainty in response to the management goals.
10. The regional ecological risk assessment approach will focus on describing the cause-and-effect relationships of multiple source (focusing on water resource users),

stressor, ecosystem/habitat relationships and how these interactions may affect stakeholders' concerns. With the risk assessment model and the social requirements for ecosystem services in the study area, the relative socio-ecological consequences of alternative water resource use can be evaluated. This is achieved using the Monte Carlo/Latin Hypercube permutation processes (Keramat and Kielbasa 1999). This approach is used to combine assigned probability risk distributions for multiple endpoints. When compared, the risk to each social and ecological feature, and the integrated social and ecological endpoints can be evaluated in a relative manner.

Conceptual model

The conceptual model was developed for the study to represent the understanding of the cause-effect linkages for the risk component (Figure 11) (Wiegers *et al.*, 1998; Ayre and Landis 2012). The model is represented as a BN (Figure 12) and acts as a framework for the Regional Risk Model. The primary focus of the conceptual model is to establish relationships between the sources and the stressors affecting the different habitats and endpoints. These relationships are then represented in a BN model (Figure 12) using the Norsys Netica software.





The causal BN model developed for this assessment includes a range of nodes that represent indicator components of the socio-ecological system, linked by arrows that demonstrate causal relationships between variables (Govender *et al.*, 2021; Adams *et al.*, 2023). The models are causal and from the left-hand side to the right-hand side input environmental variability is interpreted and integrated to represent how the endpoints respond to changes. To construct the BN and risk assessment for each site, Microsoft Excel (Microsoft Corporation, <https://office.microsoft.com/excel>) and Netica (Norsys Software, <http://www.norsys.com/>) were used.

Ranking system

A ranking scheme was incorporated for each node to calculate the relative risk to each specific endpoint. Data are converted to non-dimensional ranks in order to measure and compare the effects of different stressors on the endpoints (Landis and Wiegerts 2005). Ranks are classified as either zero, low, moderate, or high and describe the wellbeing condition or severity of impact for the respective nodes and endpoints (Table 10) (Colnar and Landis 2007; O'Brien and Wepener 2012; Hines and Landis 2014). The ranking scheme is assigned relative risk values of 0.25, 0.50, 0.75 and 1.00 respectively (O'Brien *et al.*, 2018). Each variable at each site is assessed and assigned a relative risk rank, with each rank having its own relative risk score (e.g., if habitat modification is ranked as low, its relative risk scores will be oriented towards a low rank with some uncertainty towards other ranks). Collected data, expert opinions and Google Earth imagery were used as far as possible to validate the risk ranks of the different nodes for each site (Appendix Data 4).

Table 10: Rank scores and descriptions used to rank the different nodes. Adapted from Wade *et al.*, 2020.

Rank	Description
Zero	Node is in a pristine condition and no impact or risk (less than 25%) is present compared to pre-anthropogenic establishment or reference conditions.
Low	Node is in a largely natural condition and low impact or risk (25 – 50%) is present compared to pre-anthropogenic establishment or reference conditions. Ecosystem is functioning sustainably.
Moderate	Node is in a moderate modified condition and moderate impact or risk (50 – 75%) is present compared to pre-anthropogenic establishment or reference conditions. Ecosystem functioning is on the threshold for possible failure.
High	Node is in a significantly altered condition and high impact or risk (75 – 100%) is present compared to pre-anthropogenic establishment or reference conditions. Ecosystem functioning is at failure threshold. Ecosystem can sustain functioning.

Stressors and sources

Various sources and stressors including present *in situ* habitat characteristics, water quality parameter, flow measurements and historically modified habitats, were used in this study to assess the impact of these drivers on the respective endpoints. Sources and stressors were used as input points in the BN in turn affecting the different variable receptors (Appendix Data 4).

Vision and Endpoints

The target or condition of a river needs to be aligned to the desired balance between the use and protection of water resources, or the “vision” of the resource. On a regional scale, to achieve an overall sustainable balance between the use and protection of water resources some areas may be allocated for “high but sustainable” use and other areas may be assigned a “low use and protect” scenario, where together at a regional scale allow sufficient protection of the resource for regional sustainability (DWAF 2007; Horne *et al.*, 2017). The endpoints for the study included ecological and social attributes (Table 11) to indicate the risk to fish communities at each site (Appendix Data 4).

Table 11: Study endpoints and network variable name with node descriptions.

Endpoint	Description
Ecological indicator fish (FISH_ECO_END)	Endpoint represents fish community wellbeing as an ecological indicator of ecosystem wellbeing. Endpoint is a function of potential for (1) ecologically important and sensitive fishes and (2) the physical environment for fishes.
Subsistence indicator fish (SUB_FISH_END)	Endpoint represents subsistence fish community wellbeing as an indicator of ecological wellbeing. Endpoint is a function of potential for (1) the physical environment for fishes targeted for subsistence usage and (2) the degree of demand that threatens subsistence fish.

Uncertainty

The BN accounts for some uncertainty that may influence the overall outcome for each endpoint. The Netica software has a “Sensitivity to findings” tool that is used to evaluate the outcomes of the individual variables in addition to a Monto Carlo randomization tool that integrate the effect of uncertainty (O’Brien *et al.*, 2018). When there was uncertainty regarding the confidence of the data for different input nodes for specific sites, the risk rank was assigned a zero or low rank in order not to overcompensate for the uncertainty.

Results

Bayesian model Endpoints

The risk for each endpoint in this study was determined individually for each site in the different sub-catchments and then compared to one another by considering their maximum and minimum ranges determined for the RRM.

The natural (NAT) scenario, resulted in risk scores for the ecological fish endpoint ranging from 23.47 to 49.53, with the lowest risk score recorded for the sites X31A (Sabie River at Merry pebbles) and X12K (Komati at EWR-K2) and highest risk scores recorded for the sites X40B (Uanetze SA) and X24H (Mbyamiti). All the sites were dominated by a zero to low-risk percentage distribution for the NAT scenario for the ecological indicator endpoint. For the NAT scenario for the subsistence fish endpoint, the risk scores ranged from 13.94 to 29.61, with the lowest risk score recorded at the site X33B (Sabie River at Lower Sabie) and the highest risk score recorded for sites X24H (Mbyamiti). All the sites were dominated by a zero to low-risk percentage distribution for the NAT scenario for the subsistence fish endpoint.

For the present (PRS) scenario, the risk scores for the ecological fish endpoint ranged from 34.83 to 70.94, with the lowest risk score recorded for the sites X33B (Sabie River at Lower Sabie) and X33D (Sabie River in Mozambique) and the sites X11D (Komati River at Klein Komati) and X11J (Gladdespruit) recorded the highest risk scores. All the sites were dominated by a low to moderate-risk percentage distribution for the ecological indicator endpoint for the PRS scenario. The risk scores for the subsistence fish endpoint for the PRS scenario ranged from 15.45 to 73.52, with the lowest risk at the sites X31A (Sabie River at Merry pebbles) and X24F (Crocodile River at Van Graan) and the sites X14H (Lomati River at Kleindoringkop) and X13K (Lower Komati River) recording the highest risk scores. For the PRS subsistence fish endpoint, all sites were dominated by a low to moderate risk percentage distribution.

The results for the Uanetze River sub-catchment indicated that the highest risk scores for the PRS scenario for both endpoints was observed at the X40BM (Uanetze MOZ)(PRS) site (Figure 13), while the risk percentage was dominated by a moderate to high-risk for the ecological fish endpoint and a zero-risk percentage for the subsistence fish endpoint (Figure 14B).

For the Sabie River sub-catchment, the highest risk scores for the PRS ecological fish endpoint was observed at the X32H (Sand River at Exeter) site (Figure 13A), while the risk

percentage was dominated by a low to moderate risk percentages (Figure 13B). For the Sabie River sub-catchment, the highest risk scores for the PRS subsistence fisheries endpoint was observed at the X33D (Sabie River in MOZ)(PES) site (Figure 14A), while the risk percentage was dominated by a zero and high-risk percentage (Figure 14B).

The highest risk scores for the Crocodile River sub-catchment, for the PRS ecological fish endpoint was observed at the X22H (Lower White River) site (Figure 15A), while the risk percentage was dominated by a moderate to high-risk percentages (Figure 15B). For the Crocodile River sub-catchment, the highest risk scores for the PRS subsistence fisheries endpoint was observed at the X23G (Kaap River)(PES) site (Figure 16A), while the risk percentage was dominated by a moderate to high-risk percentage (Figure 16B).

For the Komati River sub-catchment, the highest risk scores for the PRS ecological fish endpoint was observed at the X11D (Komati River at Klein Komati) site (Figure 17A), while the risk percentage was dominated by a moderate to high-risk percentages (Figure 17B). For the Komati River sub-catchment, the highest risk scores for the PRS subsistence fish endpoint was observed at the X14H (Kleindoringkop)(PES) site (Figure 18A), while the risk percentage was dominated by a moderate to high-risk percentage (Figure 18B).

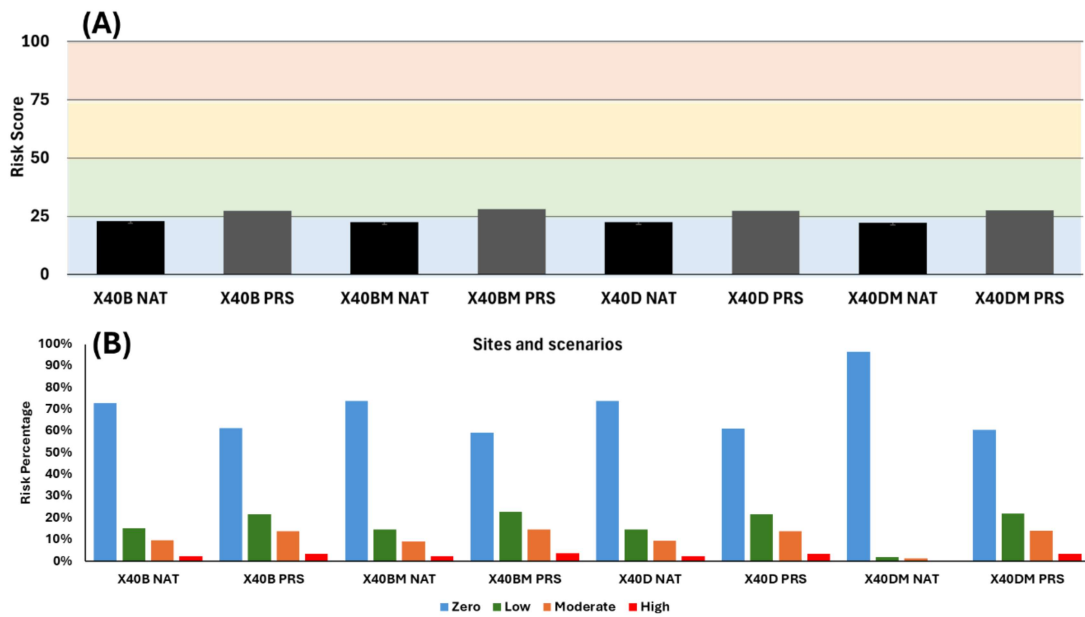
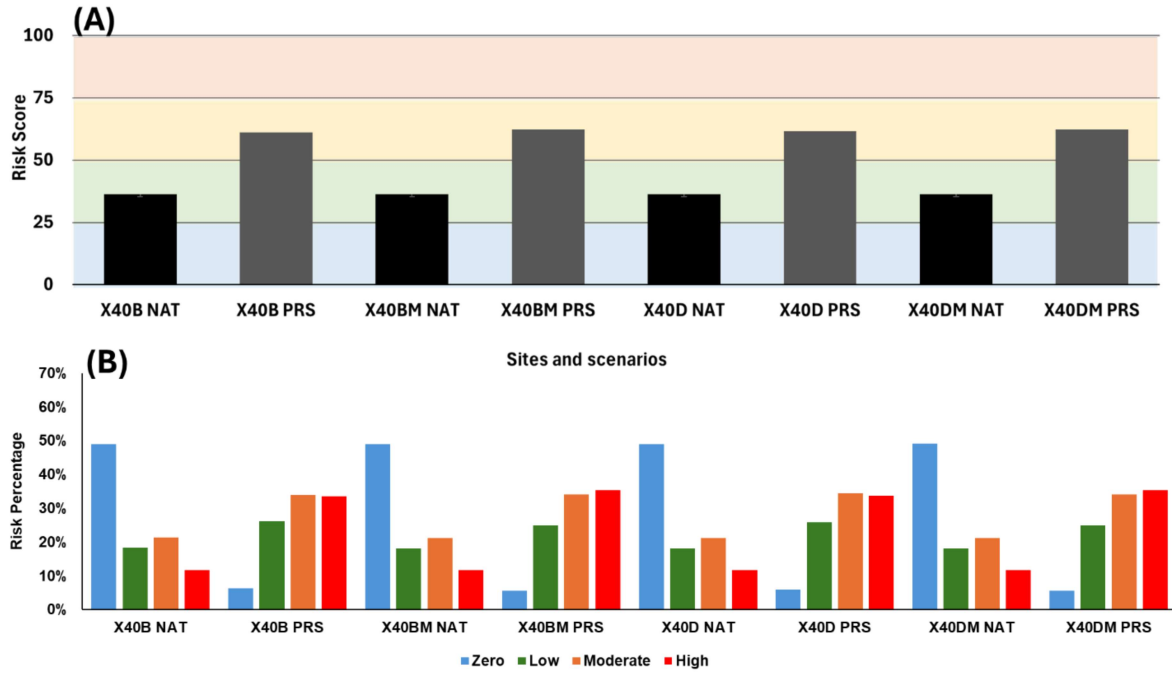
For the Incomati River sub-catchment, only one site was assessed, the X13L (Incomati Mozambique) (Figure 19 & Figure 20). The risk percentage was dominated by a zero to low-risk percentage for both the ecological and subsistence fish endpoint (Figure 19 & Figure 20).

Uncertainty analyses

The sensitivity analyses for the two endpoint nodes demonstrate that the condition of the environment had the greatest influence on the ecological fish endpoint (Table 12), while the demand for Subsistence fish affected the Subsistence fish endpoint the most (Table 12). The influence of barrier stressors had the second most influence on both the ecological influence and Subsistence fisheries endpoints.

Table 12: The percentage sensitivity obtained from the different stressor nodes for the ecological fish and subsistence fish endpoints.

Node	Ecological endpoint	Subsistence endpoint
Ecological Fish Environment	60.9	0.16
Ecological Barrier influences	20.7	0.36
Subsistence fish demand	0	51.7



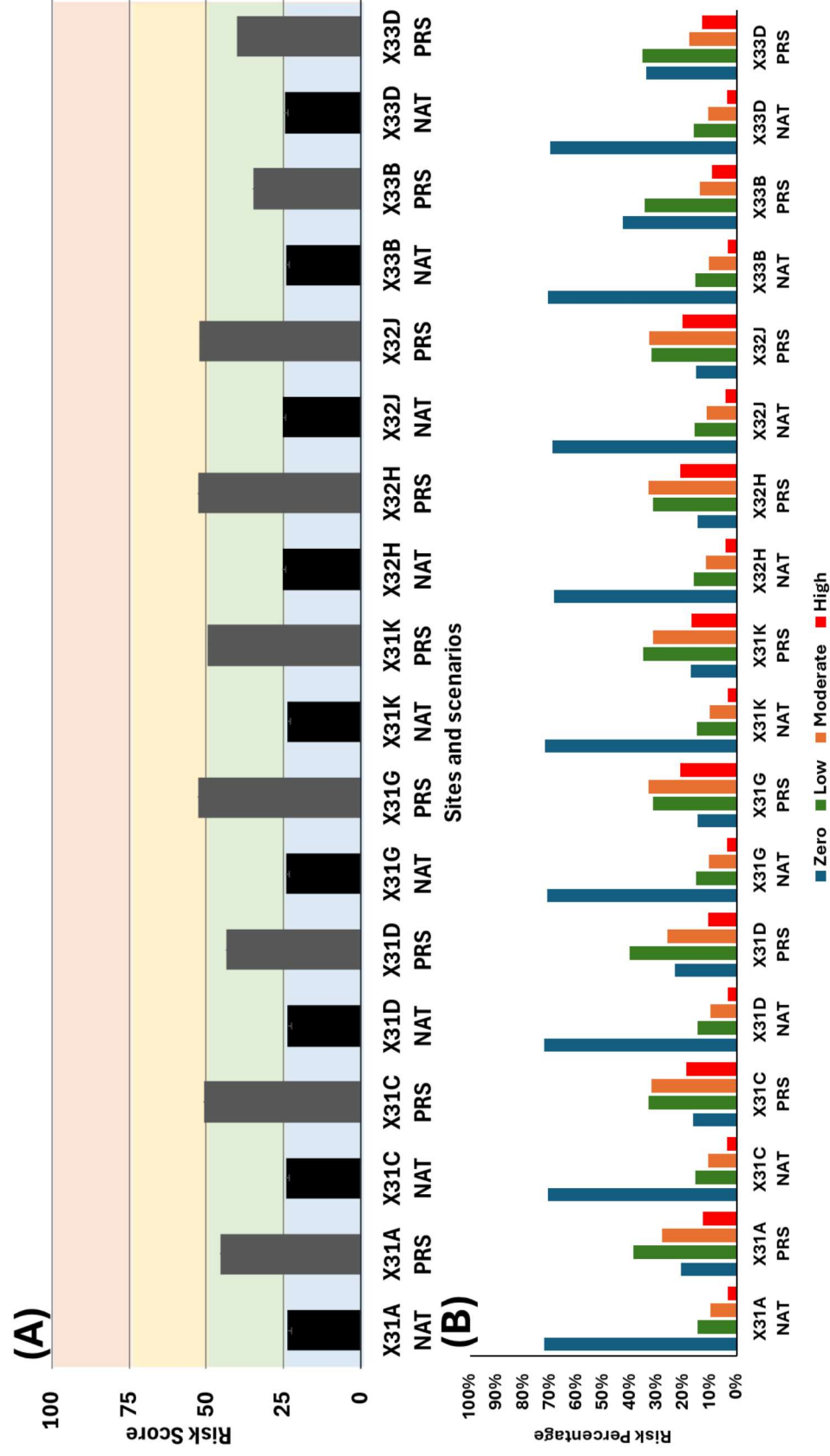


Figure 13: The Ecological indicator endpoint with the A) average relative risk scores and B) risk percentage for the hypothetical natural (NAT) and present (PRS) scenarios for the different sites in the Sabie River sub-catchment. The background colours and bars indicating the different risk ranks with Blue = Zero risk, Green = Low risk, Orange = Moderate risk and Red = High risk.

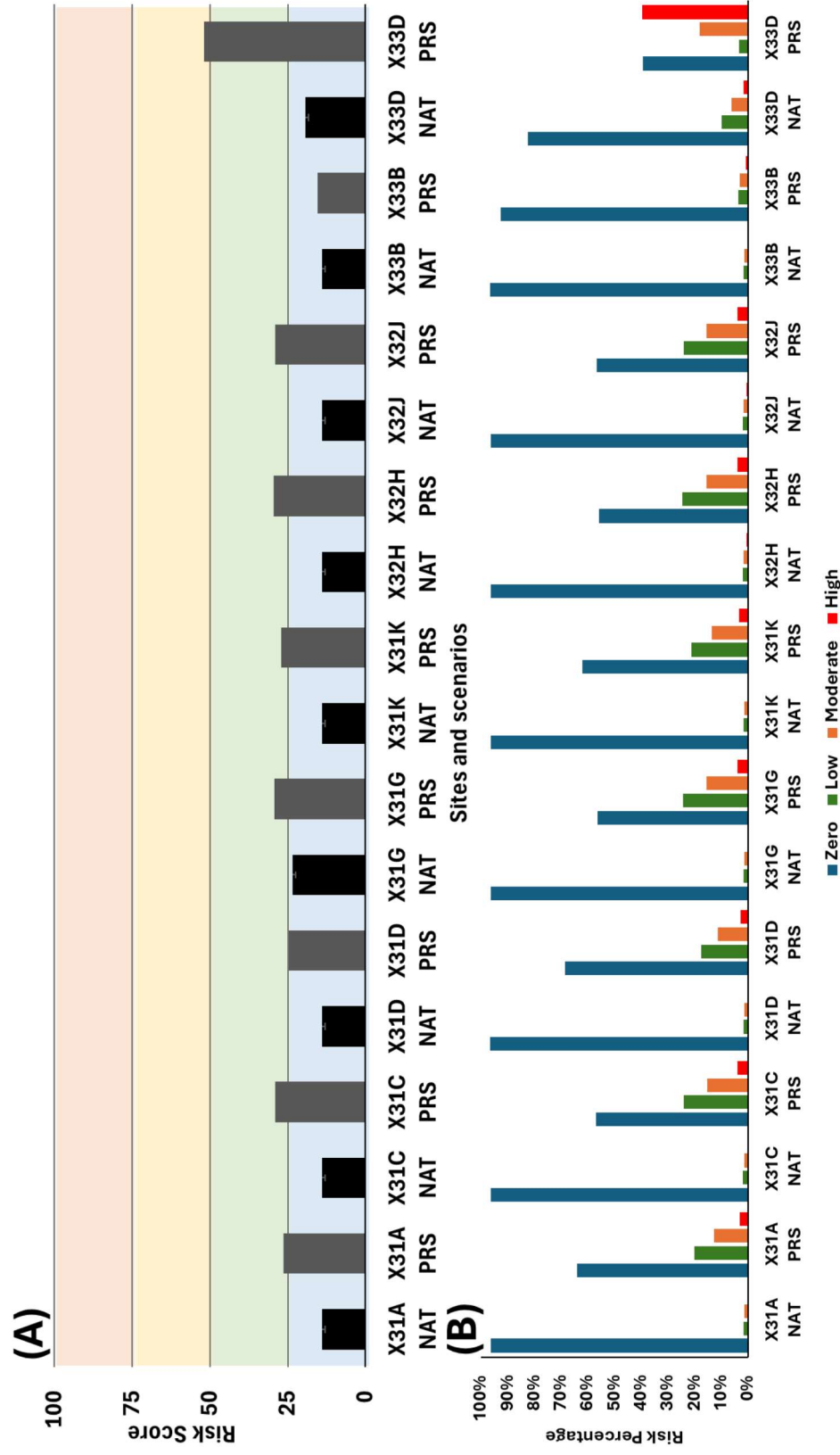
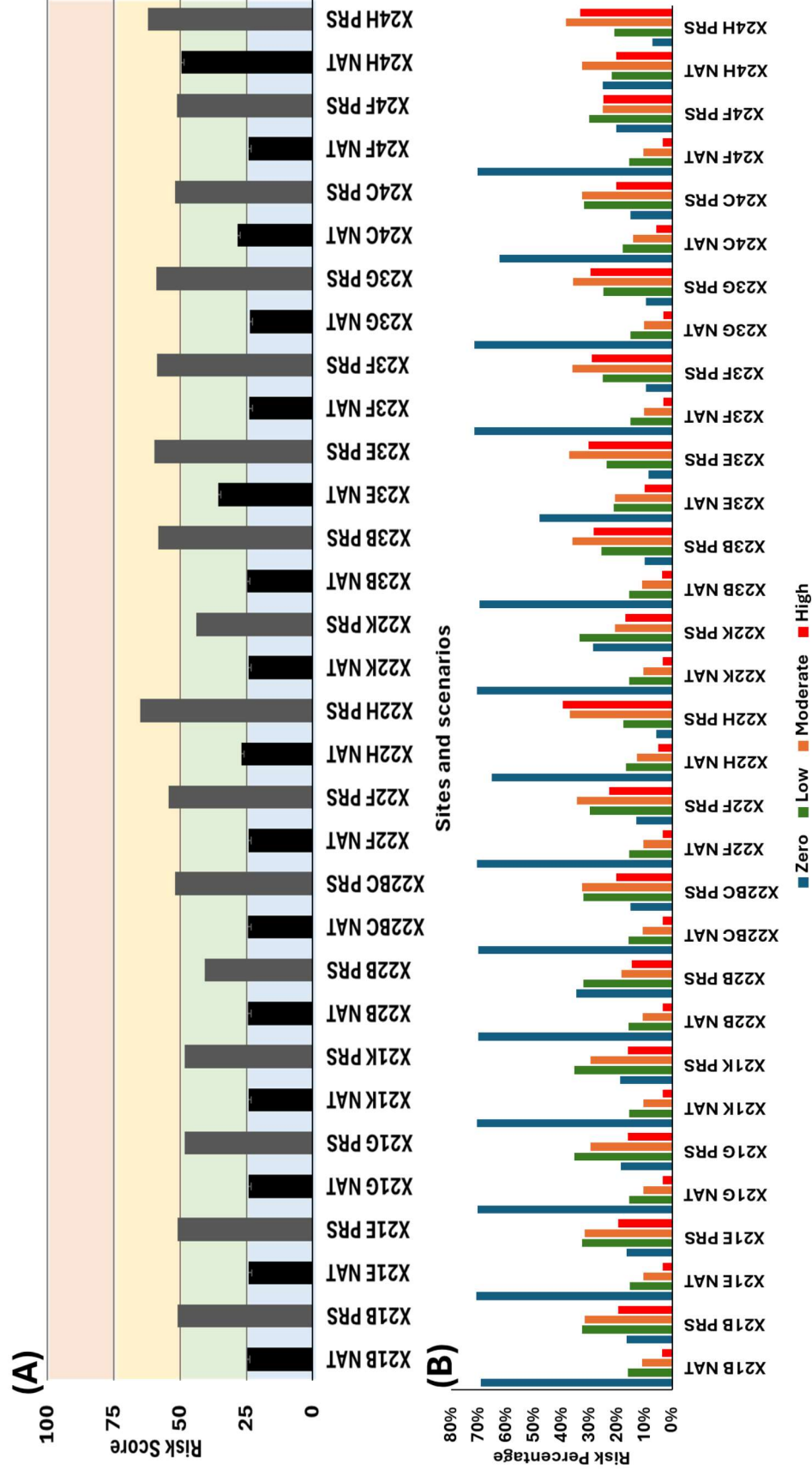
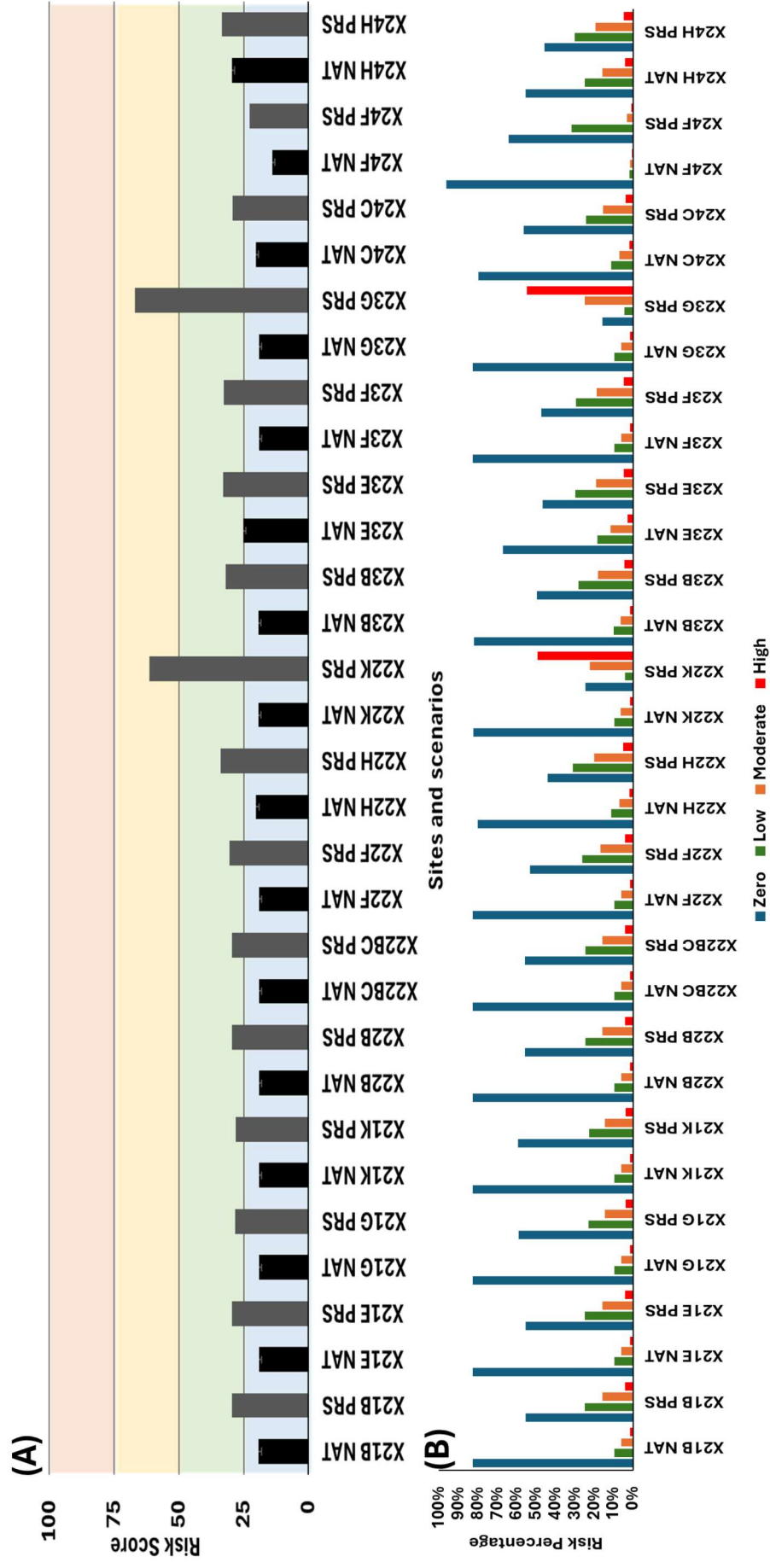
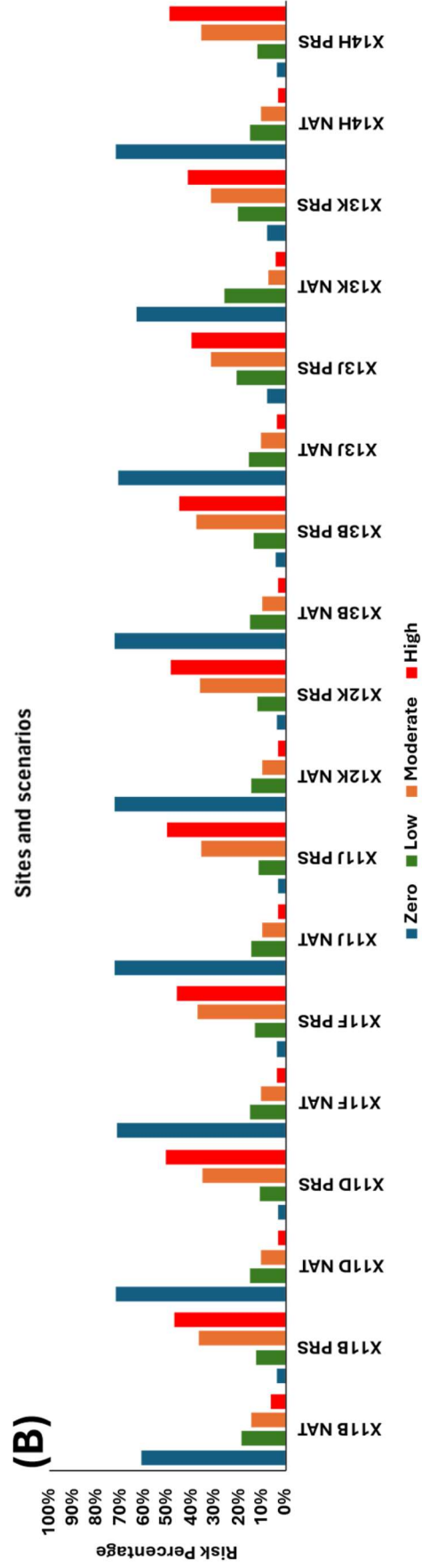
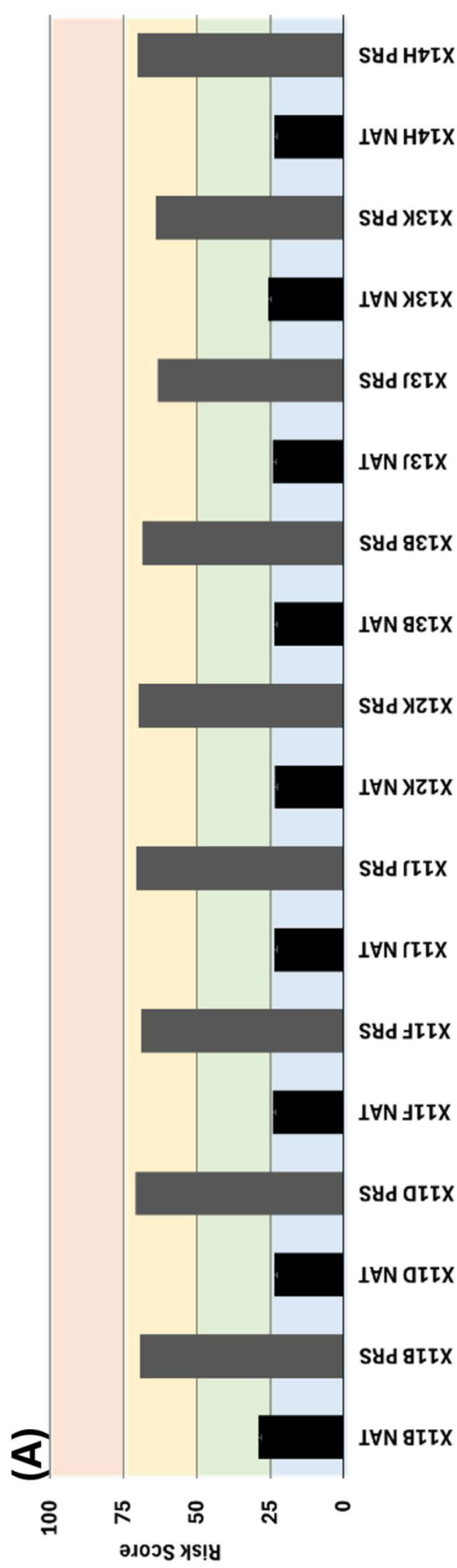
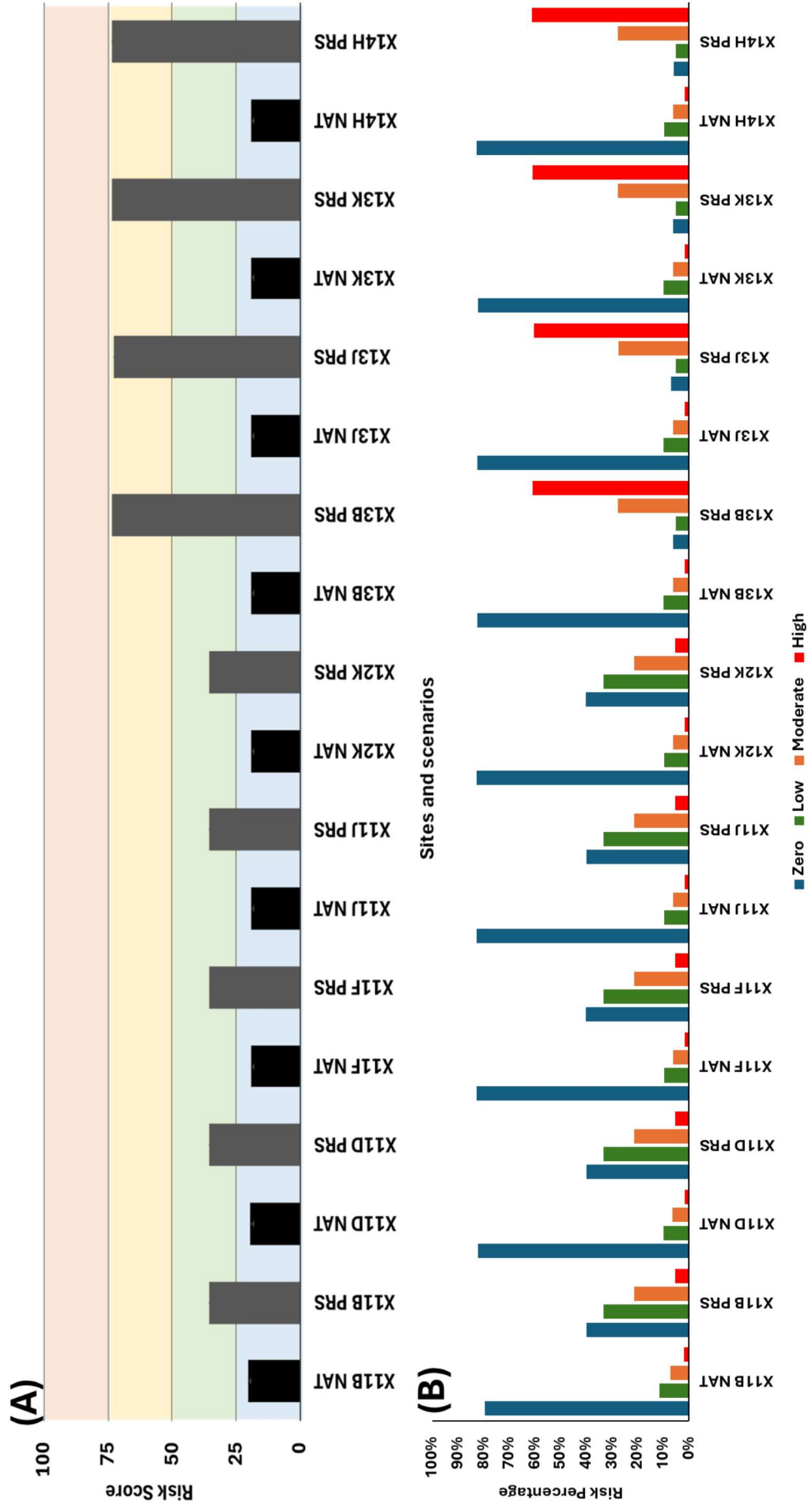


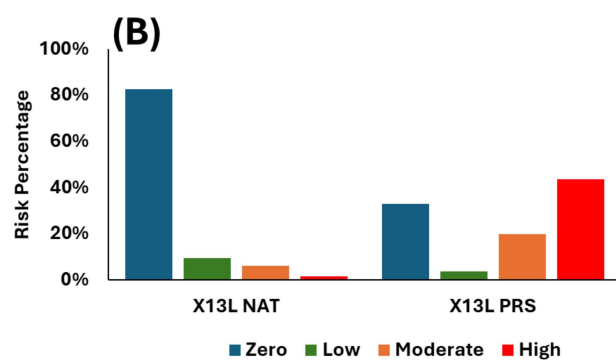
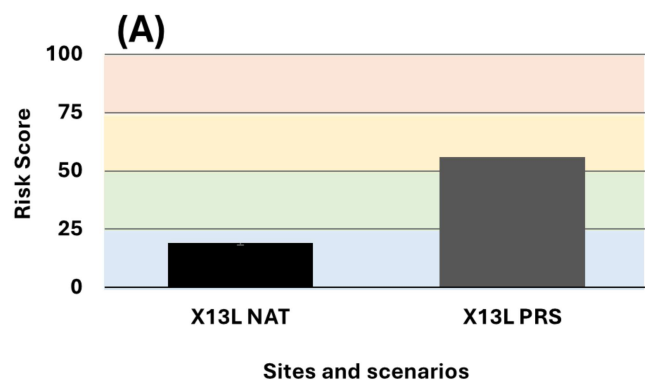
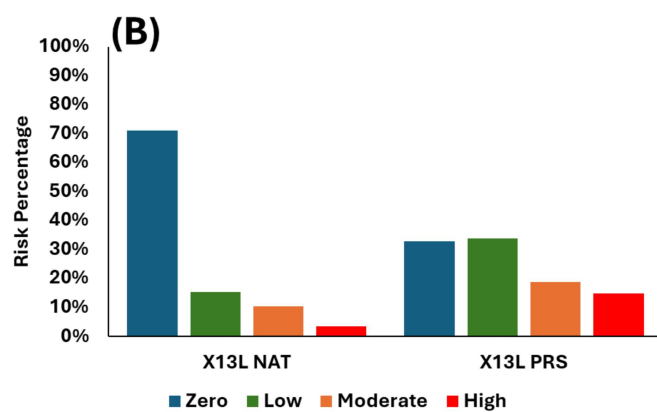
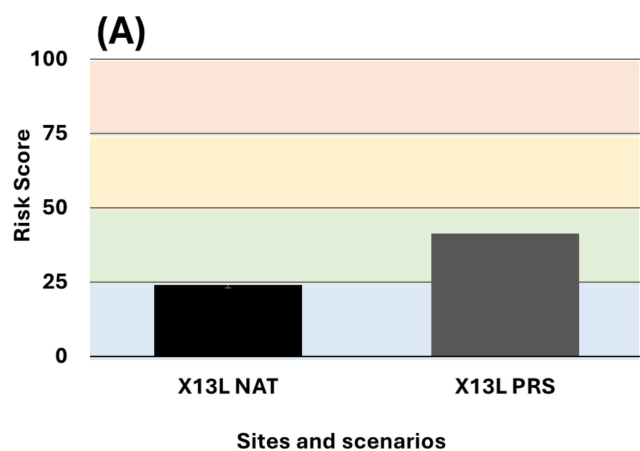
Figure 14: The Subsistence fisheries indicator endpoint with the A) average relative risk scores and B) risk percentage for the hypothetical natural (NAT) and present (PRS) scenarios for the different sites in the Sabie River sub-catchment. The background colours and bars indicating the different risk ranks with Blue = Zero risk, Green = Low risk, Orange = Moderate risk and Red = High risk.











Discussion

The freshwater ecosystems of the greater Incomati River Catchment are under threat due to multiple environmental drivers that have a synergistic effect on the wellbeing of the fish communities and subsistence fish. This study evaluated the relative risk to the ecological wellbeing of fish communities and subsistence fisheries in the greater Incomati River Catchment. The natural scenario (NAT) is a reference condition to compare the present (PRS) scenario to determine the degree of change to assist with management decisions and approaches. The sensitivity to findings indicates that firstly ecological fish are largely sensitive to the environmental influences/condition, while the subsistence fish are affected by the amount of species removal from humans (individuals taken for food).

Sub-catchment evaluations

1. Uanetze River sub-catchment

The Uanetze River sub-catchment is characterised by small seasonal river streams, some of which dry up during low flow periods (IUCMA 2020). Environmental stressors are constantly affecting the fish communities of the Uanetze River sub-catchment as all sampled sites had a moderately dominated risk score (Figure 13A). The risk percentages of all sites ranged from moderately-to-moderately high risk (Figure 13B). Environmental stressors like low-flow conditions resulting in altered water quality conditions (high temperature and low oxygen) and dis-connectivity between river reaches (stranded pools and weirs) are affecting the fish communities (Datry *et al.*, 2014; Magand *et al.*, 2020). The Uanetze River sub-catchment is situated within the South African portion of the Kruger National Park which protects it from direct pollution from local communities areas and mines (e.g., sewage/chemicals), however it is still affected by road crossing related stressors and climate stressors within the park. Within the Mozambiquan side of the sub-catchment, the river environment is subjected to stressors from local rural communities (e.g., clothes washing, road crossing, agricultural runoff).

From a subsistence fish wellbeing perspective, the risk scores (Figure 14A) and risk percentages are dominated by a zero to low-low risk conditions (Figure 14B). In the South African portion of the Kruger National Park, the targeted subsistence fish are protected as fishing is prohibited in these rivers. The same is not true for the rivers within the

Mozambican side which are fished for food. The majority of the collected fish species during the study were targeted fish species (*Clarias gariepinus*, *Oreochromis mossambicus*) as they are more tolerant to environmental changes like changing flows or water temperature (Gaigher 1973; Darwall *et al.*, 2009). Seasonal rivers like these are very important to provide refuge for protection, recruitment and migration for fish communities (Kaiser-Reichel *et al.*, 2024). However, these rivers are understudied and little is known about their fish community structure. Kaiser-Reichel *et al.*, (2024) highlighted the importance of these seasonal rivers from a conservation and ecosystem services point of view along with the understanding of better management strategies to protect and manage these seasonal rivers.

2. Sabie River sub-catchment

The Sabie River is currently classified as one of the most pristine river systems in the greater Incomati River Catchment due to high biodiversity and re-establishment of migratory pathways (Kleynhans 1986; Roux *et al.*, 2016). This system is under pressure from sedimentation, domestic water-abstraction, forestry, irrigation and illegal sand mining (IUCMA 2008; Saraiva Okello *et al.*, 2015; Roux *et al.*, 2016). In the lower reaches, physical barriers pose problems for migratory fish species, especially important catadromous migratory species such as anguillid eels (O'Brien *et al.*, 2019). In the upper reaches of the Sabie River sub-catchment (X31A, X31C, X31D, X31G, X31K, X32H) the sites have a low to moderate risk score with a low to moderately dominated risk percentage (Figure 13 & Figure 14). These river reaches are characterized by cold-water, fast flowing with diverse substrate availability (Roux *et al.*, 2016). Expected fish species in these regions are rheophilic (fast flowing) specialists and prefer cool water environments (Cortner 2000). Known environmental drivers in this region including wastewater treatment works (WWTW), Eucalyptus and pine plantations, saw mills, agricultural crop runoff and rural settlements (Roux *et al.*, 2016) are affecting the environmental condition of the upper Sabie River reaches. Water abstraction for agricultural use, saw mill usages and absorption from plantations affects the rivers' flow regime in turn affecting the preferred habitat condition and community composition of the expected fish species (Rytwinski *et al.*, 2017). Additionally, affected water quality parameters from low-functional WWTW and chemical agricultural runoff also affect the preferred environmental condition (Schinegger *et al.*, 2016). The Sabie River 'specialist' species expected in the upper Sabie River sub-catchment like *Amphilius uranoscopus*, *Chiloglanis anoterus* and the *Labeobarbus nelspruitensis* were sampled in low

abundances in these areas. This creates concern as the Chiselmouth yellowfish (*Labeobarbus nelspruitensis*) is under the ‘near threatened’ status on the IUCN red list and management towards protecting these areas are needed (Darwall *et al.*, 2009). The middle section of the Sabie River is within the Kruger National Park again ‘protected’ from external anthropogenic stressors. Upstream stressors are transported downstream and still affects the river environment throughout. As the sites moved deeper into the KNP the risk score lowers from a moderate to a low-risk score (Figure 13A). The dominated risk percentage changes from a low to moderate risk percentage to a zero to low-risk percentage. This illustrate that ‘relief’ from constant anthropogenic stressors (no added stressors in KNP) could alleviate stress form the river environment, providing a better environment for the fish communities (Burnett *et al.*, 2018). From a subsistence fisheries perspective, the sampled sites in the upper and middle reaches had a low-low to zero risk scores with a dominated zero risk percentage (Figure 14AB). Dlamini *et al.*, (no date, in press – a parallel social study) documented that fishing in the Sabie River area is limited due to limited access (river are far from settlements) or difficult finding the preferred targeted fish species. At the lowest site (X33D) the Sabie River has entered Mozambique and subsistence fishing has increased to a moderate risk score and high dominated risk percentage. Availability of slower deep flowing habitats in this area allow for larger preferred targeted fish species to inhabit the areas while the access and dependence for fishing has increased.

3. Crocodile River sub-catchment

The Crocodile River sub-catchment is ecologically one of the most important and biodiverse river systems in the greater Incomati River Catchment (Kleynhans *et al.*, 2015). The foremost influences on the river are domestic water use (South Africa and Mozambique), industrial and mining activity, forestation and irrigation (Kleynhans *et al.*, 2015; Saraiva Okello *et al.*, 2015). From an ecological indicator fish wellbeing perspective, the Crocodile sub-catchment sites range from a low to moderate risk scores (Figure 15B & Figure 16B). Throughout the Crocodile River sub-catchment the sites are affected by anthropogenic stressors that impact the environmental condition. The Elands River system (tributary), an established National Freshwater Ecosystem Priority Area (NFEPA), home to the critically endangered *Chiloglanis bifurcus* and *Kneria Spp*, is affected by the South African Palp and Paper Industry (SAPPI) along with plantations and agricultural activities (Kleynhans *et al.*, 1992; O’Brien *et al.*, 2014a). Continious monitoring and protection measures are required as

outlined by O'Brien *et al.*, (2014), to offset the impacts associated with the use of water resources (abstraction/discharge) with continued conservation efforts. The highest risk score, and highest risk percentage (Figure 15AB) was observed at the X22H (White River) site. Unmanaged WWTW effluent and chemical runoff from crops are affecting the water quality parameters of the White River, in turn affecting the suitability of the river environment through eutrophication (Carpenter *et al.*, 1998; Roux and Oelofse 2010). Additionally, the Kaap river system (X23B, X23E, X23F, X23G) follows the same trend where unmanaged WWTW effluent, mining, invasive species and agriculture is affecting the tributary systems which leads to high risks observed in the main river system.

From a subsistence fisheries perspective the Crocodile River has a zero to low risk score with a dominated zero risk percentage for the majority of the sites while, the X22K (Kanyamazane) and X23G (Kaap River) both had a moderate risk score with a dominated high risk percentage (Figure 16AB). Although fishing for subsistence use occurs throughout the catchment, these sites (X22K & X23G) are located close to large rural areas and provide a suitable environment (deep-pools) for preferred targeted species. Observational data has seen officials from Mpumalanga Park and Tourism Agency (MTPA) monitoring the Crocodile River region for unlicensed fishing and sand-mining events which could explain the low demand for subsistence fishing.

4. Komati River sub-catchment

The Komati River sub-catchment is the most stressed river system in the greater Incomati River Catchment due to irrigation, power stations, mining and industrial water use, afforestation, and domestic water usage (in both South Africa and Eswatini) (Saraiva Okello *et al.*, 2015). The water quality conditions are deteriorating (O'Brien *et al.*, 2019) and physical barriers in this river system and its tributaries affect the aquatic ecosystems and the pathways of important migratory fish (Kleynhans *et al.*, 2015)(Figure 17). From an ecological indicator fish wellbeing it is evident that all sites are at a high-moderate risk score with high risk percentages (Figure 17AB). The X11D (Klein Komati) site had the highest relative risk score to the ecological indicator fishes and management at this site should be prioritised to conserve ecological indicator fish species. Large (*Hydrocynus vittatus*) and long distance migratory fish species (anguillid eels) expected in the Komati River Catchment were absent during the sampling events. Over one-quarter of the expected fish species were not sampled during the study. This gap may reflect local population declines, which could in turn

threaten the delivery of key ecosystem services and the stability of ecological infrastructure within the catchment (Jennings *et al.*, 2005; Tim *et al.*, 2016). The lower reaches of the Komati River has high-moderate risk scores and high risk percentage for the subsistence fisheries endpoint (Figure 18AB). These subsistence fishes also experience the stressors that ecological indicator fishes experience, as well as being targeted for subsistence at these sites as local communities have a high dependence on these fishes for their livelihood (O'Brien *et al.*, 2024).

5. Incomati River sub-catchment

The Incomati River sub-catchment, located only in the Mozambican side of the greater Incomati River Catchment, is characterized by wide-sandy riverbeds with slow-deep flowing sections leading towards the floodplain environment. The Incomati River sub-catchment is mainly affected by floodplain agriculture, runoff from local rural communities, invasive species and overfishing (Basson and Rossouw 2003). From an ecological indicator fish perspective, the site had a low-risk score with a zero-to-low dominated risk percentage (Figure 19AB). The floodplain environment provides an excellent environment for breeding and feeding for ecological indicator fish species (Winemiller 2004). The management and conservation efforts for the Incomati River sub-catchment should be prioritized not only because all the sub-catchment stressors have accumulated in this section, but all amphidromous and diadromous migrations passes through or uses the Incomati River at some point to complete their life cycle. The subsistence fisheries wellbeing of the Incomati River sub-catchment is in a moderate risk score with a zero to high-risk percentage (Figure 20AB). From observations it is evident that many more species are targeted for food resources compared to any other sub-catchments. Many invasive species are targeted along with important anguillid eel species. Syndicate eel export activities are threatening these populations along with the ecological indicator species (observational data).

Conclusion

In this study a holistic approach was used to evaluate the relative risk to specific endpoints to determine the wellbeing of fishes in the greater Incomati River Catchment. The majority of the sites had fish communities that are in a low-moderate risk in terms of their persistence and wellbeing. The sites highlighted in this study should be monitored and have management prioritisation to conserve the species and prevent further degradation at the sites. The

ecological indicator fish species at these sites are at risk to local or seasonal extinction as breeding habitats, habitat preferences and connection are threatening their biology and ecology. From an ecological point of view, it is important to conserve these sites and minimise stressors that affect the regulatory and supporting ecosystem services to be obtained from the river systems.

For the subsistence fish to persist and keep up with the high demand from local communities, management strategies should aim to conserve the breeding habitats of subsistence fishes or enforce stronger requirements for subsistence fish removal for consumption (Licence enforcement). Not only are the subsistence fishes important in providing provisioning ecosystem services to local communities but ecological important fishes allow for the provisioning of other indirect ecosystem services important for a healthy ecosystem.

The use of a RRM assessment provides a cause-and-effect pathway on many spatial scales and can address complex environmental stressors. The approach now allows management authorities to directly address major environmental drivers through established resource quality objectives and set risk-based priorities. Resource allocation must be orientated to focus on risk areas that are most significant to set a balance between the use and protection of aquatic freshwater resources in South Africa. It is imperative that ecological functionality be preserved to conserve ecosystem services and through the Integrated Water Resource Management (IWRM) framework a balance between the use and protect of water resources will be achieved (Claassen *et al.*, 2001).

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Chapter 5

A regional scale risk assessment of the anguillid eels in the greater Incomati River Catchment, southern Africa, and the associated drivers affecting their life cycle ecology

The Western Indian Ocean (WIO) anguillid eels (*Anguilla* spp.) are long distance catadromous migratory fishes that use both marine and inland ecosystems to complete their life cycles. Globally, anguillid eels have a high socio-economic value and are targeted for commercial and subsistence fisheries. The anguillid eels within the greater Incomati River Catchment are under threat due to multiple environmental drivers that act in synergy to cause effect on their life cycle ecology (larvae recruitment, yellow eel maintenance, silver eel escapement & spawning). This study aimed to evaluate the relative risk and wellbeing of anguillid eels in relation to their life cycle ecology within the different life stages and for each sub-catchment within the greater Incomati River Catchment. Four scenarios (NAT – natural condition; PRS – present time, FUT1 – more barriers, FUT2 – No intervention), were evaluated to assess the risk towards the different life-cycle stages of WIO anguillid eels. The study model illustrated a noticeable increase in risk (possibility of death/extinction) towards the outcomes within the inland life cycle stages for anguillid eels at moderate to high-risk while estuarine and marine environment life cycle stages have zero to low associated risk. This study also demonstrates the probable effects that environmental drivers have on the anguillid eel populations and the ecological wellbeing within the Western Indian Ocean. Anthropogenic stressors like barriers, overfishing (exportation) and habitat destructions are greatly influencing the wellbeing of anguillid eels within the catchment with future scenarios prediction the outcomes if current management processes and strategies are not attended to. By evaluating the multiple stressors affecting Anguillids eels in the various life cycle stages will allow the continuation of anguillid eel populations within the WIO – the most ecological important region for anguillid eels.

Keywords: Western Indian Ocean, Barriers, Bayesian Network

Introduction

Globally, there are 19 known anguillid eel species (Aoyama 2009) and despite the high amount of scientific studies conducted on them, their associated ecology and biology still remain largely unknown (Arai 2014). Anguillid eels are unique long distance catadromous fish that connects the marine and freshwater environment and are an important food resource (Feunteun *et al.*, 2003). Anguillid eels possibly have the highest economical value per weight of fish (Jackson 1976; Stuart *et al.*, 2024), in turn being one of the main reasons for the drastic decline in their population numbers owing to overfishing and overexploitation (Arai 2014; Dekker & Beaulaton 2015; Dekker 2016). Additionally, by straddling the marine and freshwater environments, anguillid eels are exposed to threats and challenges within both environments including climate change, flow alterations, habitat modifications, anthropogenic barriers, water quality changes, marine currents, temperature changes and alien predators (Jacoby *et al.*, 2015; O'Brien *et al.*, 2019).

From these important anguillid eel species, four species occur within the Western Indian Ocean (WIO) region and represent the world's greatest diversity of Anguillids (Wasserman *et al.*, 2011; Schabetsberger *et al.*, 2016). These four species include the Longfin eel (*Anguilla mossambica* – Peter 1852), the African mottled eel (*A. bengalensis* – Gray 1831), the Giant mottled eel (*A. marmorata* – Quoy Gainard 1824) and the Shortfin eel (*A. bicolor* – McClelland 1844) (Darwall *et al.*, 2009; Hanzen *et al.*, 2019). It has been documented that these species migrate from the Indian Ocean, from around the Mascarene Plateau (Pous *et al.*, 2010) into East flowing rivers along the western Africa continent (Hanzen *et al.*, 2019). Within the WIO, multiple threats have led to the culminated listing of the African Longfin Eel (*A. mossambica*), African Mottled Eel (*A. bengalensis*) and the Shortfin Eel (*A. bicolor*) as being near threatened by the IUCN (Darwall *et al.*, 2009).

In South Africa, the National Environmental Management Act (NEMA) no 107 of 1998 and the National Environmental Management: Biodiversity Act (NEM:BA) no 10 of 2004 have added requirements for species conservation through the establishment of National Freshwater Ecosystem Priority Areas (NFEPA) (Driver *et al.*, 2005). With all of these systems and governmental legislations in place, South Africa's current water resources protection and fisheries management programmes does not incorporate nor consider river connectivity management and migratory species (Fausch *et al.*, 2002; Silva *et al.*, 2018). The use of migratory fishes for ecological and economic benefits in southern Africa are

understudied and undervalued and need to be considered for the sustainable vision for water resources (Stuart *et al.*, 2024).

The semi-arid greater Incomati River Catchment, southern Africa, supports an estimated 70 fish species of which several have conservation importance, including the four WIO anguillid eels (Darwall *et al.*, 2011; Kleynhans *et al.*, 2015). Growing agriculture, barrier formations, forestry and urbanisation, are the main drivers of increased water scarcity in the catchment and these intensive land cover transformations are accelerating the degradation of the catchments aquatic and terrestrial ecosystems (Saraiva Okello *et al.*, 2015; O'Brien *et al.*, 2019). Despite the presence of multiple Freshwater Environmental Protection Areas (FEPA) and designated conservation zones, the anguillid eel populations within the greater Incomati River Catchment remain poorly studied (Rahel and Magnuson 1983; Jackson *et al.*, 2001).

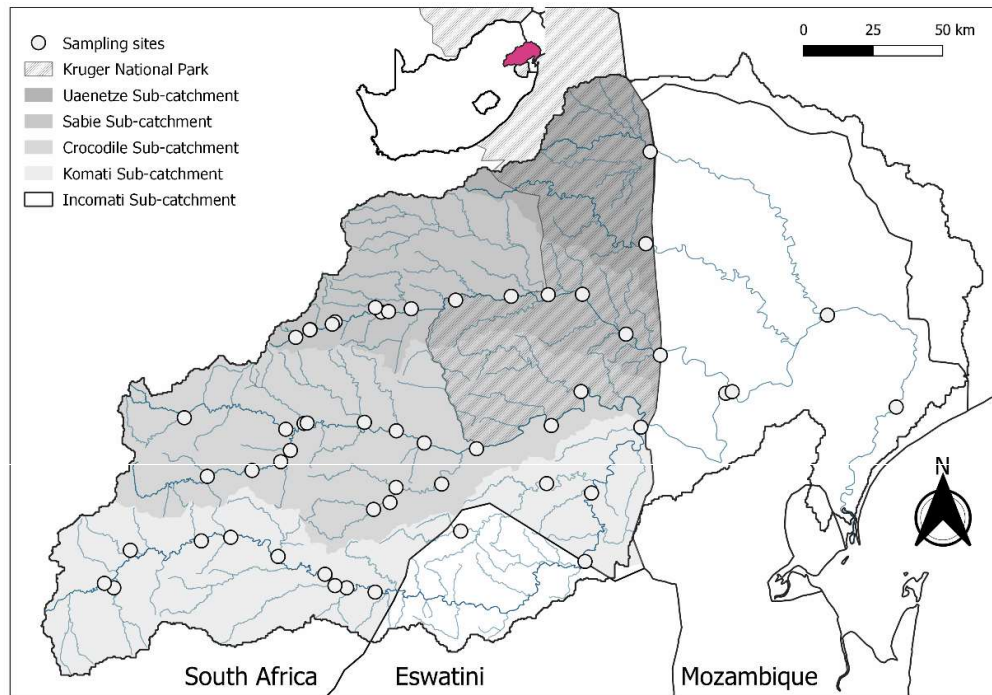
Multiple stressors can act simultaneously to alter ecosystem functioning and to understand their interactive effects, relative risk assessments are needed in adaptive management strategies (Govender *et al.*, 2021). The “South African Ecological Risk Assessment Guidelines” promote risk assessments as a process that evaluates the likelihood that adverse ecological effects occur or will occur due to the exposure of one or more stressors and endpoint (Claassen *et al.*, 2001). A Bayesian Network demonstrate how available evidence describes, sources resulting in stressors, affecting multiple receptors in the content of endpoints. The specific endpoint in the risk assessment represents the relative risk or impact associated for those specific endpoint/outcomes. Multiple ecosystem response components and multiple Lines of Evidence have been included in risk assessments (Vezi *et al.*, 2019; O'Brien *et al.*, 2021).

The aim of the study is to use a Relative Risk Model through the incorporations of Bayesian Networks (RRM-BN), to characterise the risk of multiple stressors to anguillid eels lifecycle within the greater Incomati River Catchment, southern Africa. This study incorporates source-stressor and stressor-receptor relationships established in this study and the application of hypothetical water resource use/protection scenarios, to contribute to the determination of a sustainable balance between water resources and conservation efforts for anguillid eels. Through evaluation of the risk to different life cycle stages, management decisions can be orientated to minimise or reduce the stressors, causing increased potential risk for anguillid eels at each life cycle stage.

Materials and methods

Study area

The greater Incomati River Catchment is a semi-arid transboundary catchment located in the south-eastern region of South Africa, northern parts of Eswatini and southern parts of Mozambique. Limited sampling sites were sampled throughout Mozambique and Eswatini due to assess restrictions, sampling safety in areas and the availability of historical data. Although this poses as a limitation to the study, some data needed to be included to validate the study area as the Greater Incomati Catchment and not only the Inkomati Catchment. The main sub catchments (Uanetze, Sabie, Crocodile, Komati and Incomati) descend from Highveld plateaus and flow east to the coastal plains of Mozambique; these contribute 94% of the total discharge to the greater Incomati River Catchment (Roux *et al.*, 2013). A total of 54 sites were selected throughout the larger, main river systems within the greater Incomati River Catchment to conduct the study risk assessment (Figure 21). Various habitats with different depths and velocity classes as well as environmental characteristics and anthropogenic stressors were observed throughout the study area. Sampling occurred from 2020 to 2024 (Ethics nr: AS/UMP/20220115, MTPA permit nr: MPB 5691; MPB 5849, Transboundary (Mozambique & Eswatini) access and permission letters).



Data collection

anguillid eels were collected using commercial fyke nets (19mm mesh, opening size 60cm; T&L Netmaking, Mooroolbark, Australia) deployed for 12 hours at a time in medium to deep habitats while electroshocking methods were used to sampling shallow habitats. Both methods were used at all sites. Morphological measurements were used to identify the collected anguillid eel species to species level, where after DNA analyses confirmed species identification (Pankhurst 1982; Skelton 2001). Habitat data that include three depth and velocity transects along with substrate and cover features were collected from each sampled biotope (different habitats)(Kleynhans 1996). The *in situ* water quality parameters (pH, Temperature, Electrical conductivity, Total dissolve salts and Dissolved oxygen) were also documented per site using a EUTECH PCD 650 multi-meter. A parallel study in the same study area, interviewed local community members regarding anguillid eel fishing landings and the contribution of eels to fisheries in each river and location (O'Brien *et al.*, 2024). All collected data was used to feed into the Bayesian model.

Statistical analyses and risk model construction

To construct the Bayesian Network and risk assessment for each site, Microsoft Excel (Microsoft Corporation, <https://office.microsoft.com/excel>, version 2411) and Netica (Norsys Software, <http://www.norsys.com/>, version 4.5) were used. Evidence used to represent known risk pathways between sources-stressors, stressor-receptors and receptor-endpoints is based on the understanding of the presences and abundances of anguillid eels their biology and ecology and how they respond to environmental drivers. Additional historical data from DWS (Department of Water and Sanitation 2014), and the Global Biodiversity Information Facility (GBIF) was used in the risk assessment to determine the risk to the life cycle ecology endpoints for the Natural scenario.

The methodology for the regional scale ecological risk assessment includes the well-established Relative Risk Model (RRM) from the Regional-scale Ecological Risk Assessment framework established by Wiegiers *et al.*, (1998) and optimised for application in Africa by O'Brien and Wepener (2012); Vezi *et al.*, (2019) and O'Brien *et al.*, (2021). The RRM approach incorporates the use of both Bayesian Networks (BNs) and Monte Carlo randomisation procedures that conform to current best international practice (Van Den Brink *et al.*, 2016). This regional scale ecological risk assessment approach was carried out on a spatial scale and allowed for the consideration of multiple sources of multiple stressors affecting multiple endpoints, with the inclusion of local ecosystem dynamics and the

characteristics of the landscape that may affect the risk estimate. The 10 steps of the RRM that were followed are (Chen and Landis 2005; O'Brien *et al.*, 2018):

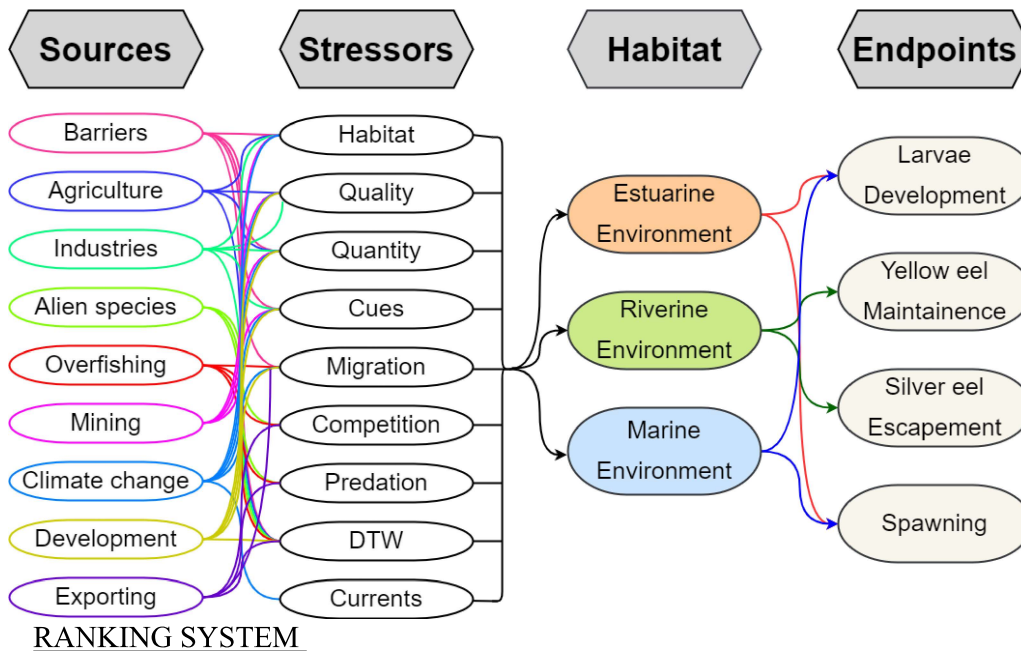
List the important management goals for the region

1. Generate a map on which the potential sources and habitats relevant to the established management goals are indicated.
2. Demarcate the map into risk regions based on a combination of the management goals, sources, and habitats.
3. Construct conceptual models from available information that represent probable risk pathways between sources, stressors, receptors in a range of habitats to the selected endpoint for the study.
4. Determine a ranking scheme to calculate the relative risk to the assessment endpoints. Ranking schemes are used to represent the state of variables, with unique measures and units to be comparable as non-dimensional ranks and combined in BN-RRMs. The outputs from these first five steps will be used to generate a holistic risk assessment framework for the study using Microsoft Excel and the freeware Nors-Netica Probability modelling software.
5. Calculate the relative risks. Preliminary risk calculations will be undertaken based on available information.
6. Evaluate uncertainty and sensitivity analysis of the relative rankings. All aspects of uncertainty associated with the entire application of the RRM-BN approach for the study including objectives and endpoint selection for the assessment, availability and use of evidence, expert solicitations, and model uncertainty for example, will be addressed.
7. Generate testable hypotheses for future field and laboratory investigations to reduce uncertainties and to confirm the risk rankings. The preliminary risk assessment will result in a range of hypotheses that need to be tested to reduce the uncertainty associated with preliminary assessments. The hypotheses will include a list of research questions that will guide future field surveys and adaptive management activities.
8. Test the hypotheses that were generated in Step 7.
9. Communicate the results in a fashion that effectively portrays the relative risk and uncertainty in response to the management goals.

10. This component of the study will focus on describing the cause-and-effect relationships of multiple source (focusing on water resource users) and stressors, and ecosystem/habitat relationships and how these interactions may affect stakeholders' concerns. This is achieved by using the Monte Carlo/Latin Hypercube permutation processes. This approach is used to combine assigned probability risk distributions for multiple endpoints to estimate the relative probability of risk for each scenario (Stephenson 2000). When compared, the risk to each life cycle stage for anguillid eels can be evaluated in a relative manner. This will allow trade-off considerations for the demand, management and conservation assessments of alternative scenarios.

Conceptual model

The conceptual model was developed to represent the understanding of the cause-effect linkages for the risk component (Figure 22) (Wiegiers *et al.*, 1998; Ayre and Landis 2012). The primary focus of the conceptual model is to establish relationships between the sources and the stressors affecting the different habitats and endpoints. The model is represented in a Bayesian model format (Figure 24) and acts as a framework for the RRM (Appendix Data 5).



A ranking scheme was incorporated for each node (stressor) to calculate the relative risk to each specific endpoint. Data are converted to non-dimensional ranks in order to measure and compare the effects of different stressors on the endpoints (Landis and Wiegiers 2005). Ranks

are classified as either zero, low, moderate, or high and describe the wellbeing condition or severity of impact for the respective nodes and endpoints (Table 13). The ranking scheme is assigned relative risk values of 0.25, 0.50 0.75 and 1.00 respectively (O'Brien *et al.*, 2018). Each variable at each site is assessed and assigned a relative risk rank of with each rank have its own relative risk score (e.g., if habitat modification is ranked as low, its relative risk scores will be oriented towards a low rank with some uncertainly towards other ranks). Expert opinions, collected data, historical data and Google Earth imagery were used as far as possible to validate the risk ranks of the different nodes for each sub-catchment.

Table 13: The different rank scores and the rank descriptions used to rank the different nodes. Adapted from Wade et al., 2020.

Rank	Description
Zero	Node is in a pristine condition and no impact or risk (less than 25%) is present compared to pre-anthropogenic establishment or reference conditions.
Low	Node is in a largely natural condition and low impact or risk (25 – 50%) is present compared to pre-anthropogenic establishment or reference conditions.
Moderate	Node is in a moderate modified condition and moderate impact or risk (50 – 75%) compared to pre-anthropogenic establishment or reference conditions. Ecosystem functioning is on the threshold for possible failure.
High	Node is in a significantly altered condition and high impact or risk (75 – 100%) compared to pre-anthropogenic establishment or reference conditions.

Stressors and sources

Various sources and stressors including present *in situ* habitat characteristics, water quality parameter, flow measurements and historically modified habitats, oceanic currents, migration barriers, and overexploitation statistics were used in this study to assess the impact of these drivers on the respective endpoints. Additionally upstream and site evaluations were also used and contributed to the wellbeing of the system and various endpoints. Sources and stressors were used as input points in the Bayesian network in turn affecting the different receptor variables.

Vision and endpoints

In this study the vision of the Bayesian Network was set to align with the current wellbeing and preferences of anguillid eels. If the vision is abided by, the river systems in each sub-catchment would have the potential to accommodate large abundances of anguillid eels. The endpoint for the study is based on the four main life cycle stages of an anguillid eel that include the Larvae development and Recruitment stage, Yellow eel maintenance stage, Silver eel escapement stage and the Spawning stage (Table 14) and will indicate the relative risk associated for each of these endpoints for an anguillid eel life cycle (Appendix Data 5).

Risk Score refer to the overall/average risk ‘amount’ for that specific endpoint. The higher the risk score, the higher the risk (probability of failure) to that specific endpoint. The risk percentage illustrate the contribution of the different risk rankings towards the risk score. E.g., which risk ranking contribute the most towards the overall risk score.

Table 14: Study endpoints and network variable name with node descriptions.

Endpoint	Description
Larvae Development and Recruitment (LARVAE_RECRUIT)	Variable is an endpoint that represent the relationship between the suitability and potential of glass eels/leptocephali to enter the estuary environment and the estuary environment that provides refuge for the glass eels.
Elver and Yellow eel Maintenance (ELV_YEL_MAINT)	Variable is an endpoint that is influenced by the relationship between the suitability of the river to maintain yellow eels, the condition/success of larvae/glass eel recruiting into the river and the river environment to maintain yellow eels. Variables represent the ability of rivers to maintain yellow eels.
Silver eel Escapement (SILVER_ESCAP)	Variable is an endpoint and represent the relationship between the suitability of the river to allow eels to escape, the number of eels (yellow) making it to the silvering stage and the river environment to maintain silver eels while they escape. Variable is a function of the amount/success rate of silver eels to successfully escape back into the ocean.
Spawning Maintenance (SPAWNING)	Variable is an endpoint that represent the relationship between the distance to spawning grounds silver eels need to travel, the number of silver eels available from the river to escape and the marine environment allowing them to reach the spawning grounds.

Scenarios

Different scenarios/hypotheses were created by altering specific node variables to historical or altered conditions to evaluate the risk for the respective endpoints (Table 15). The different scenarios aimed to assess the risk when management and monitoring strategies are aimed to

prioritise these nodes e.g., Will the risk increase or decrease for the specific life cycle stages when connectivity in the catchment is restored?

Table 15: The different scenario descriptions used in the study and the specific node variables that changed to fit each scenario.

Scenario name	Description	Node variables
Natural (NAT)	This scenario represents the natural pre-anthropogenic state of the site. Assumed that none or little modifications has occurred to the environment.	All input nodes except ESC_RIVER_SIZE, DIST_SPAWNING, CURRENTS_NvsS, EST_TYPE, DISTANCE_SEC, RIVER_LENGTH, WIO_LOCATION were all changes to have a zero or low risk rank.
Present (PRS)	This scenario represents the present state of node variables and the impacts or risk that are currently present at each site.	All input nodes are adjusted to fit the current impact rank.
Reduced connectivity (FUT1)	This scenario represents the current state (PRS) of the node variables with additional degradation to the barrier nodes (EST_BARRIERS; RIV_MAIN_BARRIERS; RIV_ESC_BARRIERS). All other node variable not associated with barriers are kept at the current present (PRS) rank. This scenario demonstrates the response if management strategies are not aimed to restore river connectivity to near natural conditions though the use of removal or fish bypasses (fishways).	The EST_BARRIERS; RIV_MAIN_BARRIERS; RIV_ESC_BARRIERS node ranks were changed to have a risk category increased in risk score for each site. E.g., if presently it is low, it is now classified as moderate (25% increased risk).
Lack of implementation (FUT2)	This scenario represents a reduction in the current state (PES) of the node variables when current legislations and governmental rules are not more strictly implemented. All nodes were changes to have the highest risk associated with the variable to represent the worst-case scenario of the environment and the different life stages. This scenario demonstrates the response if management strategies are not aimed to restore water quality, habitat alteration and connectivity conditions to near natural conditions.	All input nodes were changes to have a moderately-high to high risk scores.

Uncertainty and sensitivity analyses

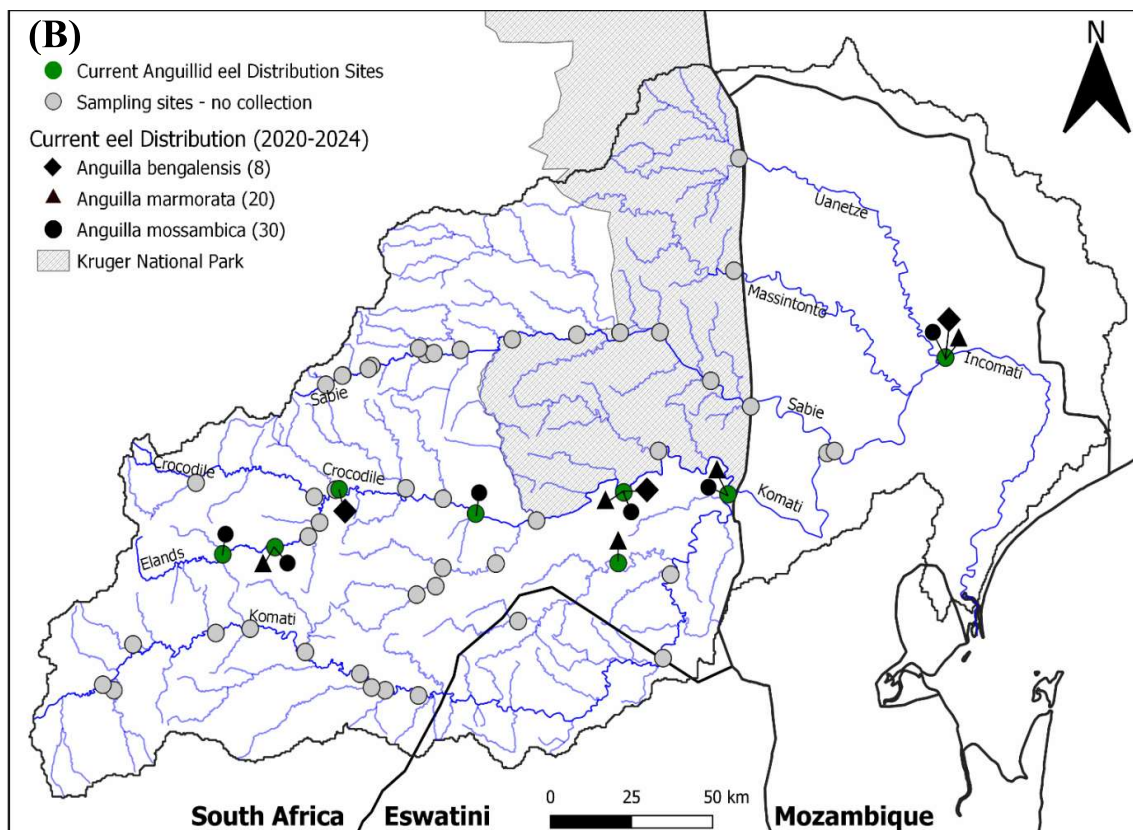
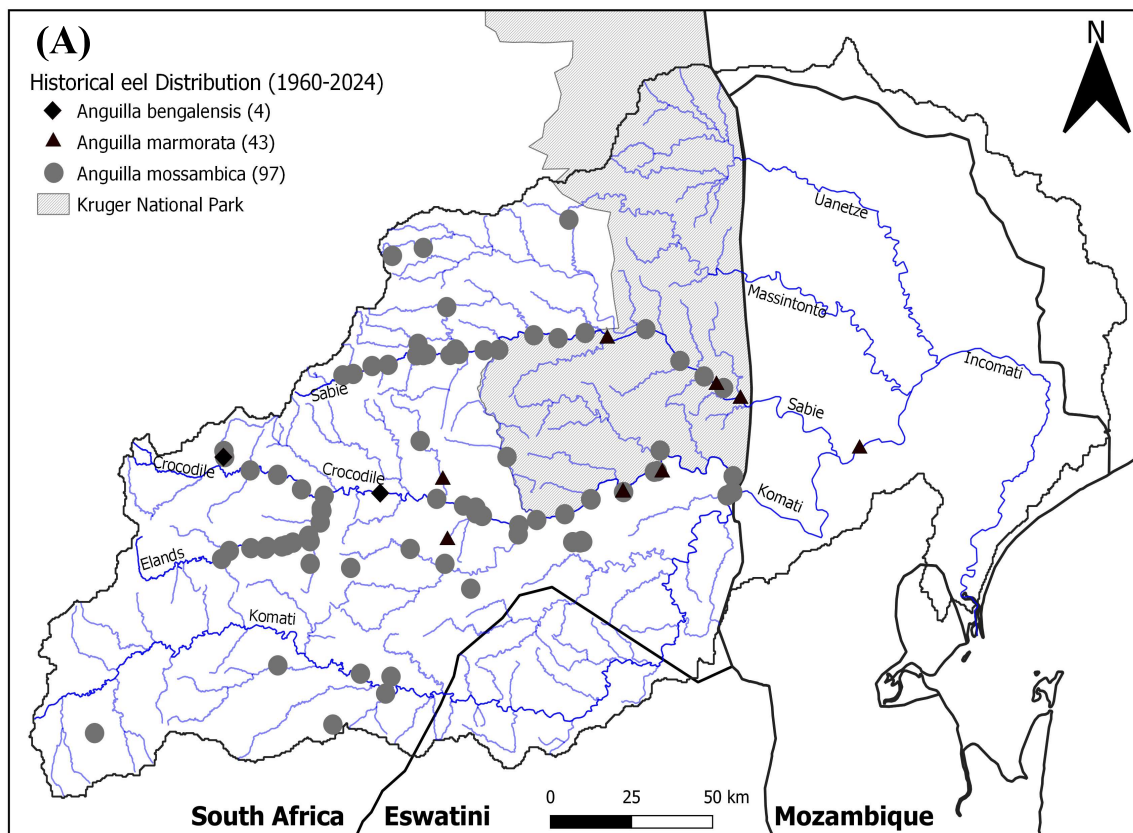
Through the implementation of the Bayesian model, uncertainties may occur that influenced the confidence of the risk outcomes and these needs to be tracked and accounted for. The results of the uncertainty analyses can be validated against the risk hypothesis to reduce the overall uncertainty. The modelling program, Netica (Norsys Software, <http://www.norsys.com/>) has a “Sensitivity to findings” tool that is used to evaluate the individual variable outcomes in addition to a Monto Carlo randomization tool that integrate the effect of uncertainty (Stephenson 2000).

Results

Distribution patterns

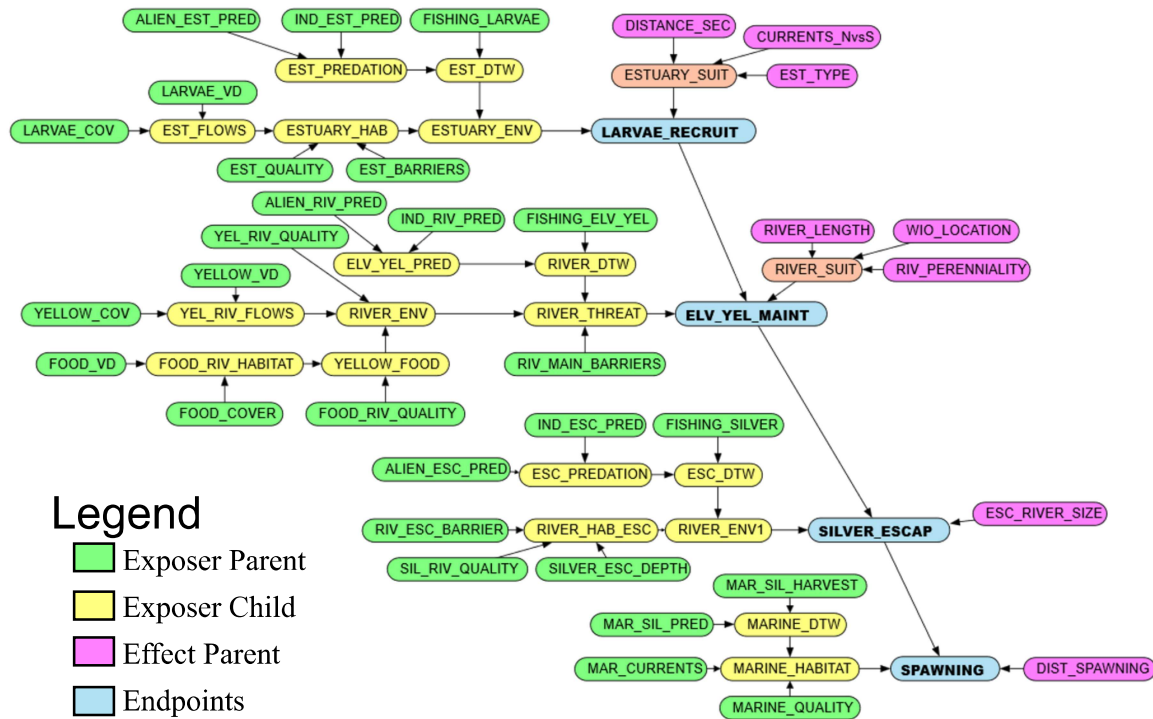
Historical distribution patterns resulted in 144 distribution points within the greater Incomati River Catchment between 1960 and 2020 (Figure 23A). The historical species collected abundances included, *Anguilla mossambica* (97), *Anguilla marmorata* (43) and *Anguilla bengalensis* (4). These historical collections were from 98 sites throughout the greater Incomati River Catchment.

A total of fifty-four sites were sampled during the low-flow and high-flow seasons between 2020-2024. During this sampling surveys, 58 eels were collected throughout the greater Incomati River Catchment (Figure 23B). A total of 28 anguillid eels were collected in South Africa while 30 were collected in Mozambique. These collected anguillid eel species were from three species namely: *Anguilla mossambica*, *A. marmorata* and *Anguilla bengalensis*. No *A. bicolor* were collected within the greater Incomati River Catchment, although it was expected in the lower reaches of the rivers. *Anguilla mossambica* was the most abundant species collected. All collected data, including species habitat usages/preferences and site environmental condition were fed into the Bayesian model to give the risk output per life cycle stage.



Bayesian model

The conceptual model was successfully adjusted using the collected environmental data, to construct the Bayesian model (Figure 24). From the Bayesian model the relative risk to the different ecological endpoints (Life stages) were determined (Appendix Data 5).



Life cycle Ecology Endpoints per risk region

The risk for each endpoint in this study was determined individually for each sub-catchment or risk region. The risks for each endpoint in the different risk regions are compared to one another by considering their maximum and minimum ranges determined for the RRM.

For the Uanetze sub-catchment risk region, the risk scores ranged from 30.4 ± 26.1 to 42.1 ± 21.8 under the natural scenario; 34.1 ± 27.5 to 49.7 ± 24.1 under the present scenario (PRS); 39.1 ± 21.0 to 55.6 ± 25.4 under the future 1 scenario (FUT1), and 39.9 ± 21.7 to 63.5 ± 26.3 under the future 2 scenario (FUT2) (Figure 25). For the natural scenario, the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest

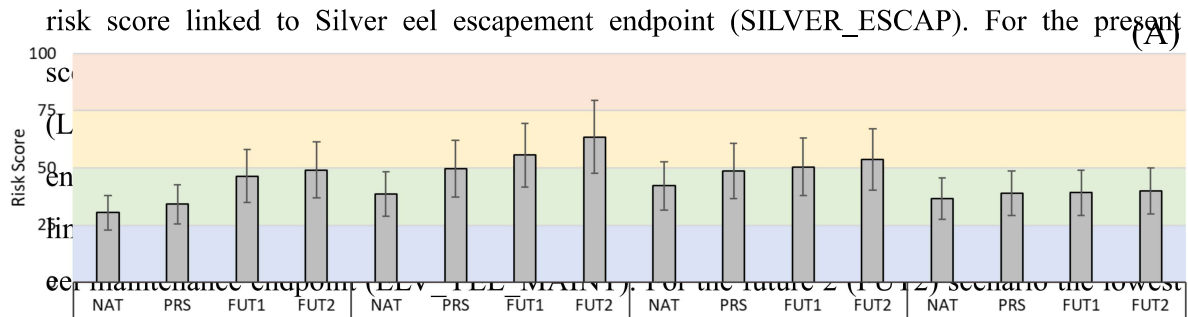


Figure 25A. Risk score linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score is linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT) (Figure 25A).

Under the natural scenario the risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk percentage for all other stages; the present scenario risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk for all other stages; the future 1 scenario is dominated by a zero-risk percentage for the larvae recruitment stage and a low-risk percentage for all other stages, and the future 2 scenario the risk percentages is dominated by a zero-risk percentage for the larvae recruitment stage, a high-risk percentage for the yellow eel maintenance stage, and a low-risk percentage for the silver escapement and spawning stages (Figure 25B).

For the Sabie River sub-catchment risk region, the risk scores ranged from 30.4 ± 26.1 to 45.2 ± 22.7 under the natural scenario; 34.1 ± 27.5 to 71.6 ± 22.2 under the present scenario (PRS); 34.1 ± 27.5 to 71.6 ± 22.2 under the future 1 scenario (FUT1), and 41.2 ± 22.8 to 74.3 ± 20.8 under the future 2 scenario (FUT2) (Figure 26A). For the natural scenario, the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest risk score linked to Silver eel escapement endpoint (SILVER_ESCAP). For the present scenario (PRS), the lowest risk score is linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 1 scenario (FUT1) the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 2 (FUT2) scenario the lowest risk score is linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score linked to the spawning endpoint (SPAWNING) (Figure 26A). Under the natural scenario the risk percentage is dominated by a

Figure 25: The associated risk for the main life cycle indicator endpoints of anguillid eels within the Uanetze River sub-catchment A) indicate the average relative risk scores for the different endpoints and scenarios and B) indicate the risk percentage for the different endpoints and scenarios. The error bars indicate the standard deviation and background colour bars indicating the different risk ranks with Blue = Zero, Green = Low, Orange = Moderate and Red = High.

zero-risk for the larvae recruitment stage and a low-risk percentage for all other stages; the present scenario risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk for the spawning stage and a high-risk percentage for the yellow eel maintenance and silver eel escapement stage; the future 1 scenario is dominated by a zero-risk percentage for the larvae recruitment stage, a low-risk percentage for the spawning stages and high-risk for the yellow eel maintenance and silver eel escapement stages; and the future 2 scenario the risk percentages is dominated by a zero-risk percentage for the larvae recruitment stage, a high-risk percentage for the yellow eel maintenance and silver eel escapement stages, and a low-risk percentage for the spawning stage (Figure 26B).

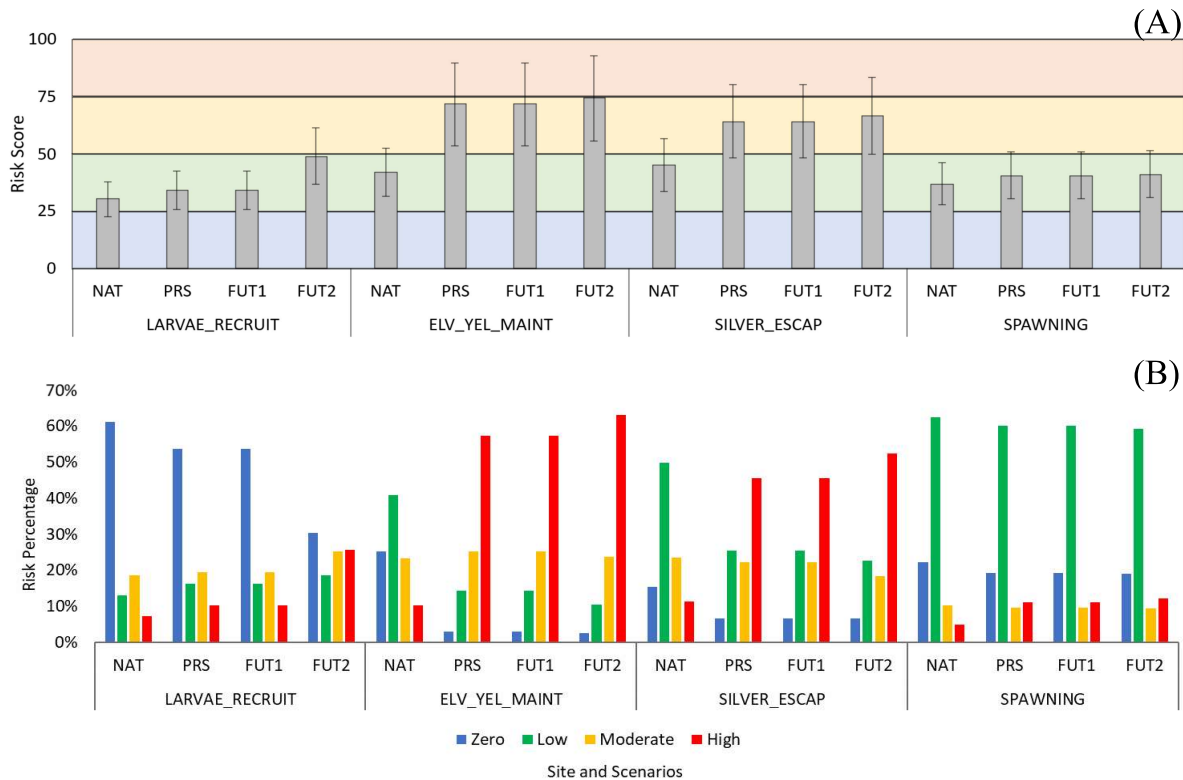


Figure 26: The associated risk for the main life cycle indicator endpoints of anguillid eels within the Sabie River sub-catchment A) indicate the average relative risk scores for the different endpoints and scenarios and B) indicate the risk percentage for the different endpoints and scenarios. The error bars indicate the standard deviation and background colour bars indicating the different risk ranks with Blue = Zero, Green = Low, Orange = Moderate and Red = High.

For the Crocodile River sub-catchment risk region, the risk scores ranged from 30.4 ± 26.1 to 45.2 ± 22.7 under the natural scenario; 33.6 ± 27.3 to 60.6 ± 23.6 under the present scenario (PRS); 46.7 ± 30.1 to 66.8 ± 23.1 under the future 1 scenario (FUT1), and 41.3 ± 22.9 to 75.1 ± 20.3 under the future 2 scenario (FUT2) (Figure 27A). For the natural scenario, the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest risk score linked to Silver eel escapement endpoint (SILVER_ESCAP). For the present scenario (PRS), the lowest risk score is linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 1 scenario (FUT1) the lowest risk score is linked to the spawning (SPAWNING) endpoint and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 2 (FUT2) scenario the lowest risk score is linked to the spawning endpoint (SPAWNING) and the highest risk score linked

to the yellow eel maintenance endpoint (ELV_YEL_MAINT) (Figure 27A). Under the natural scenario the risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk percentage for all other stages; the present scenario risk percentage is dominated by a zero-risk for the larvae recruitment stage, a low-risk for the spawning stage and a high-risk percentage for the yellow eel maintenance and silver eel escapement stage; the future 1 scenario is dominated by a zero-risk percentage for the larvae recruitment stage, a low-risk percentage for the spawning stages and high-risk for the yellow eel maintenance and silver eel escapement stages; and the future 2 scenario the risk percentages is dominated by a zero-risk percentage for the larvae recruitment stage, a high-risk percentage for the yellow eel maintenance and silver eel escapement stages, and a low-risk percentage for the spawning stage (Figure 27B).

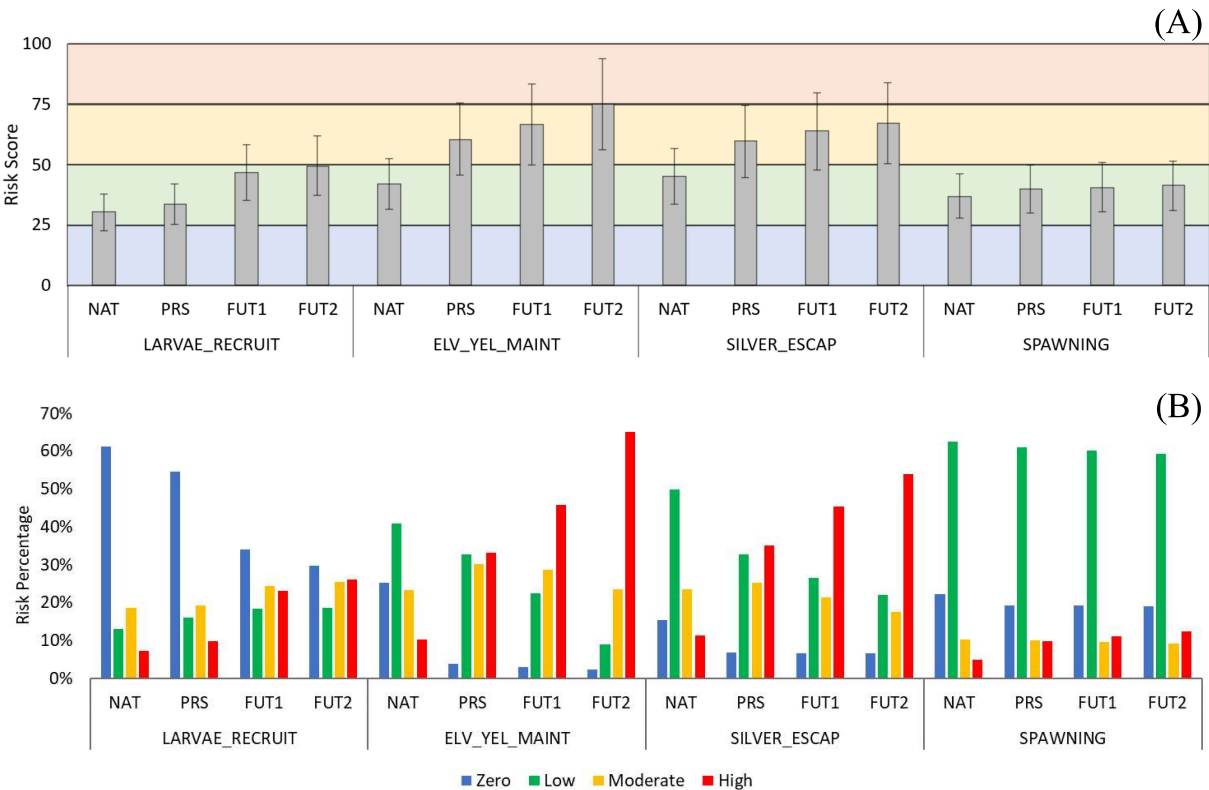
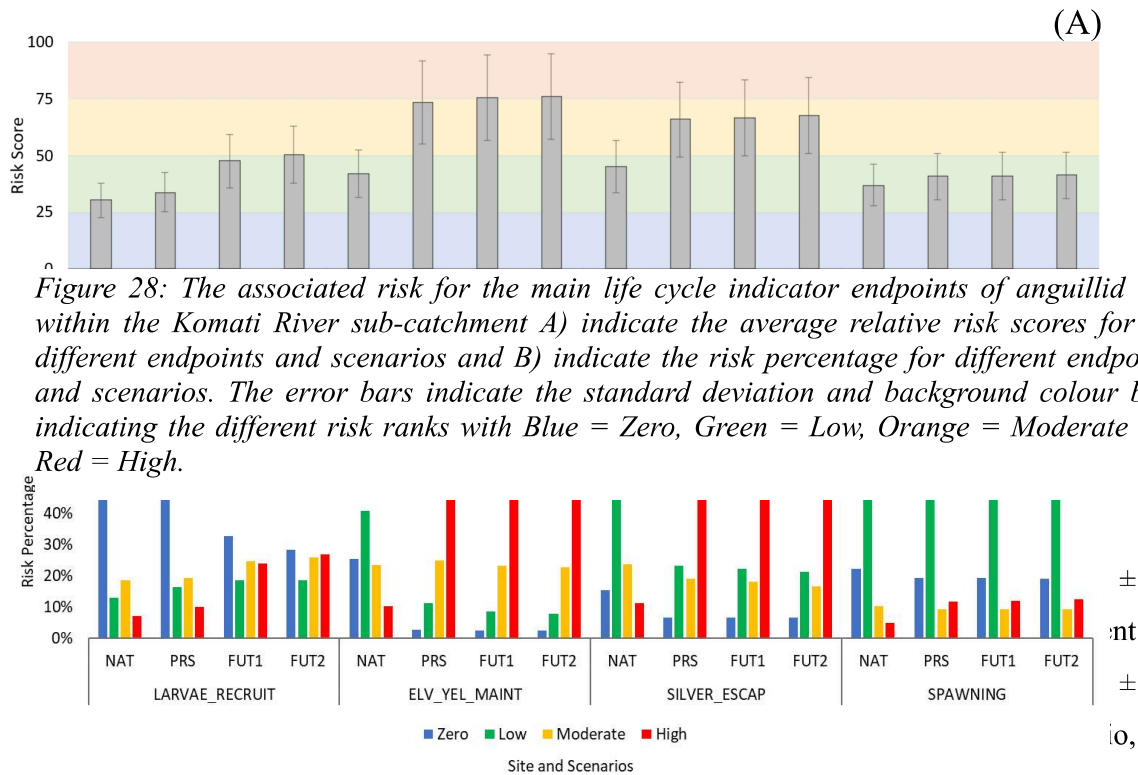


Figure 27: The associated risk for the main life cycle indicator endpoints of anguillid eels within the Crocodile River sub-catchment A) indicate the average relative risk scores for the different endpoints and scenarios and B) indicate the risk percentage for different endpoints and scenarios. The error bars indicate the standard deviation and background colour bars indicating the different risk ranks with Blue = Zero, Green = Low, Orange = Moderate and Red = High.

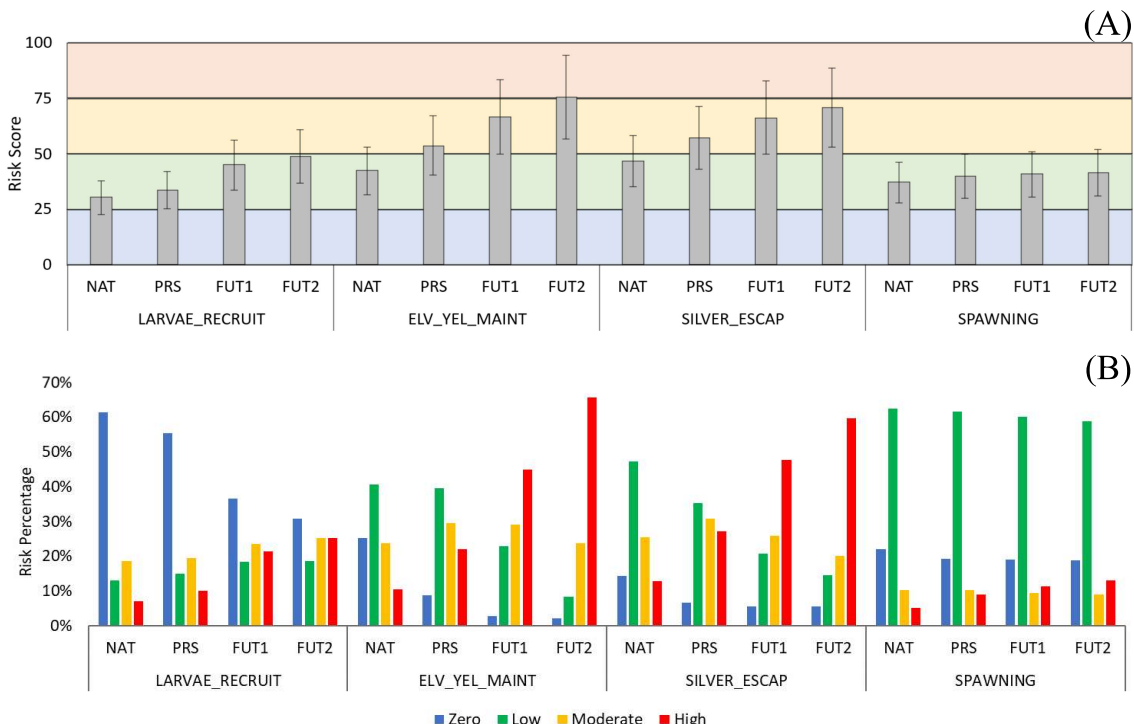
For the Komati River sub-catchment risk region, the risk scores ranged from 30.4 ± 26.1 to 45.2 ± 22.7 under the natural scenario; 33.9 ± 27.4 to 73.6 ± 21.1 under the present scenario (PRS); 40.9 ± 22.7 to 75.5 ± 20.2 under the future 1 scenario (FUT1), and 41.3 ± 22.9 to 76.0 ± 19.9 under the future 2 scenario (FUT2) (Figure 28A). For the natural scenario, the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest risk score linked to Silver eel escapement endpoint (SILVER_ESCAP). For the present scenario (PRS), the lowest risk score is linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 1 scenario (FUT1) the lowest risk score is linked to the spawning (SPAWNING) endpoint and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 2 (FUT2) scenario the lowest risk score is linked to the spawning endpoint (SPAWNING) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT) (Figure 28A). Under the natural scenario the risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk percentage for all other stages; the present scenario risk percentage is dominated by a zero-risk for the larvae recruitment stage, a low-risk for the spawning stage and a high-risk percentage for the yellow eel maintenance and silver eel escapement stage; the future 1 scenario is dominated by a zero-risk percentage for the larvae recruitment stage, a low-risk percentage for the spawning stages and high-risk for the yellow eel maintenance and silver eel escapement stages; and the future 2 scenario the risk percentages is dominated by a zero-risk percentage for the larvae recruitment stage, a high-risk percentage for the yellow eel

maintenance and silver eel escapement stages, and a low-risk percentage for the spawning stage (Figure 28B).



the lowest risk score is linked to the larvae recruitment (LARVAE_RECRUIT) endpoint and the highest risk score linked to Silver eel escapement endpoint (SILVER_ESCAP). For the present scenario (PRS), the lowest risk score is linked to the larvae recruitment endpoint (LARVAE_RECRUIT) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT). For the future 1 scenario (FUT1) the lowest risk score is linked to the spawning (SPAWNING) endpoint and the highest risk score linked to the yellow

eel maintenance endpoint (ELV_YEL_MAINT). For the future 2 (FUT2) scenario the lowest risk score is linked to the spawning endpoint (SPAWNING) and the highest risk score linked to the yellow eel maintenance endpoint (ELV_YEL_MAINT) (Figure 29A). Under the natural scenario the risk percentage is dominated by a zero-risk for the larvae recruitment stage and a low-risk percentage for all other stages; the present scenario risk percentage is dominated by a zero-risk for the larvae recruitment stage, and a low-risk for all other stages; the future 1 scenario is dominated by a zero-risk percentage for the larvae recruitment stage, a low-risk percentage for the spawning stages and high-risk for the yellow eel maintenance and silver eel escapement stages; and the future 2 scenario the risk percentages is dominated by a zero-risk percentage for the larvae recruitment stage, a high-risk percentage for the yellow eel maintenance and silver eel escapement stages, and a low-risk percentage for the spawning stage (Figure 29B).



Uncertainty analyses

The sensitivity analyses for the four life cycle endpoints were analysed using the Sensitivity to findings tool within the Netica tool. The Sensitivity to findings tool illustrate the variables that are more influential towards the specific endpoint in question (Aguilera *et al.*, 2011)(Table 16).

Table 16: The sensitivity of findings analyses for the four life cycle endpoints and the major influential nodes affecting the life cycle nodes and the percentage of influence.

Life cycle Endpoint	Major influential node	Influential node Description	Percentage contribution
Larvae Recruitment (LARVAE_RECRUIT)	Estuary Suitability (ESTUARY_SUIT)	The node represent the suitability of the estuary to be suitable to accommodate larval glass eels.	42.2
	Estuarine Environment (ESTUARY_ENV)	The node represent the condition of the estuary environment to provide an environment for	25

Elver/Yellow eel maintenance (ELV_YEL_MAINT)	River Threats (RIVER_THREAT)	larval glass eels to survive in the estuary environment. The node represent the degree of threats towards elver/yellow eels within the river environment.	29.3
Silver eel Escapement (SILVER_ESCAP)	Escapement River Size (ESC_RIVER_SIZE)	The node represent the size of the river to allow escapement for silver eels to move between the river and the marine environment.	37.4
Spawning (SPAWNING)	Distance to Spawning grounds (DIST_SPAWNING)	The node represent the distance that silver eels need to travel from the river's mouth to the area of spawning grounds.	42.8

Discussion

The anguillid eels within the greater Incomati River Catchment are at risk due to multiple environmental drivers that act in synergy to cause effect on the ecological life cycle ecology wellbeing. This study aimed to evaluate the relative risk (possibility of death/extinction) of anguillid eels in relation to their life cycle ecology within the different life stages, for each sub-catchment within the greater Incomati River Catchment. Presently (PRS) the Sabie and Komati River sub-catchments are posing the greatest risk towards the life cycle ecology of anguillid eels within the greater Incomati River Catchment with the yellow eel maintenance (ELV_YEL_MAINT) life stage carrying the greatest risk. The natural (NAT) and all future scenarios (FUT 1 – FUT 2) are hypothetical and act as a guideline to help management decisions and approaches. The sensitivity to findings indicates that the larvae Recruitment life cycle node were largely influenced by the estuarine suitability and the estuarine environment, the Elver/yellow eel maintenance life cycle node was influenced by threats within the river environment, Silver eel escapement was influenced by the size of the river to allow escapement, and lastly the Spawning life cycle node was largely influenced by the distance eels need to travel to the spawning areas (Table 16).

Natural Scenario endpoints

The Natural scenario (NAT) represent the pre-anthropogenic conditions where disturbance and stressors were at a minimal. The scenario is used as a baseline for present and future scenarios to evaluate the rate or degree of risk change (percentage changed risk). Under the natural scenario for all sub-catchments, the larvae recruitment (LARVAE_RECRUIT) endpoint indicated a zero to low-risk score with a dominated zero risk percentage, while all other nodes for the different endpoints have a low-risk score and a low risk dominated percentage. The zero risk scores and zero dominated percentage for the larvae recruitment endpoint can be attributed to the ‘natural/unchanged’ condition of the environment hundreds of years ago with zero to little human impact, or low confident levels of the model input nodes for that endpoint due to uncertainty of stressors at that time. anguillid eels are mysterious species with an understudied life cycle, especially the stages within the marine environment (Stuart *et al.*, 2024). Under natural conditions, anguillid eels are expected throughout the greater Incomati River Catchment. Naturally, in all the sub-catchments, there were still natural barriers and waterfalls restricting/hindering their movements deep into the

catchment. This could explain one reason why under the Natural scenario conditions all life cycle endpoints had a low-risk percentage had, instead of a zero-risk score, while additional reasons could be attributed to higher indigenous predators at that time. Young yellow eels are known to have the ability to overcome barrier obstacles by ‘climbing’ over the barriers to reach the top (Watz *et al.*, 2019), while unfortunately when eels become too large the ability is reduced due to their weight.

Present Scenario endpoints

The Present scenario represents the current environmental conditions within the greater Incomati River Catchment. Compared to the Natural scenario, a sharp increase in anthropogenic dominated stressors is observed namely: the number of barriers, overfishing rates, alien species and climate change related stressors (Kleynhans *et al.*, 2015; Roux *et al.*, 2013, 2016). Under the Present scenario throughout the greater Incomati River Catchment, the Larvae recruitment (LARVAE_RECRUIT) and Spawning (SPAWNING) nodes have the lowest associated risks with larvae recruitment at a zero dominated risk percentage and spawning at a low dominated risk percentage. The low-risk scores for these nodes could be attributed to the life cycle stage being within the estuarine and marine environment and knowledge or justification within this risk model regarding stressors in those environments are low and uncertain. Very little information is known about the leptocephali and silver eel spawning life stages of WIO anguillid eels as available literature only covers some threats within the marine environment (Pous *et al.*, 2010). The Incomati Estuary is a permanently open and flowing system that provides continuous access for anguillid eels from the Indian Ocean (Hoguané and Antonio 2016). Connectivity between the estuarine environment and river environment is high with no barriers within the lower reaches. Habitats for glass eels are provided by roots of many mangroves forest on the marginal edge of the estuary (Dekker 2002). The risk for anguillid eels entering (as Glass eels) or exiting (as Silver eels) the greater Incomati River Catchment are still at a minimum but increasing concerns are raised with growing bank development, mangrove die-offs, invasive species and an increased in commercial gill net and fyke net usages (O'Brien *et al.*, 2024). From social interactions with some of the locals near the estuary it seems like locals are not targeting anguillid eels (glass eels or Adults) but will utilize the eels when caught accidentally (O'Brien *et al.*, 2024).

Within the river environment for the maintenance life cycle stages of anguillid eels in the different sub-catchment a moderate to high-risk concern arise. Between the different sub-

catchments, the Uanetze sub-catchment risk region and the upper/floodplain Incomati sub-catchment risk region are the only sub-catchments with a low dominated to a moderated risk percentage. The Uanetze sub-catchment is a naturally seasonal river that reduces the initial probability of the system to maintain and host anguillid eels (Saraiva Okello *et al.*, 2015). This sub-catchment is completely within the Kruger National Park that protects it from most major anthropogenic stressors like overfishing, water quality related stressors and large barriers. Based on the preference of anguillid eel habitat usages (Kleynhans 2007) and the current condition of the greater Incomati River Catchments' habitat and fish biodiversity, shelter and food for eels are available if they do reach deep pools and survive the low-flow seasons (Taucale 2007). The upper/floodplain of the greater Incomati River also have good available habitats and food for anguillid eels with minimal barriers (temporarily barriers) (Taucale 2007). Temporarily barriers refer to barriers that only act as a barrier in low-flow seasons, while during high-flow seasons water is flowing over the barrier. Fairly good connectivity between sub-catchments and the estuary and permanent flows in the floodplain allows eels to easily move deeper into the system. A growing concern that is starting within the Incomati floodplain risk region is overfishing from local community members that target adult anguillid eels (O'Brien *et al.*, 2024). Indeed, from social interactions between local fishing community members, it is of concern that locals target anguillid eels for exporting purposes (O'Brien *et al.*, 2024). This allows them to gain nets and gear from the demanding party and export live anguillid eels in turn for financial support (O'Brien *et al.*, 2024). This growing demand for live anguillid eels might spike interest for more local fishermen to target eels for financial support, in turn reducing the viable population numbers of adult anguillid eels returning to the marine environment to spawn or moving deeper into the catchment for growing purposes. This is different from local communities in the estuary region as they do not rely on anguillid eels for financial support. Further information is needed to understand the recruitment and leptocephali life stage of anguillid eels in this region in order to increase understanding and knowledge about what affects them within that life stage.

There is a considerable risk score increase from the natural scenarios to the present scenario concerning the river systems of the Sabie-, Crocodile- and Komati sub-catchments. The risk to the wellbeing of anguillid eels in these inland river systems are dominantly high with great concern due to various anthropogenic stressors. These include flow alterations and limited water quality and habitat stressors in the Sabie River sub-catchment, considerable water quality, flow alterations, habitat changes and alien invasive species in the Crocodile

River sub-catchment, a hard-working river, and the extreme effect of barriers and altered flows with alien invasive species affecting fish communities in the Komati River sub-catchment. Stressors like these prevent or hinder the upstream and downstream migrations of anguillid eels (Silva *et al.*, 2018; O'Brien *et al.*, 2019; Wade *et al.*, 2020). Increasing anthropogenic stressors in the inland river systems of the greater Incomati River Catchment will cause further degradation of the river systems, in turn affecting the wellbeing of current anguillid eel populations. From interviews, the dependence on anguillid eels from local community members are minimal due to the difficulty and time-consuming process to collect eels because of their scarcity (O'Brien *et al.*, 2024). However, if community members do come across eels, especially near weirs or dams, they will utilise the resources for food and not exportation as in the floodplain regions (O'Brien *et al.*, 2024).

Future Scenario endpoints

The future prediction scenarios aimed to provide insight into proposed management planning and decision-making processes. The Future 1 (FUT 1) scenario indicates how the risk assessment will response when more dams are developed and build within the greater Incomati Catchment. More dam development will lead to higher high-risk score throughout the greater Incomati Catchment, except the Uanetze sub-catchment that will increase from a zero to a low risk dominated environment. The Uanetze River sub-catchment is protected within the Kruger National Park and dam development within this National Park was presently set to a minimum. Concerns are raised with the new developmental plan for the Moamba Dam, just of the confluence of the Crocodile and Komati Rivers in Mozambique (Bekele 2025). The construction of a new dam, the Moamba dam, will reduce the available habitats and river areas available for Anguillids as migration past the Crocodile – Komati River confluence will be restricted. If anguillid eels do however manage to pass over the Moamba dam, migration back to the marine environment will be further reduce as eels might easier get over barriers when they are small, but adult eels will struggle on their way back (O'Brien *et al.*, 2019; Watz *et al.*, 2019). Unless management decisions and practises already incorporate a design for a fish ladder or bypass to aid in the migration pathway of these anguillid eels (Silva *et al.*, 2018). The Sabie River sub-catchment illustrate a decline in anguillid eel population upstream of a major hydropower dam (Corumana dam). The dam is situated on the Sabie River on the South Africa border. From this study and interviewed local communities (O'Brien *et al.*, 2024) no eel have been collected or seen above the dam (in

South Africa's side) for a very long time. Corumana Dam poses as a major barrier preventing the upward migration of anguillid eels into the Sabie River sub-catchment.

The future 2 (FUT 2) prediction scenarios aimed to provide insight into proposed management planning and decision-making processes when no further management decisions or strategies are developed, and water resources are managed and protected based on the current proceedings. With the future 2 scenario predictions, all sub-catchments will have high risk dominated endpoints except for the spawning endpoints. The spawning endpoint reflects stressors within the marine environment (tidal changes, marine predators) that is not affected by inland management decisions and strategies. The future 2 predictions illustrate higher high-risk scores for all sub-catchments leading to a greater risk of the well-being of anguillid eels. Although the high-risk scores for some sub-catchments between the present and the future 2 scenarios did not increase to much ($<10\%$), it indicates that this sub-catchment is already on its max risk percentage for the model. It could get worse for those sub-catchments but the wellbeing to anguillid eels is unsustainable and neglected with the current management processes and strategies already. This is only a worst-case scenario and further future predictions can be made to aid decision making process in terms to decide what changes in a strategy is needed.

Conclusion

In this study, the RRM successfully illustrate the wellbeing of the life cycle ecology of anguillid eels within the greater Incomati River Catchment. The model illustrates a noticeable increase in risk towards the endpoint nodes within the inland life cycle sections for anguillid eels at moderate to high-risk while estuarine and marine environment life cycle sections are still at ease. The anguillid eels within the greater Incomati River Catchment are under threat due to multiple environmental drivers that act in synergy to cause effect on the ecological life cycle ecology wellbeing. Anthropogenic stressors like barriers, overfishing (for exportation) and habitat/connectivity loss are greatly influencing the wellbeing of anguillid eels within the catchment with future prediction scenarios indicating what current management processes and strategies could lead too.

The use of a RRM assessment provides a cause-and-effect pathway on many spatial scales and can address complex environmental stressors. The approach allows management authorities to directly address major environmental drivers through established resource quality objectives. The RRM assessment can contribute towards the effectiveness of

management strategies to set a balance between the use and protection of anguillid eel populations in southern Africa.

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Chapter 6

Discussion and Conclusion

This is a final discussion and conclusion chapter where findings of all data chapters are discussed and recommendations for the different outcomes are provided to aid management discussions. Reference is made back to the main aim to provide an in-depth evaluation of the anthropogenic stressors affecting fish communities and indicator species within the greater Incomati River Catchment.

Research findings

To achieve the aim of this study, five research objectives were established and completed.

The first objective was to review the knowledge of fish communities in the greater Incomati River Catchment, southern Africa along with their wellbeing associated to the different conditions of the rivers. To achieve this objective a broad literature review summarises the importance of rivers, the laws protecting them, and the different indices used in management, biomonitoring, and conservation efforts of the rivers in the greater Incomati River Catchment. Brief considerations are provided for their sustainable use, protection, and the environmental and anthropogenic threats faced by fishes. This review also included information on the historical wellbeing, conservation status and current threats to fish communities.

Objectives two was to evaluate the risk of multiple stressors affecting the rivers of the greater Incomati River Catchment, using stressor-ecosystem evidence pertaining to the state of fish in the catchment and environmental drivers of these communities. To achieve this objective, the fish communities were evaluated in terms of the multiple environmental stressors that affects them in the river environment. Multiple lines of evidence, including the Fish Response Assessment Index and Redundancy Analyses were completed to determine the current state of the fish communities as well as the environmental drivers affecting each community throughout the greater Incomati River Catchment. Of the expected 70 indigenous fishes, 50 species were collected from 53 sites located throughout the greater Incomati River Catchment between 2020 and 2024. Additionally, four invasive fish species were collected and only one of the critically endangered fish species were collected. Overall, the state of the fish communities are in a moderately modified state with a few sites that are in an un-

acceptable largely modified condition. Re-evaluations between sampling years/seasons did not indicate improvement. Various environmental drivers significantly affected the fish communities within the different sub-catchments although substrate availability and water quality alterations had the greater influence on driving all the communities. Each sub-catchment is unique to its own environmental drivers that require precise orientated monitoring and management. Monitoring of anthropogenic stressors is important to protect the valuable integrity of the greater Incomati River Catchment and its fish communities. The statistically correlated stressors affecting fish communities identified by the RDA approach are available to regulators to inform mitigation and manage strategies.

Objective three was to determine which river regions and specific endpoints are at high risk from anthropogenic activities and what this could mean for management decisions for fish communities. To achieve this objective a Relative Risk Assessment using the Bayesian Network model was used to determine the risk towards the ecological indicator fishes as well as the risk towards subsistence fisheries targeted for human usages. The risk indicate the likelihood that adverse ecological effects will occur. The outcomes provides risk regions of conservation concern and where management prioritization should be implemented. The assessment suggest low to moderate risk scores for all sites in the greater Incomati River Catchment in terms of the ecological indicator fishes with risk regions in the Komati River sub-catchment having the highest risk. The Uanetze River sub-catchment was the least affected sub-catchment with increasing risk towards the larger sub-catchments. The risk towards subsistence fisheries throughout the greater Incomati river Catchment were lower with zero to low-risk percentages. Some high populated areas had higher risk towards targeted subsistence species. This approach allows management authorities to directly address major environmental drivers through established resource quality objectives and set risk-based priorities. Resource allocation must be orientated to focus on risk areas that are most significant to set a balance between the use and protection of aquatic freshwater resources in South Africa.

The fourth objective was to determine the current distribution/status of anguillid eels within the greater Incomati River. To achieve this objective distribution maps were compared between historical collections and current collections. Historically (1960-2020), 144 anguillid eels were collected throughout the greater Incomati River Catchment with *Anguilla mossambica* being the most abundant collected species. Currently, from 54 sites, only 58 anguillid eels were collected with *Anguilla mossambica* being the most abundant. Current

anguillid eel distributions indicate a 40.2% decline in their distribution. River connectivity in the Sabie River has been disconnected with the construction of Corumana Dam in 1989. Large barriers like this prevent the bi-directional migration of anguillid eel species, hindering their future reproductive cycle. Currently, anguillid eels were only collected in the Crocodile and Incomati River sub-catchments as barriers in the lower Sabie and throughout the Komati River systems prevent their migration. Observations of illegal syndicate eel exports activities to China also threatens their current populations within Mozambique.

The last objective was to undertake a regional scale ecological risk assessment of multiple stressors affecting anguillid eel populations in the greater Incomati River Catchment and provide recommendations for sustainable management and conservation. To achieve this objective a Relative Risk Assessment using a Bayesian model were created to inform ecological and social endpoints on the relative risk involve for anguillid eels. Four scenarios, of which three were hypothetical, were evaluated to assess the risk when management and monitoring strategies are aimed to prioritise different detrimental stressors. The study model illustrated a noticeable increase in risk towards the outcomes within the inland life cycle stages for anguillid eels at moderate to high-risk while estuarine and marine environment life cycle stages have zero to low associated risk. This study demonstrates the probable effects that environmental drivers have on the anguillid eel populations and the ecological wellbeing within the Western Indian Ocean. Anthropogenic stressors like barriers, overfishing (exportation) and habitat destructions are greatly influencing the wellbeing of anguillid eels within the catchment with future scenarios prediction the outcomes if current management processes and strategies are not attended to.

Recommendations

Human and animal communities depend on the services derived directly from the quality and quantity of available freshwater sources (Holmlund and Hammer 1999). Water resources that provide these services are constantly threatened by multiple stressors that change the quality of, and abundance of these important services (Fouchy *et al.*, 2018). It is important that these systems and the fish communities be protected and/or sustainably managed. Accordingly, the following recommendations are provided:

- Management objectives should implement separate strategies for each river system due to the different anthropogenic environmental drivers. These include flow alterations and limited water quality and habitat stressors in the Uanetze and Sabie

River sub-catchments, considerable water quality, flow alterations, habitat changes and alien invasive species in the Crocodile River sub-catchment, the extreme effect of barriers and altered flows with alien invasive species affecting fish communities in the Komati River sub-catchment and lastly, invasive species, overexploitation and agricultural water abstraction in the Incomati River sub-catchment.

- Land-use changes driven by anthropogenic activities leading to sedimentation and mine drainage are responsible for the degradation of the river systems. Land rehabilitation programs should be implemented to mitigate the effect of these stressors.
- Physical barriers to migration must be mitigated by implementing fishways (Bok *et al.*, 2007) or if the barrier is considered to be redundant to be completely removed.
- In 2002, a Tripartite Agreement (South Africa, Mozambique, Eswatini), the Interim IncoMaputo Agreement (IIMA) were signed which include setting environmental flow requirements for the greater Incomati River Catchment (DWS 2002; Food and Agriculture Organization 2002; WIOMSA 2009; DWS 2019). The set environmental flows of the greater Incomati River should be revised to ensure that sufficient water supply are provided to all areas throughout the catchment.
- Illegal syndicate operations in Mozambique should be addressed and stopped to ease target pressures off vulnerable anguillid eel populations.

Conclusion

The greater Incomati River Catchment is home to several genetically unique and endangered fish species of high conservation importance, that could be better managed if existing sustainable water resource management requirements are effectively implemented. South Africa strives to balance the use and protection of its natural resources while working towards achieving the Sustainable Development Goals (SDGs) by 2030 (Dickens *et al.*, 2019). For instance, SDG 6 emphasizes the need for safe drinking water and well-managed sanitation for all.

While South Africa's water resource management framework is progressive, it remains adaptable to meet legislative needs and ensure the long-term sustainability of water resources (King and Pienaar 2011). Implementing a Multiple Lines of Evidence approach will strengthen resource management, enhance intervention strategies, and promote effective

conservation efforts. Ultimately, these efforts will help secure clean, safe water for all while ensuring the continued provision of essential ecosystem services.

In conclusion, this study successfully characterized and evaluated the effects of multiple anthropogenic stressors on the rivers of the greater Incomati River Catchment, from source to sea. By utilizing fish communities and migratory species as ecological indicators, I have provided valuable insights into the health and resilience of these aquatic ecosystems. Through multiple lines of evidence, the findings highlight the extent of human-induced pressures and their implications for riverine biodiversity and ecosystem functioning.

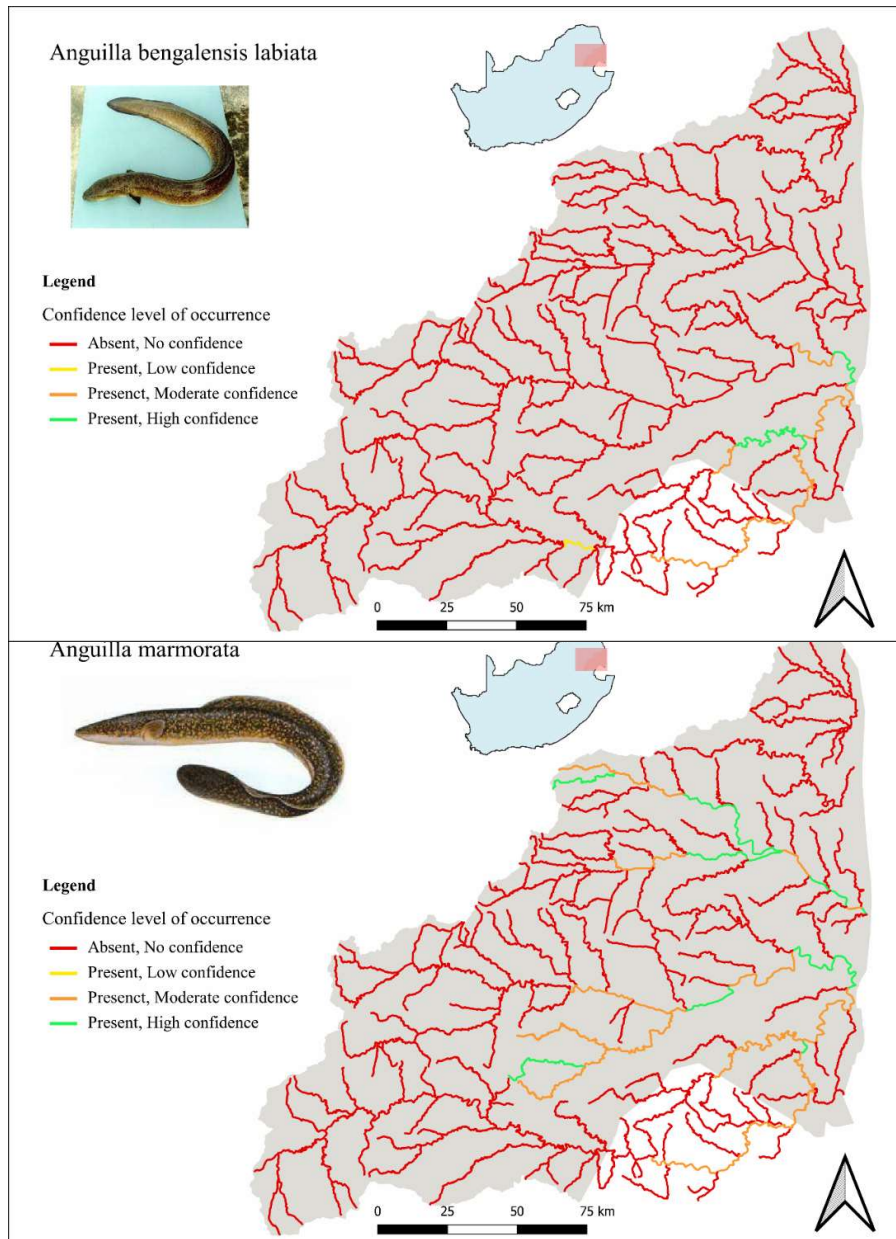
References

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- Western Indian Ocean Marine Science Association (2009) *Environmental Profile of the Inkomati River Basin*. Nairobi Convention. Available at: <https://www.nairobiconvention.org/CHM%20Documents/WIO-Lab%20Outputs/Regional%20reports/Incomati%20Environmental%20Profile%20final%20.pdf> (Accessed: [2/04/2025]).
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APPENDIX DATA

Appendix Data 1: Distribution maps of expected species in the Greater Incomati River Catchment, South Africa.

The distribution occurrence was obtained from PESEIS data (2013) based on the confidence of occurrence for the species at the specific river reach in South Africa (DWS 2016). Species names and confidence level of occurrence indicated on each map. Photos of fish are obtained from Skelton (2001) or from field collection. Data only available for South Africa rivers.



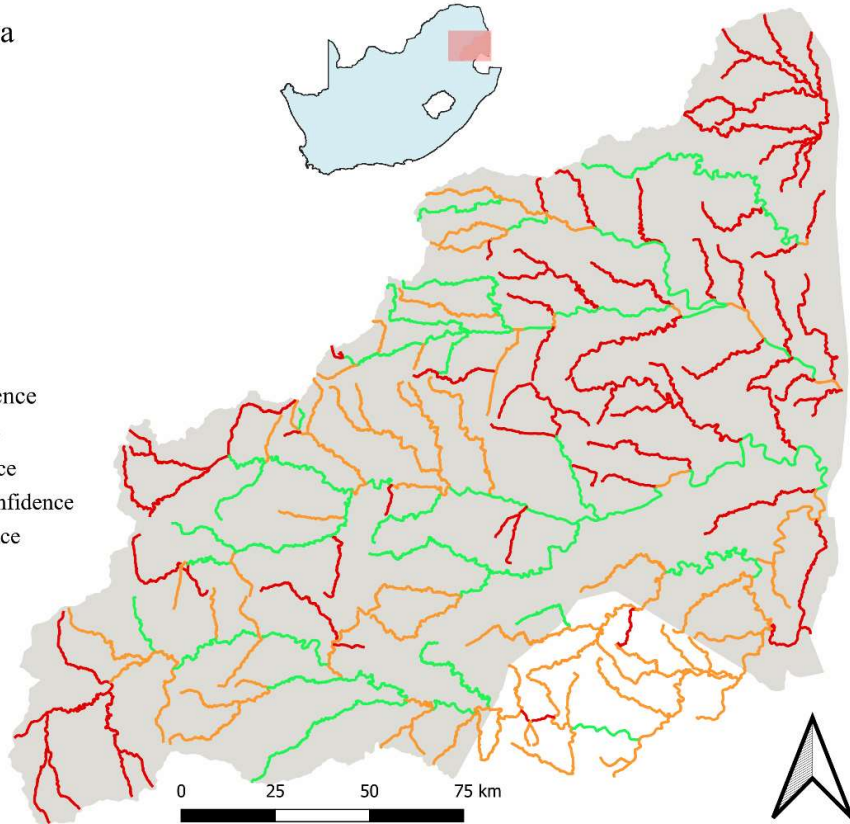
Anguilla mossambica



Legend

Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



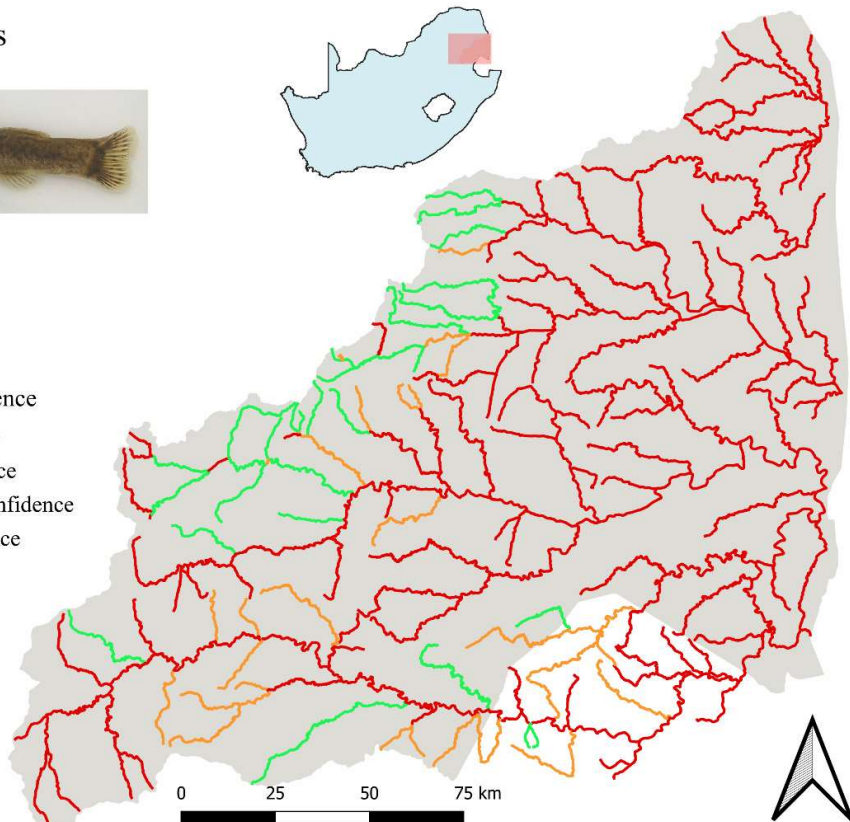
Amphilius natalensis



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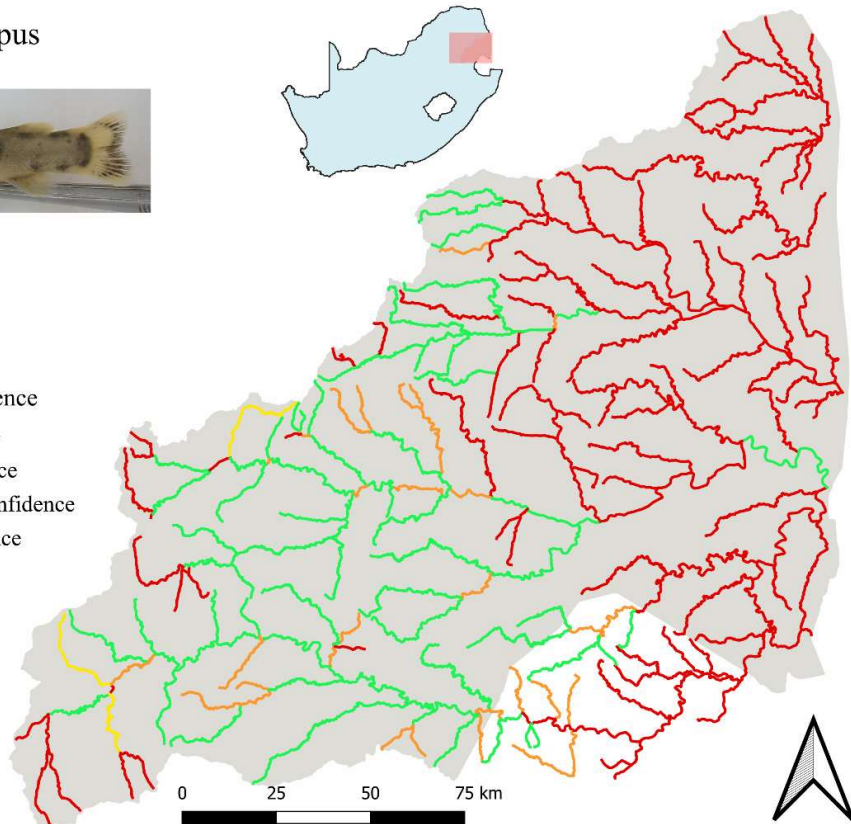
Amphilius uranoscopus



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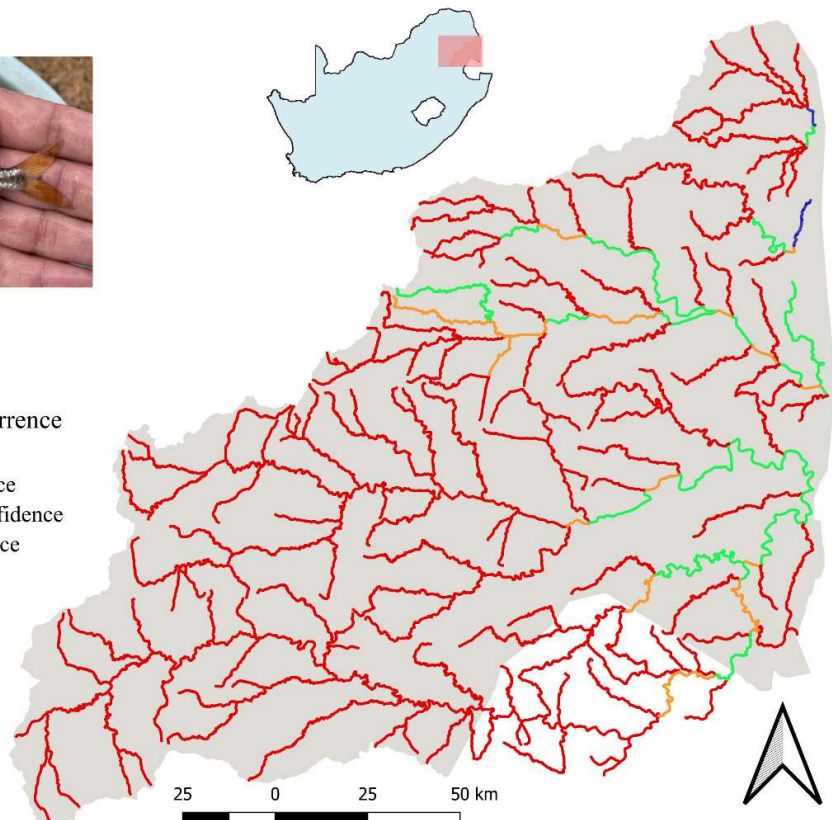
Brycinus imberi



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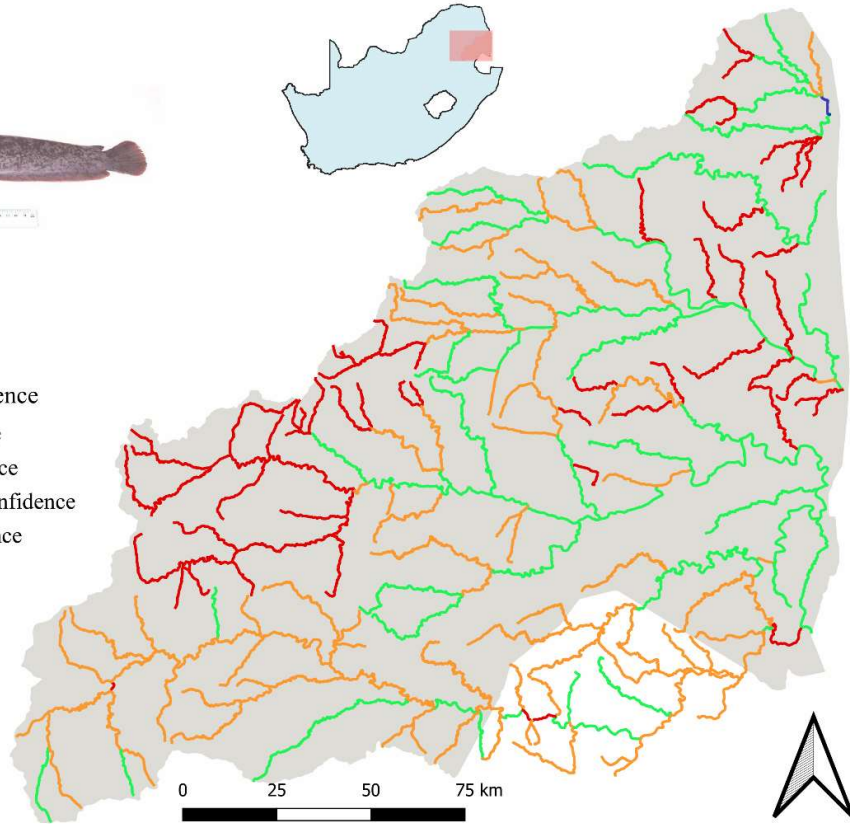
Clarias gariepinus



Legend

Confidence level of occurrence

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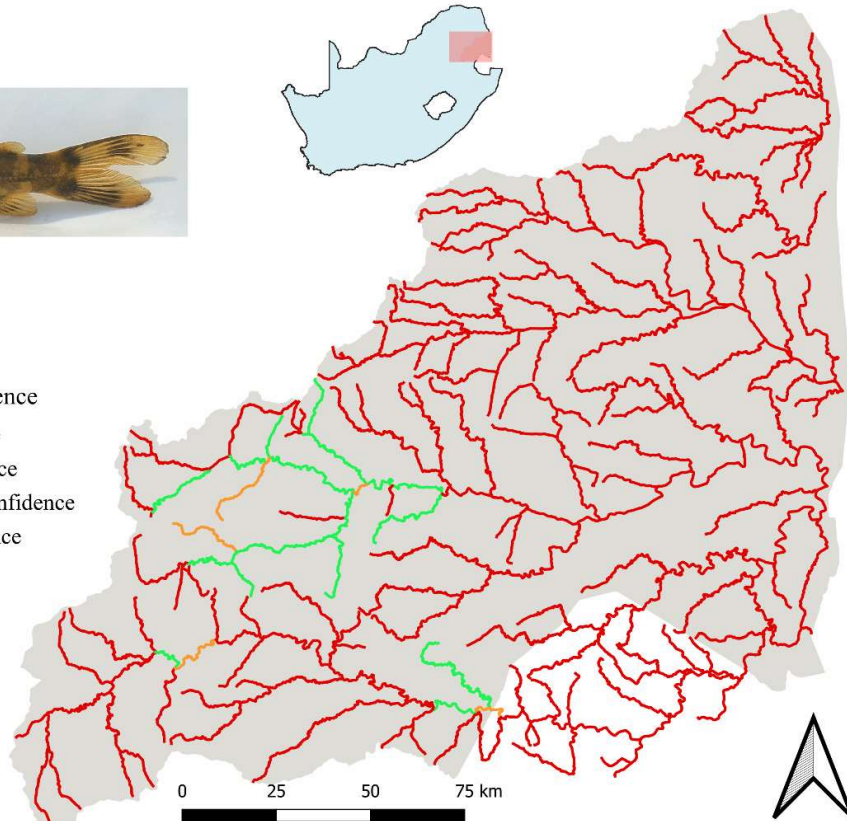
Chiloglanis bifurcus



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Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



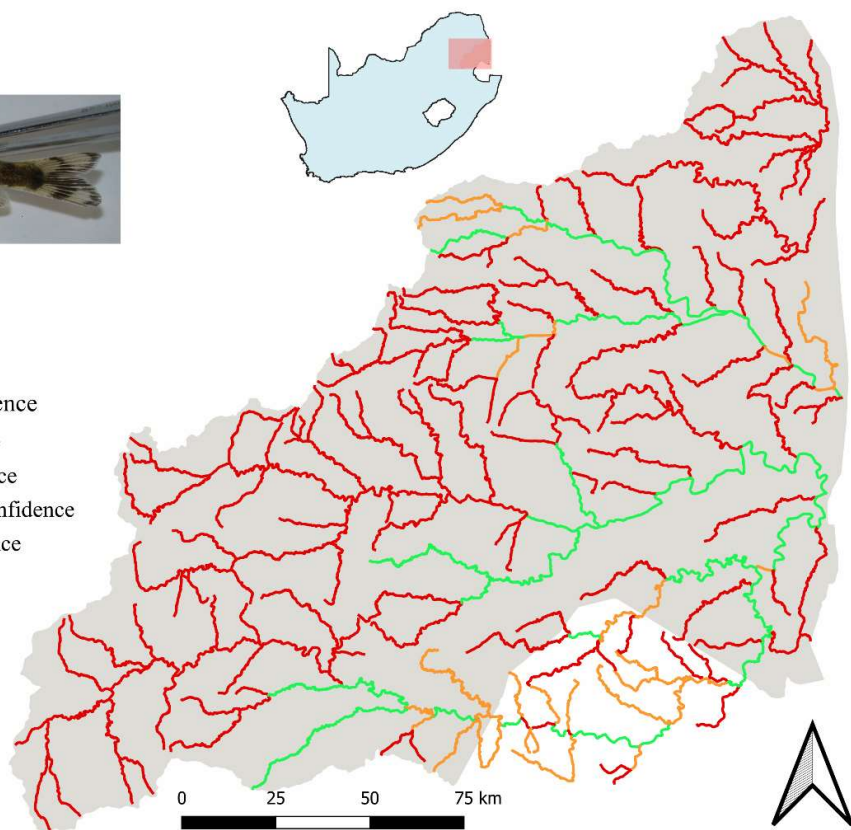
Chiloglanis paratus



Legend

Confidence level of occurrence

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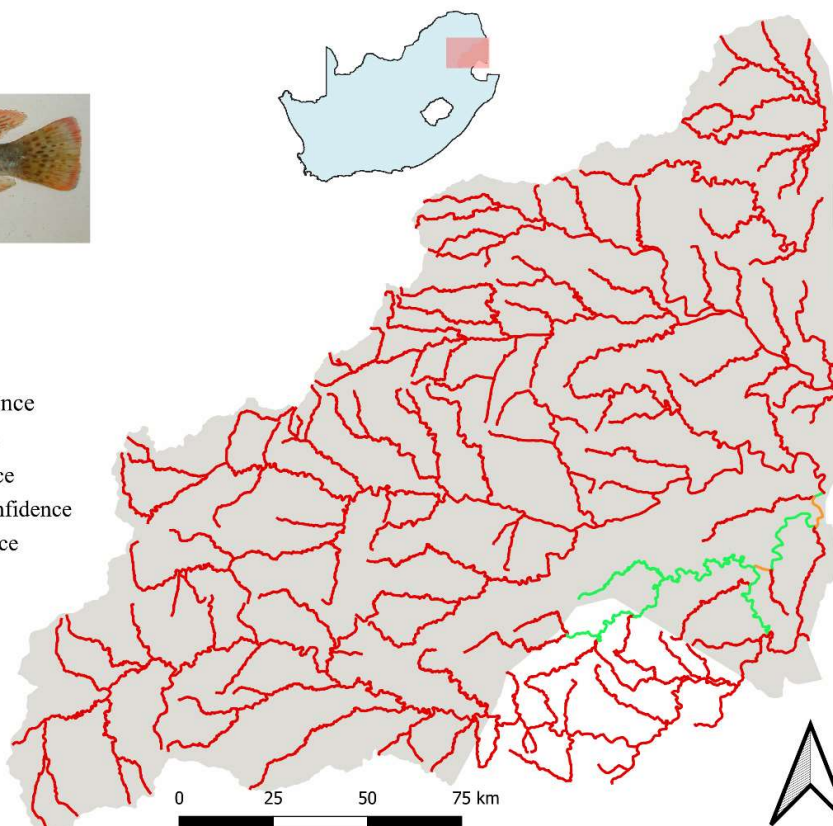
Chetia brevis



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Confidence level of occurrence

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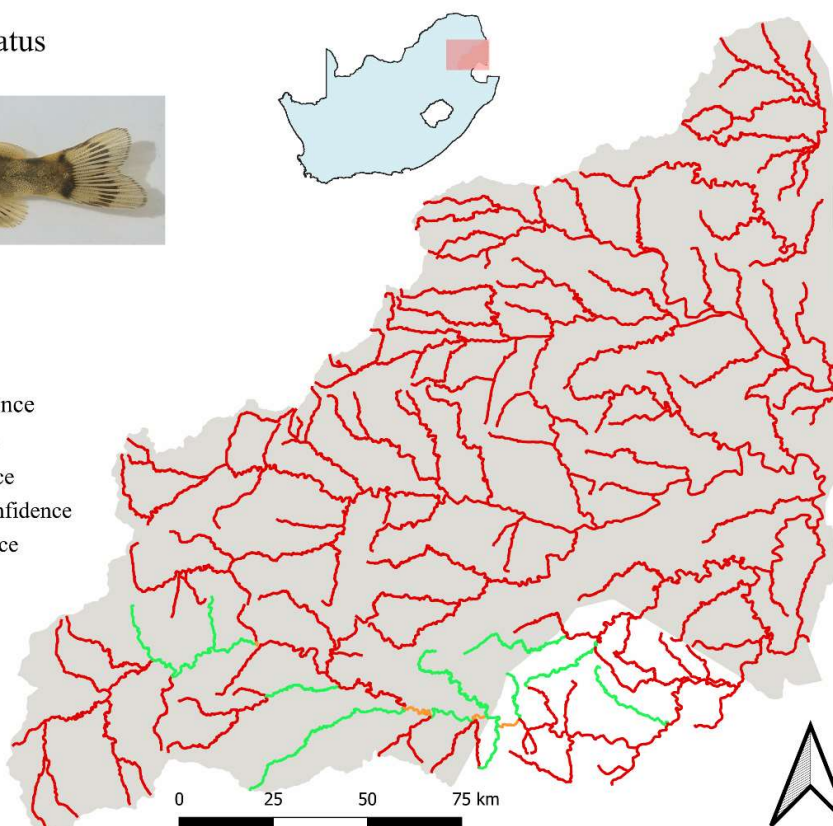
Chiloglanis emarginatus



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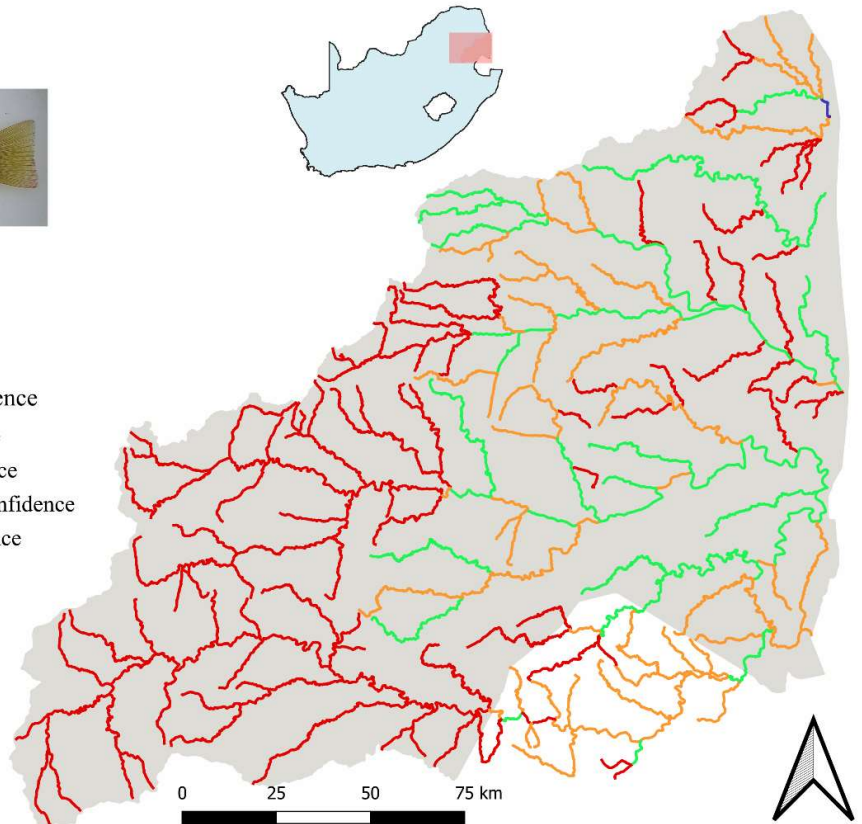
Coptodon rendalli



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Confidence level of occurrence

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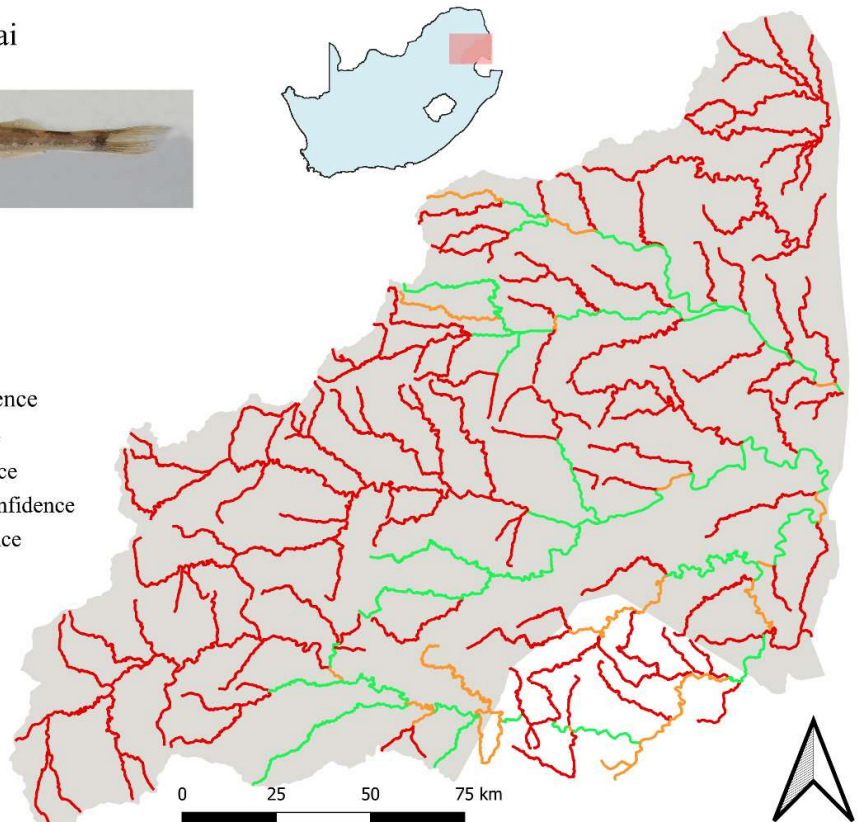
Chiloglanis swierstrai



Legend

Confidence level of occurrence

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- Present, High confidence



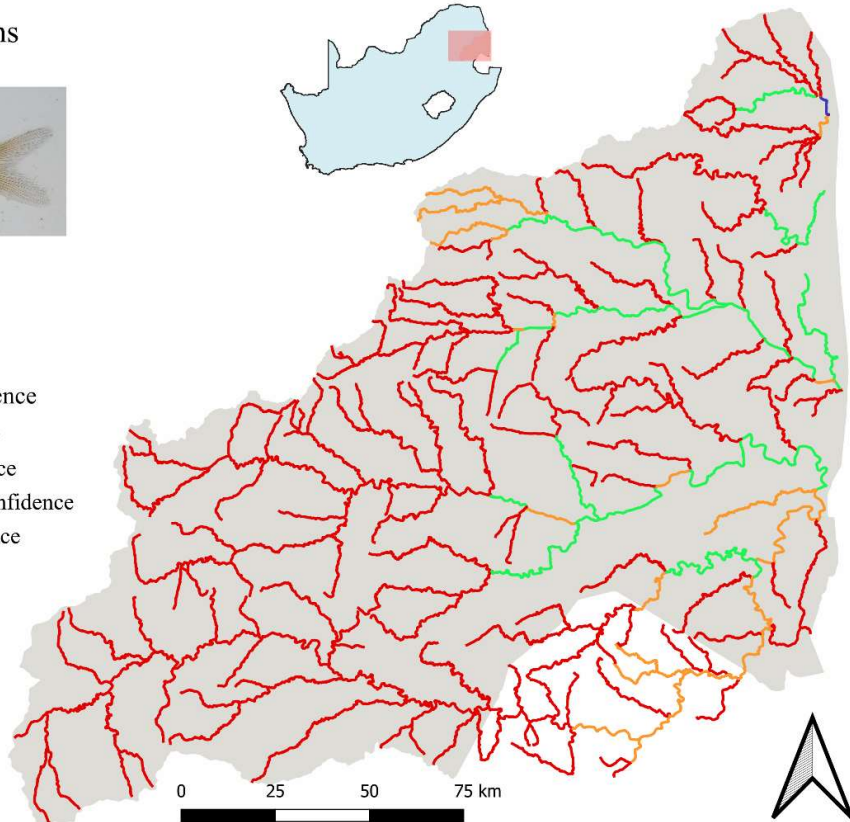
Enteromius annectens



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Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



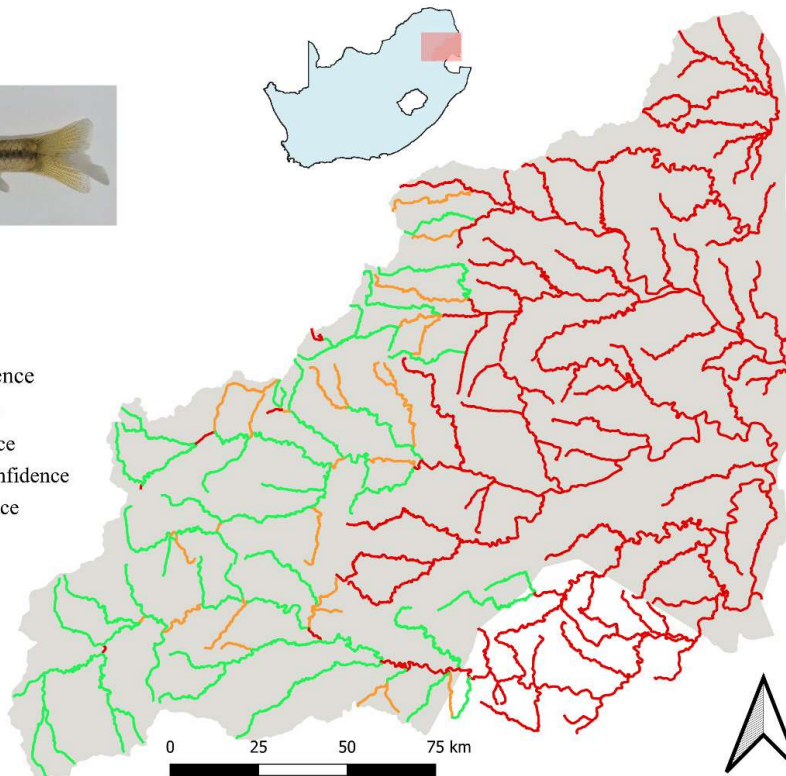
Enteromius anoplus



Legend

Confidence level of occurrence

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- Present, High confidence



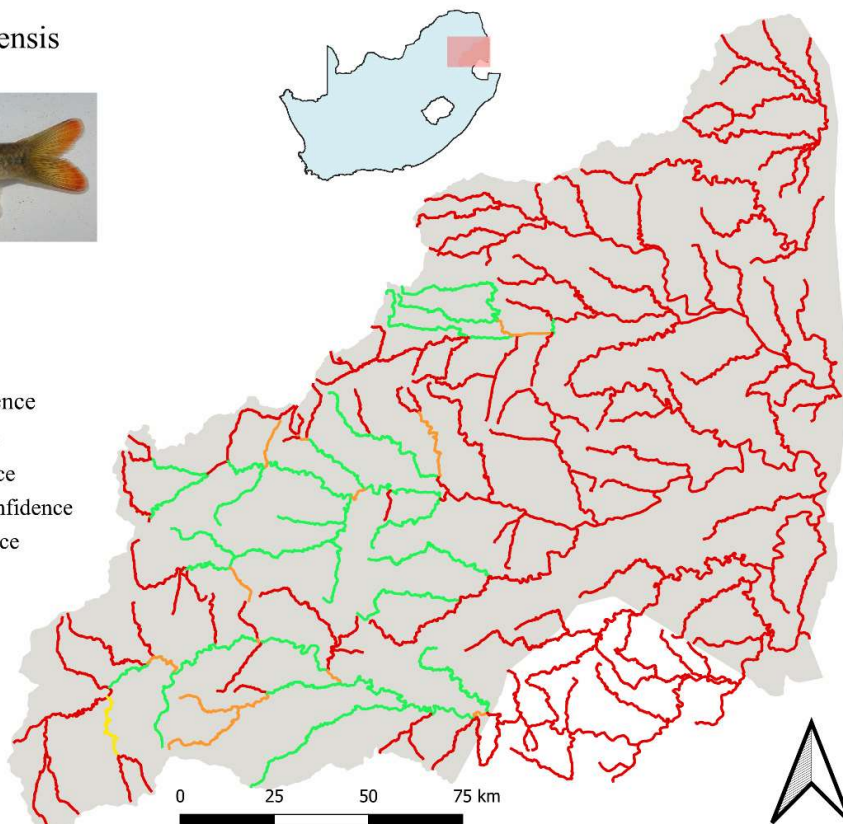
Enteromius crocodilensis



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Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



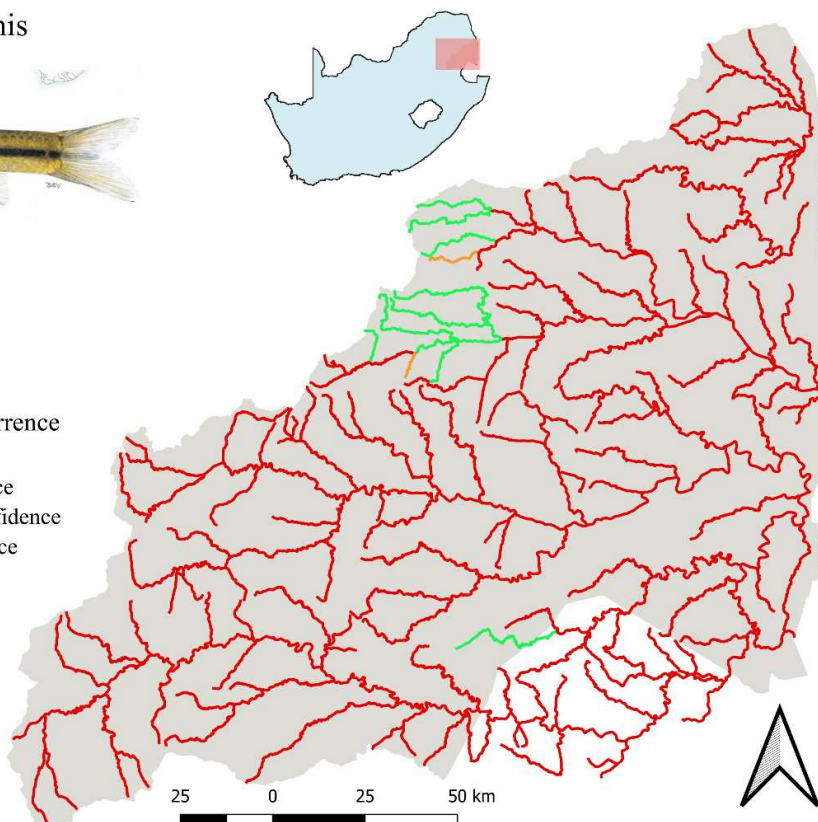
Enteromius brevipinnis



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Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



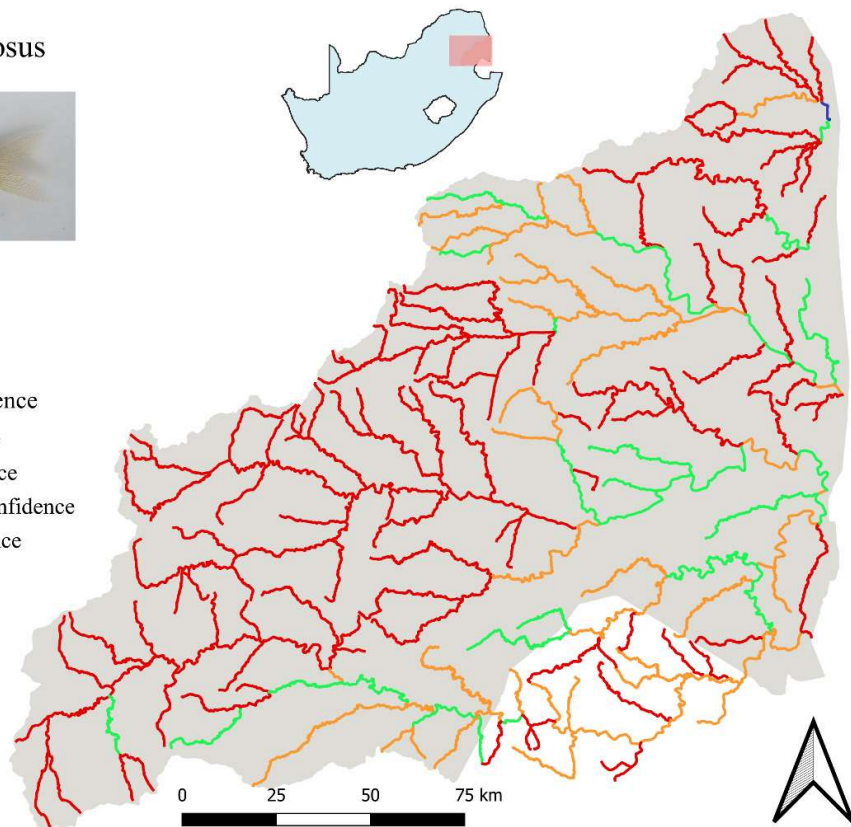
Enteromius paludinosus



Legend

Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



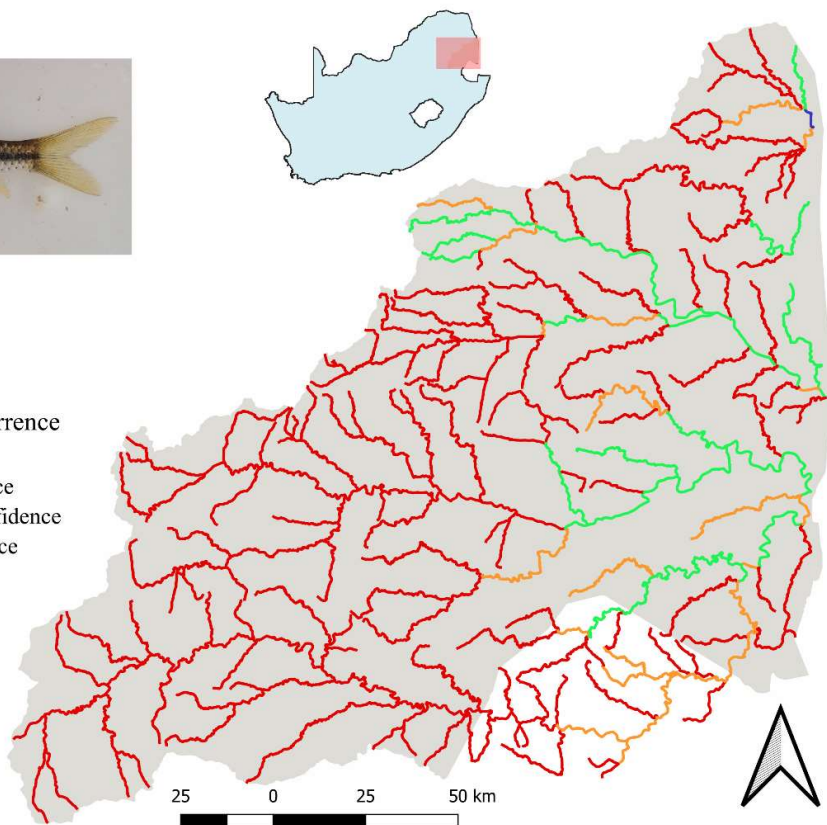
Enteromius radiatus



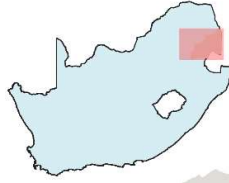
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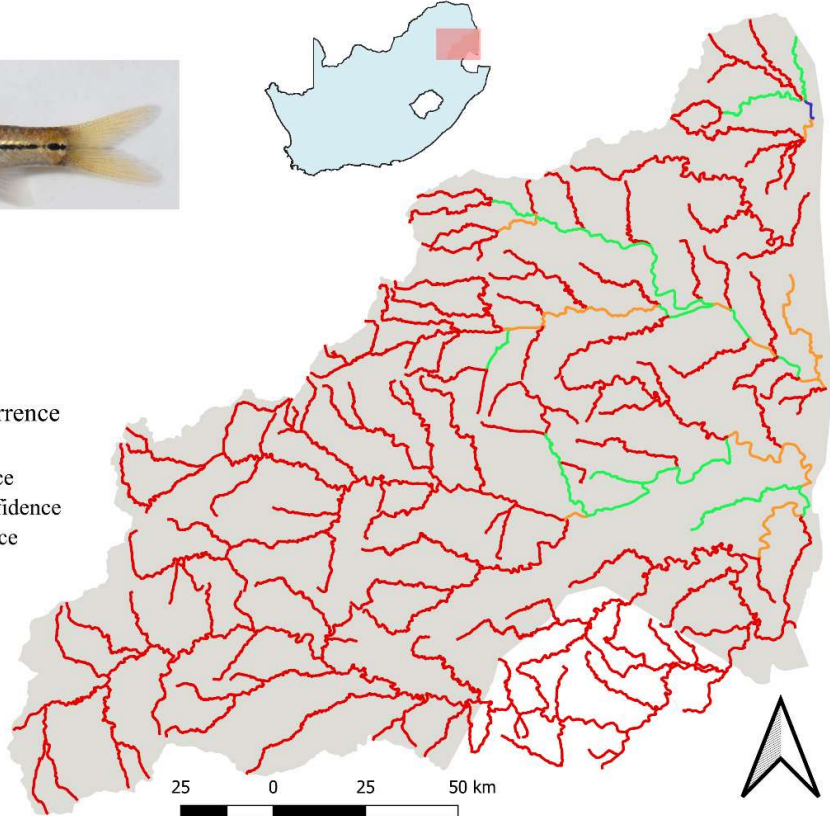
Enteromius toppini



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Confidence level of occurrence

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- Present, Moderate confidence
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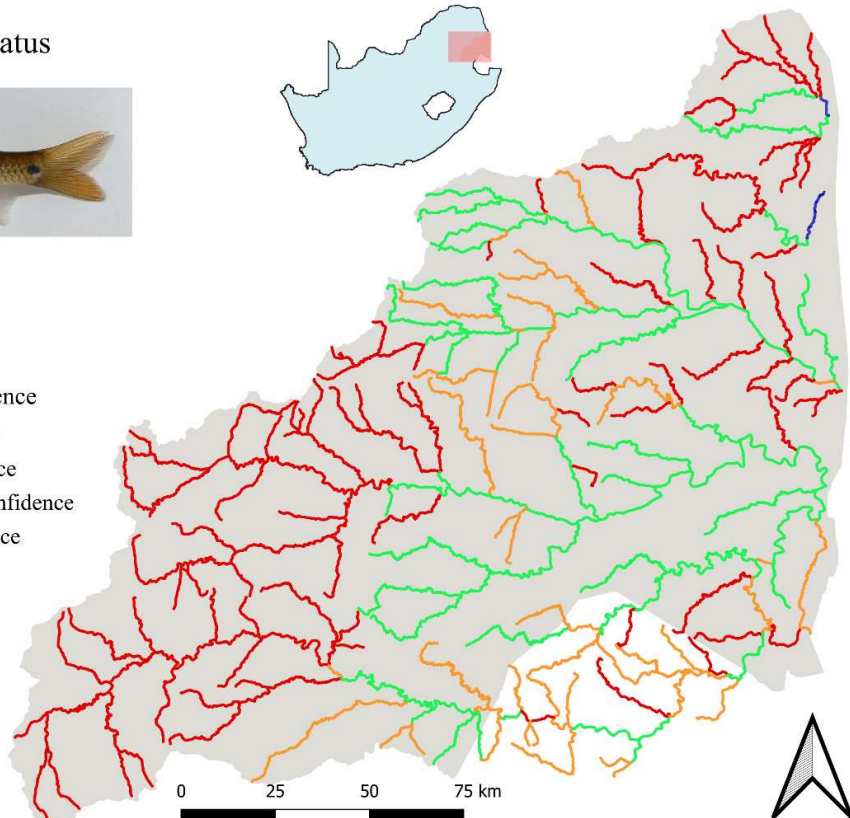
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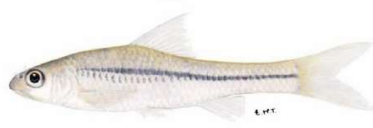
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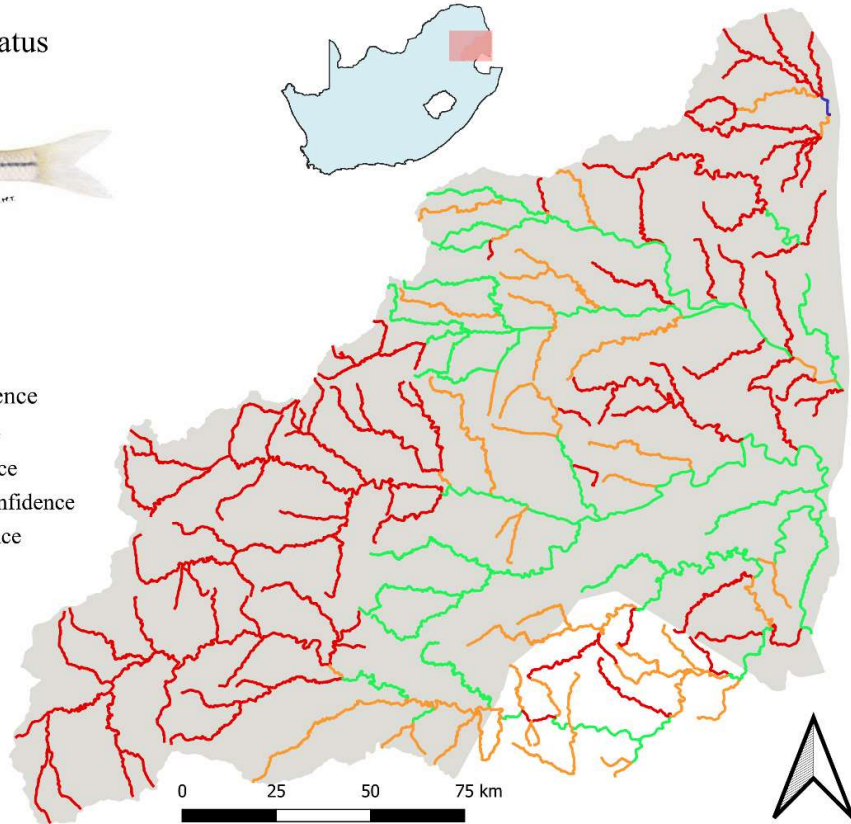
Enteromius unitaeniatus



Legend

Confidence level of occurrence

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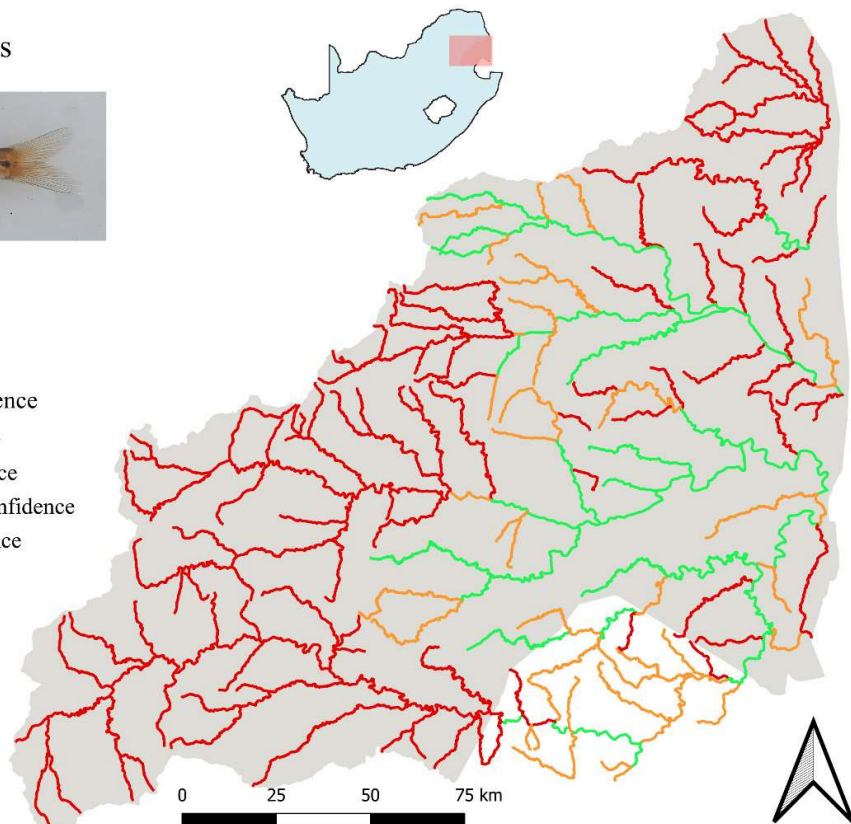
Enteromius viviparus



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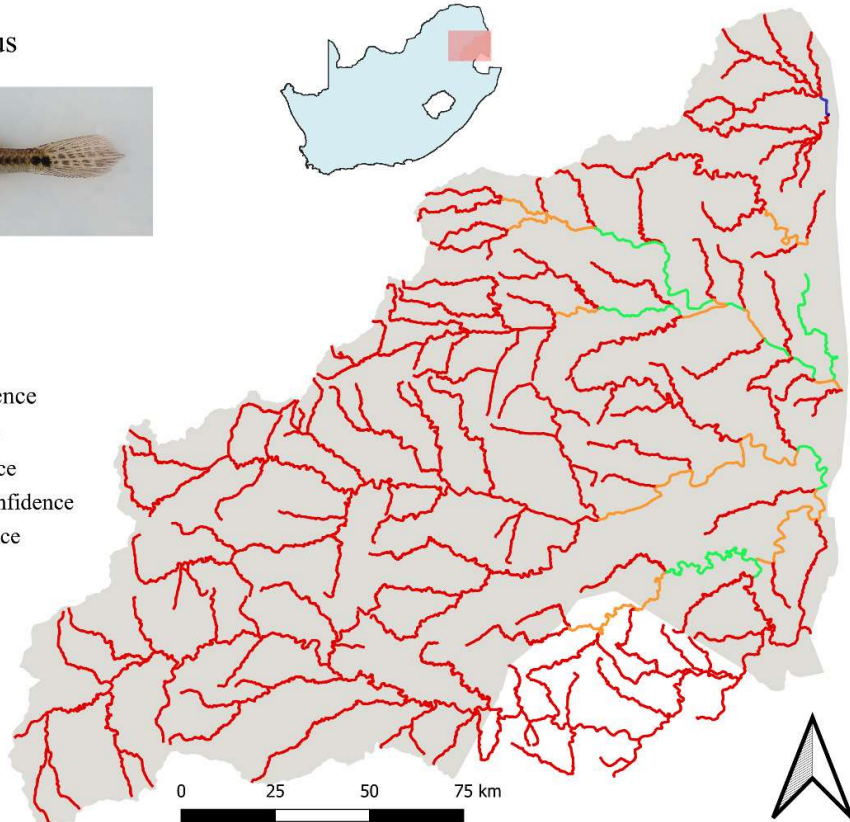
Glossogobius callidus



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Confidence level of occurrence

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- Present, High confidence



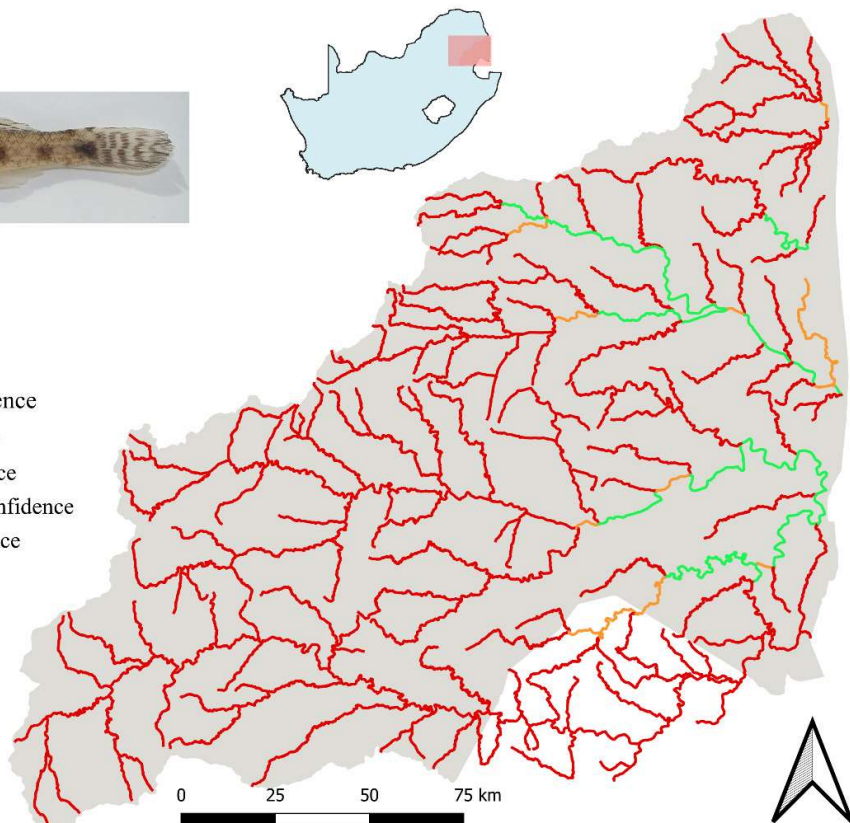
Glossogobius giuris



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Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



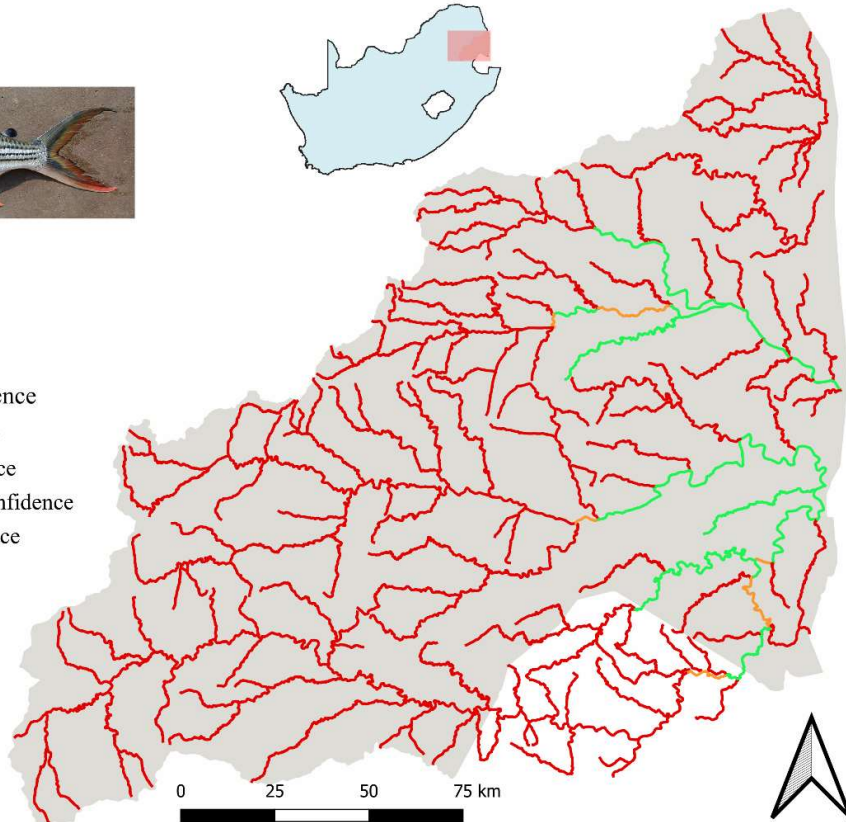
Hydrocynus vittatus



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Confidence level of occurrence

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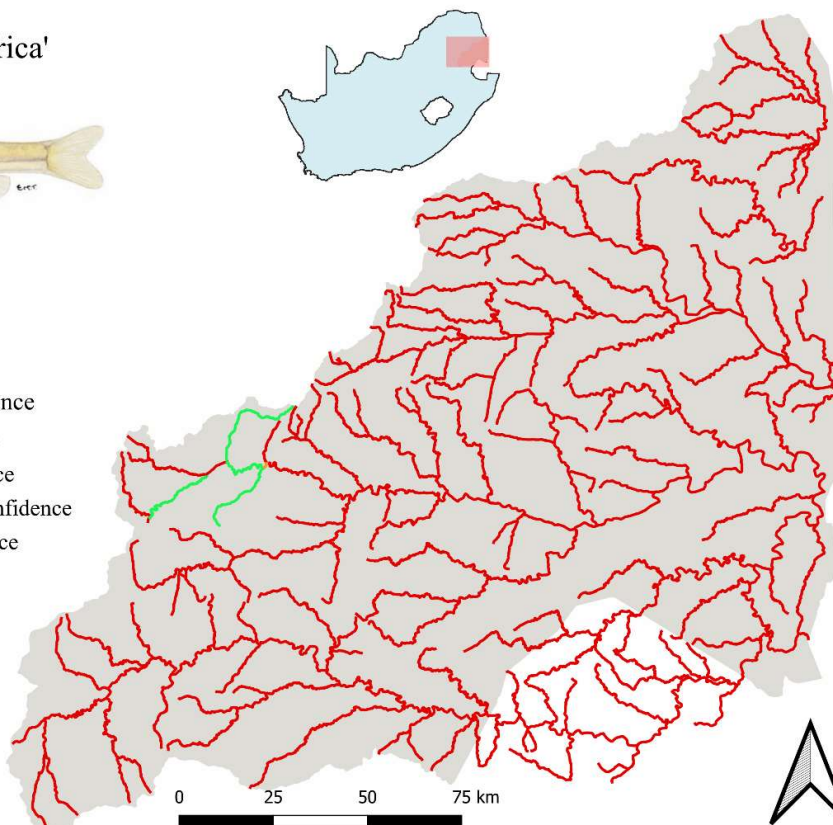
Kneria sp. 'South Africa'



Legend

Confidence level of occurrence

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- Present, Moderate confidence
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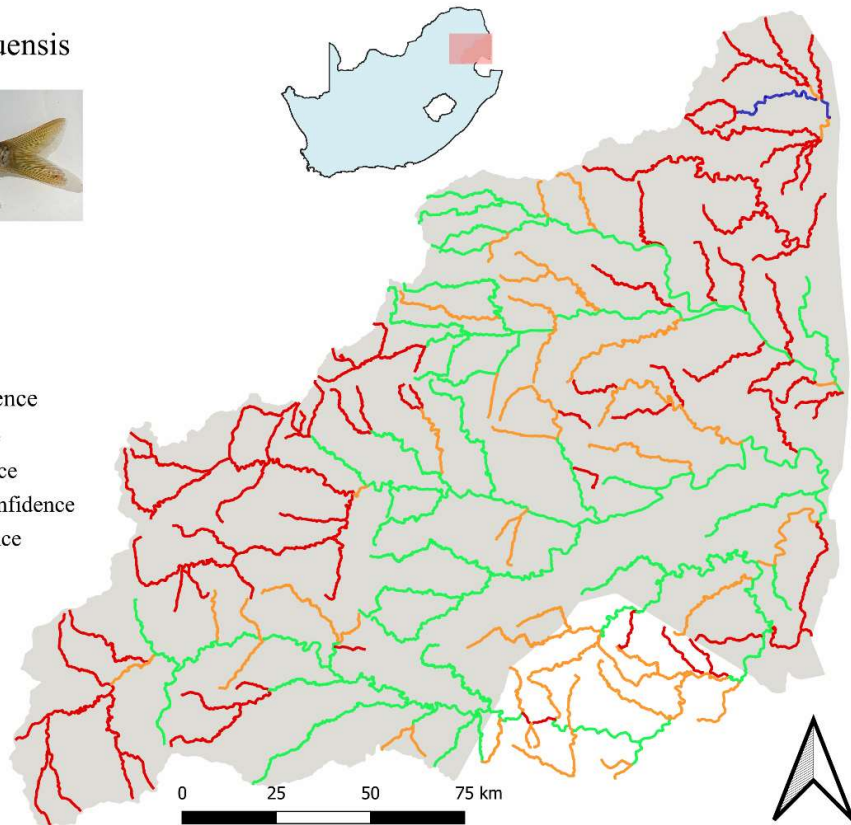
Labeobarbus marequensis



Legend

Confidence level of occurrence

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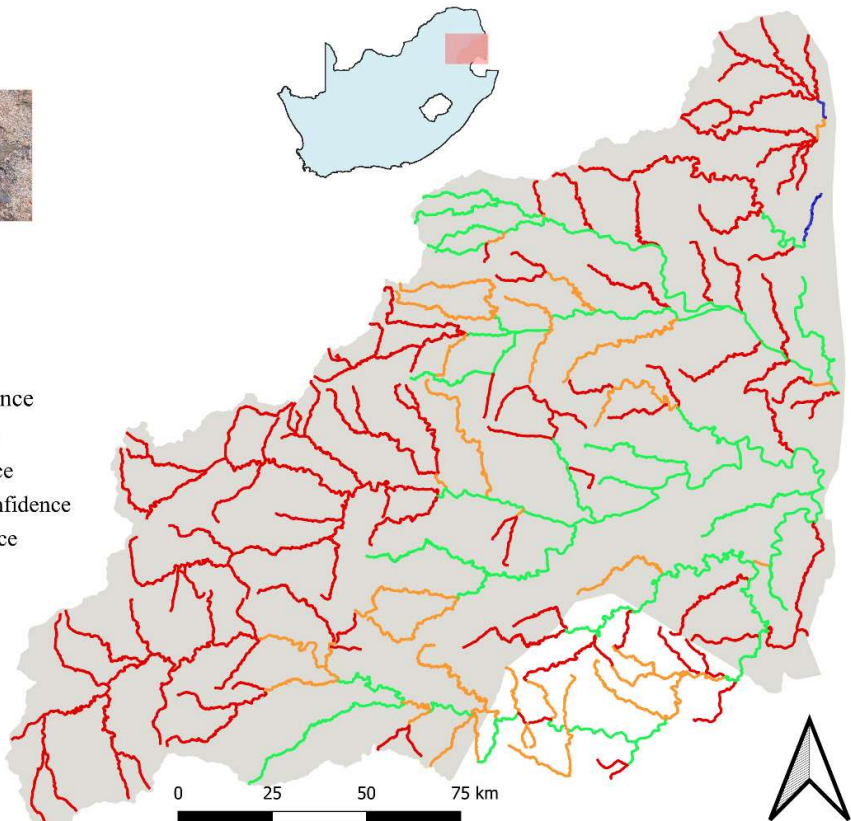
Labeo molybdinus



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Confidence level of occurrence

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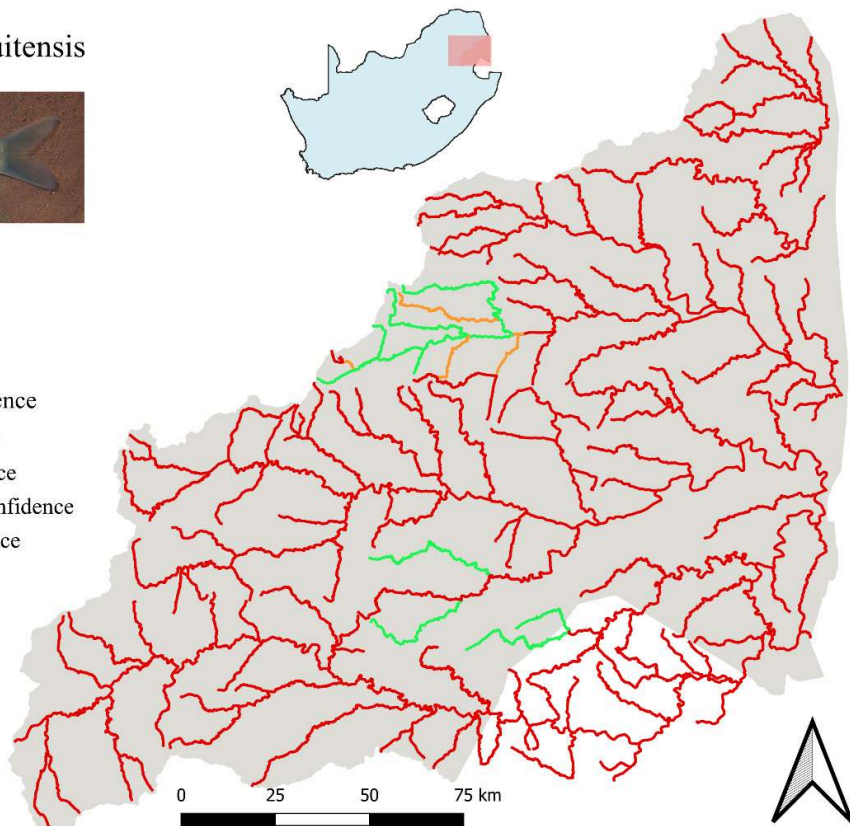
Labeobarbus nelspruitensis



Legend

Confidence level of occurrence

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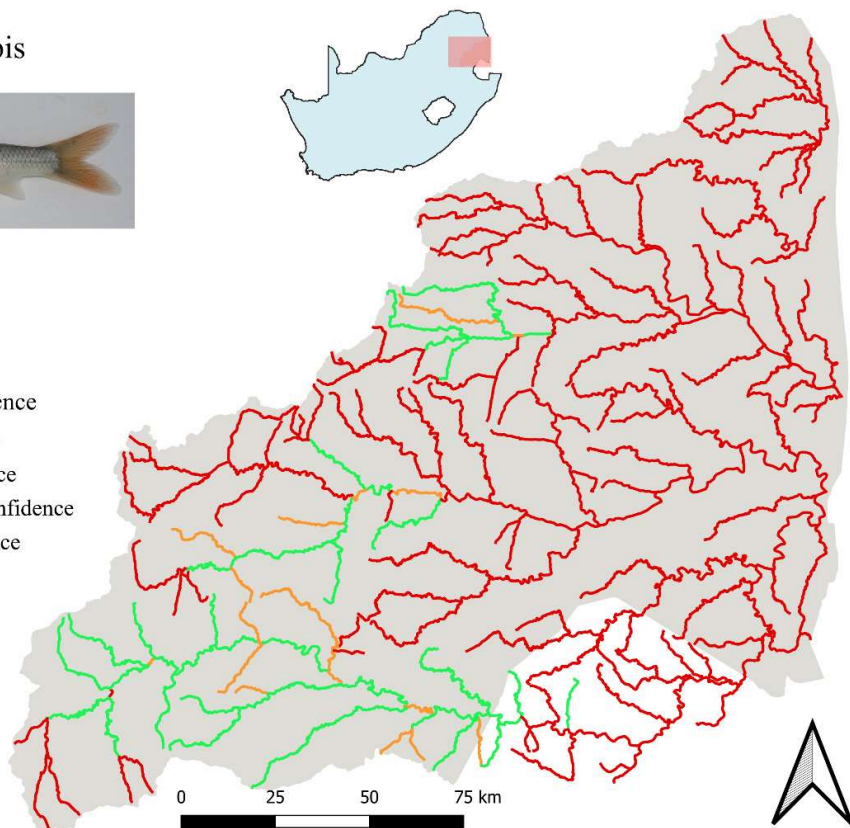
Labeobarbus polylepis



Legend

Confidence level of occurrence

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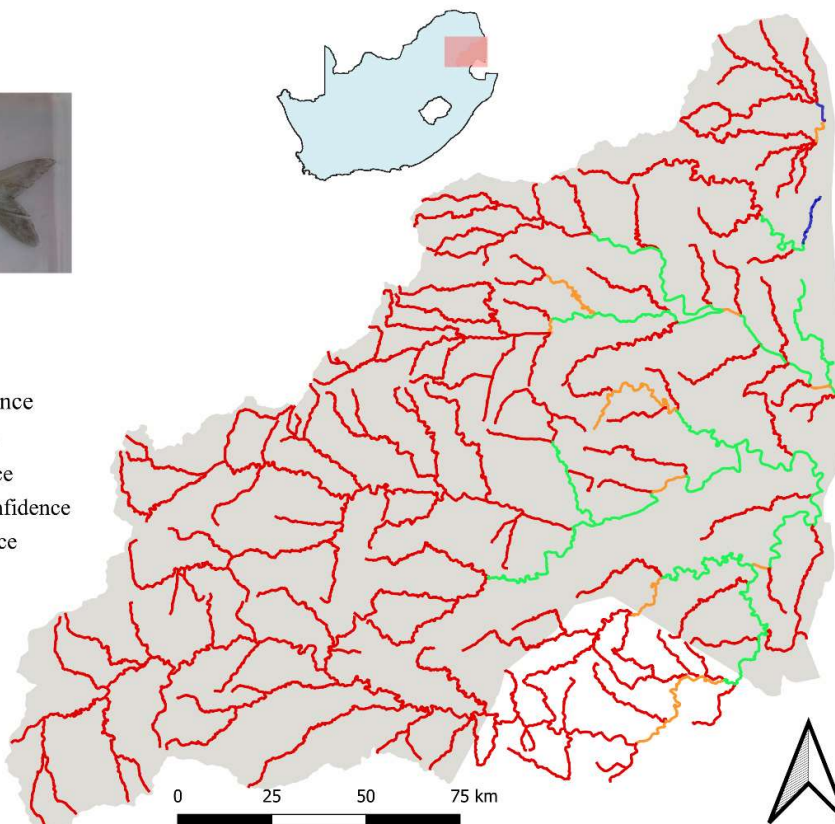
Labeo rosae



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Confidence level of occurrence

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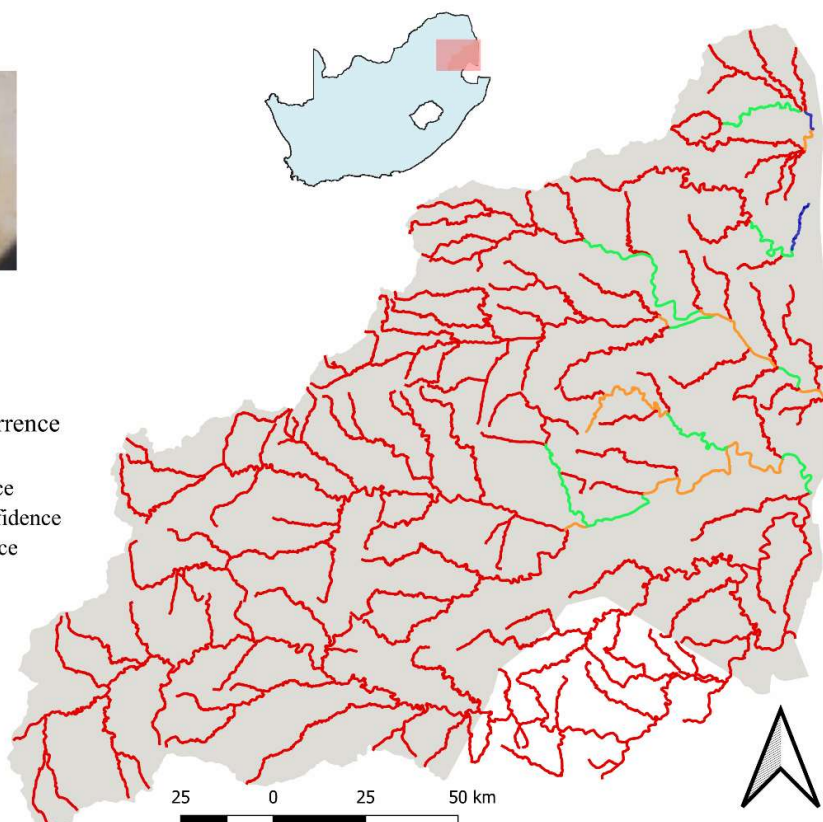
Labeo ruddi



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Confidence level of occurrence

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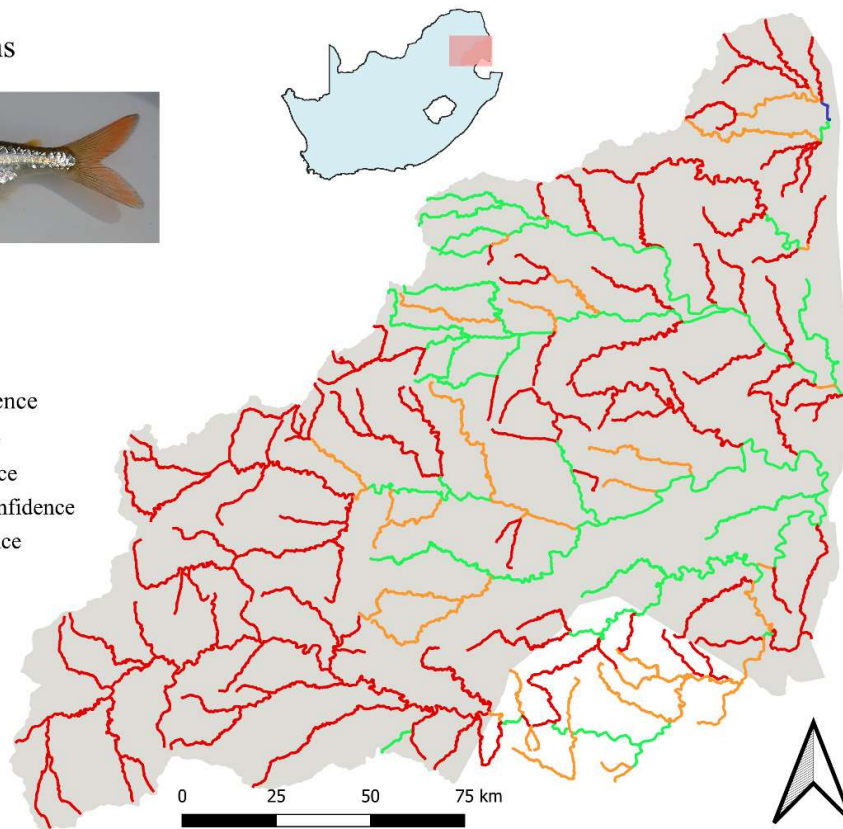
Micralestes acutidens



Legend

Confidence level of occurrence

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- Present, High confidence



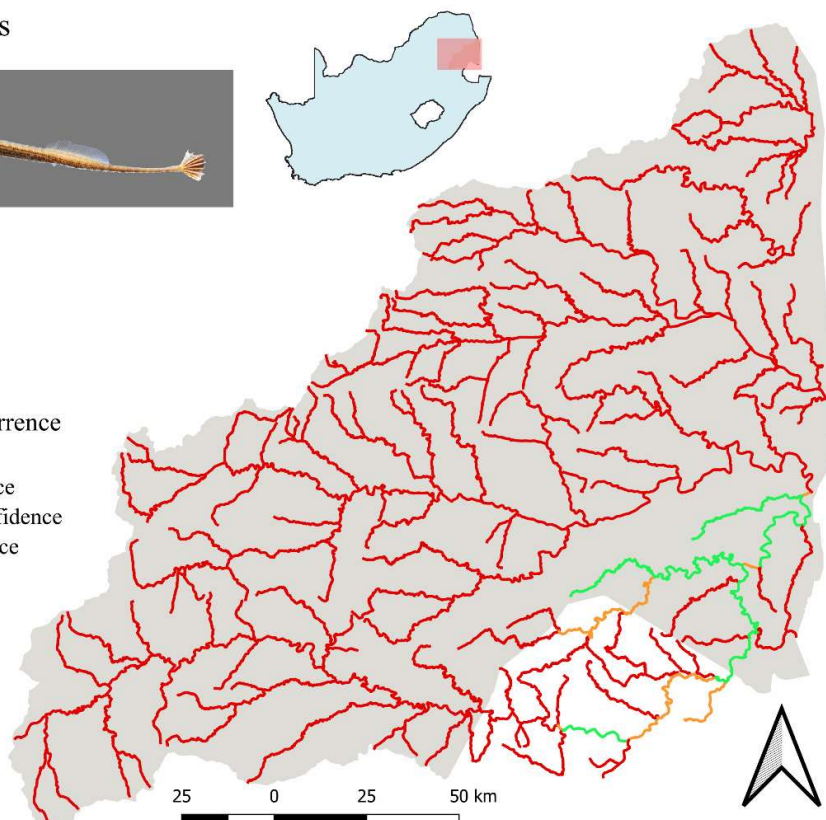
Microphis brachyurus



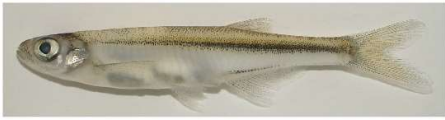
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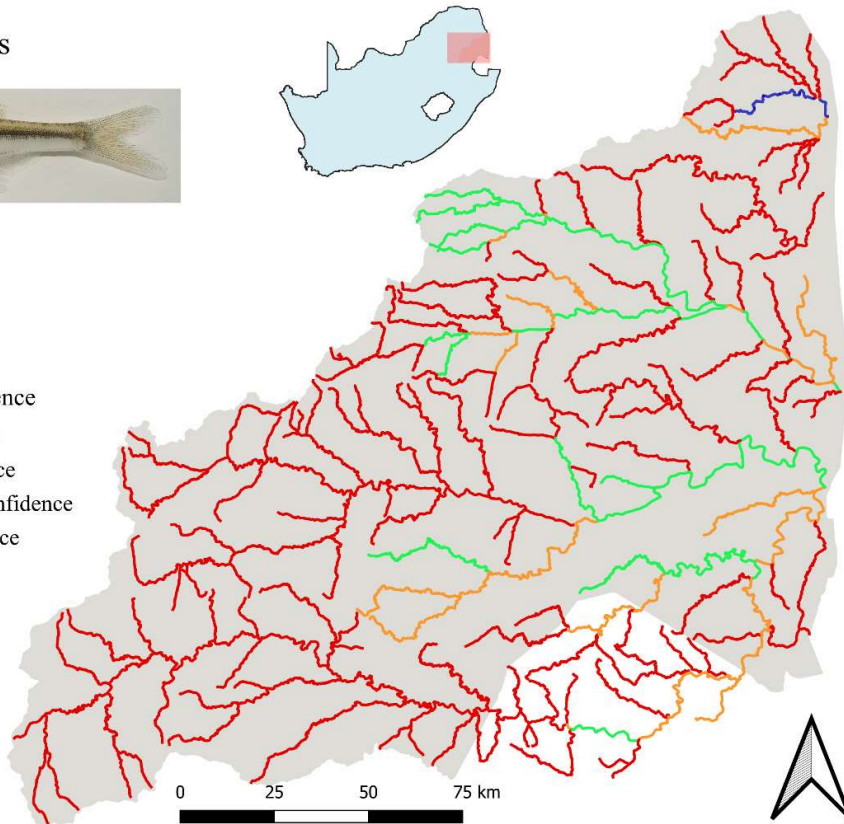
Mesobola brevianalis



Legend

Confidence level of occurrence

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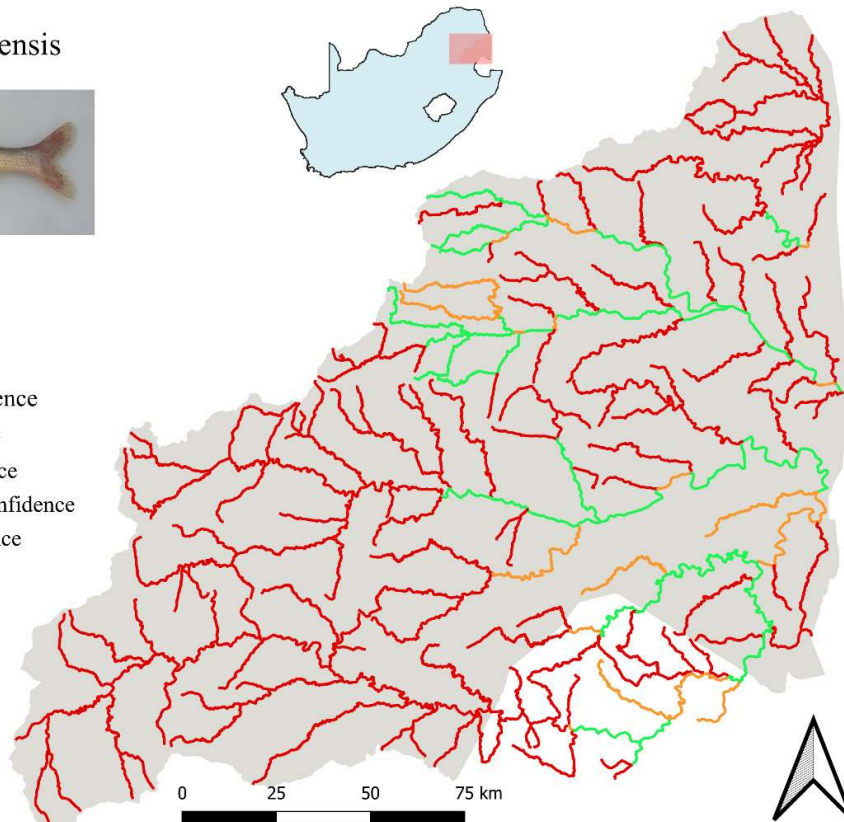
Marcusenius pongolensis



Legend

Confidence level of occurrence

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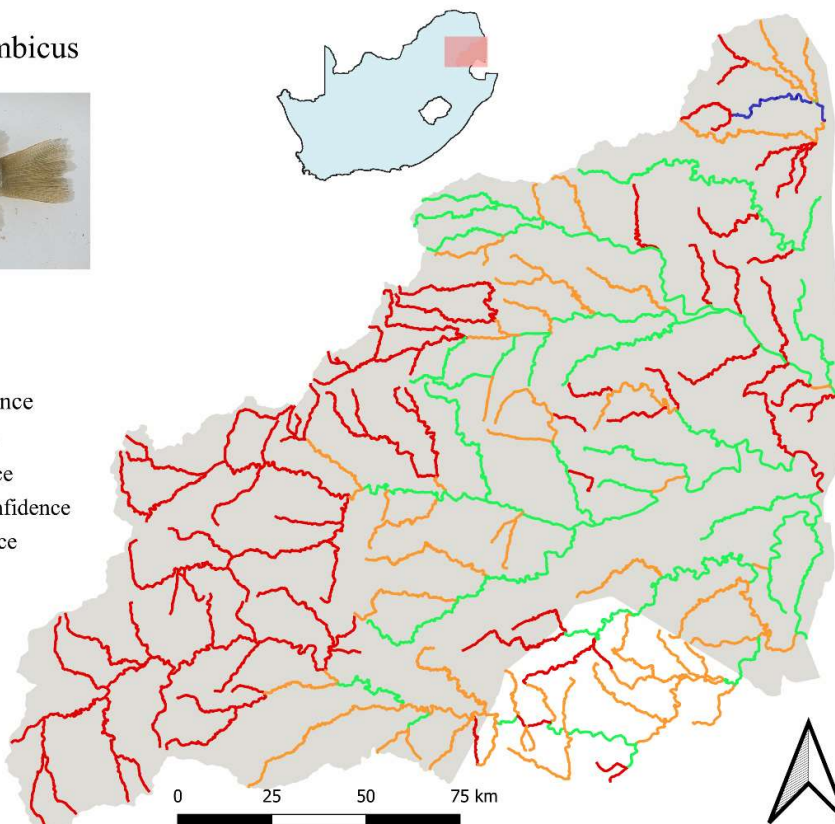
Oreochromis mossambicus



Legend

Confidence level of occurrence

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- Present, Moderate confidence
- Present, High confidence



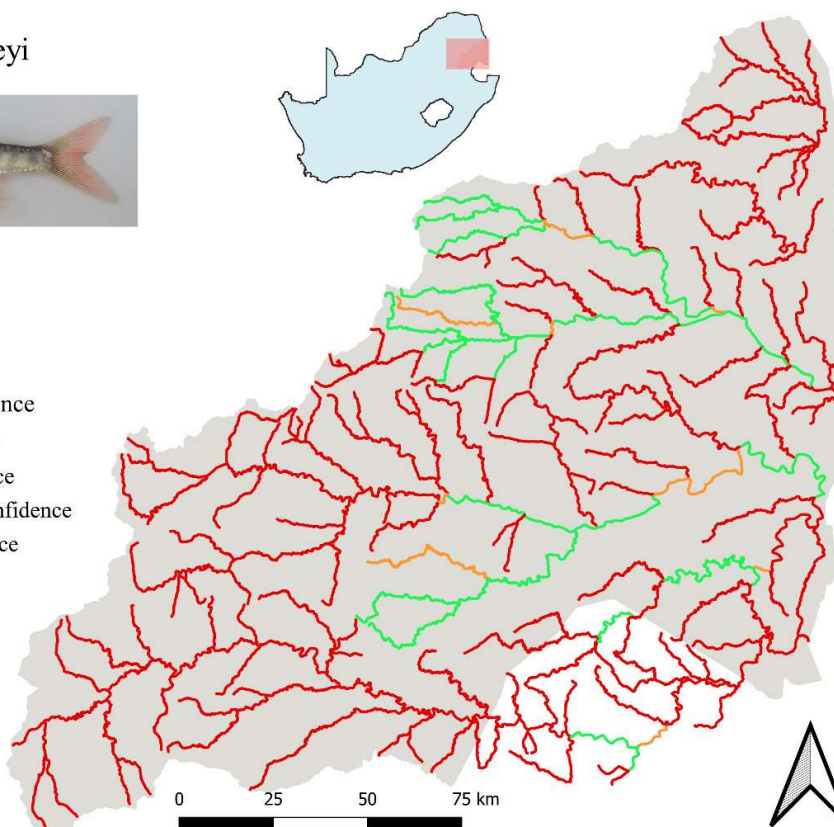
Opsaridium peringueyi



Legend

Confidence level of occurrence

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- Present, Moderate confidence
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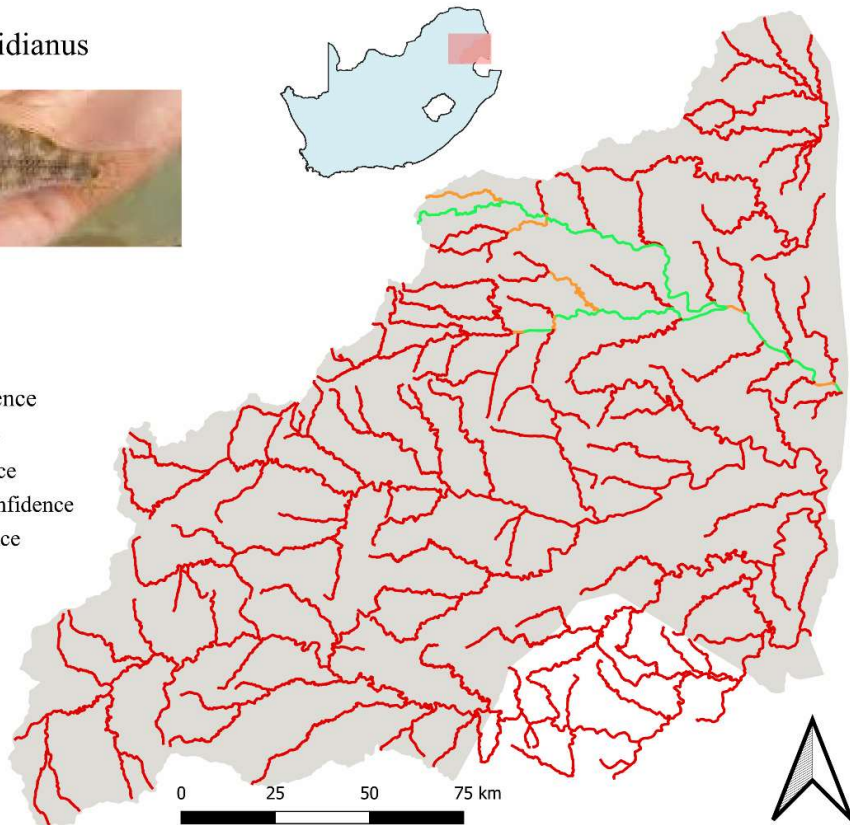
Serranochromis meridianus



Legend

Confidence level of occurrence

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- Present, Low confidence
- Present, Moderate confidence
- Present, High confidence



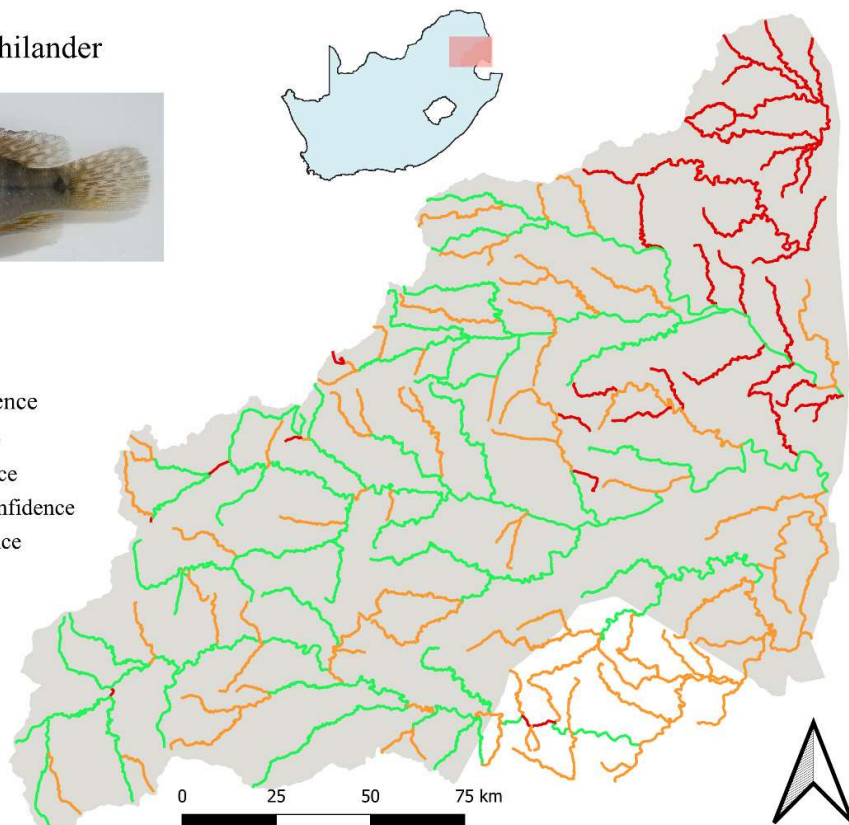
Pseudocrenilabrus philander



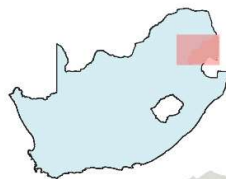
Legend

Confidence level of occurrence

- Absent, No confidence
- Present, Low confidence
- Present, Moderate confidence
- Present, High confidence



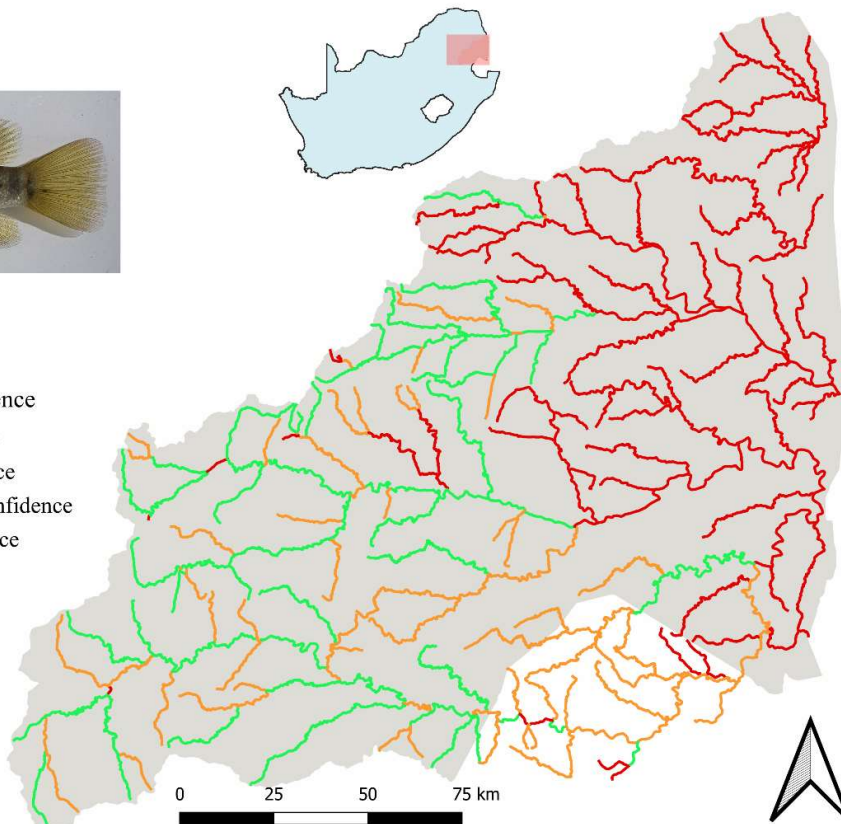
Tilapia sparrmanii



Legend

Confidence level of occurrence

- Absent, No confidence
- Present, Low confidence
- Present, Moderate confidence
- Present, High confidence

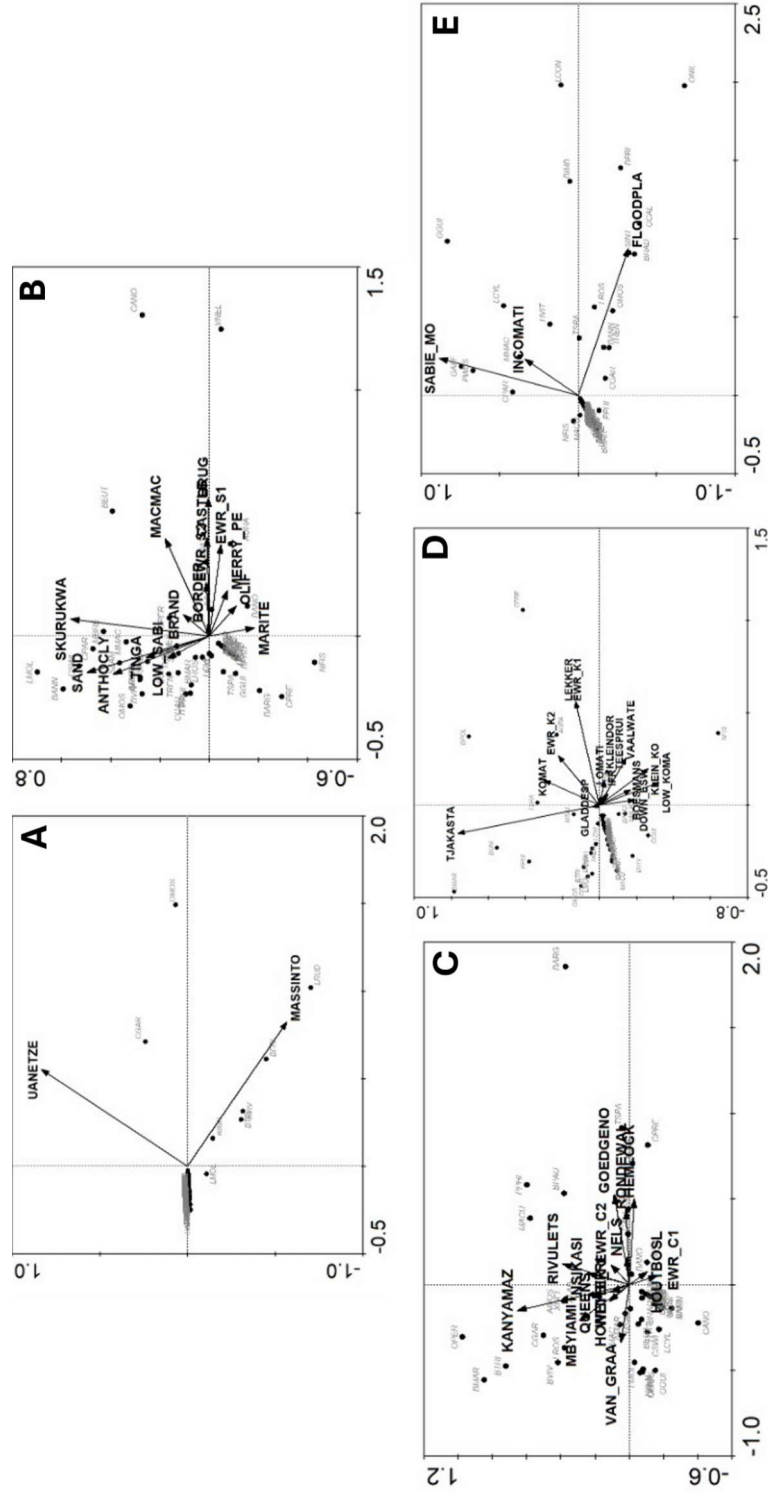


Appendix Data 2: The Preference and tolerance levels of freshwater fish species occurring within the Incomati River, South Africa. The higher the score the more sensitive or preferred the variable is for the fish species. Score range from 0 – 5 (Kleynhans 2007).

Family	Taxon	Velocity-Depth				Flow toleranc e	Cover features					Water qualit y	Migration
		FD	FS	SD	SS		OVE G	UBAN K	SUB S	AVE G	DEPT H		
Gobiidae	<i>Awaous aeneofuscus</i>	0	0	3.5	4	2	3.7	0	4.9	0	0	2.8	1
Anguillidae	<i>Anguilla bengalensis</i>	0	0	4.6	0	2.8	0	4	4.2	0	0	2.7	5
Anguillidae	<i>Anguilla marmorata</i>	0	0	4.4	0	2.8	0	3.9	4.2	0	0	2.5	5
Anguillidae	<i>Anguilla mossambica</i>	3.4	3.3	3.4	0	2.8	0	4.1	4.9	0	0	2.5	5
Amphiliidae	<i>Amphilius natalensis</i>	5	4.7	0	0	4.9	0	0	5	0	0	4.9	1
Amphiliidae	<i>Amphilius uranoscopus</i>	4.6	4.6	0	0	4.8	0	0	5	0	0	4.8	1
Cyprinidae	<i>Enteromius afrohamiltoni</i>	0	0	4.7	4.3	2.8	0	0	0	0	4	2.5	3
Cyprinidae	<i>Enteromius annectens</i>	0	0	5	0	2.8	0	0	0	0	4.7	3	3
Cyprinidae	<i>Enteromius anoplus</i>	0	0	4.1	4.3	2.3	4	0	0	3.2	0	2.6	3
Cyprinidae	<i>Enteromius crocodilensis</i>	3.7	4.3	0	0	4.6	0	0	5	0	0	4.1	1
Cyprinidae	<i>Enteromius brevipinnis</i>	0	0	3.3	4.3	1.025	4.7	4.1	0	0	0	1.025	1
Cyprinidae	<i>Enteromius eutaenia</i>	4.3	4.7	0	0	4.6	4.1	4.4	4.1	0	0	4.9	3
Characidae	<i>Brycinus imberi</i>	0	0	4.7	0	3	0	0	0	0	4.7	3.2	3
Cyprinidae	<i>Labeobarbus marequensis</i>	4.1	4.4	4.4	3.4	3.2	0	0	4.5	0	4.1	2.1	3
Cyprinidae	<i>Enteromius sp. 'neefi cf'</i>	0	0	3.3	4.7	3.4	3.9	3.3	4.4	0	0	3.4	1
Cyprinidae	<i>Enteromius paludinosus</i>	0	0	0	3.8	2.8	0	0	3.5	0	0	3.3	1
Cyprinidae	<i>Labeobarbus polylepis</i>	3.7	4.3	4.2	0	3.3	0	0	5	0	3.6	2.9	3
Cyprinidae	<i>Enteromius radiatus</i>	0	0	4.7	5	2.8	4.7	0	0	0	0	1.4	3

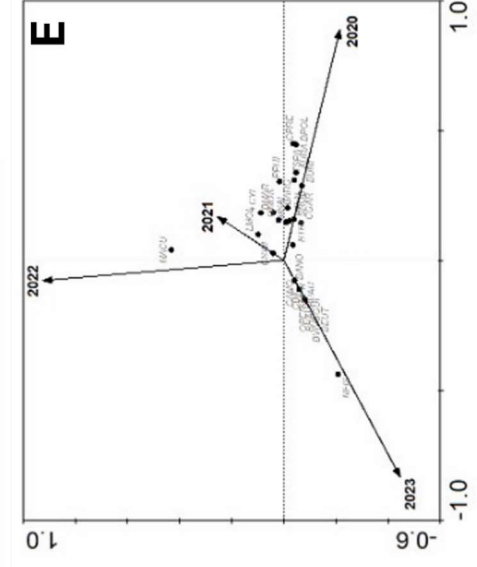
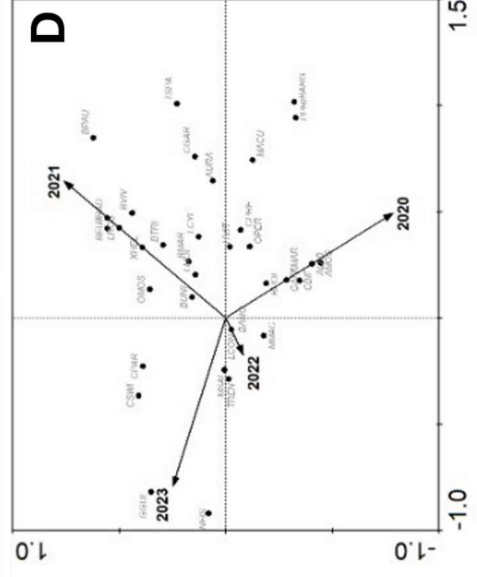
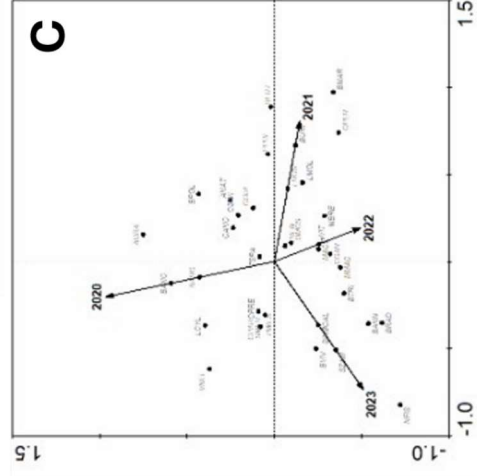
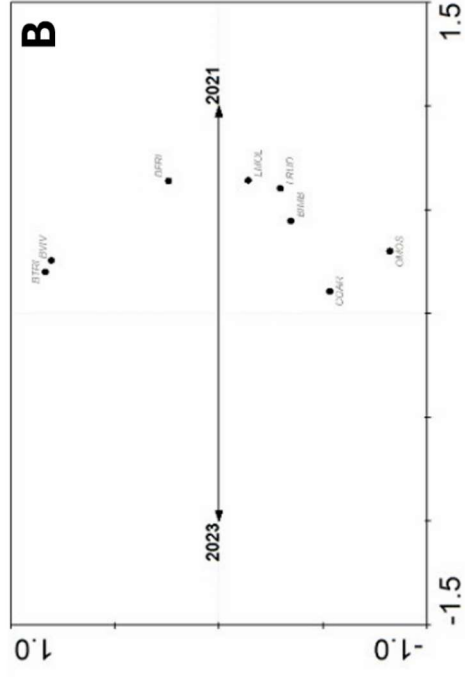
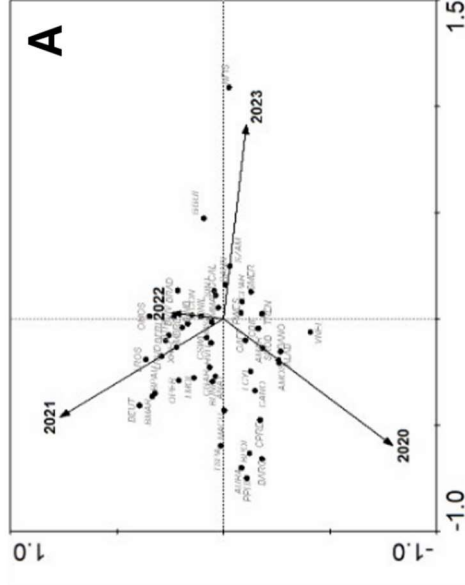
Cyprinidae	<i>Enteromius toppini</i>	0	0	3.3	4.3	1.1	4.7	0	0	0	0	0	3	3
Cyprinidae	<i>Enteromius trimaculatus</i>	0	0	3.9	3.2	2.7	3.9	0	0	0	0	0	1.8	3
Cyprinidae	<i>Enteromius unitaeniatus</i>	0	0	5	4.3	2.3	4.6	0	0	0	0	0	2.2	3
Cyprinidae	<i>Enteromius viviparus</i>	0	0	0	4.8	2.3	4.9	0	0	3.2	0	0	3	3
Mochokidae	<i>Chiloglanis anoterus</i>	4.3	4.9	0	0	4.8	0	0	4.9	0	0	0	4.7	3
Mochokidae	<i>Chiloglanis bifurcus</i>	5	3.3	0	0	4.9	0	0	5	0	0	0	4.9	3
Cichlidae	<i>Chetia brevis</i>	0	0	4.7	3.7	1.3	4.7	0	0	0	0	0	2.5	1
Mochokidae	<i>Chiloglanis emarginatus</i>	5	3.2	0	0	5	0	0	5	0	0	0	5	3
Clariidae	<i>Clarias gariepinus</i>	0	0	4.3	3.4	1.7	0	0	0	0	0	0	1	3
Mochokidae	<i>Chiloglanis paratus</i>	4.2	4.9	0	0	3.2	0	0	4.9	0	0	0	3.1	3
Mochokidae	<i>Chiloglanis pretoriae</i>	4.3	4.9	0	0	4.8	0	0	4.9	0	0	0	4.5	3
Mochokidae	<i>Chiloglanis swierstrai</i>	0	4.7	0	0	4.8	0	0	4.9	0	0	0	3.3	3
Gobiidae	<i>Glossogobius callidus</i>	0	0	0	4.7	1.5	0	0	4.9	0	0	0	2.3	1
Gobiidae	<i>Glossogobius giuris</i>	0	0	0	4.6	1.7	0	0	4.9	0	0	0	2.5	1
Characidae	<i>Hydrocynus vittatus</i>	3.6	0	4.7	0	2.7	3.4	0	0	0	0	4.9	3.1	3
Kneriidae	<i>Kneria</i> sp. 'South Africa'	0	0	0	4.2	2.5	4.7	0	3.6	0	0	0	4.1	1
Cyprinidae	<i>Labeo congoro</i>	5	0	5	0	3.3	0	0	5	0	0	3.4	3	3
Cyprinidae	<i>Labeo cylindricus</i>	3.4	4.8	0	0	3.1	0	0	4.9	0	0	0	3.1	3
Cyprinidae	<i>Labeo molybdinus</i>	3.3	4.3	3.7	0	3.3	0	0	4.7	0	0	0	3.2	3
Cyprinidae	<i>Labeo rosae</i>	0	0	4.7	0	2.5	0	0	5	0	0	0	3	3
Cyprinidae	<i>Labeo ruddi</i>	0	0	4.7	0	2.9	0	0	4.7	0	0	0	3	3
Characidae	<i>Micralestes acutidens</i>	0	0	4.3	4.3	3.1	3.1	0	0	0	0	4	3.1	3
Syngnathidae	<i>Microphis brachyurus</i>	0	0	4	0	1	0	0	0	4	0	0	4	0
Cyprinidae	<i>Mesobola brevianalis</i>	0	0	4.3	4.2	1.1	0	0	0	0	0	5	2.8	3
Mormyridae	<i>Marcusenius</i>	0	0	4.2	3.7	3	3.8	5	0	0	0	0	3.4	3

Appendix Data 3: The Redundancy Analyses results for Chapter 3. Each graph is a different analysis. More detail about the significance of each analysis (graph) is provided in Chapter 3.



Figure

Redundancy analysis biplot based on the Intra-river catchment analyses for each of the sub-catchments sites in the main river catchment for all sampling efforts and fish communities. A) The Uanetze River sub-catchment with 93.4% presented on axis 1 and 6.6% on axis 2; B) The Sapie River sub-catchment with 30.1% presented on axis 1 and 19.7% on axis 2; C) The Crocodile River sub-catchment with 36% presented on axis 1 and 22.1% on axis 2; D) The Komati River sub-catchment with 73.5% on axis 1 and 18.6 on axis 2.



-1.5
-0.4
1.0

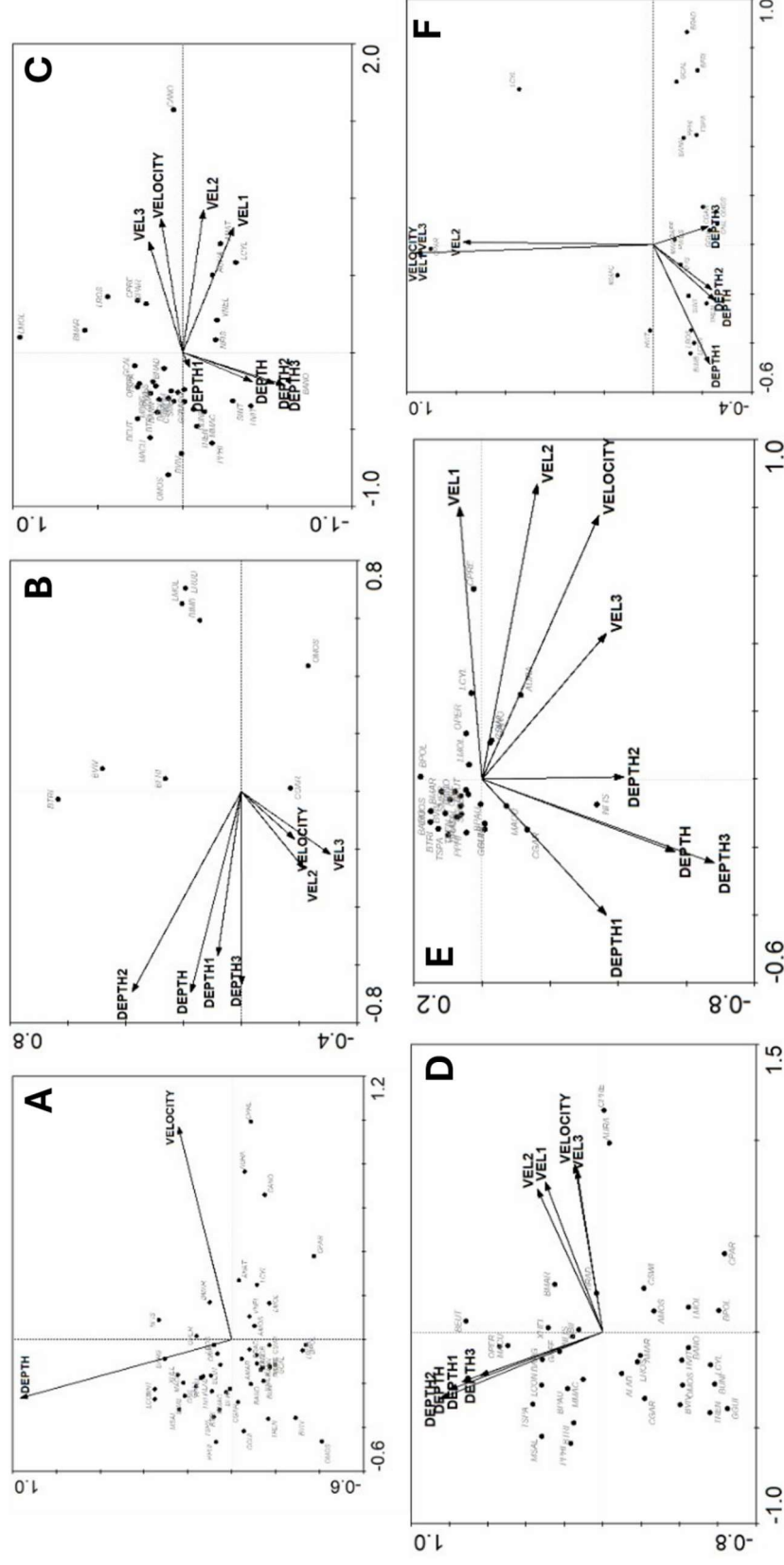


Figure 33: Redundancy analysis biplot based on the Velocity-Depth analyses for all sampling efforts and fish communities per sub-catchment. A) The combined evaluation of all sites in the greater Incomati River Catchment with 59.0% presented on axis 1 and 26.6% on axis 2; B) The Uanetze River sub-catchment with 52.3% presented on axis 1 and 36.4% on axis 2; C) The Sabie River sub-catchment with 58.2% on axis 1 and

16.9% on axis 2; D) The Crocodile River sub-catchment with 40.7% presented on axis 1 and 26.1% on axis 2; E) The Komati River sub-catchment with 73.9% presented on axis 1 and 12.3% on axis 2 and F) The Incomati River sub-catchment with 56.6% presented on axis 1 and 22.1% on axis 2.

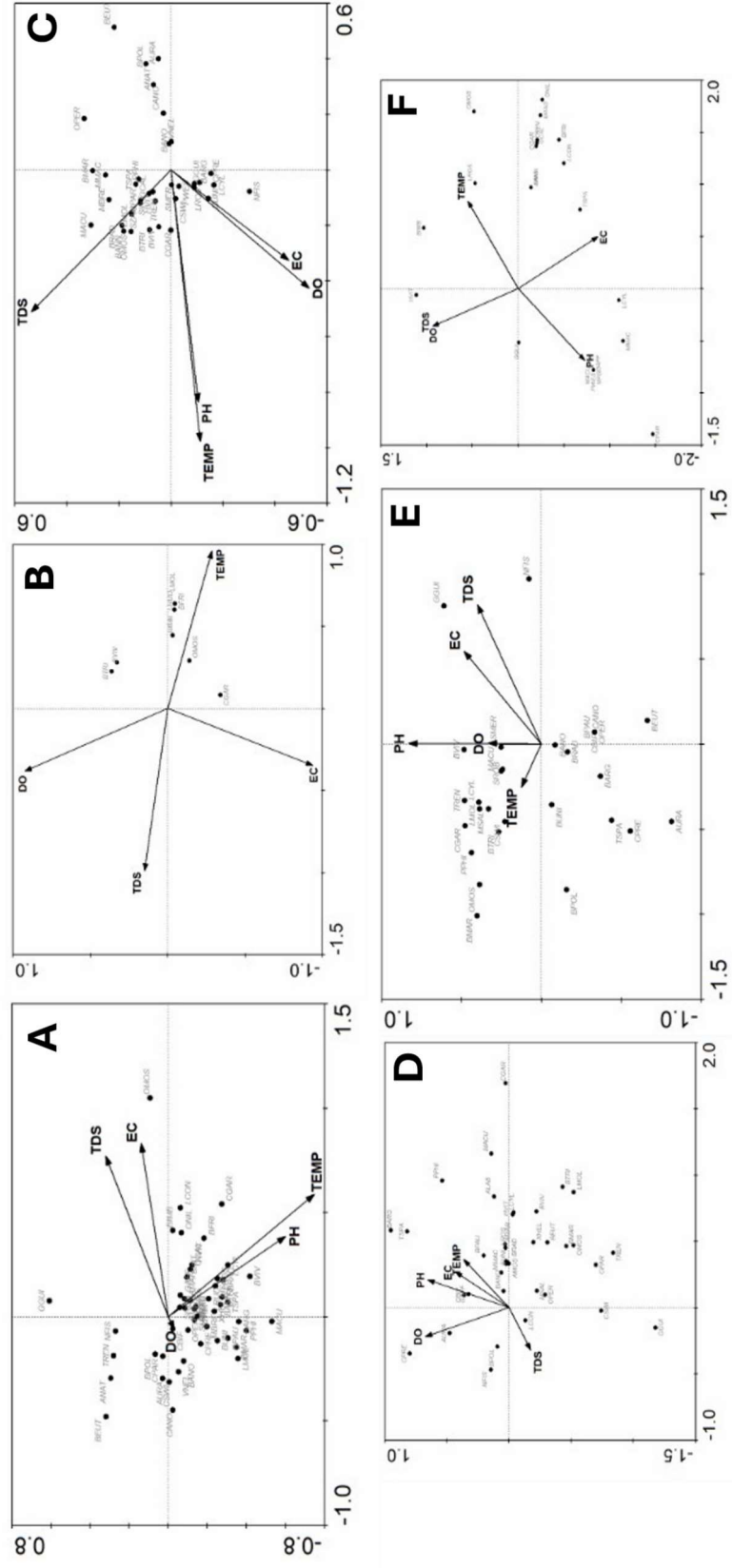


Figure 34: Redundancy analysis biplot based on the Water Quality analyses for all sampling efforts and fish communities per sub-catchment. A) The combined evaluation of all sites in the Incomati River Catchment with 42.3% presented on axis 1 and 28.8% on axis 2; B) The Uanetze River sub-catchment with 77.1% on axis 1 and 22.9% on axis 2, no significant relationship; C) The Sabie River sub-catchment with 43.8% presented on axis 1 and 23% on axis 2; D) The Crocodile River sub-catchment with 47.6% presented on axis 1 and 27.2% on axis 2; E) The Komati River sub-catchment with 59.6% presented on axis 1 and 26.9% on axis 2, and F) The Incomati River sub-catchment with 79.1% presented on axis 1 and 20.9% on axis 2.

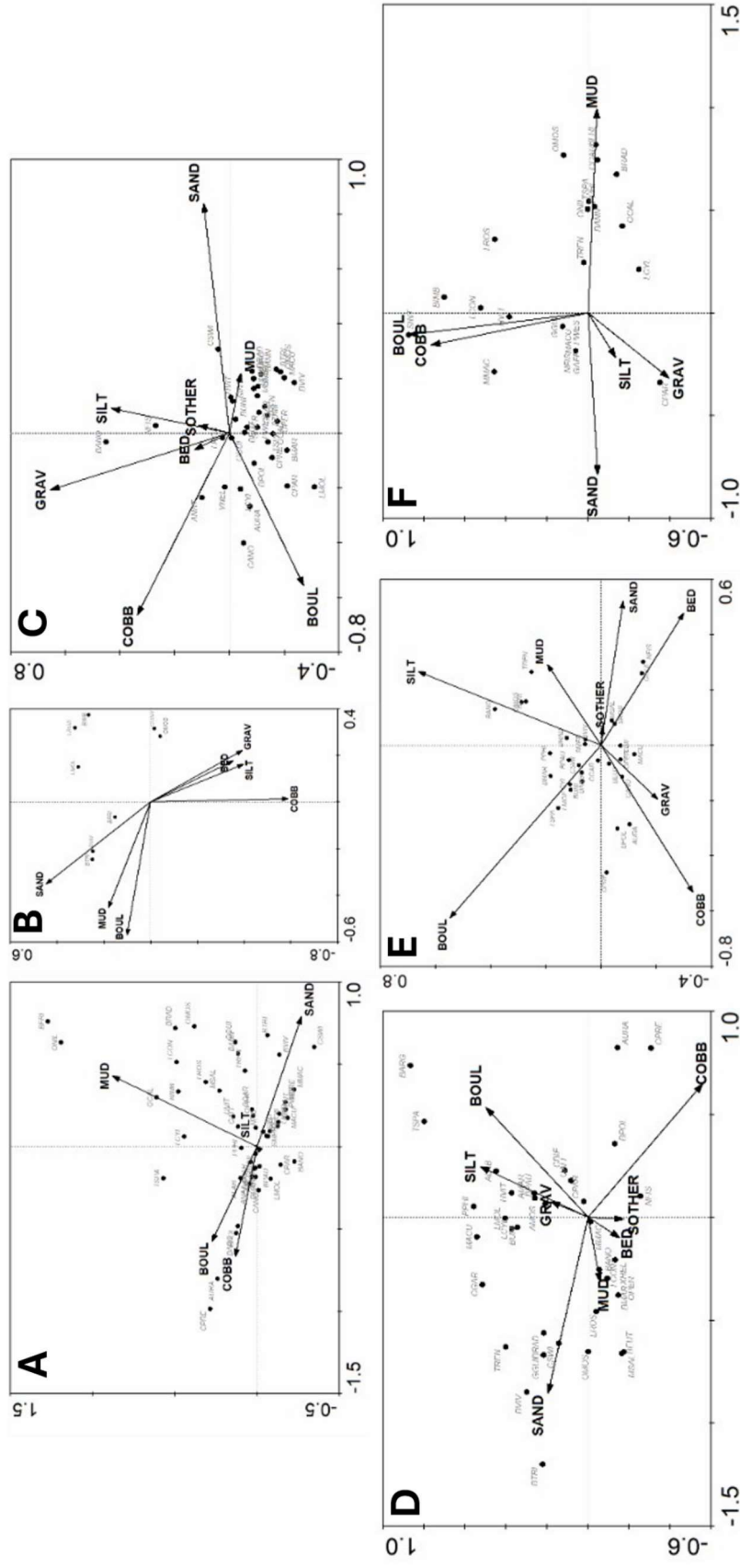


Figure 35: Redundancy analysis biplot based on the Substrate diversity analyses for all sampling efforts and fish communities per sub-catchment. A) The combined evaluation of all sites in the Incomati River Catchment with 52% presented on axis 1 and 15.8% on axis 2; B) The Uanetze River sub-catchment with 51.2% presented on axis 1 and 30.9% on axis 2, no significant relationship; C) The Sabie River sub-catchment with 41.1% on axis 1 and 25.4% on axis 2; D) The Crocodile River sub-catchment with 45.2% presented on axis 1 and 25.2% on axis 2; E) The Komati River sub-catchment with 61.6% presented on axis 1 and 15.4% on axis 2, no significant relationship; F) The Incomati River sub-catchment with 56.7% presented on axis 1 and 23.1% on axis 2, no significant relationship.

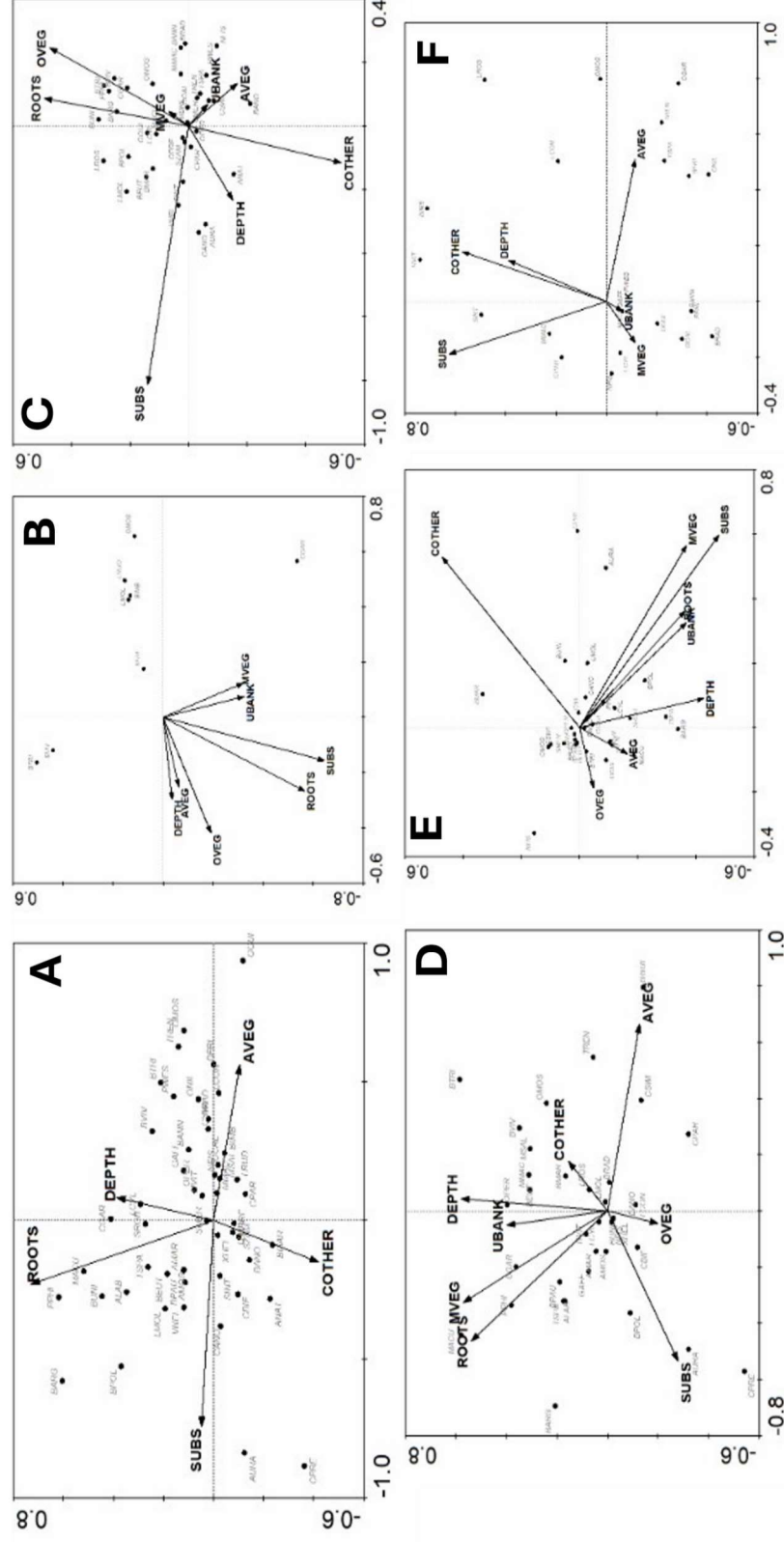


Figure 36: Redundancy analysis biplot based on the Cover feature analyses for all sampling efforts and fish communities per sub-catchment. A) The combined evaluation of all sites in the greater Incomati River Catchment with 46.6% presented on axis 1 and 21.5% on axis 2; B) The Uanetze River sub-catchment with 58.8% presented on axis 1 and 23.9% on axis 2, no significant relationship; C) The Sabie River sub-catchment with 34.9% on axis 1 and 23.1% on axis 2; D) The Crocodile River sub-catchment with 41.1% presented on axis 1 and 31% on axis 2; E) The Komati River sub-catchment with 61.6% presented on axis 1 and 15.4% on axis 2; and F) The Incomati River sub-catchment with 40.7% presented on axis 1 and 23.4% on axis 2, no significant relationship.

Appendix Data 4: Justification table per node used in the Bayesian Network for Chapter 4. Different nodes carry different relationships with different input data and ratios.

AMMONIUM	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 1.2mg/L; Low 0.3-3.5mg/L; Moderate 0.9-4mg/L; High > 4mg/L.
ANIONS	This is a conditional probability node which take into account the Sulphate and Chloride concentrations to give a combined outcome of the effects of anion variables in the system. The conditional probability table is orientated towards a rank degree of CHLORIDE (55%) > SULPHATE (45%).
CATIONS	This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.
CHLORIDE	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 85.3mg/L; Low 3.8-102.5mg/L; Moderate 14.5-141.5mg/L; High > 163.2mg/L.
Cl_mg/L	Chloride concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.
COD_mg/l	Chemical Oxygen Demand concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.
CONDUCTIVITY	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 482.3uS/cm; Low 17-897.9uS/cm; Moderate 35.6-1072.6uS/cm; High > 1266.6uS/cm.
DISCHARGE_FD	Node represent the available discharge for the river reach during months of flood. This is an input node where the actual recorded discharge from the day of sampling is inserted into the model and used.
DISCHARGE_HF	Node represent the available discharge for the river reach during high flow months. This is an input node where the actual recorded discharge from the day of sampling is inserted into the model and used.
DISCHARGE_LF	Node represent the available discharge for the river reach during low flow months. This is an input node where the actual recorded discharge from the day of sampling is inserted into the model and used.
DO_mg/l	Dissolved oxygen concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted

	into the model for assessment.			
EC_uScm	Electrical conductivity concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.			
FISH_ALIEN_COMP	The presences and abundance (measure and unit) of known competing alien fauna (fish)(e.g., <i>Micropterus salmoides</i> ; <i>Serranochromis robustus</i>) on indigenous fishes are considered. Ranks represents the potential threat/impact of alien fauna on native species.	Zero	No threat/potential impact or no competing alien fauna present.	van Wilgen <i>et al.</i> , 2020
		Low	If the occurrence of alien (not invasive - extralimital) species that will not significantly affect life cycle of any indigenous species.	Weyl <i>et al.</i> , 2020
		Med	If the occurrence and abundance of alien species with potential to affect indigenous populations significantly (invasive) occur in low to moderate abundances.	
		High	The occurrence and presence of alien species is in high abundances and poses a high threat to indigenous and subsistence fisheries populations.	
FISH_ALIEN_PRED	The node represents the presence and abundance (measure and unit) of predacious known alien fauna (fish)(e.g., <i>Micropterus salmoides</i> ; <i>Serranochromis robustus</i>) on indigenous and subsistence fish communities. Ranks represent the severity of potential impact of alien invasive fishes.	Zero	There are no threats/potential impact of predacious alien fauna.	van Wilgen <i>et al.</i> , 2020
		Low	If the occurrence of predacious alien (not invasive - extralimital) species that will not significantly affect life cycle of any indigenous species.	Weyl <i>et al.</i> , 2020
		Med	If the occurrence and abundance of alien species with potential to predate on indigenous populations significantly (invasive) occur in low to moderate	

			abundances.	
		High	The occurrence and presence of predacious alien species is in high abundances and poses a high threat to indigenous and subsistence fisheries populations.	
FISH_ECO_ALI			The node incorporate the conditional probability of the fish response to the threat of alien and or invasive species (usually fish but can include invertebrates and plants etc.) predating and or competing with indigenous fishes. The node run from a set out conditional probability table that is a function of the severity of the presence of alien invasive species competing or predating on the present ecological fish species. E.g., Alien invasive species predating on ecological fish individuals (for food) poses a larger risk then competitive alien invasive species.	van Wilgen <i>et al.</i> , 2020 Weyl <i>et al.</i> , 2020
FISH_ECO_BAR			Variable is a function of the potential effect of the presence of barriers and the percentages of indicator fish species affected by the presence of barriers. Combined it gives an indication on the threat's barriers pose to the indicator's fishes. Node is measured as the percentage of indicator fishes at each site that is influenced/intolerant to the presence of barriers. E.g., if there are high number of barriers (NO_BAR_FISH node) along with high number of indigenous fish affected/intolerant to changes due to barriers the conditional probability table is orientated towards a high risk.	DWS, 2014
FISH_ECO_COV			The node represent the fish response to changes in potential for cover features required by fish associated with river depth and potential for suitable habitats associated with sufficient depth of water, inundation of substrates including cobbles and boulders undercut bank inundation and water level interaction with current marginal and riparian vegetation which may provide fish with cover requirements. The node represent the combine effects of the available cover features during low flow and high flow months. The conditional probability table of the node is orientated towards a rank degree of FISH_ECO_COV_LF (50%) = FISH_ECO_COV_LF (50%).	
FISH_ECO_COV_HF			This node represent the fish guild/trait response to changes in depth associated cover features for fish during high flow periods. This is a conditional probability table that functions from the DISCHARGE_HF <i>in situ</i> data. The node is a transitional node to convert input data from the input node into a probability % node. Node is specific to the	

	different river reaches. E.g., Zero > 1.2m ³ /s; Low 0.4-0.8m ³ /s; Moderate 0-0.8m ³ /s; High <0.4m ³ /s.
FISH_ECO_COV_LF	This node represent the fish guild/trait response to changes in depth associated cover features for fish during high flow periods. This is a conditional probability table that functions from the DISCHARGE_HF <i>in situ</i> data. The node is a transitional node to convert input data from the input node into a probability % node. Node is specific to the different river reaches. E.g., Zero > 1.2m ³ /s; Low 0.4-0.8m ³ /s; Moderate 0-0.8m ³ /s; High <0.4m ³ /s.
FISH_ECO_CUEQ	The node incorporate the conditional probability of the fishes response to changes in timing (seasonality), volume and duration of rivers flows required for fish migrations. The node run from a set out conditional probability table that is a function of the discharge during high flow months. If the discharge is too low to provide sufficient habitat, flow, depth and cover features for migrating fish, the conditional probability table is orientated to a high risk for the node.
FISH_ECO_DTW	The node variable (Disturbance to Wildlife) represents the potential for alien species (FISH_ECO_ALL) and targeted overfishing from people to affect the availability and abundance of indigenous fishes. FISH_ECO_DTW is independent of physical characteristics of the environment as well as the population density of the surrounding community members depended on fish for food/money.
FISH_ECO_END	Endpoint represents fish community wellbeing as an ecological indicator of ecosystem wellbeing. Endpoint is a function of potential for (1) ecologically important and sensitive fishes, and (2) the physical environment for fishes to survive. The node represent the final component (endpoint) of the wellbeing of the ecologically important fish species within the river reach. The node will contribute to the determination of the risk of multiple stressors to the ecologically important ecosystem services provided by the fish community of the river reach. This node represent the final output data. The outcome risk percentage and score is used to determine the overall risk assessment evaluation of the river reach.
FISH_ECO_ENV	This is a conditional probability node that represents the total threat to the supply of fish or physical environmental condition (risk) associated with multiple anthropogenic stressors that will pose a risk to fish. The node is a function of the ecological fish instream condition, ecological influences due to barriers, and the disturbance to wildlife (demand for subsistence fishing). The conditional

	probability table is orientated towards a rank degree of FISH_ECO_DTW (40%) > FISH_ECO_BAR (35%) > SUB_FISH_DEMAND (25%).	
FISH_ECO_GEOM	The node is a conditional probability node that represent the fish response to physical habitat template or substrates based on threat of changes or loss of substrates and increase (usually sedimentation) or decrease (armouring) of habitats through sediment supply. The node is a function of the probable sediment supply and sediment flow within the river reach. The conditional probability table is orientated towards a rank degree of FISH_ECO_SSUP (65%) > FISH_ECO_SFLO (35%).	
FISH_ECO_HAB	The node is a conditional probability condition variable that represents the condition of the physical habitat for ecological indicator fishes. The node also considers habitat to facilitate recruitment of indicator species (such as knowledge of substrates and habitats for Anguillids, Cyprinids, and Chiselids observed in the study). The requirements of habitat are based on species preferences in relation to natural (known historical) habitat conditions and associated stress of indicator species. The habitat availability for ecological fish is a function of the available cover features for refuge/protection, the available geomorphology (substrate) of the reach for breeding and protection and the available flow-depth relationship. The conditional probability table is orientated towards a rank degree of FISH_ECO_VD (40%) > FISH_ECO_COV (33%) > FISH_ECO_GEOM (27%).	DWS, 2014
FISH_ECO_INS	The node is a conditional probability node that represent the fish instream ecosystem condition as a function of the potential threat of multiple stressors to instream ecosystem for fish integrating potential threats of water quality, physical habitat and ecological cue availability for fish. The conditional probability table is orientated towards a rank degree of FISH_ECO_HAB (37%) > WQ_ECOSYSTEM (33%) > FISH_ECO_CUEQ (30%).	
FISH_ECO_POT	The node is a potential input node that represents the potential for an important (consider ecological importance and sensitivity) fish communities in this reach of river to occur, and the potential for habitat (ecoregion comparisons) to be available, and river order to represent the size of that river	There is a high potential for important fish communities to occur in the river reach with variable available habitat diversity. The river order is high to facilitate large abundance of species. There is a moderate potential for important fish communities to occur in the river reach

	and potential. This node contributes to the understanding of the supply of fish and allows for comparison between river reaches (risk regions) considered in the study.		with variable available habitat diversity. The river order is still high to facilitate large abundance of species.	
		Med	There is a low potential for important fish communities to occur in the river reach with low available habitat diversity. The river order is low to facilitate moderate abundance of species.	
		High	There is almost zero potential for important fish communities to occur in the river reach with zero available habitat diversity. The river order is low to facilitate low abundance of species.	
FISH_ECO_SFLO	The node represent the sediment flow supply characteristics for habitats associated with increases (usually sedimentation) or decreases (armouring) of habitats through sediment supply associated with land use changes. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.			
FISH_ECO_SSUP	The node represent the sediment supply characteristics for habitats associated with increases (usually sedimentation) or decreases (armouring) of habitats through sediment supply associated with land use changes. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.			
FISH_ECO_VD	The node is a conditional probability node that represent the fish response to changes in velocity-depth distributions, specifically rheophilic and limnophilic species, their requirements and available instream habitats. The node represent the combine effects of the available velocity depth features during low flow and high flow months. The conditional probability table of the node is orientated towards a rank degree of FISH_ECO_VD_HF (50%) = FISH_ECO_VD_LF (50%).			
FISH_ECO_VD_HF	The node represent the fish guild/trait response to availability of and changes in velocity-depth instream habitat characteristics during high flow periods. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.			

FISH_ECO_VD_LF	The node represent the fish guild/trait response to availability of and changes in velocity-depth instream habitat characteristics during low flow periods. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.			
LANDUSE_SSUP	The node represent the Land use sediment supply from the riparian zone. Characteristics for habitats associated with increases (usually sedimentation) or decreases (armouring) of habitats through sediment supply associated with land use changes. This is a transition node to convert the in situ data into a conditional probability node for it continuous use.			
Na_mgL	Sodium concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.			
NH4_mgL	Ammonium concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.			
NITRATES	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 1.2mg/L; Low 0.3-2.6mg/L; Moderate 0.9-3.2 mg/L; High > 3.5mg/L.			
NO_BAR_FISH	Variables represent the number of barriers for fish that occur within that river reach. The node is selected as some fishes may be sensitive or intolerant to the presence of barriers as it could influence their migrations for life cycle, feeding ecology and behavioural activities. Node is measured as the number of artificial barriers that occur in the river reach. Artificial structures that is classified as barriers include: bridges with supporting structures within the river, in-channels small (farm dams) or large dams,	Zero	No Artificial barriers occur within the river reach. Present of natural barriers like waterfalls may be present.	DWS, 2014 Kleynhans, 2007
Low		Less than five artificial barriers occur within the river reach.	Jackson, Woodford, and Weyl, 2016;	
Med		Between five and 10 artificial barriers occur within the river reach.	O'Brien <i>et al.</i> , 2019	
High		More than 10 artificial barriers occur within the river reach.		

	gauging weirs (big or small), culverts.		
NO2_NO3_mgL	Nitrate and Nitrite concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.		
NUTRIENTS	This is a conditional probability node that takes into account the outcomes from the AMMONIUM, PHOSPHATE and NITRATES nodes. The node gives a combined outcome of the effects of all nutrient variables in the system. The conditional probability table is orientated towards a rank degree of AMMONIUM (55%) > PHOSPHATE (30%) > NITRATES (15%).		
OXYGEN	This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.		
OXYGEN_DO	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero 4.5-11.7mg/L; Low 1.9-6.1 & 10.1-14mg/L; Moderate 1.6-6.9 & 10.9-14.7mg/L; High < 1.2 & > 15.5mg/L.		
pH	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero 6.1-8.2; Low 4.9-8.7; Moderate 2.5-6.6 & 8.2-9.7; High < 2.5 & > 10.2.		
pH_UNIT	pH concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.		
PHOSPHATE	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 1.5mg/L; Low 0.3-1.9mg/L; Moderate 0.6-2.3mg/L; High > 2.6mg/L.		
PO4_mgL	Phosphate concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.		
SALTS	This is a conditional probability node that takes into account the outcomes from the ANIONS, CATIONS and CONDUCTIVITY nodes. The node gives a combined outcome of the affects of all salt variables in the system. The conditional probability table is orientated towards a rank degree of CONDUCTIVITY (40%) > SODIUM (30%) > ANIONS (30%).		
SO4_mgL	Sulphate concentration data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the		

	model for assessment.		
SODIUM	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 55.4mg/L; Low 3.8-85.3mg/L; Moderate 22.4-163.2mg/L; High > 186.5mg/L.		
SUB_FISH_ALI	The node incorporate the conditional probability of the Subsistence fish response to the threat of alien and or invasive species (usually fish but can include invertebrates and plants etc.) predating and or competing with targeted Subsistence fishes. The node run from a set out conditional probability table that is a function of the severity of the presence of alien invasive species competing or predating on the present Subsistence fish species. E.g., Alien invasive species predating on targeted Subsistence fish individuals (for food) poses a larger risk then competitive alien invasive species.		
SUB_FISH_COV	The node represent the Subsistence fish response to changes in potential for cover features required by fish associated with river depth and potential for suitable habitats associated with sufficient depth of water, inundation of substrates including cobbles and boulders undercut bank inundation and water level interaction with current marginal and riparian vegetation which may provide Subsistence fish with cover requirements. The node represent the combine effects of the available cover features during low flow and high flow months. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.		
SUB_FISH_CUEQ	The node incorporate the conditional probability of the Subsistence fishes response to changes in timing (seasonality), volume and duration of rivers flows required for fish migrations. The node run from a set out conditional probability table that is a function of the discharge during high flow months. If the discharge is too low to provide sufficient habitat, flow, depth and cover features for Subsistence migrating fish, the conditional probability table is orientated to a high risk for the node.		
SUB_FISH_DEMAND	The node represent the potential for people to harvest fishes for subsistence use (food). The node is a degree of the potential harvesting or overuse of removal of fish species by people. Harvesting of fish for food is considered to be acceptable while to overutilization and	Zero	The degree of subsistence fishing is very low with minimal local community members dependent on fish for food. No commercial equipment is used to fish.
		Low	The degree of subsistence fishing is low with minimal local community members

	specific methods used (gill nets) are unacceptable. The degree of demand is calculated from observed data (seeing people fish in the river) and the size of the nearby local communities who might be dependent on fish for food.		dependent on fish for food. No commercial equipment is used to fish.	
		Med	The degree of subsistence fishing is moderate with local community members dependent on fish for food. Some commercial equipment is used to fish.	
		High	The degree of subsistence fishing is very high with the majority of the local community members dependent on fish for food. Commercial equipment is also used to fish.	
SUB_FISH_END	Endpoint represents the wellbeing of the targeted subsistence fish species within a river reach. Endpoint is a function of potential for (1) the physical environment for Subsistence fishes to survive and (2) the negative demand for Subsistence fishes to be targeted within that river reach. The node represent the final component (endpoint) of the wellbeing of the targeted Subsistence fish species within the river reach. The node will contribute to the determination of the risk of multiple stressors to the ecologically important ecosystem services provided by the Subsistence fish community of the river reach. This node represent the final output data. The outcome risk percentage and score is used to determine the overall risk assessment evaluation of the river reach.			
SUB_FISH_ENV	This is a conditional probability node that represents the total threat to the supply of Subsistence fish or physical environmental condition (risk) associated with multiple anthropogenic stressors that will pose a risk to targeted Subsistence fish. The node is a function of the Subsistence fish instream condition, ecological influences due to barriers, and the disturbance of alien invasive fish species. The conditional probability table is orientated towards a rank degree of $SUB_FISH_INST(40\%) > FISH_ECO_BAR(35\%) > SUB_FISH_ALI(25\%)$.			
SUB_FISH_GEOM	The node is a conditional probability node that represent the Subsistence fish response to physical habitat template or substrates based on threat of changes or loss of substrates and increase (usually sedimentation) or decrease (armouring) of habitats through sediment supply. The node is a function of the probable sediment supply and sediment flow within the river reach. The conditional probability table is orientated towards a rank degree of			

	SUB_FISH_SFLO (65%) > SUB_FISH_SSUP (35%). The node is a conditional probability condition variable that represents the condition of the physical habitat for targeted Subsistence fishes. The node also considers habitat to facilitate recruitment of Subsistence species (such as knowledge of substrates and habitats for Catfish, Tilapia, Labeos, Anguillids observed in the study). The requirements of habitat are based on species preferences in relation to natural (known historical) habitat conditions and associated stress of Subsistence species. The habitat availability for targeted Subsistence fish is a function of the available cover features for refuge/protection, the available geomorphology (substrate) of the reach for breeding and protection and the available flow-depth relationship. The conditional probability table is orientated towards a rank degree of SUB_FISH_VD (40%) > SUB_FISH_COV (33%) > SUB_FISH_GEOM (27%).
SUB_FISH_HAB	The node is a conditional probability node that represent the target Subsistence fish instream ecosystem condition as a function of the potential threat of multiple stressors to instream ecosystem for Subsistence fish integrating potential threats of water quality, physical habitat and ecological cue availability for Subsistence fish. The conditional probability table is orientated towards a rank degree of SUB_FISH_HAB (37%) > WQ_ECOSYSTEM (33%) > SUB_FISH_CUEQ (30%).
SUB_FISH_INST	The node represent the sediment flow supply characteristics for habitats associated with increases (usually sedimentation) or decreases (armouring) of habitats through sediment supply associated with land use changes. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.
SUB_FISH_SFLO	The node represent the sediment supply characteristics for habitats associated with increases (usually sedimentation) or decreases (armouring) of habitats through sediment supply associated with land use changes. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.
SUB_FISH_SSUP	The node is a conditional probability node that represent the Subsistence fish response to changes in velocity-depth distributions, specifically rheophilic and limnophilic species, their requirements and available instream habitats. The node represent the combine effects of the available velocity depth features during low flow and high flow months. This is a transition node to convert the <i>in situ</i> data into a conditional probability node for it continuous use.
SUB_FISH_VD	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero < 55.4mg/L; Low 3.8-141.5mg/L; Moderate 22.4-163.2mg/L; High > 186.5mg/L.
SULPHATE	

SYSTEM_VAR	This is a conditional probability node that takes into account the outcomes from the pH, TEMPERATURE and OXYGEN nodes. The node gives a combined outcome of the effects of all <i>in situ</i> variables in the system. The conditional probability table is orientated towards a rank degree of OXYGEN (55%) > TEMPERATURE (30%) > pH (15%).
TEMP_oC	Temperature data for the river reach. This is a direct input node where the <i>in situ</i> data is inserted into the model for assessment.
TEMPERATURE	This is a conditional probability node that incorporate the <i>in situ</i> data reading for this node and give an output based on the risk severity score. The <i>in situ</i> concentrations are ranged according to the following acceptable ranged for freshwater systems and fish. Zero 161.4-26.9°C; Low 14.4-29.1°C; Moderate 8.8-18.4°C & 24.7-29.1°C; High < 5.4°C & > 33.5°C.
WQ_ECOSYSTEM	This is a conditional probability node that takes into account the outcomes from the SYSTEM_VAR, NUTRIENTS and SALTS nodes. The node gives a combined outcome of the effects of all water quality variables in the system. The conditional probability table is orientated towards a rank degree of SYSTEM_VAR (55%) > NUTRIENTS (30%) > SALTS (20%).

Appendix Data 5: Justification table per node used in the Bayesian Network for Chapter 5. Different nodes carry different relationships with different input data and ratios.

Node name	Rank (score)	Rank definition and measure for variable	Justification	References
Alien or extralimital vertebrates that predate on escaping silver eels. (ALIEN_ESC_PRED)	Zero	Absence of alien fishes and or no extralimital fishes that predate on silver eels in estuaries.	This node considers the presence of, and abundance of alien and or extralimital vertebrates (fish and crocodiles considered) that are known to predate on Western Indian Ocean migrating silver eels escaping estuaries, in areas where natural predators either do not occur and or where new alien animals now dominate the communities. This should be considered in context of natural predation in rivers and estuaries throughout the distribution area of eels where many indigenous predators already threaten the successful escapement of eels from rivers into the Indian Ocean. Note it is hypothesised that eels do not generally reside in estuaries during their escapement migrations and consider (based on references from <i>Anguilla anguilla</i> L.) that at least 50% of eels might not successfully escape. The unit of measure considered here includes the presence (diversity) and abundance (dominance of community) of alien and or extralimital vertebrates know to predate on eels or similar large growing fishes. In the context of the natural occurrence of predators on eels it is hypothesised that these aliens cannot contribute to a "High" risk to escaping eels but can contribute to a "Zero" risk if they do not occur in the system, "Low" risk if they occur but in	Chen, Chang and Han (2018) Westerberg <i>et al.</i> , (2021)
	Low	Presence of alien and or extralimital vertebrates in low abundances (abundance does not dominate community (<25%)).		
	Med	Presence of alien and or extralimital vertebrates in high abundances (abundance dominate community (>25%)).		
	High	NA		

			low abundances (< 25%) where they do not dominate communities and "Moderate" risk if they occur in large abundance (>25%) where they dominate vertebrate communities in rivers.	Musumeci <i>et al.</i> , (2014) Miyake <i>et al.</i> , (2018)
Alien or extralimital biota that predate on recruiting glass eels. (ALIEN_EST_PRED)	Zero	Absence of alien and or no extralimital biota that predate on recruiting eels in estuaries.	This node considers the presence of, and abundance of alien and or extralimital biota that are known to predate on Western Indian Ocean migrating glass eels recruiting into estuaries, in areas where natural predators either do not occur and or where new alien animals now dominate the communities. This should be considered in context of natural predation in rivers and estuaries throughout the distribution area of eels where many indigenous predators already threaten the successful recruitment of eels from the Indian Ocean. Note it is hypothesised that the threat cannot be high due to the high abundances of recruiting eels with relatively long residence time as they generally recruit through estuaries. For this node it is hypothesized that the predation rate of estuarine predators on <i>Anguilla rostrata</i> , can be applied and units used for the predation of other <i>Anguilla</i> species. The unit of measure includes the presence (diversity) and abundance (dominance of community) of alien biota (includes vertebrates and invertebrates) know to predate on glass eels or similar larval fishes. In the context of the natural occurrence of predators on glass eels it is hypothesised that these aliens cannot contribute to a "High" risk to recruiting eels but can contribute to a "Zero" risk if they do not occur in the system, "Low" risk if they occur but in low abundances (<25%) where they do not dominate communities	
	Low	Presence of alien and or extralimital biota in low abundances (<25%) (abundance do not dominate community).		
	Med	Presence of alien and or extralimital biota in high abundances (>25%) (abundance dominate community).		
	High	NA		

			and "Moderate" risk if they occur in large abundance (>25%) where they dominate vertebrate communities in rivers.	Jellyman (1977) Van den Berg <i>et al.</i> , (1987) Arai (2016)
Alien or extralimital biota that predate on elver and yellow eels in the river environment. (ALIEN_RIV_PRED)	Zero	Absence of alien fishes and or no extralimital fishes that predate on elvers or yellow eels in the river environment.	This node considers the presence of, and abundance of alien and or extralimital biota that are known to predate on Western Indian Ocean elver and yellow eels that reside in rivers (fish, crocodiles, birds), in areas where natural predators either do not occur and or where new alien animals now dominate the communities. This should be considered in context of natural predation in rivers throughout the distribution area of eels where many indigenous predators already threaten the survival of resident eels. The unit of measure includes the presence (diversity) and abundance (dominance of community) of alien biota (includes vertebrates and invertebrates) know to predate on elvers and yellow eels. In the context of the natural occurrence of predators on eels, it is hypothesised that these aliens can pose a "High" risk to elvers and yellow eels only if known significant impacts of aliens affecting eels exists (>50%). These aliens can however contribute to "Zero" risk if they do not occur in the system, "Low" risk if they occur but in low abundances where they do not dominate communities (25%) and "Moderate" risk if they occur in large abundance where they dominate (>25%) vertebrate communities in rivers.	
	Low	Presence of alien and or extralimital vertebrates in low abundances (abundance does not dominate community (<25%)) that predate on elvers or yellow eels in the river environment.		
	Med	Presence of alien and or extralimital vertebrates in high abundances (abundance dominate community (>25%)) that predate on elvers or yellow eels in the river environment.		
	High	Presence of alien and or extralimital biota in high abundances (abundance dominate community (>25%)) with knowledge of significant impacts on		

	eel communities.			
The North vs South currents determining the recruitment of larvae. (CURRENTS_NvsS)	Zero	Marine currents are strong enough to support the movement/ migration of anguillid eels to spawning grounds.	This node considers the direction of the oceanic currents and how they could influence the drifting/recruitment of leptocephali from the spawning grounds. It is hypothesized that currents in the direction towards the western Africa side will be more beneficial to anguillid eels than currents moving away from the western Africa region. It is hypothesized that eels will follow warmer currents during recruitment due to faster metabolic rates (grow faster/stronger) compared to cold temperatures where they might enter torpor.	Pous <i>et al.</i> , 2010 Low confident node as little data is available.
	Low	Marine currents are moderately strong enough to support the movement/ migration of anguillid eels to spawning grounds.		
	Med	Marine currents are low and does not fully support the movement/ migration of anguillid eels to spawning grounds.		
	High	Marine currents do not assist in the migration of anguillid eels to the spawning grounds.		
Distance needed to travel to spawning grounds. (DIST_SPAWNING)	Zero	Need to travel < 100 km	This node considers the distance (units in km) that silver eels need to travel to the spawning ground from the mouth of the estuary. It is considered that silver eels that need to travel considerably greater distances to spawning sites use more energy and will	Tsukamoto <i>et al.</i> , (2011) Arai (2020)

			have reduced potential to contribute to the recruitment of populations, due to the deletion of fat stores and a longer exposure time to predators and environmental stressors. Individuals with depleted fat stores and reduced health conditions might reach spawning grounds but will be unfit to produce viable eggs. Note that it is known that the migration distance for <i>A. marmorata</i> overlaps with <i>A. japonica</i> , a known long distance migrator of between 2000 - 4000 km. The variable has been estimated using measured hypothetical straight lines that migrating eels might follow from the mouth of the estuary to the recruitment area of the South Equatorial Current (15.4° - 17.0°S and 60.0°E). For this node it is hypothesised that the location of the escaping estuary (mouth) can contribute to a "Zero" risk if the estuary is in close proximity to the recruitment area (<100km), a "low" risk is proposed if the estuary mouth is location within a 100 - 2000km, a "moderate" risk is proposed if anguillid eels need to travel between 2000-4000 km and a "high" risk for distances greater than 4000km.	Tsukamoto <i>et al.</i> , (2011) Arai (2020)
	Low	Need to travel > 100 km but < 2000 km		
	Med	Need to travel > 2000 km but < 4000 km		
	High	Need to travel > 4000 km		
	Zero	Need to travel < 100 km		
Distance needed to travel from spawning grounds. (DISTANCE_SEC)	Low	Need to travel > 100 km but < 2000 km	This node considers the distance (units in km) that Leptocephali/larva need to travel/drift from the spawning ground to the mouth of an estuary. It is hypothesised that larva needs to travel/drift considerably great distances from the spawning sites to the mouth of an estuary and that the greater the distance the more predator exposed and energy depletion is experienced. The variable has been estimated using measured hypothetical straight line migrating eels follow from the \ recruitment area of	

				the South Equatorial Current (15.4° - 17.0°S and 60.0°E) to the mouth of the estuary/catchment. For this node it is hypothesised that the location of the recruiting estuary (mouth) can contribute to a "Zero" risk if the estuary is in close proximity to the recruitment area (<100km), a "low" risk is proposed if the estuary mouth is location within a 100 - 2000km, a "moderate" risk is proposed if anguillid eels need to travel between 2000-4000 km and a "high" risk for distances greater than 4000km.	
	Med	Need to travel > 2000 km but < 4000 km			
	High	Need to travel > 4000 km			
Size of the river to allow escapement and assess the marine environment. (ESC_RIVER_SIZE)	Zero	NA		This node represent the size of the river and mouth of the estuary to allow anguillid silver eels to successfully escape the marine environment into the Indian Ocean from the river environment. This node considers that larger rivers, in relation to the size of other rivers in the catchment, will be able to support and produce more anguillid eels since it will be more capable to sustain healthy fish and invertebrate communities that act as food resources for eels and have more a more variety of habitats. It is hypothesized that a river in question is classified as a large river based on their flows (seasonal/permanent), ability to hold/sustain relative high levels of water and its ability to have diversity of co-occurring species utilised by eels for resources and habitat diversity. All of this is taken in terms of	
	Low	If the river in question is classified as the largest river in the catchment with the highest carrying capacity for other species and anguillid eels			

The number of barriers in the estuary. (EST_BARRIERS)			relevance to the other rivers within the same catchment. A "low" risk is proposed for the escapement of anguillid eels if the river in question is classified as the largest river in the catchment with the highest carrying capacity for other species and anguillid eels; A "moderate" risk is proposed for the escapement of anguillid eels if the river in question is classified as the second or third largest river in the catchment in terms of carrying capacity for other species and anguillid eels; A "high" risk is proposed for the escapement of anguillid eels if the river in question is classified as the fourth or lower classified river in the catchment in terms of carrying capacity for other species and anguillid eels. No specifications are assigned to the "zero" risk class because all of the above variables could be in place to classify the rivers as large enough for the escapement of eels irrespective of other stressors e.g. barriers and then these variables are not what determines the successful escapement of eels.	O'Brien <i>et al.</i> , (2019) Hanzen <i>et al.</i> , (2019) Watz <i>et al.</i> , (2019)
	Med	If the river in question is classified as the second or third largest river in the catchment in terms of carrying capacity for other species and anguillid eels		
	High	If the river in question is classified as the fourth or lower classified river in the catchment in terms of carrying capacity for other species and anguillid eels.		
	Zero	There are no barriers preventing the migrations of anguillid eels upstream and river blockage is comparable to historic conditions.	This node considers the number of barriers (natural/anthropogenic/permanent/temporally) in a catchment or river that will partially or fully block the migration of anguillid eels during the upstream migration of glass eels. This variable is estimated from the number of barriers that poses a blockage during all seasons (High flow & low flow) within the estuarine environment. It is hypothesized that barriers that overflows during the high flow season and barriers with functional bypasses classify as	
	Low	There are a few (<5) temporary barriers preventing the migration		

The water quality of the estuarine environment that affect recruiting eels. (EST_QUALITY)		of anguillid eels while the majority of anguillid eels could potentially pass though.	temporary barriers while large dam walls without overflow or bypasses classify as permanent barriers. This node considers estuaries with no barriers or temporary barriers pose "zero" risk; estuaries with a few (<5) temporary barriers to pose a "low" risk; an estuary with large dams with or without bypasses, hydropower turbines and temporary barriers (>5) poses a "moderate" risk and estuaries with large dams or weirs with no bypasses and hydropower turbines poses a "High" risk for the migration of anguillid glass eels upstream.	
	Med	There are a few (> 5) temporary barriers and large dams with functional bypasses preventing the migration of anguillid eels while anguillid eels could potentially still pass though.		
	High	There are large amounts of barriers with no bypasses (>5) posing the complete blockage of the migration of anguillid eels.		
	Zero	The estuary environment or estuarine shores/zones are less than 15% transformed; this transformation/degradation n include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries.	This node represents the degree of water quality impairment given by a body of water, in this case the estuarine environment. Estuarine environments receives and process land water discharges potentially filled with anthropogenic pollutants and high nutrient loads caused by different land use activities. This node represent the degree of impact water quality degradation has on anguillid eel populations. This node hypothesize that eels in general are quite resilient to water quality degradations but that too much chemical stress/accumulations could affect their lipid stores that in turn will impair their successful recruitment.	Whitfield and Elliott (2002) Belpaire and Goemans (2007) Arai (2016) Prosser <i>et al.</i> , (2018) Expert specialist consultation
	Low	The estuary environment or estuarine shores/zones		

		are between 15 - 45 % transformed; this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries.	This node considers that large areas of transformation along the estuarine shore/zone will contribute to the degradation of the water body. This node hypothesize that anguillid eels recruiting into estuaries with less than 15% transformation or estuarine shores/zones degradation are at "zero" risk; this transformation/degradation include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries. anguillid eels are at a "low" risk if the estuarine shore/zone is transformed/degraded between 15 - 45%, this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries. anguillid eels are at a "moderate" risk if the estuarine shore/zone is transformed/degraded between 45 - 75%, this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries, and if estuary environment are used as a docking shore for large vessels. anguillid eels are at a "high" risk if the estuarine shore/zone is transformed/degraded by > 75%, this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries, and if estuary environment are used as a docking shore for large vessels.	
	Med	The estuary environment or estuarine shores/zones are between 45-75 % transformed; this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries, and if estuary environment are used as a docking shore for large vessels.		
	High	The estuary environment or estuarine shores/zones are more than 75 % transformed; this degradation/transformation include <4 identified sources of sand mining, >		

Type of estuary mouth for accessibility for eel larva into the estuary from the Indian Ocean (open/closed). (EST_TYPE)		70% urban or agricultural development, > than 8 large established factories/industries, and if estuary environment are used as a docking shore for large vessels.	This node represent the timing/duration the mouth of the estuary is open to allow anguillid eel larva to successfully recruit into the estuary environment from the Indian Ocean. This node considers that estuarine mouths that are year roundly open/accessible will allow a larger successful recruitment rate of anguillid eel larva, while estuarine environments that are temporary closed daily or seasonally will have less successful recruitment numbers from anguillid eels larva. It is hypothesized that a year round open estuary mouth (constant flow) pose a "zero" risk to the recruitment of anguillid eel larva; an estuary mouth with a daily/tidally open mouth will pose a "low" risk; estuaries with seasonally closed mouths (a few months per year) will pose a "moderate" risk to the successful recruitment of anguillid eels into the estuary environment. No condition is assigned for the "high" risk variable as all estuarine mouths have the ability to recruit eels (daily/seasonally/permanently) and that it is just the timing and challenges involve of getting into the estuary mouth that will affect recruitment success.	Perissinotto <i>et al.</i> , (2010)
	Zero	Year round open estuary		
	Low	Partially open-closed estuary, tidally/daily.		
	Med	Seasonally closed estuary mouth (few months per year).		
	High	NA		

Fishing of elver and yellow eels. (FISHING_ELV_YEL)				Robinet <i>et al.</i> , 2007 Study (questionaries)
	Zero	No harvesting is taking place, reference to historical conditions/pre-anthropogenic conditions.	This node considers the intensity of elvers and yellow eel harvest based on the area or abundance of people that could potentially harvest eels in the region. It is hypothesized that larger harvest yield will not allow less elvers or yellow eels to grow large and be able to return to the estuarine environment. For this node the area of influence will be based on the area of urban, peri-urban and rural areas (determined from the number of people in the area) surrounding the river/estuary reach within 30km from the edge of the river banks and their dependence (questioners) on fisheries (including eels). It is known that in some areas Chinese syndicates work with locals to catch and sell eels without the locals depending on eels for food. It is hypothesized and propose a "zero" risk if the region (within 30km) around the estuary is not developed or there is no dependence on eels from local communities and comparable to natural, pre-anthropogenic conditions; a "low" risk if the area (within 30km) around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally	
	Low	The around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally		
	Med	The area (within 30km) around the estuary region are classified as peri-urban, have between 40-60 individuals/hectare, small and large scale (>5 hectare per person) agriculture/farming, less than 3 large factories/industries, some syndicate operations (<20 eels per month), and the		

Fishing for larvae. (FISHING_LARVAE)		dependence on eels from community members are monthly	factories/industries, some syndicate operations (<20 eels per month), and the dependence on eels from community members are monthly; a "high" risk if the area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the dependence on eels from community members are weekly. Not all criteria per condition needs to be met, but the condition with the most overlap needs to be chosen.	Braind <i>et al.</i> , (2007) Study (Questionnaires)
	High	The area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the dependence on eels from community members are weekly	This node considers the intensity of glass eel/larvae harvest based on the area or abundance of people that could potentially harvest glass eels in the region. It is hypothesized that larger harvest yield will not allow eels to grow large and be able to return to the estuarine environment. For this node the area of influence will be based on the area of urban, peri-urban and rural areas (determined from the number of people in the area) surrounding the river/estuary reach within 30km from the edge of the estuary banks and their dependence (questioners) on fisheries (including eels). It is known that in some areas Chinese syndicates work with locals to catch and sell eels without the locals depending on eels for	
	Zero	No harvesting is taking place, reference to historical conditions/pre-anthropogenic conditions.		
	Low	The area around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big		

		factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally	food. It is hypothesized and propose a "zero" risk if the region (within 30km) around the estuary is not developed or there is no dependence on eels from local communities and is comparable to natural, pre-anthropogenic conditions; a "low" risk if the area (within 30km) around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally; a "moderate" risk if the area (within 30km) around the estuary region are classified as peri-urban, have between 40-60 individuals/hectare, small and large scale (>5 hectare per person) agriculture/farming, less than 3 large factories/industries, some syndicate operations (<20 eels per month), and the dependence on eels from community members are monthly
	Med	The area (within 30km) around the estuary region are classified as peri-urban, have between 40-60 individuals/hectare, small and large scale (>5 hectare per person) agriculture/farming, less than 3 large factories/industries, some syndicate operations (<20 eels per month), and the dependence on eels from community members are monthly	
	High	The area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the	the area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the dependence on eels from community members are weekly. Not all criteria per condition needs to be met, but the condition with the most overlap needs to be chosen.

Rate of fishing for silver eels escaping the river environment. (FISHING_SILVER)		dependence on eels from community members are weekly		This node considers the intensity of silver harvest based on the area or abundance of people that could potentially harvest silver eels in the region. It is hypothesized that larger harvest yield will not allow eels to grow large and be able to return to the estuarine environment. For this node the area of influence will be based on the area of urban, peri-urban and rural areas (determined from the number of people in the area) surrounding the river/estuary reach within 30km from the edge of the estuary banks and their dependence (questioners) on fisheries (including eels). It is known that in some areas Chinese syndicates work with locals to catch and sell eels without the locals depending on eels for food. It is hypothesized and propose a "zero" risk if the region (within 30km) around the estuary is not developed or there is no dependence on eels from local communities and is comparable to natural, pre-anthropogenic conditions; a "low" risk if the area (within 30km) around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally	Dekker <i>et al.</i> , (2018) Study (Questionnaires)
	Zero	No harvesting is taking place, reference to historical conditions/pre-anthropogenic conditions.			
	Low	The around the estuary region are classified as rural, have less than 40 individuals/hectare living there, only small scale farming/agriculture and no major big factories/industries, no syndicate operations, and the dependence from the communities on eels as food is seasonally			
	Med	The area (within 30km) around the estuary region are classified as peri-urban, have between 40-60 individuals/hectare, small and large scale (>5 hectare per person) agriculture/farming, less than 3 large factories/industries, some			

The superstructural features required for the miniatous of fish/invert communities (food for eels). (FOOD_COVER)		syndicate operations (<20 eels per month), and the dependence on eels from community members are monthly	agriculture/farming, less than 3 large factories/industries, some syndicate operations (<20 eels per month), and the dependence on eels from community members are monthly; a "high" risk if the area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the dependence on eels from community members are weekly. Not all criteria per condition needs to be met, but the condition with the most overlap needs to be chosen.	
	High	The area (within 30km) around the estuary region are classified as urban, have more than 60 individuals/hectare, small and large scale (>15 hectare per person) agriculture/farming, more than 3 large factories/industries, syndicate operations (>20 eels per month), and the dependence on eels from community members are weekly		
	Zero	Distribution and abundances that will support pristine/natural community of indicator species	This node represents the availability of physical cover features for fish/invertebrate (as a food resource for eels) communities expected to occur in the river environment/reach/region. There is a strong relationship between riparian vegetation, instream habitat and community structure in aquatic ecosystems. The availability of marginal vegetation and associated cover features are important environmental variable which affects the sustainability of instream habitats for fish communities. Different cover features included overhanging vegetation, marginal vegetation,	Allanson and Read 1995; Dala-Corte <i>et al.</i> 2016
	Low	Occurrence (but altered) of cover distributions for indicator species		

The water quality conditions for the miniatous of fish/invert communities (food for eels). (FOOD_RIV_QUALITY)	Med	Modified cover distributions but majority of indicator species is provided for.	aquatic vegetation, undercut bank, and substrate. Different fish/invertebrate species have different preferences for different cover features. This variable will be based on what the fish community require as a cover feature with the assumption that this will be sufficient for the invertebrate community. This node hypothesize that a large reduction in the physical cover features within a reach will negatively affect fish/invertebrate communities that in turn will cause a reduction in the available food resources for eels. Ranks include distribution and abundances that will support pristine/natural community of indicator species (Zero), occurrence of (but altered) of cover distributions for indicator species (Low), modified cover distributions for at least one fisheries indicator species while majority of indicator species is provided for (Mod) and significant alteration of cover characteristics that affects fish community significantly.	Koehnken <i>et al.</i> 2020; Wepener <i>et al.</i> 2011
	High	Significant alteration of cover characteristics that affects fish community significantly.		
	Zero	The river environment reach/zones are less than 15% transformation/degradation n include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries.	This node represents the degree of water quality impairment given by a body of water, in this case the river environment. River environments receives and process land water discharges potentially filled with anthropogenic pollutants and high nutrient loads caused by different land use activities. This node represent the degree of impact water quality degradation has on fish communities and invertebrate communities (resources of food for eels) expected to occur within the reach/catchment in question. This node hypothesize that some fish/invertebrate species in general are quite resilient	
	Low	The river environment reach/zones are between		

The velocity-depth classes		15 - 45 % transformed; this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries.	to water quality degradations, but that some however are affected by degraded water quality parameters. This node considers that large areas of transformation along the river reach/zone will contribute to the degradation of the water body. Water quality conditions comparable to pre-anthropogenic activities (Zero). Water quality conditions altered but have no impact on fish communities (Low). Water quality conditions altered sensitive fish species not present (Moderate). Water quality conditions heavily altered , limited survival of fish communities (High).	
	Med	The river environment reach/zones are between 45-75 % transformed; this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries.		
	High	The river environment reach/zones are more than 75 % transformed; this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries.		
The velocity-depth classes			This node represents the availability of velocity-	Kleyhans

conditions for the maintenance of fish/invertebrate communities (food for eels). (FOOD_VD)		distribution abundances that support pristine/natural community of indicator species.	depth classes for fish/invertebrate (as a food resource for eels) communities expected to occur in the river environment/reach/region. Changes to the flow regimes affect resources and habitat availability. Hydrological variability influences the physical habitat of riverine systems and thus shapes the structure and diversity of aquatic communities. Different fish species can be used as indicators for different velocity depth classes (Slow-deep, slow-shallow, fast-deep, fast-shallow). Hydraulic data was used to determine at what discharges the different velocity-depth classes become activated for specific study areas (where hydraulic data was available). Based on the preferences for the indicator species the different discharge ranks were determined. The parent nodes were curve were adjusted based on the availability and diversity of different velocity depth classes as preferred by indicator species. Relative distribution and abundance (unit m2) of instream velocity-depth habitat (slow-deep, slow-shallow, slow-very shallow, fast-intermediate, fast-shallow and fast-deep) distributions (measure) associated with requirements of indicator fishes (large growing cyprinids). Limited information is available to relate the flow in the whole WIO to velocity depth profiles which can be related to habitat requirements of local fish communities. Qualitative evaluation of the available velocity depth profiles has however been carried out using the application of the fish responses assessment index. In addition to the above mentioned for the general assessment of whole WIO area and the regions where hydraulic data was not	2007; Cattaneo 2005;
	Low	Velocity-depth distribution abundances altered but habitat distributions are sufficient for indicator species.		
	Med	Modified velocity-depth habitat distributions for but majority of indicator species are provided for		
	High	Significant alteration of velocity-depth habitat characteristics that affects fish community significantly.		

The abundances of indigenous predators on larva eels in the estuary environment. (IND_EST_PRED)			available a desktop review was conducted to determine the water abstraction/use (dams, pipelines, transformation) in the basin as a whole. Zero: All velocity depth classes are available in high abundances and distribution to support all indicator fish species , pristine condition, Low: There are changes in the abundances and distribution of different velocity depth classes. All indicator fish species are still present, medium: Some of the velocity depth classes abundances are reduced, majority of indicator fish species are still present, High: There are significant alteration of velocity depth classes which affect fish communities, most of the indicator fish species are absent.	Miyake <i>et al.</i> , (2018) Evans <i>et al.</i> , (2022)
	Zero	Absence of indigenous fishes and or native fishes/vertebrates that predate on larva in estuaries.	This node considers the presence of, and abundance of indigenous or native vertebrates (fish and crocodiles considered) that are known to predate on Western Indian Ocean recruiting glass eels within the estuaries, in areas where natural predators do occur and where no alien animals are present in the communities. This should be considered in context of natural predation in estuaries throughout the distribution area of eels where many indigenous predators already threaten the successful recruitment of eels from the Indian Ocean into estuaries (e.g., Catfish, birds). Note it is hypothesised that eels larva generally reside in estuaries during their recruitment migrations. The unit of measure considered here includes the presence (diversity) and abundance (dominance of community) of indigenous or native vertebrates know to predate on glass eels or similar fishes. In the context of the natural occurrence of	
	Low	Presence of indigenous fishes and or native vertebrates in low abundances (abundance do not dominate community) that predate on eel larva.		
	Med	Presence of indigenous fishes and or native vertebrates in high abundances (abundance dominate community) that		

The abundances of indigenous predators on silver eels in the estuary and marine environment. (IND_ESC_PRED)		predate on eel larva.	predators on eels it is hypothesised that these vertebrates cannot contribute to a "High" risk to recruiting eels but can contribute to a "Zero" risk if they do not occur in the system, "Low" risk if they occur but in low abundances where they do not dominate communities (<25%) and "Moderate" risk if they occur in large abundance where they dominate vertebrate communities in rivers (>25%). This node considers the presence of, and abundance of indigenous or native vertebrates (fish and crocodiles considered) that are known to predate on Western Indian Ocean escaping silver eels within the estuaries and marine environments, in areas where natural predators do occur and where no alien animals are present in the communities. This should be considered in context of natural predation in estuaries throughout the distribution area of eels where many indigenous predators already threaten the successful escapement of silver eels into the Indian Ocean (E.g., Catfish, Crocodiles, large birds). Note it is hypothesised that silver eels generally do not reside in estuaries during their escaping migrations. The unit of measure considered here includes the presence (diversity) and abundance (dominance of community) of indigenous or native vertebrates known to predate on silver eels or similar large growing fishes. In the context of the natural occurrence of predators on eels it is hypothesised that these vertebrates cannot contribute to a "High" risk to escaping silver eels but can contribute to a "Zero" risk if they do not occur in the system, "Low" risk if they occur but in low abundances where they do not	Miyake <i>et al.</i> , (2018) Evans <i>et al.</i> , (2022)
	High	NA		
	Zero	Absence of indigenous fishes and or native fishes/vertebrates that predate on silver eels in estuaries and marine environment.		
	Low	Presence of indigenous fishes and or native vertebrates in low abundances (abundance do not dominate community) that predate on silver eels in estuaries and marine environment.		
	Med	Presence of indigenous fishes and or native vertebrates in high abundances (abundance dominate community) that predate on silver eels in estuaries and marine environment.		
	High	NA		

			dominate communities and "Moderate" risk if they occur in large abundance where they dominate vertebrate communities in rivers.	
The abundances of indigenous predators on yellow eels in the river environment. (IND_RIV_PRED)	Zero	Absence of indigenous fishes and/or native fishes/vertebrates that predate on yellow eels in the river environment.	This node considers the presence of, and abundance of indigenous or native vertebrates (fish and crocodiles considered) that are known to predate on Western Indian Ocean yellow eels within the river environments, in areas where natural predators do occur and where no alien animals are present in the communities. This should be considered in context of natural predation in rivers throughout the distribution area of yellow eels where many indigenous predators already threaten the successful growth of yellow eels in rivers (E.g., tigerfish, crocodiles, otters, large birds). Note it is hypothesised that yellow eels generally reside in river environments during their growth periods. The unit of measure considered here includes the presence (diversity) and abundance (dominance of community) of indigenous or native vertebrates known to predate on yellow eels or fishes. In the context of the natural occurrence of predators on eels it is hypothesised that these vertebrates cannot contribute to a "High" risk to escaping silver eels but can contribute to a "Zero" risk if they do not occur in the system, "Low" risk if they occur but in low abundances where they do not dominate communities and "Moderate" risk if they occur in large abundance where they dominate vertebrate communities in rivers.	Miyake <i>et al.</i> , (2018) Evans <i>et al.</i> , (2022)
	Low	Presence of indigenous fishes and/or native vertebrates in low abundances (abundance do not dominate community) that predate on yellow eels in the river environment.		
	Med	Presence of indigenous fishes and/or native vertebrates in high abundances (abundance dominate community) that predate on yellow eels in the river environment.		
	High	NA		

The cover/structural features required for eel larvae to survive within the estuary. (LARVAE_COV)	Zero	The cover/ structure features within the estuarine environment are less than 15% transformed and are comparable to historical/pre-anthropogenic conditions.	This node represents the availability of physical cover features for anguillid eel larva to successful migration/recruitment within the estuarine environment/reach/region. The availability of marginal vegetation and associated cover features are important environmental variable which affects the sustainability of instream habitats for fish communities and it is particular associated with maintenance habitat for fish recruitment and fish refugia. Different cover features included overhanging vegetation, marginal vegetation, aquatic vegetation, undercut bank, and substrate. Different fish species have different preferences for different cover features. This variable will be based on what the fish community require as a cover feature with the assumption that this will be sufficient for the migration/recruitment of glass eels. This node hypothesize that a large reduction in the physical cover features within a reach will negatively affect fish communities and thus anguillid eel larvae migration/recruitment. Ranks include distribution and abundances that will support pristine/natural community of indicator species (Zero), occurrence of (but altered) cover distributions for indicator species (Low), modified cover distributions for at least one fisheries indicator species while majority of indicator species is provided for (Moderate) and significant alteration of cover characteristics that affects fish community significantly (High).	Allanson and Read 1995; Whitfield 1998; Whitfield and Elliott 2002
	Low	The cover/ structure features within the estuarine environment reach/zones are transformed/degraded between 15 - 45 %.		
	Med	The cover/ structure features within the estuarine environment reach/zones are between 45-75 % transformed.		
	High	The cover/ structure features within the estuarine environment reach/zones are more than 75 % transformed.		
The velocity depth required	Zero	The average yearly water	Node represents the suitable depth (in relation to	

for the larvae entering the estuary. (LARVAE_VD)		column depth is deeper than 1.3m.	historical depths, using hydrology models where available) requirements of anguillid eel larva species that need access between different habitats in the estuarine environment/reach/region for successful maintenance and recruitment of anguillid eels (glass/larva life stage). This node hypothesize that the low water depth between habitats will restrict or hinder the movement and connectivity of anguillid eel larvae in estuarine and river environment. This variable will also be based on what the different reach scale migratory fish communities require as suitable depths with the assumption that this will be sufficient for the migration/recruitment of glass eels. Different reach scale migratory larvae There are no changes in the depth required for access between reaches (Zero). There are some changes in depth requirements but reach scale migrator larvae especially the anguillid eel larvae have access between reaches (Low). There are changes in depth requirements but some reach scale migrator larvae and therefore some anguillid eel larvae have access between reaches (Moderate). There are changes in depth requirements, no reach scale migrator larvae especially the anguillid have access between reaches (High).	O'Brien <i>et al.</i> , 2009
	Low	The average yearly water column depth is between 0.7 - 1.3m.		
	Med	The average yearly water column depth is between 0.3 - 0.7m.		
	High	NA		
	Zero	Marine currents are strong enough to support the movement/ migration of anguillid eels to spawning grounds.	This node represent the strength of the oceanic currents that could influence the energy expenditure of anguillid eels during escapement periods towards the spawning grounds. It is hypothesized that stronger currents will allow anguillid eels to use less energy travelling to the spawning grounds as stronger currents could "help anguillid eels move	
Marine current that influence the escapement to spawning grounds. (MAR_CURRENTS)				Tsukamoto 2009; Low confident node with little data available.

	Low	Marine currents are moderately strong enough to support the movement/ migration of anguillid eels to spawning grounds.	faster and further distances at a time"	Chen, Chang and Han (2018) Westerberg <i>et al.</i> , (2021)
	Med	Marine currents are low and does not fully support the movement/ migration of anguillid eels to spawning grounds.		
	High	Marine currents do not assist in the migration of anguillid eels to the spawning grounds.		
The abundances of predators on silver eels in the marine environment (continental sheath). (MAR_SIL_PRED)	Zero	Absence of indigenous/Alien/native fishes and or vertebrates that predate on silver eels in the marine environment within the continental sheath.	The exposed distance (km) within the continental sheath, as recorded that predation numbers are higher within the continental sheath. This node considers the presence of, and abundance of indigenous or native vertebrates (fish, crocodiles, sharks, whales considered) that are known to predate on Western Indian Ocean silver eels within the marine environments, in areas where natural predators do occur and where no alien animals are present or dominate the communities. This should be considered in context of natural predation in marine environments throughout the distribution	
	Low	Presence of indigenous/alien/native fishes and or vertebrates in low abundances (abundance do not		

The water quality of marine environment that can affect spawning. (MARINE_QUALITY)		dominate community, <50% occurrence rate) that predate on silver eels in the marine environment within the continental sheath.	area of silver eels where many indigenous predators already threaten the successful escapement/migration of silver eels in oceans. Note it is hypothesised that silver eels generally reside in ocean environments during their escaping periods towards the spawning grounds. The unit of measure considered here includes the presence (diversity) and abundance (dominance of community) of indigenous/alien or native vertebrates know to predate on silver eels or such large growing fishes within a set distance of the continental sheath. Consider the assumption that more predators occur within the continental sheath. In the context of the natural occurrence of predators on eels it is hypothesised that these vertebrates cannot contribute to a "High" risk to escaping silver eels but can contribute to a "Zero" risk if no natural predators occur in the system or the continental sheath, "Low" risk if they occur but in low abundances (low occurrence rates), small ranges within the continental sheath and "Moderate" risk if they occur in large abundance (high; > 50 % of the time), large home/predation ranges within the continental sheath.	Low confident node. Data not available.
	Med	Presence of indigenous/native/ alien fishes and or vertebrates in high abundances (abundance dominate community, > 50% occurrence rate) that predate on silver eels in the marine environment or within the continental sheath.		
	High	NA		
	Zero	The water quality of the marine environment is considered to be good and no anthropogenic affects are visible to influence the migration of anguillid eels.		
	Low	The water quality of the marine environment is	This node considers the water quality conditions of the oceanic environment in terms of salinity fronts/measurements. It is hypothesized that anguillid eels use/follow salinity fronts/currents to find spawning grounds.	

The number of barriers in the river affecting migration. (RIV_ESC_BARRIER)		considered to be good with some anthropogenic affects visible to influence the migration of anguillid eels.			O'Brien <i>et al.</i> , (2019) Hanzen <i>et al.</i> , (2019) Watz <i>et al.</i> , (2019)
	Med	The water quality of the marine environment is considered not to be good and anthropogenic affects are visible to influence the migration of anguillid eels.			
	High	Water quality of the marine environment is considered to be very bad with consistent influences from anthropogenic issues/influences.			
	Zero	There are no barriers preventing the migrations of anguillid eels downstream and river blockage is comparable to historic conditions.		This node considers the amount of barriers (natural/anthropogenic/permanent/temporally) in a catchment or river that will partially or fully block the downstream migration of anguillid eels. This variable is estimated from the amount of barriers that poses a blockage during all seasons (High flow & low flow) within the river environment. It is hypothesized that barriers that overflows during the high flow season and barriers with functional bypasses classify as temporary barriers while large dam walls without overflow or bypasses classify as permanent barriers. This node considers rivers with no barriers as "zero" risk; rivers with a few (<5) temporary barriers to pose a "low" risk; an river with	
	Low	There are a few (<5) temporary barriers preventing the migration of anguillid eels while the majority of anguillid eels could potentially pass through.			

	Med	There are a few (> 5) temporary barriers and large dams with functional bypasses preventing the migration of anguillid eels while anguillid eels could potentially still pass though.	large dams with or without bypasses, hydropower turbines and temporary barriers (>5) poses a "moderate" risk and rivers with large dams or weirs with no bypasses and hydropower turbines poses a "High" risk for the migration of anguillid eels downstream.	
	High	There are large amounts of barriers with no bypasses (>5) posing the complete blockage of the migration of anguillid eels.		
The length of the river. (RIVER_LENGTH)	Zero	Strahler river order 5 and 6	This node represent the length and size of the river based on the river order (Strahler data). Strahler data defines the potential of a river to support higher fish diversities and anguillid eel abundances while it is hypothesized that streams with higher orders are ranked with higher risk. Streams with high rank orders (1 and 2) are smaller streams, usually tributaries that might not have the capacity to carry higher abundances of fish species and anguillid eels while lower rank orders (6 and 7) are larger, bigger streams that have higher species carrying capacities. A "zero" risk is proposed for streams with a stream order of 5 and 6 as they are large deep flowing rivers known to support a high diversity of fish species; a "low" risk is proposed for streams with a stream order of 3 and 4 as there are less large deep flowing areas known to support a high diversity of fish	
	Low	Strahler river order 3 and 4		
	Med	Strahler river order 1 and 2		

The water quality required for the maintenances of silver eels in the river/estuary environment. (SIL_RIV_QUALITY)			species; a "moderate" risk is proposed for streams with a stream order of 1 and 2 as these are considered tributaries, seasonally or permanent, known to have reduced variety and Availability of different habitats and cant support large amounts of fish species. No criteria has been assigned for a "high" risk as other stressors/factors in have a strong relationship with river order and could be the determining factors for anguillid eel distributions.	Whitfield and Elliott (2002) Belpaire and Goemans (2007) Arai (2016) Prosser <i>et al.</i> , (2018) Expert specialist consultation Drouinau <i>et al.</i> , (2018)
	High	NA		
		Zero	The estuary environment or estuarian shores/zones are less than 15% transformed; this transformation/degradation include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries.	
	Low		The estuary environment or estuarian shores/zones are between 15 - 45 % transformed; this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries.	

	Med	The estuary environment or estuarine shores/zones are between 45-75 % transformed; this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries, and if estuary environment are used as a docking shore for large vessels.	transformed/degraded between 15 - 45%, this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries. anguillid eels are at a "moderate" risk if the river reach/zone is transformed/degraded between 45 - 75%, this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries. anguillid eels are at a "high" risk if the river reach/zone is transformed/degraded by > 75%, this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries.	Kleynhans 2007
	High	The estuary environment or estuarine shores/zones are more than 75 % transformed; this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries, and if estuary environment are used as a docking shore for large vessels.	This node represent the average year round depth within the lower river and estuarine environment/reach/region that is needed to have	
The required depth for silver eel escapement. (SILVER_ESC_DEPTH)	Zero	The average yearly water column depth is deeper than 1.3m.		

			connectivity between different habitats for successful maintenance and escapement of anguillid eels (silver life stage). This node hypothesize that to low depths between habitats will restrict or hinder the movement of anguillid eels in the river/estuarine environment. No velocity requirement/preference are observed in eels as their preference are dominated by all velocity depth classes (Kleynhans 2007). This node hypothesize that the movement hindering/restrictions for anguillid eels are at "zero" risk when the average yearly water column depth in the lower river reach/region and estuarine environment is deeper than 1.3m. The movement hindering/restrictions for anguillid eels are at "low" risk when the average yearly water column depth in the lower river reach/region and estuarine environment is between 0.7 - 1.3m and the movement hindering/restrictions for anguillid eels are at "moderate" risk when the average yearly water column depth in the lower river reach/region and estuarine environment is between 0.3-0.7m deep. Yearly average water column depth below 0.3m could pose a "high" risk for escaping anguillid eels. Yearly water depth is calculated or obtained from available hydrological and discharge data or specialist knowledge.	
	Low	The average yearly water column depth is between 0.7 - 1.3m.		
	Med	The average yearly water column depth is between 0.3 - 0.7m.		
	High	NA		
The Location in the Western Indian Ocean (tropical vs temperate). (WIO_LOCATION)	Zero	The river is located within the tropical region that is within 10° from the equator, either North or South.	This node considers the location of the estuary mouth in relation to its location within the Western Indian Ocean. This node considers that river environments within a tropical region will have more preferred habitats/food resource quantities or temperature climates for eels (irrespective of the life	

The water quality required for the maintenances of yellow eels. (YEL_RIV_QUALITY)	Low	The river is located within the tropical region that is within 10° - 20° from the equator, either North or South.	stage), while rivers further away from the tropical environment will still allow eels to successfully recruit, grow and escape within these rivers but might not have the preferred climate, habitat or food resources. The tropical areas are known to support 11 species, while the temperate regions support 5 species of which the southern Africa anguillid eels overlaps in both these area. This node hypothesize that rivers located within 10° from the equator will pose a "zero" risk to anguillid eels due to having all of the preferred habitats, climate and food resources. While moving further away from the equator (North and South) a river will pose a "low" risk if it is located between 10° - 20° from the equator, rivers more than 20° from the equator (North and South) is hypothesize to pose a "moderate" risk , all due to a decrease in preferences for climate, habitat and food resources. A "high" risk is not assigned as all rivers (irrespective of all other stressors) can still successfully recruit, grow and escape anguillid eels. This node represents the degree of water quality impairment given by a body of water, in this case the river environment. River environments receives and process land water discharges potentially filled with anthropogenic pollutants and high nutrient loads caused by different land use activities. This node represent the degree of impact water quality degradation has on anguillid eel populations. This node hypothesize that eels in general are quite resilient to water quality degradations (see references) but that to much chemical stress/accumulations could affect their lipid stores	Prosser, D.J., Jordan, T.E., Nagel, J.L. <i>et al.</i> 2007. Arai, T., 2016. Drouineau, H., Durif, C., Castonguay, M., Mateo, M., Rochard, E., Verreault, G., Yokouchi, K.
	Med	The river is located further away from the tropical region that is more than 20° from the equator, either North or South.		
	High	NA		
	Zero	The river environment reach/zones are less than 15% transformation/degradation n include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries.		
	Low	The river environment reach/zones are between		

The cover (structural)		15 - 45 % transformed; this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries.	that in turn will impair their successful recruitment/escapement. This node considers that large areas of transformation along the river reach/zone will contribute to the degradation of the water body. This node hypothesize that anguillid eels within rivers with less than 15% transformation or degradation are at "zero" risk; this transformation/degradation include agricultural activities and urban development (<10% transformed) and/or < 2 large/established factories/Industries. anguillid eels are at a "low" risk if the river reach/zone is transformed/degraded between 15 - 45%, this degradation/transformation include <2 identified sources of sand mining, between 10 - 40% urban or agricultural development and between 2 - 4 large established factories/Industries. anguillid eels are at a "moderate" risk if the river reach/zone is transformed/degraded between 45 - 75%, this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries. anguillid eels are at a "high" risk if the river reach/zone is transformed/degraded by > 75%, this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries.	and Lambert, P., 2018.
	Med	The river environment reach/zones are between 45-75 % transformed; this degradation/transformation include <4 identified sources of sand mining, between 40 - 70% urban or agricultural development, between 4 - 8 large established factories/Industries.		
	High	The river environment reach/zones are more than 75 % transformed; this degradation/transformation include <4 identified sources of sand mining, > 70% urban or agricultural development, > than 8 large established factories/industries.		
	Zero	The cover/ structure	This node represents the availability of physical	

feature) required for the maintenance of yellow eels. (YELLOW_COV)		features within the river environment are less than 15% transformed and are comparable to historical/pre-anthropogenic conditions.	cover features for anguillid eels (Yellow life stage) to successfully migrate/grow within in the river environment/reach/region. There is a strong relationship between riparian vegetation, instream habitat and community structure in aquatic ecosystems. This variable will be based on what the fish community require as a cover feature with the assumption that this will be sufficient cover for the anguillid eels (Yellow life stage). The availability of marginal vegetation and associated cover features are important environmental variable which affects the sustainability of instream habitats for fish communities including anguillid eels. Different cover features included overhanging vegetation, marginal vegetation, aquatic vegetation, undercut bank, and substrate. Different fish/invertebrate species have different preferences for different cover features. This node hypothesize that a large reduction in the physical cover features within a reach will negatively affect fish communities and the success rate of anguillid eels (Yellow life stage) to migrate and grow within the river environment/reach/region. Ranks include distribution and abundances that will support pristine/natural community of indicator species and anguillid eels (Zero), occurrence of (but altered) of cover distributions for indicator species and anguillid eels (Low), modified cover distributions for at least one fisheries indicator species while majority of indicator species is provided for and anguillid eels (Mod) and significant alteration of cover characteristics that affects fish community and	Allanson and Read 1995; Dala-Corte <i>et al.</i> 2016; Thrush <i>et al.</i> 2004
	Low	The cover/ structure features within the river environment reach/zones are transformed/degraded between 15 - 45 %.		
	Med	The cover/ structure features within the river environment reach/zones are between 45-75 % transformed.		
	High	The cover/ structure features within the river environment reach/zones are more than 75 % transformed.		

			anguillid eels significantly.	
The velocity depth requirement of yellow eels. (YELLOW_VD)	Zero	There are no changes in the depth required for access between reaches. The average yearly water column depth is deeper than 1.3m.	Node represents the suitable depth (in relation to historical depths, using hydraulic models where available) requirements of anguillid eel (yellow life stage) species that need access between reaches within the river environment/reach/region. This node hypothesize that low water depth between habitats will restrict or hinder the movement of anguillid eels in the river environment. No velocity requirement/preference are observed in eels as their preference are dominated by all velocity depth classes from Kleynhans 2007. There are no changes in the depth required for access between reaches (Zero) with an average yearly water column depth that is deeper than 1.3m. There are some changes in depth requirements but most potadromous or reach scale migrators especially the eels have access between reaches (Low) with an average yearly water column depth is between 0.7 - 1.3m. There are changes in depth requirements but some potadromous or reach scale migrators have access between reaches (Moderate) with an average yearly water column depth is between 0.3 - 0.7m. Note that no condition is assigned to the "high" risk node as it is known that eels could potentially 'climb' over structure.	Jellyman and Chisnall 1999; Kleynhans 2007; Baumgartner <i>et al.</i> 2014; Drinkwater and Frank 1994; Kleynhans, Louw and Moolman 2007; Petts 1984
	Low	There are some changes in depth requirements but most potadromous or reach scale migrators have access between reaches. The average yearly water column depth is between 0.7 - 1.3m.		
	Med	There are changes in depth requirements but some potadromous or reach scale migrators have access between reaches. The average yearly water column depth is between 0.3 - 0.7m.		
	High	NA		
	Zero	There are no fishing practises or syndicate		
Fishing for silver eels in the marine environment.			This node considers the intensity of silver eel harvest based on the frequency of marine	Aalto <i>et al.</i> , (2016)

(MAR_SIL_HARVEST)		operations within the area of anguillid eel migrations and no removal of eels occur	trolling/line fishing/spear-fishing events/large fishing vessels and the demand for silvers eels from syndicate markets. This unit includes the frequency (seasonally, monthly, weekly, daily) that fishing vessels fish around the migration pathway of anguillid eels and either catch eels to sell or have eels in the bycatch, also consider the amount/demand for fishing for silver eels from syndicates (locals catching eels and selling to syndicates) to sell and export eels. It is hypothesized that larger yield will allow less silver eels to reach the spawning ground and not reproduce. A "zero" risk category is considered when there are no fishing practises or syndicate operations within the area of anguillid eel migrations and no removal of eels occur; a "low" risk; is considered in the present of large fishing vessels (line fishing/trolling/spear fishing)(<50) that fish in the migration pathway area seasonally or syndicate operations only occur seasonally (e.g., locals only go out in rainy season to catch eels); a "moderate" risk; is considered in the present of large fishing vessels (line fishing/trolling/spear fishing) (50-100) that fish in the migration pathway area monthly or syndicate operations that occur monthly; a "high" risk; is considered in the present of large fishing vessels (line fishing/trolling/spear fishing) (>100) that fish in the migration pathway area weekly or syndicate operations that occur weekly. If no syndicate operations occur within the region in question, the focus is placed on the amount of fishing vessels and their fishing/trolling intensity for eels or potential bycatch.
	Low	The present of large fishing vessels (line fishing/trolling)(<50) that fish in the migration pathway area seasonally or syndicate operations occur seasonally	
	Med	The present of large fishing vessels (line fishing/trolling) (50-100) that fish in the migration pathway area monthly or syndicate operations that occur monthly	
	High	The present of large fishing vessels (line fishing/trolling) (>100) that fish in the migration pathway area weekly or syndicate operations that occur weekly	

			the intensity of harvest of silver eels are between 5 - 10 kg/hectare/year; and node are at a "high" risk if more than 10 kg/hectare/year of silver eels are harvested from the marine environment especially around the spawning grounds.	
The perennality condition of the river. (RIVER_PERENNIALITY)	Zero	A river system with constant drought periods	This nodes considers the perennality condition of the river system in question. Full perennality is defined as a river system that is constantly flowing even though water levels are fluctuating due to the different seasons, importantly there is always flowing water within the system. River systems with full perennality have a greater potential to accommodate more eel, fish, invertebrate and plant species that in turn will provide a better environment for anguillid eel species. The lower the risk the less potential there is to accommodate anguillid eels. A river system with constant drought periods are classified at "Zero" risk, a river system with seasonal drought or seasonal perennality is classified at a "low" risk, large river systems with season perennality changes but water levels never reach zero is ranked at a "moderate" risk, and river system that are large with no perennality changes and constant flow are ranked at "high" risk.	
	Low	A river system with seasonal drought or seasonal perennality		
	Med	Large river systems with season perennality changes but water levels never reach zero		
	High	A river system that are large with no perennality changes and constant flow		

Barriers in the river environment influencing the upstream migration in rivers. (RIVER_MAIN_BARRIER)		Zero	There are no barriers preventing the migrations of anguillid eels upstream and river blockage is comparable to historic conditions.	This node considers the amount of barriers (natural/anthropogenic/permanent/temporally) in a catchment or river that will partially or fully block the migration of anguillid eels during the upstream migration of yellow eels. This variable is estimated from the amount of barriers that poses a blockage during all seasons (High flow & low flow) within the river environment. It is hypothesized that barriers that overflows during the high flow season and barriers with functional bypasses classify as temporary barriers while large dam walls without overflow or bypasses classify as permanent barriers. This node considers rivers with no barriers or temporary barriers pose "zero" risk; rivers with a few (<5) temporary barriers to pose a "low" risk; a river with large dams with or without bypasses, hydropower turbines and temporary barriers (>5) poses a "moderate" risk and rivers with large dams or weirs with no bypasses and hydropower turbines poses a "High" risk for the migration of anguillid glass eels upstream.	O'Brien <i>et al.</i> , (2019) Hanzen <i>et al.</i> , (2019) Watz <i>et al.</i> , (2019)
		Low	There are a few (<5) temporary barriers preventing the migration of anguillid eels while the majority of anguillid eels could potentially pass through.		
		Med	There are a few (> 5) temporary barriers and large dams with functional bypasses preventing the migration of anguillid eels while anguillid eels could potentially still pass through.		
		High	There are large amounts of barriers with no bypasses (>5) posing the complete blockage of the migration of anguillid eels.		
	ELV_YEL _MAINT Maintenance of elvers and yellow eels		RIVER_SUIT (20) LARVAE_RECRUIT (70) RIVER_ENV (10)	Note: Variable is an endpoint that is influenced by the relationship between the suitability of the river to maintain yellow eels, the condition/success of larvae/glass eel recruiting into the river and the river environment to maintain yellow eels. Variable represent the ability of	

			rivers to maintain yellow eels. Ratio: If eels don't recruit into the river, they won't maintain the population: secondary to that is where the catchment is the river suitability and then only once it is there will the environment effect it.
ELV_YEL_PRED	Predation on elvers and yellow eels in the river environment	ALIEN_RIV_PRED (70) IND_RIV_PRED (30)	Note: Variable represent the relationship between alien and indigenous (aquatic/terrestrial) predators on yellow eels during the miniatous period in the rivers. Ratio: Alien invasive predators if present have a greater impact than natural predators to the survivability of eels. Alien predators do not have natural predators that will cause them to be in higher abundances
ESC_DTW	DTW during escapement in the river environment	FISHING_SILVER (65) ESC_PREDATION (35)	Note: Variable (Disturbance to Wildlife) represents the potential for predation from alien and indigenous species (competition and predation) and overfishing from people to affect the availability of silver/adult eel to escape. Ratio: Predation does not cause mass mortality (35), and fishing exploit harvesting (65) can be more severe.
ESC_PREDATION	Predation during escapement	ALIEN_ESC_PRED (70) IND_ESC_PRED (30)	Note: Variable represent the relationship between alien and indigenous (aquatic/terrestrial) predators on silver eels during the escapement period in the rivers. Ratio: Alien invasive predators if present have a greater impact than natural predators to the survivability of eels. Alien fauna do not have many natural enemies and could occur in larger abundances
EST_DTW	DTW in the estuary environment	FISHING_LARVAE (60) EST_PREDATION (40)	Note: Variable (Disturbance to Wildlife) represents the potential for predation from alien and indigenous species (competition and predation) and overfishing from people to affect the availability of glass eel to enter the estuary during recruitment.

			Ratio: Predation does not cause mass mortality, and fishing exploit harvesting can be more severe. Fishing for larvae are being more developed to increase catch per unit efforts.
EST_FLOWS	Flows to maintain estuary environment	LARVAE_VD (40) LARVAE_COV (60)	Note: Variable is a function of the relationship between the velocity-depth classes and the cover/substrate features (instream habitat and depth to link classes) available for glass eels to enter the river from the estuary. Ratio: Velocity-depth class variability (as long as there is water) are less important than cover as cover provides refuge for glass eels to hide and follow to recruit into the rivers.
EST_PREDATION	Predation in the estuary	ALIEN_EST_PRED (70) IND_EST_PRED (30)	Note: Variable represent the relationship between alien and indigenous (aquatic/terrestrial) predators on leptocephali/glass eels during the recruitment period into the rivers. Ratio: Alien invasive predators if present have a greater impact than natural predators to the survivability of eels. Alien invasive predators might not have natural enemies in the river and might be in higher abundances in the rivers.
ESTUARY_ENV	Estuarine environment	EST_DTW (65) ESTUARY_HAB (35)	Note: Variable is a function of the severity of disturbance of wildlife and the ability of the river habitat environment to allow glass eels to migrate in and maintain them in terms of the river access, water quality and flow variables. Ratio: DTW could influence the amount of glass eels entering the river more than the river environment as it more important in this stage to get into the river then to find refuge.
ESTUARY_HAB	Estuarine habitat for the maintenance/refuge for glass eels	EST_BARRIERS (50) EST_QUALITY (20) EST_FLOWS (30)	Note: Variable is a function of the relationship between the amount of barriers in the estuary, the quality of the water for glass eels and the flows/biotopes available for eels to find refuge. Ratio: Access into the estuary is the most important as eels need to go up

			into estuaries to complete their life cycle (there are some exceptions) and they then need to find refuge. From what have observed eels are locations in some bad water quality conditions so water quality is ranked last.
ESTUARY_SUIT	Estuary suitability for Leptocephali to enter estuary.	DISTANCE_SEC (30) CURRENTS_NvsS (20) EST_TYPE (50)	Note: Variable represent the relationship between the distance leptocephali needs to drift to the estuary mouth, the current strenght taking them to the estuary mount (weaker currents cause more time exposed in marine environment) and the estuary month type the larvae drifts towards. Ratio: The estuary month type is most important as a closed mouth will not allow the larvae to enter into any estuary while the distance they need to travel is second important and the current direction can take them to any estuary.
FOOD_RIV_HABITAT	River habitat in terms of food for eels	FOOD_VD (55) FOOD_COVER (45)	Note: Variable represent the relationship between fish/invertebrate communities that act as food for yellow eels and the velocity-depth classes and cover/substrate feathers available in the river environment to maintain these communities. Ratio: Variables are nearly equal, although the present of velocity-depth classes could ensure the present of some cover/substrate feathers.
LARVAE_RECRUIT	Recruitment of larvae	ESTAUARY_SUIT (60) ESTUARY_ENV (40)	Note: Variable is an endpoint that represent the relationship between the suitability of glass eels/leptocephali to enter the estuary environment and the estuary environment that provides refuge for the glass eels. Ratio: Estuary suitability is ranked more important as glass eels/larvae need to enter the estuary environment before the actual estuary environment could influence them.

MARINE_DTW	DTW in marine environment	MAR_SIL_HARVEST (65) MAR_SIL_PRED (35)	Note: Variable is the function of the amount/frequency of silver eel harvested in the marine environment and the predators predating of silver eels on their way to the spawning grounds. Ratio: Accidental Harvest would have a greater impact that natural predation (eels are a greater extent adapted to natural predation than large/small-scale accident harvesting).
MARINE_HABITAT	Marine habitat to maintain eels	MAR_CURRENT (25) MARINE_QUALITY (40) MARINE_DTW (35)	Note: Variable is a function of the relationship between the strength of the marine currents, the quality of the marine environment (eg salinity) and the disturbance to wildlife influencing the ability of silver eels to escape and migrate through the marine environment. Ratio: Salinity front would be more important than current flow as silver eels use it to find its spawning ground. Eels as strong swimmers and DTW (harvesting) is more severe as their removal at this stage would be devastated.
RIVER_DTW	Disturbance to wildlife in the river environment	FISHING_ELV_YEL (50) ELV_YEL_PRED (30)	Note: Variable (Disturbance to Wildlife) represents the potential for predation from alien species (competition and predation) and indigenous species present in the river, and the overfishing potential from people that affect the availability of eel in rivers. Ratio: Predation does not cause mass mortality, and fishing exploit harvesting can be more severe.
RIVER_ENV	River environment to maintain yellow eels	RIVER_DTW (20) RIVER_HABITAT (40) YELLOW_FOOD (40)	Note: Variable is a function of the severity of disturbance of wildlife, the ability of the river habitat environment to maintain eels and the condition/health of fish/invertebrate communities as food for eels. This node represent the ability of the river environment to maintain yellow eels. Ratio: Habitat and food are equal drivers of the system, with DTW less so.

RIVER_ENV1	River environment during escapement as a function of habitat and stressors.	ESC_DTW (40) RIVER_ESC_HAB (60)	Note: The variable represent the relationship between the amount of disturbance to silver eels and the river environment to provide habitat for silver eels while they migrate back to the ocean. Ratio: If the environment does not provide for escapement (Barrier in the system, large waterfall drops etc...) this is more severe than the presence of predators
RIVER_HAB_ESC	River habitat during escapement	RIV_ESC_BARRIER (50) SIL_RIV_QUALITY (10) SILVER_ESC_DEPTH (40)	Note: Variable is a function of the relationship between the amount/type of barriers in the river, the water quality that could influence silver eels and the depth of water to allow silver eels to escape back to the ocean. Ratio: Barriers are a major stressors to the downstream migration of eels and is rank the highest as many eels get trapped behind barriers, secondly the depth between habitats are important for connectivity and migration. Water quality is ranked lowest as eels are tolerant to most water quality parameters.
RIVER_HABITAT	River habitat to maintain eels	RIV_MAIN_BARRIER S (40) YEL_RIV_QUALITY (20) YEL_RIV_FLOWS (40)	Note: Variable is a function of the relationship between the amount of barriers in the river, the quality of the water for yellow eels and the flows/biotopes available for eels to find refuge. Ratio: Barriers and flows are ranked equal as barriers and flows (Velocity-depth) allows yellow eels to migrate up the river and over some barriers while it is known that eels are tolerate to water quality parameters.
RIVER_LS	River suitability for the maintenance of	RIVER_LENGTH (30) RIVER_ALT (25) WIO_LOCATION (45)	Note: Variable represent the relationship between the length of the river, the altitude range of the river and the location of the river in relation to the WIO. Variable represent the suitability of the river to maintain yellow eels.

	yellow eels		Ratio: Locality in the tropical-temperate zone will impact the distribution of yellow eels more than the length and altitude. The locality in the tropical-temperate zone will influence the climate, temperatures, and habitat possibilities.
SILVER_ESCAP	Escapement of silver eels into the marine environment	ESC_RIVER_SUIT (20) ELV_YEL_MAINT (60) RIVER_ENV1 (20)	Note: Variable is an endpoint and represent the relationship between the suitability of the river to allow eels to escape, the amount of eels (yellow) making it to the silvering stage and the river environment to maintain silver eels while they escape. Variable is a function of the amount/success rate of silver eels to successfully escape back into the ocean. Ratio: Eels must reach the silvering stage in order for them to escape so the maintenance of yellow eels are most important, while the river size, environment are weighed equal important.
SPAWNING	Spawning probability of eels	DIST_SPAWNING (10) SILVER_ESCAP (60) MARINE_HABITAT (30)	Note: Variable is an endpoint that represent the relationship between the distance to spawning grounds silver eels need to travel, the number of silver eels available from the river to escape and the marine environment allowing them to reach the spawning grounds. Ratio: If there are no eels escaping spawning won't happen, eels are strong swimmers across its range and would be limited more by the marine environment (current strengths) than the distance to spawn.
YEL_RIV_FLOW	River flows to maintain yellow eels	YELLOW_VD (70) YELLOW_COV (30)	Note: Variable is a function of the relationship between the velocity-depth classes and the cover/substrate (instream habitat and depth to link classes) available for yellow eels to be maintained/grow in the river. Ratio: anguillid eels have strong preferences for undercut banks and substrate features and slow deep habitats. Velocity-depth classes is ranked higher as the present of deep habitats will ensure the availability of

			undercut banks and refuge substrates.
YELLOW_FOOD	Maintenance of fish/inverts in term of food for eels	FOOD_RIV_HABITAT (55) FOOD_RIV_QUALITY (45)	Node: Variable is a function of the relationship between the available habitat (Cover/substrate and flow classes) and the water quality to maintain fish/invert communities that act as a food source for yellow eels in the rivers. Ratio: Habitat is ranked more important as tolerant species will still occur in bad water quality conditions and eels could feed on them.

THE END