Fish and habitat present ecological state in the Mooi River catchment in the North West Province

MFM Tele

orcid.org/0000-0002-5510-7815

Dissertation accepted in partial fulfilment of the requirements for the degree *Master of Environmental Management with Ecological Water Requirements* at the North-West University

Supervisor:	Dr CW Malherbe
Co-supervisor:	Dr RJL Gerber

Graduation July 2022

29686938

ACKNOWLEDGEMENTS

I would like to thank the Almighty for the strength He has given me to complete the research.

Also, words of gratitude to my supervisors, Dr Wynand Malherbe and Dr Ruan Gerber for their patience, comments, inputs and motivation during my studies.

To my children, Kabelo Mpadi and Oratile Nakedi Dzuvha (Orandzu), thank you for their support, especially Kabelo for taking care of his younger sister when I had to attend classes (block weeks). He is an angel. Thank you so much, my dearest.

A special thank you to my parents; my mom for being such a blessing in my life, a woman of prayer. Without her prayers, I wouldn't have been able to make it.

Thank you to my friend, Ms. Daphney Lethabo Ramashala and my classmates for their motivation.

Thank you Ms. Khanyisa Baloyi and Mr. Tendani Mphaga for assisting with maps and Ms. Grietjie Stander and Dr Wynand Malherbe, my supervisor for assisting with sampling.

To my sponsors, the North-West University (NWU) and Ekurhuleni Water Care Company (ERWAT), for entrusting me with the finances.

DECLARATION

I, Mosima Tele, hereby declare that this dissertation submitted by me has never been submitted for a review at this or any other institution. From the knowledge that I have, I also confirm that this dissertation has documented no previously published content written by another person except where due reference is made in this project.

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ABSTRACT

Anthropogenic activities are important for various purposes such as economic activities, recreation and to partially safeguard food and energy across the world. In a semi-arid country such as South Africa, where the freshwater resources are already vulnerable and threatened by drought and deteriorating water quality, the catchment conditions are a major concern contributing to ecological status decline. The potential impact on fish species and habitat associated with anthropogenic activities that change catchment conditions are extensive and devastating. Therefore, the aim of this study was to assess the fish and habitat integrity representing the ecological status of the Mooi River located in the North-West Province. The fish species were assessed during austral spring in October 2018 together with the habitat integrity. The Fish Response Assessment Index and Index of Habitat Integrity (IHI) were used to determine Present Ecological State (PES) and habitat indicators linked to fish species. A Principal Component Analysis (PCA) and unconstrained ordination were used to determine the temporal and spatial variation of landscape and soil properties for fish species. The results showed that there is an abundance of anthropogenic activities which change catchment conditions and the in-channel and bank conditions are modified. The in-stream and riparian zone integrity scores were between 40-59% and 60-79% placing it to category D or C/D. The results for overall fish PES were 52, placing it in category D, therefore, largely modified. The results of the habitat integrity and PES are within the recommended limit of Resource Quality Objectives (RQOs). The results of the PCA and unconstrained ordination indicated temporal variation at Sites 2 and 3 as well as sites 4, 5 and 6 which were grouped together. Site 1 varied spatially and grouped separately due to increased changes in landscape. The PES across all sampling sites is moderately to largely modified, therefore there is a great need to continuously monitor the catchment and alert community and stakeholders on possible activities that may trigger a critical modified environment.

Key Words: Catchment, Habitat integrity, Resource Quality Objectives, Present Ecological Status.

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LIST OF ABBREVIATIONS

DWS: Department of water and sanitation

RHP: River Health Programmes

AEHMP: Aquatic Ecosystem Health Monitoring Programme

WRC: Water Research Commission

PES: Present Ecological State

FRAI: Fish Response Assessment Index

HAI: Heath Assessment Index

RIVPACS: River Invertebrate Prediction and Classification System

SASS5: South African Scoring System 5

ASPT: Average Score per Taxa

HQI: Habitat Quality Index

IHAS: Habitat Assessment System

IHI: Intermediate Habitat Integrity

FAII: Fish Assemblage Integrity index

IBI: Fish Community Index

IWB: Fish Base Index Wellbeing

WMA: Water Management Area

PCA: Principal Component Analysis

CHAPTER 1: INTRODUCTION

1.1 Background to the study

Water is the most important natural resource on earth because of its importance for the survival of all living organisms (WHO, 2011. Although it covers 70% of our planet, only 3% is considered freshwater, of which 1% is easily accessible, making it a very scarce resource and one of the biggest challenges worldwide (Nairizi, 2017). It is estimated that there will be a 40% shortfall of water supply by 2030 if no changes are made to water demand management (Cavicchioli *et al.,* 2019). South African surface water quality is a threatened due to discharged industrial effluents, partially treated sewage effluent sewage spillages as well as illegal discharge of water containing pollutants (Edokpayi *et al.,* 2017). Water resources are prone to pollutants such as heavy metals, nutrients and emerging pollutants of concern. As a result of continuous accumulation of pollutants in the rivers, water quality deteriorates; therefore creating unfavourable conditions for aquatic ecosystems (Dube, 2020). Although water quality is a challenge in South Africa, the aquatic habitat has become vulnerable due to various anthropogenic factors such as agriculture, urbanisation, mining and industrialisation (Hammond *et al.,* 2021).

Water quality changes and modification of aquatic systems are posing a great threat to water security and freshwater systems. Growth in sewage connections from urban developments also contributes significantly to wastewater effluents which may end up in the environment (Hammond *et al.,* 2021). There are various indicators of water quality deterioration measures such as invertebrates used to determine toxicity (Bird, 2010).

Due to concerns over aquatic ecosystem deterioration in South Africa, the Department of Water and Sanitation (DWS) established the River Health Monitoring Programme (RHP) that looks at the Present Ecological Status (PES) of these systems (Louw & Kleynhans, 2007). Although the RHP was developed across many water systems, it has not been implemented across all catchments in South Africa (Nel & Driver, 2015). The PES was traditionally conducted through the use of invertebrates' assessments; however, the habitat and fish assemblage also form a critical part of RHP (Kleynhans, 2007). Riverine ecosystems have suffered from intense human intervention resulting in habitat loss and degradation and, consequently, many fish species have become highly endangered, particularly in areas where heavy demand is placed on freshwater (Galib *et al.,* 2013). In any catchment, there are fish species that are expected to occur across all trophic levels; however, the migration and extinction of fish species in the aquatic systems are attributed to habitat changes and water quality deterioration (Smith *et al.,* 2009).

Mooi River is one of the south African River that are experiencing the migration and extinction of fish species. Mooi River is located in the west of Gauteng and it's a tributary of the Vaal River. It is surrounded by informal settlements, mines and agricultural activities which are the problematic area as they are the main contributor of pollution in the Mooi River catchment (Venter et al., 2013). It is also underlain by outcrops of dolomite which results in only half of its total area yielding significant runoff. It is consist of sub-catchments such as Loop Spruit and Wonderfontein Spruit which receives pollution from the mines polluting with metals and Acid Mine Drainage and also from the surrounding communities (Winde, 2010; Barnard et al., 2013). There are a lot of anthropogenic activities taking place in the Mooi River which results in the water quality deterioration and the water quality deterioration is one of the reason fish migrate or become extinct, hence it is very important to study their state as a results of deterioration in water quality in the catchment.

The Mooi River catchment has a total of 14 fish species; however, there are concerns over changes in the aquatic ecosystem due to natural and anthropogenic activities which pose a detrimental impact on the habitat and fish species (Levin *et al.,* 2019).

Fish have been used in river health monitoring in South Africa, however; in certain catchments, there is a lack of information on fish communities (Rashleigh *et al.,* 2009), especially in the hard-working river catchments like the Mooi River. The Mooi River is one of the catchments which is dominated by water quality problems due to surrounding natural and anthropogenic activities (Potgieter, 2019). Freshwater fish are one of the most threatened taxonomic groups because of their high sensitivity to the quantitative and qualitative alteration of aquatic habitats (Mohammad *et al.,* 2017). Therefore, they are often used as bio indicators for the assessment of water quality, river network connectivity or flow regime (Marzin, 2013). The ecological status of the river is determined using aquatic biota as ecosystem health indicators of an aquatic environment River Health Programme (RHP), to monitors the present ecological state in the rivers (Dallas, 2007; Lowe & Murphy, 2010)..

1.2 Problem statement

Surface water, more specifically the perennial rivers and the in-stream and riparian zones serve and represent a large portion of the biodiversity. The overall loss of biodiversity in aquatic systems includes the loss of flora and fauna (Irfan & Alatawi, 2019). Native fish species have always been viewed as important aspects of biodiversity, similarly to flora which form part of the fish habitat (Milardi *et al.,* 2019). Due to the growth of anthropogenic factors such as industrialisation, urbanisation, agriculture, mining and related activities, and the aquatic environment is more prone to changes (Khatri & Tyagi, 2015). In South Africa, there are wide spread changes in water quality attributed to man-made factors. Natural disturbances form a smaller portion of the changes in the ecosystem; however, their effects are noticeable (Dallas & Rivers-Moore, 2014).

Although some fish species migrate to find spawning grounds and the return to grounds, Arthington *et al*. (2016) explained that migration and extinction of fish species may also be caused by the changes in the aquatic systems. In recent years, bank modifications such as the construction of bridges and damming structures have resulted in modification of the habitat and migration of fish species (Slingenberg *et al.,* 2009). Over the past years in the Mooi River catchment, there have been concerns over water quality deterioration and changes in aquatic habitat. This may result in the fish species migrating or becoming extinct (Bach *et al.,* 2020; Dube, 2020). To date it is not very clear if all fish communities are still present due to ecological changes. The availability and migrating of native species have always been linked with problems such as water quality deterioration, destruction of hydrophytes, loss of longitudinal connectivity of rivers as well as habitats (Wasserman *et al.,* 2011).

According to literature such as Arevalo *et al.* (2020) and Avenant (2010), the migration and extinction of fish species represent changes in the habitat and ecological integrity of the river. There has been reports that in South Africa, native fish species are declining in most rivers, therefore the species are listed on the red list as an indication of risk to extinction attributed to changes in aquatic environmental conditions (Henning *et al*., 2009; Musil *et al*., 2007). The assemblage is very sensitive to the changes in aquatic and environmental systems; therefore, their distinctive characteristics of assemblages can be used as indication of their present ecological status (Rashleigh *et al.,* 2009).

In addition, the ecological assessments of the Mooi River have not included fish and habitat integrity in previously conducted studies. This has led to a lack of information and knowledge on fish and habitat structures in the study area. The Mooi River catchment and its fish were not included in previous reports such as:

- The ecosystem health and water quality of the Mooi River and associated impoundments (Pelser, 2015a).
- Plankton algae and cyano prokaryotes as indicators of ecosystem quality in the Mooi River system in the North West Province (Venter *et al.,* 2013).
- Challenges in using fish communities for assessing ecological integrity (Avenant, 2010).
- Report of the national survey on metal accumulation in fish on six major river catchments of South Africa (Heath & Claasen, 1999).

Fish species can represent the ecological status across all trophic levels (Jia *et al.,* 2021). Therefore, the current study used fish and habitat to assess the ecological status of the Mooi River. The results will serve as a baseline for future studies and development in the catchment.

This research will benefit the stakeholders of the Mooi River as it gives an indication of the integrity class, indicates compliance or non-compliance against the Resource Quality Objectives of the Vaal Water Management Area (WMA) and it also outlines the measures to be taken to improve the deteriorated ecological class.

1.3 Aims and objectives

1.3.1 Aim

The study aimed to determine the Present Ecological Status (PES) and habitat integrity of the Mooi River through assessments of fish communities, habitat and supplementary variables.

1.3.2 Objectives

- To determine the diversity and abundance of habitat and fish species present in the Mooi River.
- To determine fish responses to physicochemical, hydrological and geomorphological changes.
- To assess the fish integrity (ecological category) of the Mooi River using the Fish Response Assessment Index and to compare the results with the Vaal WMA RQOs.

1.4 Hypothesis

The fish and habitat ecological status in the Mooi River has deteriorated from the reference condition.

1.5 Dissertation breakdown

Chapter 1: Introduction

This section provides the background information of the research, the research problem statement, the research question and the objectives of the study.

Chapter 2: Literature review

This section critically assesses the literature reviewed on ecological assessment, ecological state of rivers and ecological integrity of the Mooi River in the North West Province.

Chapter 3: Research methodology

This section outlines the road map taken during the study period. This covers the research methodology adopted to achieve the aim of this research. Lastly, it discusses the limitations of the study.

Chapter 4: Results and discussion

This chapter aims to interpret and discuss all the data gathered in relation to the research questions and objectives of this study.

Chapter 5: Conclusion and recommendations

This section summarises and provides conclusions drawn from the results found. Based on the conclusions made from this study, the recommendations for future studies were outlined.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter focuses on reviewing the literature relevant to the study. Literature on the global and South African perspectives towards habitat and fish species and its influence on ecological status, the diversity and dynamics of fish species in the Mooi River, the measurements and indicators of ecological status as well as techniques and methods used for ecological status assessments is reviewed. This is done through analysis of literature from studies previously conducted on the subject under investigation.

2.2 Habitat and fish species' effect on ecological status

Globally, rivers, estuaries, lakes, streams and other freshwater systems are regarded as habitat for aquatic organisms and their main sources are aquifers, glaciers as well as rainfall (Gozlan *et al.,* 2019). The fauna and flora in freshwater represent the biodiversity system which is dependent on water either during their entire life or part thereof (Balian *et al.,* 2010). In Asia and Europe, the freshwater habitat is highly diverse; however due to growing anthropogenic activities, native freshwater fish species are threatened. Lake Baikal in Russia covers almost 20% of the world's freshwater resources (Gozla*n et al.,* 2019). In order to sustain the biodiversity in freshwater systems in Europe, the red list of habitat integrity with stipulated criteria from A to D was established, similar to the South African in-stream and riparian zone habitat integrity (Holland *et al.,* 2012). The implementation is due to rising concerns on anthropogenic factors which leave the habitat at threat for modification.

Worldwide, there is an alarming behaviour of migratory fish which requires a better understanding in the causes and challenges faced in the ecological drivers (Tamario *et al.,* 2019). Many developed countries in Europe, Asia, South America and North America have placed threatened fish species on the red list which can be attributed to ecological modification caused by anthropogenic and natural factors such as urbanisation, agriculture, pollution, human intrusions, pollution and geologic events that leave the habitat vulnerable to threat (Grzybowski & Glińska-Lewczuk, 2019).

The continuous modification of the habitat has adverse effects on the extinction of fish species and relocation which represent changes in habitat integrity (Tamario *et al.,* 2019). Besides the migration and extinction impact globally, there are long-term effects associated with shifts in behaviours, production and growth trajectories which affect fish populations (Jacquin *et al.,* 2020).

More than 20% of freshwater fish have been extinct (Jordaan *et al.,* 2020). The world's freshwater resources are vulnerable to pollution. Globally, due to threats posed to ecosystems, programmes have been established to protect the freshwater systems and fish species such as regulation, education and stewardships as well as the red list to protect endangered species (Carpenter *et al.,* 2011). In Romania, many estuaries, including the Danube River, have faced degradation in habitat due to water pollution and activities such as river engineering and land use (Schiemer *et al.,* 2004). This, over the years, has caused declining trends in many species and has led to native fish taxa becoming endangered, resulting in deteriorated ecological status. In order to determine the ecological status, Schiemer *et al.* (2004) assessed the correlation between habitat and fish species with environmental variables. There was a positive correlation between habitat changes and environmental variables and noticeable migration of species attributed to the changes.

Lijiang is one of the largest rivers in China and provide habitat for 37 native fish species. Over the years, research has shown that the Lijiang River has been the habitat of species such as *Pseudogastromyzon fangi*, *Zacco platypus*, *Acrossocheilus parallens* and *Erromyzon sinensis.* Their declining population has been linked to biophysical factors such as turbidity, dissolved oxygen, temperature and other environmental variables affecting ecological requirements (Huang *et al.,* 2019). The world's freshwater systems are also vulnerable due to the modification of flow regimes. In areas like the northern part of Australia, during dry season the stream flow is very low. This has threatened the aquatic systems and biodiversity which include native fish species (Keller *et al.,* 2019).

Africa is a developing continent as opposed to Europe, Asia and North America. In Africa, there is a lot of development in infrastructures such as roads, cities and urbanisation which are likely to affect the remainder of the natural habitat and its biodiversity (Anderson *et al.,* 2013). Although Africa is known for its abundance in biodiversity, the habitat destruction and declining in protected fish species are major concerns which affect ecological integrity (Berrahmouni, 2020). The surface water resources of South Africa are deemed to be severely under pressure due to man-made factors and climate change (Bills & Impson, 2013). Due to a continuous decline in habitat and fish species, the IUCN red list was established in South Africa to protect the extinction of native species (SANBI, 2010). In 2005, the river health monitoring programme was established with the inclusion of habitat and fish assessment to determine the ecological status in South Africa (Louw & Kleynhans, 2007). Although assessments of ecological status were conducted in some of the catchments, the majority of South African rivers have not been assessed using habitat and fish species (Shelton *et al.,* 2019).

According to Dlamini (2019), the fish community structures and habitat integrity represent ecological status. In the study by Dlamini (2019), environmental variables and yellowfish (*Labeobarbus natalensis*) were used to assess the present ecological status of the Umngeni River in Kwazulu Natal, South Africa. The migratory behaviour and habitat integrity showed that the ecosystem is declining from its natural environment. This explains why freshwater fish are the most threatened species in South Africa. Over 172 fish species are native to the South African surface water system; however, there is a high level of endemism with approximately 22% of species only occurring in South Africa (Bills & Impson, 2013). The migration and extinction of these fish species can easily describe the changes in habitat which affect the Present Ecological Status (PES) (Duplisea *et al.,* 2016).

2.3 Ecological status of South African rivers

Since the inception of RHP programmes in 2005 in South Africa, it was expected that, after decades of implementation, the majority of rivers will have a known status on their ecological integrity (Roux & Nel, 2013). The challenges regarding assessments of ecological status were mainly capacity and accessibility of data and sites. Although there were challenges regarding ecological assessments in South Africa, more than 112 rivers were assessed amounting to 84% of the total rivers being threatened. The percentage split between critically endangered, endangered and vulnerable is alarming (Roux & Nel, 2013). According to literature, 54% of South African rivers are critically endangered, while 18% and 12% are endangered and vulnerable (Dallas &

Rivers-Moore, 2014; Nel *et al.,* 2007). The ecological assessments conducted show that, of 112 rivers assessed, only 16% of the rivers are not threatened which is a very small proportion of the total (King *et al.*, 2008).

Perennial rivers have also been noted to be the most endangered freshwater systems; more than rivers with intermittent flow or a highly variable stream flow (Day *et al*., 2020). The most protected rivers are those lying within sensitive areas; however, most main rivers are threatened freshwater systems (Roux & Nel, 2013). In the last assessment by DWS, the Mooi River's ecological status was not assessed due to issues underlying capacity and accessibility (DWS, 2018). The threatened ecosystem requires the conservation of main rivers and their tributaries (Sabater & Elosegi, 2014).

2.4 General description of the Mooi River catchment and its anthropogenic activities

The origin of the Mooi River is in Gauteng and extends to the North West Province to the eastern part of Koster and the south-east of Stilfontein and Potchefstroom in South Africa (Bezuidenhout, 2013). The Mooi River catchment covers an estimated surface area of 4 500 km^2 of which 1 800 km^2 is in the North West Province. The Mooi River and its tributaries receive contamination from various points and diffuse sources (DWS, 2016). Growing communities located in the catchment include Kagiso, Mohlakeng, Toekomsrus, Rietvlei and Bekkersdal which contribute to the diffuse sources of pollution through uncontrolled sewage spillages (Bach *et al.,* 2020).

The Mooi River catchment was assessed through different studies and over the years, it has shown a declining trend in water quality with major pollution emanating from activities in the catchment such as agriculture, mining, industrialisation and human settlement (Barnard *et al.,* 2013). The Mooi River has two major dams located in the catchment serving as major sources of commercial activities and drinking water (Kleynhans, 2007). The Klekskraal Dam is one of the dams located along the Mooi River with agricultural activities as a major source of pollution (Venter *et al.,* 2013). Although impoundments pose a threat to downstream water requirements due to water demand in the area, the Boskop Dam exists with reported water quality deteriorations (Bach *et al., 2020)*.

The major tributary contributing to pollution is the Wonderfontein Spruit, which originates in Krugersdorp and Randfontein in the Gauteng province within South Africa (Nealer, 2020). There are existing abandoned gold mines, which have had concerns of acid mine drainage and contribute to chemical water pollution and residue deposits within the catchment (McCarthy, 2011). Increasing water pollution is set out to have major impacts on water security in the future, as both Boskop and Klerkskraal Dam supply water to Potchefstroom.

The major activities contributing to the economy and linked to water pollution are the South African Brewery Depot, Abattoir, Nestle and fertilizer manufacturers. A phosphor heap is located outside Potchefstroom, contributing to the pollution of the river (Pelser, 2015b). The Wasgoed Spruit is a canalised tributary to the river which contains industrial effluents from the above-mentioned industries, along with urban and storm water runoff flowing into the river without being treated (Wendel, 2010). Trompie Kitsgrass is situated along the banks of the river and produces different types of grass (Pelser, 2015b). The wastewater treatment works are situated on the Southern town edge discharging final effluent into the Mooi River. During the rainy season, the plant/wastewater treatment works may experience high flows, which might cause semi-treated or raw water to be discharged into the river (Wendel, 2010).

The Loop Spruit is another tributary of the Mooi River and is located downstream of Potchefstroom (McCarthy, 2011). The activities in the Loop Spruit are mainly agricultural in nature, namely livestock watering and irrigation of crops. There are two goldmines and the Kulusi informal settlement, which is situated between the two goldmines and the Klipdrift Dam. The Loop Spruit receives mine dewatering upstream of the Klipdrift Dam which contributes to the high nutrient levels in the dam, ultimately affecting the Mooi River (Bach *et al.,* 2020). Figure 2.1 below details all the anthropogenic activities and sources of potential stressors at the Mooi River Catchment.

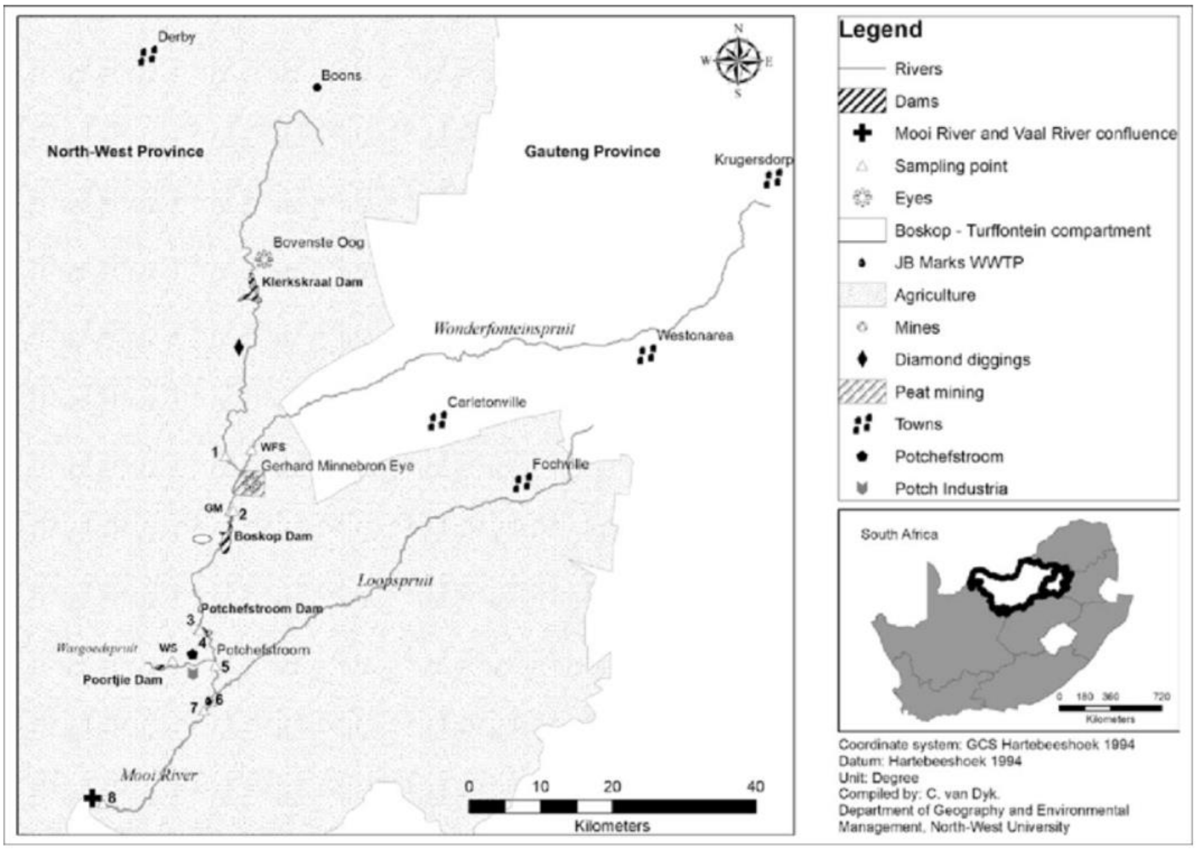

Figure 2.1: Map of Mooi River catchment and sources of potential stressors

2.4.1 Hydrology

Hydrology is the study of science that deals with the presence, availability and conservation of water resources on the globe and other planets (Sivapalan, 2017). It deals with the movement of water throughout the hydrological cycle (Balasubramanian & Nagaraju, 2017). It is important to describe the hydrological features as they play a crucial part to sustain the ecological status of the catchment (Pelser, 2015b). The Mooi River is a perennial river, which is subdivided into three quaternary catchments: Loop Spruit located on the eastern part, Wonderfontein Spruit located on the north-eastern part and the Mooi River on the northern reach (Bezuidenhout, 2013). The origin of the Mooi River is in close proximity to a town known as Derby and from there it flows alongside the southern part towards the Klerkskraal Dam, Boskop Dam and Potchefstroom Dam to join the Vaal River (DWS, 2018).

The mean annual runoff at the Boskop Dam and the confluence with the Vaal River have been documented as 72 Mm^3/a and 122 Mm^3/a respectively for the period 1920 to 1994 (Wendel, 2010). The Mooi River is complex due to constant interaction between ground and surface water (Fosso-kankeu *et al.,* 2015). The wastewater treatment works discharges the biggest portion of the return flow and the mine dewatering also returns to the river system. Without the dewatering of the mines, the water supply would be a challenge (Opperman, 2008). There is a slight impact of low rainfall on the ecosystem as pollutants become too concentrated.

2.4.2 Geohydrology

According to Winde (2010), dolomites cover most of the Mooi River catchment acting as eyes and storing groundwater. The dolomitic underground is divided into several compartments separated by impermeable vertical syenite dykes, which were introduced during the Mokolian age (Vander Walt *et al.,* 2002). Many dolomitic compartments have been dewatered to prevent flooding because of the extensive mining activities in the region and the nature of the gold mining (Winde, 2010).

2.4.3 Climate

The climate of the Mooi River is typical of the southern African Highveld with maximum temperatures in January and minimum temperatures in July. The annual temperature ranges between 16°C in the west to 12°C in the east and an average of about 15°C for the catchment as a whole (Chumchean *et al.,* 2016).

South African rainfall is strongly seasonal with the most rain occurring in the summer period (October to April). The annual average rainfall of 507mm is experienced in the area mostly during mid-summer and only 44.2% of the catchment yields a significant runoff due to extensive dolomite outcrops (SAWS, 2020). The annual rainfall of the Upper Vaal WMA decreases uniformly westwards from the eastern escarpment regions across the central plateau area with the peak rainfall months being December and January (SAWS, 2020).

2.4.4 Topography and vegetation

The Mooi River is part of the upper Vaal WMA which slopes gently from about 1 800m in the east to 1 450m in the west in the vicinity of the Vaal Barrage, with some steep areas in the headwaters of the Wilge tributary on the south-eastern border closer to the Orange River (Winde, 2010). The water from the Upper Vaal WMA flows across the Middle Vaal, Lower Vaal and Lower Orange WMAs before reaching the Atlantic Ocean near the town of Alexander Bay in the western corner of the country (Hobbs *et al*., 2013).

The upper Vaal WMA is mainly covered with pure grassveld and the Mooi River mainly supports gazing and crop farming. The upper reaches of the Wonderfontein Spruit are covered with exotic tree species and grassland with minor shrub manifestations (Winde, 2010).

2.4.5 Economics

The economy of the North West Province is dominated by the mining industry which contributed around 32.5% in 2018, followed by 5.1% in manufacturing, 2.8% in agriculture and 2.5% in construction (Vander Walt *et al.,* 2002). The upper WMA is economically one of the most important in the country and nearly 20% of the GDP of South Africa originates from the Upper Vaal WMA (Clapham, 2012). Mining is the main contributor to the GDP in Potchefstroom contributing R7814.7 million. It has created more than 32 946 employment opportunities for households (Kritzinger, 2017).

2.5 Measurements and indicators for ecological status

Ecological measurements are methods used to determine the present ecological state of the river that are done by monitoring the biotic and abiotic components in the ecosystem (Haase *et al.*, 2018). Biotic is the living components of an ecosystem and they are sorted into three groups: autotrophs, heterotrophs and detritivores while abiotic are non-living chemical and physical parts of an ecosystem (Snow, 2020). In this section, the specific components' importance is discussed together with the methods used to define them.

2.5.1 Water quality

Water quality monitoring is one of the most used methods in determining the integrity of an ecosystem. Water quantity – flow quantities and variability - is regarded as the most important driver of aquatic ecosystem health. Continuous monitoring is necessary to obtain data that is of value to determine aquatic ecosystem health (Chen *et al.,* 2013).

An ecosystem rests on water quality and habitat integrity. Poor water quality will result in loss of sensitive species in the ecosystem (Bond *et al.,* 2008). The Department of Water and Sanitation (DWS) published its 1st edition of South African Water Quality Guidelines in 1996 which included guidelines specific to the ecological/aquatic health (Department of Water Affairs and Forestry, 1996). Water quality monitoring is executed by measuring the most important parameters: temperature, dissolved oxygen, hardness, alkalinity, pH, turbidity, nutrients, metals and organic compounds (Dey *et al.,* 2021).

Both seasonal and daily temperature changes can be experienced by rivers and all organisms found in the river survive best at a certain temperature (Dallas, 2008). Daily temperature changes can be caused by thermal pollution caused by either heated industrial discharge or returning irrigation water. Upstream water resources can have significant effects on thermal conditions downstream and changes in riparian vegetation (Dallas, 2008).

River temperature increase results in decreased oxygen solubility that can also cause an increase in the toxicity of certain chemicals (Li *et al.,* 2013). Temperature changes in the river can further cause an increase in organism stress and can also affect life cycle and metabolic patterns by changing reproductive periods, development rate and emergence times of aquatic organisms (Dallas & Ross-Gillespie, 2015). Most of the organisms depend on dissolved oxygen in water and water temperature which can indirectly control local biodiversity and ecosystem health (Weiskopf *et al.,* 2020).

Atmospheric re-aeration, atmospheric pressure increase and photosynthesis are some of the factors that can cause an increase in dissolved oxygen while on the other hand, temperature increase, aquatic organisms' respiration and chemical breakdown of pollutants are some of the factors that can decrease dissolved oxygen levels (Dick *et al.,* 2016). Oxygen requirements of fish and aquatic organisms in the water may vary with the type of species, life stages, size and different life processes (Wu & Yu, 2021).

Plant nutrients such as nitrogen and phosphorus are essential elements responsible for normal plant growth and reproduction (Razaq *et al.,* 2017). Nitrogen and phosphorus are most commonly implicated in excessive plant growth resulting from nutrient enrichment of aquatic systems (Guignard *et al.,* 2017). Nutrient enrichment may result in an imbalance in biological communities, particularly an increase in plant communities and associated water quality problems (Adams *et al.,* 2020). The discharge of insufficiently treated wastewater effluent results in a long-term impact for most of the rivers and contributes to the degradation of in-stream habitat integrity (Lin *et al.,* 2017).

Metals can naturally be found in the aquatic ecosystem as a result of human activities and their concentrations vary depending on the types of metals detected (Briffa *et al.,* 2020). Their effects on the aquatic ecosystem range from troublesome (Ca, Zn) to dangerously toxic (Pb, Hg) while their level of toxicity depends on the concentrations (Algül & Beyhan, 2020). The discharge of mine and agricultural water into the Mooi River contributes to enhanced toxic metals affecting the fish community negatively (Dube, 2020).

2.5.2 Sediment

Sediment is a natural earth material which consists of soil particles of all sizes. Based on its topography, geology and precipitation rate, a river can consist of a variety of sediment (Lintern *et al.,* 2018). Although sediment is a response indicator at a different level, it has a great impact on response indicators such as fish and is thus considered a driver of ecosystem health (Berhanu *et al.,* 2016).

2.5.3 Habitat

Habitat integrity is the ability of a location to support native species with the resources acceptable to complete their life cycle (Chambers *et al.,* 2019). Amis *et al*. (2007) speak of the habitat integrity of a river as the maintenance of a balanced composition of physicochemical and habitat characteristics on a temporal and spatial scale that is comparable to the characteristics of the natural habitats of the region.

The aquatic biota present in the river is determined by the availability and diversity of habitats. Within the RHP, the index of habitat integrity is developed to gauge the impact of human disturbance in stream habitats and the riparian (Dallas *et al.,* 2010).

According to Kleynhans *et al*. (2009), the habitat integrity considers the overall state of the river ecosystem, considering both the riparian and the in-stream as well as the stresses that are being placed on the river, which also helps to gain an understanding of issues and stresses at the site. There are two levels of assessment that can be used for the IHI, namely an aerial assessment of a river reach or Entire River and a site or ground-based assessment (Kleynhans *et al*., 2009).

2.5.2 Riparian vegetation

Riparian vegetation is referred to as a key indicator of the condition of the channel, which aids to link the in-stream aquatic ecosystem to the adjacent terrestrial ecosystem (Day & Malan, 2012). It plays an important role in the functioning of the riparian zone. The Riparian Vegetation Index (RVI) is designed as part of the bio monitoring techniques utilised in the River Health Programme to assess ecological status (Kleynhans & Louw, 2007). According Kleyhans & Louw (2007), the RVI was designed for the following purposes:

- To assist with providing an indicator of riparian vegetation health and ecological status.
- To assist with decision making by identifying sites of different riparian vegetation status.
- To provide clear indications of the type and extent of disturbances present.

2.5.3 Macroinvertebrates

Macroinvertebrates are any animal lacking a backbone and large enough to see without the aid of a microscope (Bate & Sam-Uket, 2019)[i]. They are exothermic and may be aquatic or terrestrial; the aquatic organisms often being larval or nymph forms of otherwise terrestrial species (Tennesse Department of Environment & Conservation, 2021). Aquatic macroinvertebrates live in many different types of aquatic habitats such as stony beds, in-stream vegetation, mud and sand and they form a major component of the biota of the aquatic ecosystem (Khudhair *et al.,* 2019).

Venter (2013) stated that the ecological status of the Mooi River should be monitored continuously due to the presence of mining activities, agricultural practices and informal settlements linked to increasing water quality trends (Venter *et al.,* 2013). Pelser (2015) used bioindicators such as diatoms and macroinvertebrates to evaluate the ecosystem health of the Mooi River. Pelser (2015) also confirmed a declining trend in the ecosystem health upstream and downstream of the Mooi River. The same scientific approach was used; however, this study was aimed at using fish and habitat as an indication of the ecological health of the Mooi River.

2.6 Aquatic organisms as indicators of ecological status

Several methods are used for assessment of river health across the world, of which the most common is the use of organisms such as invertebrates, periphyton and fish species (Dallas *et al.,* 2010). According to literature, most studies conducted at the Mooi River focused on invertebrates and periphyton organisms for the assessment of ecological health in the catchment (Venter *et al.,* 2013, Pelser, 2015b). Several factors need to be taken into consideration when selecting organisms for river health monitoring.

As stated by Dietrich *et al.* (2020), these factors are:

- They should be able to respond rapidly to physical and chemical conditions if exposed.
- They should be able to show bioaccumulation of contaminants and the effects of pollution.
- Study organisms must show responses to habitat loss and impact therein.
- They should be easy to sample and the mortality rate should correspond with contaminant exposure.
- The population of the organism should be high and represent the ecological state of the river.

2.6.1 Invertebrates

Invertebrates are small organisms that lack a vertebral column but they are large enough to be seen without the use of microscopic evaluation (Bird, 2010). The most used invertebrates are macroinvertebrates which are mostly submerged under rocks and vegetation in running water (Dallas & Rivers-Moore, 2014). The most common types of macroinvertebrates include insects, crayfish, snails and worms (Hussain, 2012). Macroinvertebrates have been used to present conditions of river health as an attribute to water quality conditions such as organic or inorganic pollution, metals and nutrients contamination (Gresens *et al.,* 2010). Habitat loss and sedimentation also affect the biodiversity of macroinvertebrates (Bird, 2010). Azis and Abas (2021) and Lowe and Murphy (2010) listed the most used types of macroinvertebrates in river health assessments as follows:

Table 2. 1: Macroinvertebrates common in freshwater

Phylum	Type
Ephemeroptera	Mayfly
Plecoptera	Stonefly
Megaloptera	Dobsonfly
Coleoptera	Aquatic Beetles
Diptera	True flies
Odonata	Dragonfly and Damselfly
Pelecypoda	Clams
Gastropoda	Snails
Hemiptera	True bugs

Source: Adopted from Lowe and Murphy (2010)

Macroinvertebrates are interpreted through biotic indices such as multimetric and multivariate techniques (Jun *et al.,* 2012). The multimetric technique includes the holistic integrated approach of physical and functional communities of macroinvertebrates described as metrics to the composite index (Lowe & Murphy, 2010). Human interference and anthropogenic factors are the most notorious factors influencing metrics of change (Lagabrielle *et al.,* 2018). Another index based on the estimation and prediction of taxa is the multivariate technique (Musonge *et al.*, 2020). The multivariate approach is used in line with considerations of environmental factors in sites where samples are to be taken (Musonge *et al.,* 2020). As opposed to the multimetric approach, this approach considers species' class, biotic index, taxa levels and ASPT predicted values (Desrosiers & Pinel-alloul, 2020). It is widely used in the United Kingdom in association with the River Invertebrate Prediction and Classification System (RIVPACS) model (Kral *et al.,* 2017).

Pelser (2015a) used macroinvertebrates in the Mooi River such as *Aeshnidae, Leptophlebiidae, Baetidae, Gomphidae, Naucoridae, Chlorocyphidae and Porifera*. These study organisms had shown adverse effects with high sensitivity scores posed by elevated nutrients and physical contaminants such as pH. From a South African perspective, it is common to use the South African Scoring System 5 (SASS5) and the Average Score per Taxa (ASPT) as macroinvertebrates indices (Dickens & graham, 2002). Sensitivity figures and values represent SASS5 scores and the ASPT is dependent on the results of the assessment of SASS5 (King *et al.*, 2008). Other indices used for invertebrates' assessments are richness, signal index, PET Taxa richness, percentage of sensitivity taxa index and percentage of tolerant taxa index. These are well described by Suhaila and Che Salmah (2017).

2.6.2 Periphyton

Ecological assessments are not limited to invertebrates. Periphyton has been used for decades in the field of River health assessments (Culp *et al.,* 2020). Most organisms under the periphyton classification include soft algae, diatoms, fungi, protozoa and other heterotrophic bacteria (Dallas *et al.,* 2010). Unlike macroinvertebrates, periphyton is persistent and abundant on the surface of rocks, rivers, streams and submerged wood, debris and organic matter. A common use of periphyton was considered due to their ability to represent water pollution and availability of contaminants (Abdel-Satar *et al.,* 2017). They are normally associated with wastewater and nutrient contamination in rivers, streams and stagnant water (Bhateria & Jain, 2016).

The most common used periphyton in the Mooi River is diatoms. Studies such as Pelser (2015) and Venter (2013) have confirmed the use of diatoms as an indication of environmental health problems and eutrophication in the Mooi River catchment. There are various metrics and indices associated with diatoms. This has led to several authors and researchers using diatoms as biological indicators (Tyree *et al.,* 2020). In a selection of periphyton, more specifically diatoms, factors such as the ability to attach to the substrate, high taxa number, ease to sample and susceptibility to changes in the environment are taken into account (Richards *et al.,* 2020). Due to advantages of diatoms, most indices such as the percentage of live diatoms, percentage of sensitive diatoms, percentage of aberrant diatoms, Shannon diversity index, percentage of motile diatoms, percentage of community similarity diatoms, pollution tolerance index for diatoms and simple diagnostic metrics have been offered over other indices (Falasco *et al.,* 2021). This does not rule out the use of other periphyton indices such as simple weighted average and aetiological indices as they present scientific significance in ecological assessments (Chakandinakira *et al.,* 2019).

According to Hao *et al.* (2020), more than 80 species of periphyton can be studied belonging to different classifications and genera. These include *Bacillariophyta, Chlorophyta, Cyanophyta, Pyrrophyta, Euglenphyta* and other periphyton in genera and classes not listed (Singh *et al.,* 2017). Studying periphyton can indicate if the ecology and aquatic ecosystem are under environmental stressors such as nutrients which promote their growth (Gubelit & Grossart, 2020).

2.6.3 Fish species

The most widely used method in the study area are seen to be macroinvertebrates and diatoms in the Mooi River catchment. This is confirmed by literature. The use of macroinvertebrates and diatoms in ecological assessments alone in the Mooi River has created different challenges such as the fair description of river health status and lack of information on fish species and habitat structures. The assessment of ecological health using water quality variables such as physicochemical water quality parameters only does not represent a true reflection of habitat and biological indicators (Gubelit & Grossart, 2020). This has led to a shift in the research paradigm and more interest in the utilisation of fish and habitat in the river health assessments.

Fish species are important in ecological assessments as they present a clear picture across the trophic levels (Xu *et al.,* 2021). Fish species are susceptible to problems posed by water pollution such as mining activities, agriculture and contaminants from both industrial and domestic wastewater (Levin *et al.,* 2019). The ability of fish species to accumulate contaminants in their cells, mobility and relocation due to harsh conditions as well as the ease with which they can be identified and captured have made them popular for use in river health programmes across the world (Neves, 2012).

Fish are good indicators of the ecological status of the river, as they are easy to collect and responds well to a wide range of environmental changes. As a reference to studies done by Swemmer (2011) and Malherbe *et al.* (2008), there was a great need to cover a large scope of rivers in KZN, as the rivers are interconnected. More than 23 rivers were assessed using the Fish Response Assessment Index (FRAI). This method was not employed in other studies done around KZN. It was further noted that there was a gap in the utilisation of indigenous fish species in South Africa, more specifically the *Labeobarbus natalensis* which is known as the Kwazulu Natal (KZN) yellowfish or scaly (Swemmer, 2011; Evans, 2017; Malherbe & Wepener, 2008; Levin *et al*., 2019).

Fish species are used as indicators to assess river health in South Africa. The table below lists those fish species that are used to assess river health (Swemmer, 2011; Evans 2017; Malherbe & Wepener, 2008; Levine *et al.,* 2019). These fish species are likely to form part of the fish population of the Mooi River.

Table 2. 2: List of fish species used for river health monitoring in South Africa

Scientific Name	Common name
Labeobarbus aeneus	Small yellowfish
Labeo capensis	Orange River Mudfish
Labeo umbratus	Moggel
Clarias gariepinus	sharptooth catfish
Tilapia sparrmanii	Banded Tilapia
Pseudocrenilabrus philander	Southern Mouth brooder

Enteromius trimaculatus	Three spot barb
Micropterus dolomieu	Smallmouth bass
Xiphophorus hellerii	Swordtail
Micropterus salmoides	Largemouth bass
Ctenopharyngodon idella	Grass carp
Austroglanis sclateri	Rock catfish
Chetia flaviventris	Canary Kurper

Source: Adopted from Smith *et al.* (2009)

Yellowfish are indigenous and present in most rivers in South Africa. It was noted that water quality problems in the catchments had resulted in the migration of yellowfish communities into other catchments (Swemmer, 2011; Evans, 2017; Malherbe *et al*., 2008; Levine *et al.,* 2019; Watson *et al.,* 2012).

2.7 Methods and programmes used in SA for fish and habitat assessments to determine the ecological status

2.7.1 River Health Programme

The National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) is a programme that comprises the river health programme in assessing ecological health in water resources (Dallas & Rivers-Moore, 2014). The river health programme is the national programme established by DWS, WRC and DEAT to monitor the condition of the rivers in South Africa and was designed to evaluate, measure and report on the ecological state of the river (Louw & Kleynhans, 2007). It is also used to outline an overview of the ecological health and present ecological state of the rivers in South Africa (Shelton *et al.,* 2019).

Aquatic macroinvertebrates, riparian vegetation and fish community are used as the main indicators of ecological integrity in the river health programme (Dallas, 2007). This programme has been used to assess several valuable State of the River Reports in South Africa, which includes various environmental aspects of rivers and the bioassessment of the resource (Dallas, 2007).

The National River Health Programme assists in assessing the health of rivers by measuring aquatic macroinvertebrates, riparian vegetation and fish community that represent the condition of the larger ecosystem (Braune *et al.,* 2010). This assessment enables reports on the ecological state of river systems to be produced in an objective and scientifically sound manner.

The majority of research undertaken in the Mooi River focussed on invertebrates and microorganisms to assess the river health. According to Levin *et al.* (2019), fish communities and habitat can be used effectively for the evaluation of environmental health due to their ability to cover a wide range of processes involved in ecological integrity such as trophic levels.

2.7.2 Eco classification

Ecological classification is a process used to determine and categorise the present ecological state of various biophysical attributes of a river to gain understanding and insight of the source (Grizzetti *et al.,* 2016). It is an important part of environmental flow requirements and the ecological reserve determination method (Avenant, 2010).

2.7.3 Fish Response Assessment Index

The Fish Response Assessment Index (FRAI) is a habitat-based and cause-and-effect index aimed at interpreting the deviation of a fish assemblage from the reference condition (Kleynhans, 1999, 2007). FRAI is an assessment index based on:

- The preference and environmental intolerances of the reference fish assemblage.
- The response of the constituent species of the assemblage to particular groups of environmental determinants or drivers.

These preferences and intolerance attributes are categorised into metric groups with constituent metrics that relate to the environmental requirements and preferences of individual species (Chambers *et al.,* 2019).

2.7.4 Health Assessment Index

The Heath Assessment Index is a quantitative index that allows statistical comparisons of fish health among data sets (Watson *et al*., 2012). It uses external and internal microscopic examination and a blood constituent analysis to obtain HAI scores per fish (Crafford & Avenant-Oldewage, 2009). Health Assessment Index is used to characterise the health of fish in the ecosystem and is a simple and inexpensive means of rapidly assessing general fish health in field situations (Watson *et al*., 2012).

Health Assessment Index has not used a diagnostic tool but is an alert to investigators to be aware of possible problems with the health of fish under investigation so that more specific tests can be performed (Assefa & Abunna, 2018).

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

This chapter provides a detailed discussion of the research methodology and description of the study area, which include study locations and sampling sites. Research methodology entails the research approach, method of data collection and sampling as well as data analysis and interpretation (Gounder, 2012). This refers to a systematic way of data collection and dissemination in order to answer research questions and achieve the objectives of the research (Sivarajah *et al.,* 2017). It is also important to describe the features of the study area as they play a vital role in the ecological status of rivers (Dutta *et al.,* 2017).

3.2 Location of sampling sites

The Mooi River is located in the JB Marks Local Municipality in the Highveld eco-region within the North West Province in South Africa. A perennial river, it falls under the upper Vaal River WMA, catchment C23 and drains into the Vaal River (Fosso-Kankeu *et al.,* 2015). The geographic coordinates covering the catchment are 26°52′28″S and 26°57′6″E with elevation between 1 305m (4281 ft.) and 1 550m (5090 ft.). Details are depicted and given in Figure 3.1 and Table 3.1 below.

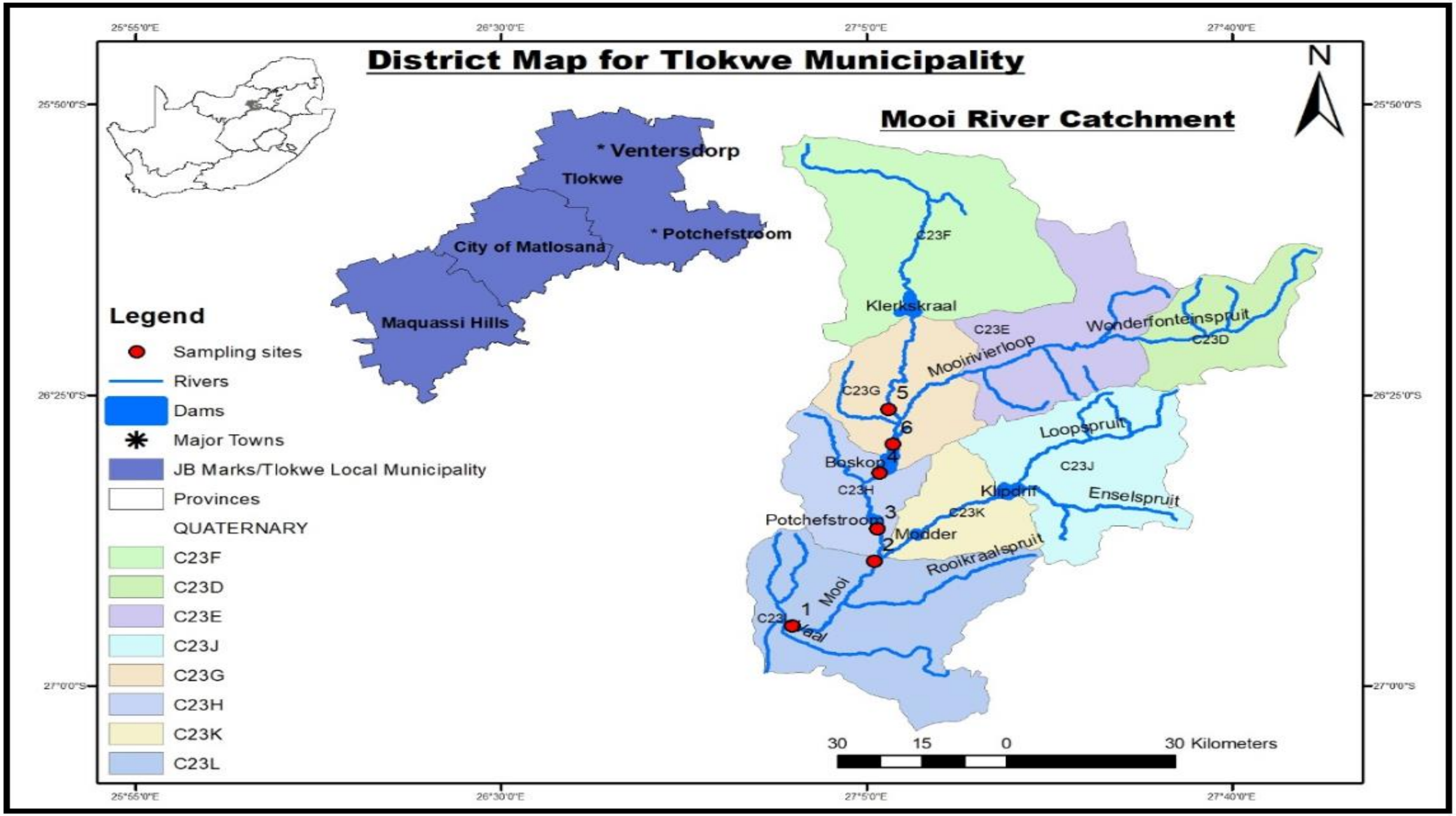

Figure 3. 1: Location of sampling sites 1- 6 for fish and habitat assessments

Six sampling sites were selected within the Mooi River catchment. The sampling sites are between southeast of the Mooi River (upstream) and the southern part of the Mooi River (downstream). Table 3.1 and Figure 3.1 show site names and coordinates of the sampling sites.

All six sites fall within the Mooi River catchment that is a tributary of the Vaal River within the Upper Vaal River catchment. The sites fall within the low land area and the catchment is classified as a low land river within the Highveld Eco region. Six sites were considered low lands river because they fall within a gradient of 0.001-0.005; thus, this is regarded as a lower gradient mixed with bed alluvial channel which is confined as stated by Rowntree *et al.* (2000). Site 1 which is located downstream of Kromdraai bridge within the Mooi River catchment in quaternary catchment C23L is the only sampling point which has a gauging station. A wetland was observed within a 50m radius of quaternary catchment C23G downstream of Boskop Dam in Site 4 as depicted in Table 3.1. Rainfall is seasonal across all sites, i.e. Site 1-6 with an average of 300-700mm per year across North West. This is below the world average of 850mm (DWS, 2018). Details of site 2, 3, 5 and 6 features are provided below on site-specific pictures/photographs and in the data and discussion section under habitat integrity assessments.

Table 3. 1: Summary of selected sampling site

Site No	Site name	Quaternary Catchment	Coordinates	Altitude (m)	Longitudinal Zone	Hydrological	Associated systems
1	Kromdraai	C23L	26°52′49″ S 26°57′52″ E	1320	Lowland river	Perennial	
2	Potchefstroom South Bridge	C23K	26°45′01″ S 27°05′44″ E	1340	Lowland river	Perennial	Wastewater treatment works
3	Potchefstroom North Bridge	CL23H	27°05′59″ S 26°41′05″ E	1340	Lowland river	Perennial	
4	Boskop Dam	C23G	26°34′21″ S 27°06′11″ E	1380	Lowland river	Perennial	Wetland 50 m radius
5	Muiskraal	C23E	26°26′42″ S 27°07′04″ E	1300	Lowland river	Perennial	Farm
6	Boskop	C23 E/G	26°30′52″ S 27°07′30″ E	1400	Lowland river	Perennial	

Source: Adopted from Louw and Kleynhans (2007)

3.3 Photographs taken during site assessment at the Mooi River

Site photographs of all sampling sites upstream and downstream were taken during the site assessments. These pictures provide evidence of site-specific features such as rocky and shallow parts of the stream or river where water flows (Figure 3.2: a-h). Site 1 in Figure 3.2 (a) and (b) was assessed in October 2018 and was found to have a Department of Water and Sanitation gauging station for stream flow measurements as well as the Kromdraai bridge which cuts across the river bank. Site 2 in Figure 3.2 (c) and (d) was predominated by vegetation along the river bank and is located downstream of South Bridge. Site 3 in Figure 3.2 (e) does not have an abundance of vegetation and is located downstream of Potchefstroom North Bridge that cuts across the river bank. Site 4 on the downstream of Boskop Dam presented in Figure 3.2 (f) is comprised of dry woody vegetation as well as rocky habitat within and outside riparian zone. Muiskraal, identified as Site 5 presented in Figure 3.2 (g) is dominated by rocky habitat and shallow waters with vegetation on the river banks. Site 6 on the upstream of Boskop Dam is located a few meters alongside the bridge (see Figure 3.2 (h)). The physical and environmental features identified were crucial in determining the channel and bank assessment modifications and catchment. Details of site-specific features are given in Sections 5.3.1 and 5.3.2 describing in-channel and bank assessments modifications and catchment conditions.

Figure 3. 2: (a) Site 1 upstream Kromdraai gauging station, (b) site 1 downstream of Kromdraai bridge, (c) site 2 downstream of Potchefstroom North bridge, (d) site 2 upstream Potchefstroom South bridge, (e) site 3 upstream of Potchefstroom North bridge, (f) site 4 downstream Boskop dam, (g) site 5 Meiskraal, (h) site 6 upstream Boskop dam

3.4 Research approach

For this research, both a qualitative and quantitative research approach were used for data collection, i.e. fish collection and the FRAI model as well as habitat assessment. The use of both these approaches in a single study is referred to as a mixed method approach. The collection of fish species entails counting, observing as well as modelling the FRAI. This is regarded as a quantitative approach. A quantitative research method is described as a deductive technique which is aimed at testing theories using facts and a statistical approach (Valunaite Oleskeviciene & Sliogeriene, 2020). The major components of quantitative research entails using a method such as an experimental approach and surveys to generate data and analyses through a statistical approach (Pandey, 2016). According to Sekaran and Bougie (2016), a quantitative research involves a numerical analysis of data and is deemed as a scientific and investigative way of conducting research and measurements and verification are involved in this type of research.

The observations and assessment of a habitat feature was regarded as the qualitative approach. A qualitative approach is a type of research method that is more concerned with the inductive approach to generate theory (Snelson, 2016). Verbal description and explanations are involved in a qualitative research method and were relied on for the habitat assessment.

The primary purpose of a qualitative research method is to obtain a complete and detailed description of the situation and phenomenon under study to interpret the situation and understand the different contexts of the different ideas (Tariq & Woodman, 2013).

3.5 Sampling

3.5.1 Site habitat observations

Data was collected from six sampling sites at the Mooi River during October 2018. Details of the sampling sites can be found in Table 3.1 and Figure 3.1. Habitat data was collected using the qualitative method in the form of field observation and recording of data on the sheet (see Appendix A). Field observation sites were chosen based on the maximum variation sampling method where personal judgment is used. This is also known as a judgmental sampling technique. This type of technique involves a selection of the number of samples, location and timing based on the researcher's expertise on the subject matter and the situation under assessment (Creswell, 2013. Data was collected using the River Health Programme field data sheet in Appendix A.

3.5.2 Fish sampling

All fish communities were sampled in October 2018 during Austral spring. Sampling was done at the Mooi River across six sampling sites. A list of expected fish at the Mooi River was adapted from the report on the status and distribution of freshwater diversity in Southern Africa (Smith *et al.,* 2009). The sampling method for fish species was done according to prescribed guidelines in the monitoring programme as stated by Nel *et al. (*2007). Fish identification was done following the guide by Skeleton (2012) which describes the characteristics and taxonomy of fish species.

The 12V battery operated electro shocker (Samus electro-shocker) was used to temporarily shock fish into an unconscious state for a limited time to ensure that they can be resuscitated after unconsciousness This was done across the different velocity depth classes present at each of the sites, namely slow deep, slow shallow, fast deep, and fast shallow velocity, as shown in the table in Appendix A. The average times recorded for the six sites ranged from 30–45 minutes during electroshocking. After electric stunning of the fish species, the 5mm size fine mesh fish net was used to collect the unconscious fish species from the river. The captured fish species were observed and identified as listed in Appendix A. All fish were returned to the river using the fine mesh fish net.

Figure 3. 3: Picture of fish expected in the Mooi River taken in 2018/10/10

3.6 Data analysis method

Data analysis is a process that entails reducing a large volume of data collected to organise, provide structure and to make conclusive evidence from them (Lewis, 2015). Data analysis consists of three steps which are organising the data, reducing the data using either summarisation or categorisation and linking the data use patterns and themes (Maxwell & Reybold, 2015). According to Conrad *et al.* (2014), several approaches can be used to analyse the data, namely ethnographic analysis, narrative analysis, phenomenological analysis and constant comparative methods. The following data analysis tool was used to analyse the collected data.

3.6.1 Habitat assessments

The conditions of the catchment across all sites were assessed based on the descriptive classes for assessment of catchment conditions as stated by Kleynhans (2007) in the Water Affairs and Forestry Manual for site characterisation for the River Health Programme (see Table 3.2).

Table 3. 2: Descriptive classes for assessment of catchment conditions and land-use impact

Impact class	Description	Score
None	None in the proximity of site, no discernible impact	0
Limited	Limited to a few localities, minimal impact	1
Moderate	Land-use is present, impact noticeable	2
Extensive	Land-use widespread, impact significant, small areas unaffected	3
Entire	Land-use predominantly, impact significant, larger area affected	4

Source: Adopted from Kleynhans (2007)

3.6.2 Index of Habitat Integrity (IHI)

The IHI habitat index was used to measure the degree to which the Mooi River had been modified from its natural state. It involves a qualitative assessment of the number and severity of disturbances on the Mooi River and the damage they potentially impose upon the system. Each impact's severity can be ranked using a scale from 0, which means no impact and 25, which means critical impact. This assessment focusses on the impacts of the riparian zone and the in-stream habitat (Graham & Louw, 2008). To obtain the actual in-stream Habitat integrity classes, the following formula was utilised as per parameters in Appendix B:

$$IHI\ impact\ score = \frac{Actual\ Rating\ score}{maximum\ rating\ criteria} * Weight\ (Wgt)\% \quad \textbf{..............................1}$$

Where IHI impact score is the in-stream or riparian impact score, actual rating score is the value obtained from rating criteria in Table A presented in appendix B; maximum rating criterion is the highest value in rating criteria (25) in Table A presented in Appendix B and Weight is each value assigned to each in-stream or riparian weighting criterion in Table B presented in appendix B. The Index of Habitat Integrity (IHI) was determined following the guidelines in Table 3.3, as stated by Kleynhans (2007).

Table 3. 3: Intermediate Habitat Integrity categories (IHI)

Category	Description	Score (% of total)
A	Unmodified, natural	90-100
B	Largely natural with few modifications. A small change in Natural habitats and biota may have taken place but the ecosystem functions are essentially unchanged.	80-90
C	Moderately modified. A loss and change of natural habitat and biota have occurred but the basic ecosystem functions are still predominantly unchanged.	60-79
D	Largely modified. A large loss of natural habitat, biota and basic ecosystem functions has occurred.	40-59
E	The loss of natural habitat, biota and basic ecosystem functions is extensive.	20-39
F	Modifications have reached a critical level and the lotic system has been modified completely with almost complete loss of natural habitat and biota. In worst instances, basic ecosystem functions have been destroyed and changes are irreversible.	0-19

Source: Adopted from Kleynhans (2007)

3.6.3 Frequency of Occurrence (FROC)

The Frequency of Occurrence (FROC) ratings, as stated by Kleynhans (2007), were used to score the species availability (see the FROC rating standard table in Appendix D). To obtain the FROC, the number of each site species sampled was divided by the sampling site points and results were multiplied by the maximum FROC (5) as depicted in Appendix D (Table D).

3.6.4 Fish Response Assessment Index (FRAI)

The Fish Response Assessment Index (FRAI) was used to assess the integrity of fish communities found in the Mooi River. This was done by using the MS Excel FRAI model which generates and integrates data collected as per Appendix A datasheet and Table 3.4.

The steps and procedures in Table 3.4 were followed in order to compute FRAI for the current study. A list of reference species is presented in Table 3.5 which was adopted from a report on the status and distribution of freshwater biodiversity in Southern Africa (Smith *et al.,* 2009). The reference species were used to determine reference fish assemblage in line with species FROC in the FRAI model. The present state of rivers was determined using the Index of Habitat Integrity following the procedure by Dallas (2005). The field data sheet in Appendix A was used for determination of fish habitat conditions at site as stated by Dallas (2005) following the procedure and steps by Kleynhans (2007). The velocity depth classes were determined using the RHP monitoring data sheet in appendix A at slow shallow, fast deep and fast shallow, considering covers such as vegetation, roots, wads, substrate and water columns. Each sample was taken from these different locations and transformed into fish FROC. Thereafter, the FRAI model was executed in order to determine fish category and present ecological status.

Table 3. 4: Steps and procedures be used in calculating the Fish Response Index model (FRAI)

Step	Procedure
River section earmarked for assessment	● As for study requirements and design
Determine reference fish assemblage: species and FROC	● Use historical data & expert knowledge ● Model: use Eco region and other environmental information ● Use expert fish reference FROC database if available
Determine the present state for drivers	● Hydrology ● Physico-chemical ● Geomorphology ● Index of habitat integrity
Select representative sampling sites	● Field survey in combination with other survey activities
Determine fish habitat condition at site	● Assess fish habitat potential ● Assess fish habitat condition
A representative fish sampling at the site or in river section	● Sample all velocity depth classes per site if feasible ● Sample at least three-stream sections per site
Collate and analyse fish sampling data per site	● Transform fish sampling data to the FROC ratings
Execute FRAI model	● Rate the FRAI metrics in each metric group ● Enter species reference FROC data ● Enter species observed FROC data ● Determine weights for the metric groups ● Obtain FRAI value and category ● Present both modelled FRAI & adjusted FRAI.

Source: Adopted from Kleynhans (2007)

Table 3. 5: Expected fish species to determine Fish Response Index (FRAI)

No.	***Expected species (Scientific name)***	**English common name**	**ABBREVIATION**
1	*Austroglanis sclateri*	Rock catfish	*Ascl*
2	*Labeobarbus aeneus*	Smallmouth yellow fish	*L. Aeneus*
3	*Enteromius anoplus*	Chubby head barb	*Bano*
4	*Labeobarbus kimberlyeyensis*	Largemouth yellowfish	*Bkim*
5	*Enteromius trimaculatus*	Threepot barb	*L. Trimaculatus*
6	*Enteromius neefi*	Sidespot barb	*Bnee*
7	*Enteromius paludinosus*	Straightfin barb	*E. Paludinosus*
8	*Clarias gariepinus*	Sharptooth catfish	*Cgar*
9	*Labeo capensis*	Orange river labeo	*Lcap*
10	*Labeo umbratus*	Moggel	*Lumb*
11	*Pseudocrenilabrus philander*	Southern mouth broader	*Pphi*

Source: Adopted from Smith *et al.* (2009)

3.6.5 Present Ecological Status (PES)

Present Ecological Status (PES) of the Mooi River was used by assessing in-stream and riparian habitat and fish community integrity. The results of the indices are presented in the form of one of the six PES categories. The categories range from an "A" to an "F" state. The categories and state descriptions are represented in Table 3.6 (Louw & Kleynhans, 2007).

Table 3. 6: Categories and state description of PES

PES Category/Class	Description	Score %
A	Unmodified or approximate natural conditions. High diversity of taxa with numerous sensitive taxa.	90 to 100
B	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modification	80 to 89
C	Moderately Modified. A lower-than-expected species richness and presence of most intolerant species. Some impairment of health may be evident at the lower limit of this class.	60 to 79
D	Largely Modified. A lower-than-expected species richness and absence or much-lowered presence of intolerant and moderately intolerant species. Impairment of health may become more evident at the lower limit of this class	40 to 59
E	Seriously Modified. A strikingly lower than expected species richness and the general absence of intolerant and moderately intolerant species. Impairment of health may become very evident.	20 to 39
F	Critically Modified. An extremely lowered species richness and absence of intolerant species. Only tolerant species May be present with a complete loss of species at the lower limit of the class. Impairment of health generally very evident	0 to 19

Source: Adopted from Louw and Kleynhans (2007)

3.6.6 Statistical analysis

In order to do statistical analysis, the Principal Component Analysis (PCA) was used to determine the temporal or spatial variations between six selected sites using the information on catchment conditions, in-channel and bank modifications, and habitat conditions as well as fish species availability (Canoco Version 5). The ordination technique was considered using a linear PCA which determined the variations of the composition of the six selected sites at the Mooi River catchment. In order to do the analysis, the accurate fit values on the plot were used, coupled with the catchment conditions, in-channel and bank modification as well as the fish data as a function of secondary matrix as opposed to the primary data. The environmental variables were split into dependent and independent variables in order to determine the relationships.

The ordination used for analysis was a linear PCA, more specifically an unconstrained technique (Smilauer & Leps, 2014).

3.7 Ethical clearance

Ethical issues must be considered during the formulation of the evaluation plan during research. These issues relate to qualitative, quantitative and mixed-methods research approaches and all stages of research (Creswell, 2013. Researchers are expected to communicate the purpose of their studies to the participants. This is done to ensure that there is no confusion among participants. Participants need to understand their involvement in the proposed research (Creswell, 1994). The ethical considerations taken into account during this research are outlined below.

Ethical clearance for the study was obtained from the North-West University Department of Environmental Sciences following the college ethics procedures. The ethics approval letter is attached in Appendix C with ethics number: NWU-01654-20-A9. As part of the ethics clearance requirements, consent was obtained from the departmental lecturers to use the required equipment on site. There was no consent required to access the riparian zone as it falls outside owned premises.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Catchment assessment

4.1.1 Catchment conditions

Catchment conditions for site 1-6 are summarised in Table 4.1. Site 1 was characterised by activities such as livestock, agricultural irrigation, sewage, aquaculture and prevalence of alien invasive species. There were also traces of littering in the vicinity of the river. There was extensive growth of alien invasive species dominated by *Salix babylonica* woodland community and some areas with *Phragmites australis* (giant reeds). The threat to the river health was the wastewater treatment works located 20kms upstream of Site 1. There was also a prevalence of sewage treatment works in Site 2 which affected water quality. According to literature, (Conley *et al*., 2019; Wear *et al*., 2021), sewage treatment works contribute to contaminants such as nutrients, metals and emerging pollutants which may ultimately cause water quality problems such as eutrophic conditions.

Site 3 was not characterised by many activities; however, from Table 4.1 it is clear that there was a prevalence of alien invasive species and infestation of vegetation such as *Phragmites australis*, *Rubus* bramble and *Salix babylonica* woodland community (Weeping Willow). Alien invasive plants consumed increased quantities of water as opposed to indigenous plants species; thus also affecting ecological conservation (Chamier *et al.,* 2012). There was also littering taking place in close proximity to the riparian zone. Site 4 is located 300m downstream of Boskop Dam. This is an impoundment with extensive effects on river health given a rating impact score of 3. The prevailing alien invasive species were similar to those in Sites 1-3, as explained above. Site 5 did not have major ongoing activities; however, the presence of agricultural livestock posed a threat to water quality. At Site 6 there was a prevalence of agricultural livestock, agriculture and alien invasive species. According to a study by Bartz and Kowarik (2019), alien invasive species outcompete native plants, which are the habitat and source of food for fish species. Bruton (2021) further highlighted that alien invasive species can also accelerate water quality problems that can create unfavourable conditions to fish species.

Table 4. 1: Summary of catchment conditions (Impact scale: 0=none, 1=limited, 2=moderate, 3 Extensive, 4=entire)

Land-use	Within Riparian zone	Beyond Riparian Zone	Potential impact on River Health	Level of confidence (H=High, M=Medium, L=Low)
Site 1 catchment conditions				
Agricultural-crops	X		3	M
Agricultural-livestock	X		2	M
Agricultural-irrigation	X		2	M
Alien vegetation infestation	X		3	M
Aquaculture	X		2	M
Construction		X	2	M
Roads	X		2	M
Sewage works	X		2	H
Litter/debris	X		2	H
Site 2 Catchment Conditions				
Roads	X		2	M
Sewage works		X	3	H
Litter/debris	X		1	H

Land-use	Within Riparian zone	Beyond Riparian Zone	Potential impact on River Health	Level of confidence (H=High, M=Medium, L=Low)
Site 3 Catchment Conditions				
Alien vegetation infestation	X		2	M
Aquaculture	X		1	M
Roads	X		2	M
Urban development	X			M
Litter/debris	X		3	H
Site 4 Catchment Conditions				
Agricultural-irrigation	X		2	M
Alien vegetation infestation	X		2	M
Aquaculture	X		1	M
Roads	X		2	M
Impoundment (Weir/dam)	X		3	H
Recreational	X		1	H
Site 5 Catchment Conditions				
Agricultural-livestock	X		2	
Roads	X		2	M

Land-use	Within Riparian zone	Beyond Riparian Zone	Potential impact on River Health	Level of confidence (H=High, M=Medium, L=Low)
Site 5 Catchment Conditions				
Afforestation-general	X		2	M
Agricultural-livestock	X		2	M
Alien vegetation infestation	X		2	M
Aquaculture	X		1	M
Roads	X		2	M

4.1.2 Assessment of in-channel and bank modification

Table 4.2 illustrates the modifications that affected the in-channel and bank conditions using the RHP data sheet in Appendix A and following the impact rating score presented in Table 4.2. The channel and banks on all six sites were moderately modified according to the rating scores. The prevailing modification was posed by Boskop Dam which is 300m upstream of sampling Site 4 and was given an extensive impact score of 3. Channel modification had adverse effects on the environment because it altered the rivers and associated wetlands destroying the floodplain vegetation and hardwood (Lewin, 2014). Channel modification also influenced reduced base flow, which affected groundwater levels, ultimately affecting stream flow and recharge and impacts biodiversity and reduction in fish species (Robertson, 2018).

Table 4. 2: In-channel and bank condition for Sites 1-6 (rating on scale of 0-4: 0=no impact, 1=limited impact, 2=moderate impact, 3= extensive impact, 4= impact across entire area)

	Impact score on river health						
In channel and bank modifications	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Distance**
Bridge elevated in channel support	1	2		2		2	0
Bridge elevated in side-channel support			2	2			0
Causeways/low-flow bridges			2		2		0
Canalization-concrete/gabion				2			0
Gabions/reinforce			1	2			0
Fences-in channel						2	0
Gravel, cobble or sand extraction	2		2			2	0
Roads in riparian zone/tar			2		2	2	200 m
Roads in riparian zone/gravel	1	1					200 m
Dams (large)				3			300 m
Dams (small) weir	1						

4.2 In-stream habitat integrity assessments

In-stream habitat integrity forms part of habitat integrity assessments in river health assessments. The in-stream habitat assessments for Sites 1-6 were completed according to Kleynhans (2015). Table 4.3 presents the in-stream habitat integrity for Sites 1-6 against the habitat integrity classes as stated by Dallas (2005). The result calculations are presented in Table 4.4.

The results for the in-stream integrity index for Sites 1-6 are provided in Table 4.3. Sites 1 and 2 which are located in quaternary catchment C23L and CL23H show that they were largely modified according to the in-stream habitat integrity classification. The classification of these sites indicates that there was a loss of natural habitat and biota with a prevailing reduction in ecosystem components and functioning. This is an attribute to land-use impact assessed such as agriculture, alien vegetation, constructions, solid waste pollution and issues around impoundments and channel modifications.

Site 2 is located within quaternary catchment C23K, with minimal land-use impact identified. This site was moderately modified, i.e. there was a change in natural habitat and biota; however, the basic components and functioning of the ecosystem were largely in its natural state.

Sites 4, 5 and 6 had limited modifications, i.e. the habitat was still in its natural conditions within quaternary catchment C23G, C23E and C23E/G. This is because it was noted that there had been a minimal modification of natural habitat and biota and the functioning of the ecosystem was partially changed. There was minimal impact from the land-use identified. Channel modification and the in-stream parameters show that they were minimally impacted as well. According to a study by Cooper *et al.* (2013), the rivers respond to alteration by human activities, which are linked to the destruction of vegetation, hydrology and activities that trigger water quality problems.Rytwinski *et al.* (2017) confirmed that land-use changes promote lengthy dry season flow regimes, increasing contaminant concentrations which may lead to extinction of native fish species.

Table 4. 3 Classification of in-stream habitat integrity for Sites 1-6 in the Mooi River

IHI Class	Description	IHI Scores (%)	Site 1 score	Site 2 score	Site 3 score	Site 4 score	Site 5 score	Site 6 score
A	Unmodified	90-100						
B	Limited Modification	80-89		80		81	89	85
C	Moderately modified	60-79						
D	Largely modified	40-59	49		59			
E	Seriously modified	20-39						
F	Critically modified	0-19						

Table 4. 4: Determination of in-stream index of habitat integrity scores for Sites 1-6 in the Mooi River

		IHI Impact Scores						
in stream Criteria	**Weight**	**Site 1 score**	**Site 2 score**	**Site 3 Score**	**Site 4 Score**	**Site 5 Score**	**Site 6 Score**	**Level of confidence**
Water Abstractions	14	9	0	11	6	0	0	Medium
Extent of inundation	10	0	0	6	0	0	0	Medium
Water quality	14	11	11	8	0	3	3	High
Flow modification	7	7	1	6	7	5	5	Medium
Bed modification	13	8	0	3	0	0	0	Medium
Channel modification	13	8	0	0	0	0	0	Medium
Exotic macrophytes	9	4	4	4	4	4	4	Medium
Exotic fauna (E.g. Fish)	8	0	0	0	0	0	0	Medium
Solid waste disposal	6	4	4	4	2	0	4	Medium
	In-stream Habitat Integrity Class	**49**	**80**	**59**	**81**	**89**	**85**	

4.3 Riparian zone integrity assessments

The riparian zone integrity index was calculated based on the scoring and weighting criteria in Appendix B (Table A & B). The results for riparian integrity scores are presented in Table 4.6.

According to the results in Tables 4.5 and 4.6, sites 1 and 3 were largely modified within the riparian zone. This is due to land-uses that were happening in the vicinity to the river as described in the conditions of the catchment. There was a general loss of habitat with prevailing reduced ecosystem function within these sites. The loss of habitat was also attributed to modified channel and deforestation, which had occurred at these sites.

Sites 2, 4, 5 and 6 were moderately modified as highlighted in the results in Table 4.5. Although these sites were moderately modified, due to large number of land-use, the system is most likely to be vulnerable to changes in future. According to studies by Rytwinski *et al*. (2017 and Scott *et al*. (2014), the alteration of riparian vegetation, habitat and soil quality increases soil erosion, acts as pollutants pathways and ultimately impacts the fish communities.

Table 4. 5: Classification of riparian zone habitat integrity scores for Sites 1-6 in the Mooi River

IHI Class	Description	IHI Scores (%)	Site 1 score	Site 2 score	Site 3 score	Site 4 score	Site 5 score	Site 6 score
A	Unmodified	90-100						
B	Limited Modification	80-89						
C	Moderately modified	60-79		64		76	69	64
D	Largely modified	40-59	41		53			
E	Seriously modified	20-39						
F	Critically modified	0-19						

Table 4. 6: Determination of riparian zone integrity index scores for Sites 1-6 in the Mooi River

		IHI Impact scores						
Riparian Zone Criteria	**Weight**	**Site 1 Score**	**Site 2 Score**	**Site 3 Score**	**Site 4 Score**	**Site 5 Score**	**Site 6 Score**	**Level of confidence**
Water Abstraction	13	5	0	8	3	0	0	Medium
Extent of inundation	11	0	0	0	0	0	0	Medium
Water quality (Clarity, odour, macrophytes)	13	8	8	6	3	3	3	High
Flow modification	7	6	1	5	0	3	3	Medium
Channel modification	12	6	0	2	0	7	7	Medium
Decreased indigenous vegetation	13	13	10	8	8	8	13	Medium
Exotic vegetation encroachment	12	8	8	7	5	5	5	Medium
Bank erosion	14	14	9	11	5	5	5	Medium
	Instream Habitat Integrity Class	**41**	**64**	**53**	**76**	**68**	**64**	

4.4 Resource quality objectives (RQOs) for in-stream and riparian zone habitat

Resource quality objectives for in-stream and riparian habitat were established by the Department of Water and Sanitation to manage the habitat in the ecosystem for catchment C23F (Government Gazette No: 39943, 2016). According to Dickens *et al*. (2014), the Mooi River in-stream and riparian limit is >D and >42 respectively as shown in Table 4.7. The in-stream results show a limited to largely modified habitat (B/D), while the riparian zone habitat integrity results range between moderately to largely modified (C/D). According to the results, the Mooi River is still within RQOs limit as stipulated in Table 4.7. Although the habitat integrity status is within the required limits of RQOs, it is necessary to ensure that continuous monitoring is done to conserve the biodiversity that supports the ecosystem.

In 2012, the Mooi River was assessed amongst other catchments and it was found to be critically modified (E) (Dickens *et al.,* 2014). Most issues were around the anthropogenic factors such as bank and channel modifications, decreased vegetation and increased exotic species. Other factors were water abstractions, agriculture and water quality deterioration. This shows that the Mooi River requires continuous monitoring as the habitat status is not stable and there seems to be intermittent changes over short period of time. The results by Dickens *et al.* (2014) are different from the current results which show an improved status from critically modified (E) in 2014 to limited to largely modified (B/D & C/D) in the current study. The differences in the findings are attributed to interventions such as the implementation of RQOs which limit the impact of land-uses and promote ecological conservation. There has also been a lot of ongoing research from DWS and NWU over the years which serves as environmental alert to all stakeholders involved. Although current findings are not similar to Dickens *et al.* (2014), there is similarity to the findings of Louw *et al.* (2018) for the Letaba River catchment, showing limited to largely modified habitat status across seven sites.

Table 4. 7: RQOs for in-stream and riparian zone habitat for C23F

A	**Class**	**River**	**RU**	**REC**	**RQO**	**Limit**
UL	**III**	**Mooi River**	**71 & 73**	**D**	**-**The in-stream habitat must be in a largely modified or better state to support ecosystem. **-**The riparian zone must be largely modified or better state	-in-stream habitat integrity category >D (>42) -Riparian zone habitat integrity category >D (>42)

Source: Government Gazette no: 39943 (2016) and Dickens *et al.* (2014)

4.5 Fish Response Assessment Index (FRAI)

The fish habitat segments based on velocity depth classes, cover, fish habitat and sampled features were used to create the fish FROC references and FRAI was determined in this section.

4.5.1 Fish Habitat Segments: velocity depth classes and cover

Fish habitat segments were recorded onsite using the field data sheet in Appendix A and standard rating table for fish habitat segments as described by Kleynhans (2007) and depicted in Table 4.8.

Site 1 is characterised by 5-25% of overhanging vegetation and 0% of undercut banks and root wads within the slow shallow and fast shallow habitats. The site is characterised by 25-75% of bedrock substrate, providing enough habitat for species availability. There was a significant availability of fish species with five *PPHI*, 1 *L. TRIMACULATUS* and *TSPA*. The velocity depth class was between 0.5m in-depth and 0.3 m/s velocity.

Site 2 is characterised by 90-100% of overhanging vegetation within slow deep and 75-95% of undercut banks and roots. There was minimal habitat substrate for species with only 5% availability. Only one specimen of *PPHI* was caught. The velocity depth class was between 0.5m and 0.3 m/s.

At Site 3 there was only two species sampled within slow shallow and fast shallow with minimal overhanging vegetation and available habitat (5%). The velocity depth was ranging between 0.3 - 0.5 m and 0.3 m/s. Substrate availability was 5% with one *CGAR* and one *PPHI* species sampled. The site did not provide sufficient substrate and habitat for fish.

Site 4 is characterised by 25-75% of overhanging vegetation and undercut banks within the fast deep reach. The substrate was common providing 25-75% of habitat availability. Only one *PPHI* and *L. AENEUS* were caught. This habitat was suitable for species response to very fast deep conditions.

At site 5, the slow shallow habitat is characterised by 0-5% of overhanging vegetation and undercut root wads. There was no suitable substrate for *PPHI* species, thus only one was caught. Within the fast shallow reaches, only one *E. paludinosus* species was caught.

There was insufficient habitat to support fish species at Site 6. The area was free from overhanging vegetation's, undercut banks and root wads in the slow shallow, fast deep and fast shallow velocity depth classes. Site 6 did not have sufficient substrate for fish species such as. The velocity depth ranged between 0.3 m and 0.3 m/s, thus suitable for fast deep and fast shallow response species. Only one *E. paludinosus* and one *L. AENEUS* were caught.

According to a study by Evans (2017), the unavailability of yellowish species in most rivers in KZN is due to the inability of habitat to sustain their life; therefore, they relocate from their native habitat. One of the expected fish species that was not caught in the Mooi River is *Austroglanis sclateri,* which occurs in rocky habitats with flowing water. The results in the current research shows that the sites are not predominantly rocky, therefore indicates the inability of habitat to support the present of *Austroglanis sclateri* (FAO, 2004). The habitat for Sites 1-6 is characterised by overhanging vegetation in some areas that could hinder the species movement. Other expected species which were not caught include *Enteromius anoplus, Labeobarbus kimberlyeyensis, Labeo umbratus* and others from the list. This could be attributed to the fact that certain species hibernate in seasons that are not favourable as well as changes in the habitat (Ngesa *et al.,* 2018). The study was only conducted during spring; therefore, the other three seasons were not covered. A study by Spence *et al.* (1999) used the fish communities and habitat to determine the ecological health of Oklahoma streams in the summer. The study uncovered that, during the drought period, the flow dependent habitat is severely affected which leads to poor habitat availability. Results showed fish community index scores of 10 to 28 with habitat integrity ranging from fair to poor. The deviation of scores from inference scores represents the modification of habitat and water quality challenges during low flows.

Table 4. 8: Standard table for habitat segment rating

Description	Relative Ecological value	Occurrence (%)
None	0	0
Rare	1	0-5
sparse	2	5-25
common	3	25-75
abundant	4	75-90
Very abundant	5	90-100

4.5.2 Fish Frequency of Occurrence (FROC)

Total of 16 fish species across six sampling sites were caught in the Mooi River catchments. The 16 species caught represented six taxa, as listed in Figure 4.1 and Table 4.9. The standard rating table for FROC in Table 4.10 was used to describe the fish occurrence across all sampling sites in the Mooi River. A total of six taxa of indigenous (native) fish species were not detected across six sampling sites.

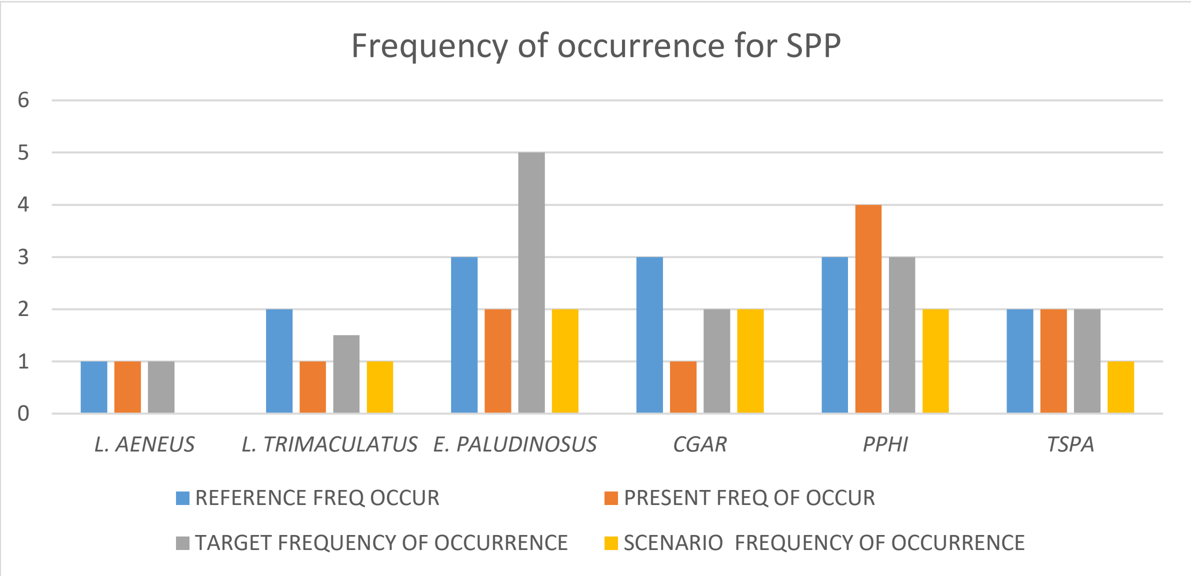

Figure 4. 1: Fish species frequency of occurrence

According to Table 5.9, six species identified were rated according to Table 5.10 in order to describe the presence of the species. The types of species identified within sampling Sites 1-6 in the Mooi River include Labeobarbus aeneus (*L. AENEUS), Enteromius trimaculatus (E. trimaculatus),* Enteromius *paludinosus (E. paludinosus), Clarius Gariepinus (CGAR*), *Pseudocrenilabrus Philander (PPHI) and Tilapia Sparrmaiil (TSPA).*

The FROC for *L. AENEUS* was 10%, showing that the species was present in very few sites (see Table 5.10). According to Gerber *et al*. (2012), the ideal habitat for *L. AENEUS* is strong flowing water in the mainstream sections. The *L. AENEUS* feed on a variety of food across the food chain which include plant material, insects in the water, crabs and other fish of smaller sizes. The absence of a variety of food and the inability of the habitat to sustain the fish species result in their lesser occurrence

(Temesgen *et al.,* 2022). The reference FROC for *L. AENEUS* is 1 as depicted in Table 5.9. This is the list of known fish species to be present at the specific site of Mooi River and therefore expected to be present at very few sites. The target FROC is what is expected to be caught in the sites while scenario FROC is what could be a possible number of fish to be caught in the site. The fish species of *L. AENEUS* presents 0 scenario FROC due to its sensitivity to habitat changes and food requirements. According to the IUCN red list, it is regarded as a species of least concern.

The FROC for *E. trimaculatus* is also 10%, also showing species availability in very few sites across the sampling sites. The reference FROC is 5, meaning the species should be present across all sites with an abundance of greater than 70%, as presented in Table 4.10. The fish species *E. trimaculatus* prefers a vegetative habitat and feeds on small insects (Desai *et al.,* 2019). Most sites assessed lack vegetation on slow shallow, fast deep and fast shallow depth velocity classes; therefore, the abundance of the species could not be supported. According to the IUCN red list, it is regarded as a species of least concern.

The FROC for *E. PALUDINOSUS* was determined to be 10-25%. With reference to the rating Table 5.10, the species is present at few sites. The reference FROC and the target FROC are 3. Although reference was not met, the target fish species occurrence of 2 was met. The species is not considered threatened by IUCN and its habitat is flowing rivers, freshwater marshes and inland delta (Ngesa *et al.,* 2018).

The FROC for *CGAR* was 10%; therefore, according to the standard Table 5.10, the species is present at very few sites. The reference FROC as well as the target are 3. They main habitat is inland waters and they normally feed on fruits, seeds, invertebrates and vertebrates (FAO, 2012). According to ICUN, *CGAR* is the species of least concern.

The FROC for *PPHI* was greater than 75%. According to the standard rating Table 4.10, .the fish species of *PPHI* is present at almost all sites. This means that it is the most abundant species across six sampling sites. The reference FROC as well as the target are 3. This is surpassed by the present FROC of 4, showing that there was abundant species availability. They occupy vegetated zones and are found in flowing waters and feed on small insects, shrimps as well as small fish (Sayer *et al.,* 2018. According to the IUCN red list, *PPHI* is the species of least concern.

Finally, the *TSPA* FROC was 10-25%; therefore, the species was present at a few sites similar to *E. PALUDINOSUS.* The fish reference FROC and target are 2 which is met by the present FROC ranging between 10-25%. The fish species of *TSPA* prefers a quite environment with standing water comprised of submerged vegetation. According to IUCN, it is the species of least concern.

Table 4. 9: Fish species FROC

Reference species	**Scientific names**	**Reference FROC**	**Present FROC**	**Description**	**Target FROC**	**Scenario FROC**
L. AENEUS	*LABEOBARBUS AENEUS (BURCHELL, 1822)-YELLOW FISH*	1	2	10%	1	0
L. TRIMACULATUS	*ENTEROMIUS TRIMACULATUS (PETERS, 1852)*	5	1	10%	5	2
E. PALUDINOSUS	*ENTEROMIUS PALUDINOSUS (PETERS, 1852)*	3	2	10-25%	2	2
CGAR	*CLARIAS GARIEPINUS (BURCHELL, 1822)*	3	1	10%	3	2
PPHI	*PSEUDOCRENILABRUS PHILANDER (WEBER, 1897)*	3	4	>75%	2	2
TSPA	*TILAPIA SPARRMANII (SMITH, 1840)*	2	2	10-25%	2	1

Table 4. 10: Standard table for FROC of fish caught

Frequency of Occurrence Rating	Description
0	Absent
1	Present at very few site (<10%)
2	Present at few sites (>10-25%)

3	Present at about 25-50%
4	Present at 50-75%
5	Present at almost all sites (>75%)

4.5.3 Fish ecological category

To obtain fish category, the present ecological state (PES) classes were compared against the standard table for ecological category. The RQO for in-stream the fish integrity category should be >D (>42) for the Mooi River quaternary catchment (Dickens *et al.,* 2014).

Table 4.11 presents the results of the fish ecological category from six sampling sites and the overall PES over the catchment. Sites 1-6 ecological category was between 40-59 and 60-69, placing it in ecological category C and D. According to the PES standard table category, ecological status falling within C and D category represents a largely and moderately modified PES (Kleynhans, 1999). The ecological fish category across all sampling sites was within the RQOs guidelines for the Mooi River of >D (>42) a. The overall FRAI is 52%, placing the catchment at category D. Therefore, the studied catchment is within the recommended limit of fish ecological state as per RQOs, although largely modified.

Although limited research is ongoing at the Mooi River on the application of FRAI, it is on its inception the current study results can also be compared to studies done outside the Mooi River catchment. According to a study by Levin *et al.* (2019), the evaluation of freshwater fish can be used as bio-indicators and present ecological status. Samples of fish were taken and habitat assessment was done across the Crocodile West River catchment. The modelled FRAI results showed fish category ranging between B, D, E and F; therefore indicating largely natural, largely modified, seriously modified to critical PES.

The average FRAI scores across six sampling sites are similar; however, there is an indication that the ecological status is largely modified. According to a study completed by Roux and Selepe (2013), fish species assemblages in most South African catchments are affected by changes in habitat integrity attributed to anthropogenic activities. It was evident across the sampling site that the in-stream and riparian habitat was largely modified owing to increasing anthropogenic activities such as agriculture, urbanisation, flow modifications through damming and the presence of solid waste as well as exotic species.

Table 4. 11: Computed FRAI and Ecological Category (EC) for site 1-6 in Mooi River

Site	FRAI (%)	EC: FRAI
1	53	D
2	73	C
3	51	D
4	58	C/D
5	58	C/D
6	55	D
Overall fish ecological category	52	D

4.6 Spatial variation of fish species in the Mooi River

4.6.1 Effects of landscape parameters on fish communities

Figure 4.2 and Table 4.12 below represent the statistical analysis of landscape parameters as and influence on the fish community across six sampling sites in the Mooi River. The triplot in Figure 4.2 explains 85% of the variance on the first axis and 8% on the second axis, 5% on the third axis and 2% on fourth axis. The summary of the variation within the triplot is presented in Table 4.12 below.

According to Figure 4.2, the spatial variation was indicated for Sites 3 and 2 which were grouped together. This was due to the abundance of *CGAR* fish species. The increase in litter and debris as well as presence of a sewage works could be linked to the changes in the abundance of PPHI at these sites. The spatial variation also indicated that Sites 4, 5 and 6 were grouped together due to the abundance of *L. AENEUS* and *E. PALUDINOSUS.* Catchment conditions such as recreation, afforestation and weir/dam are landscape variables identified at these sites. Spatial variation indicated that Site 1 was the only sampling site that grouped separately. The species present in the site were *E. TRIMACULATUS* and *TSPA* and the decline in species such as *PPHI, CGAR, L. AENEUS* and *E. PALUDINOSUS* could be attributed to increased alien vegetation, aquaculture, agricultural activities and construction which are landscape variables impacting on catchment conditions.

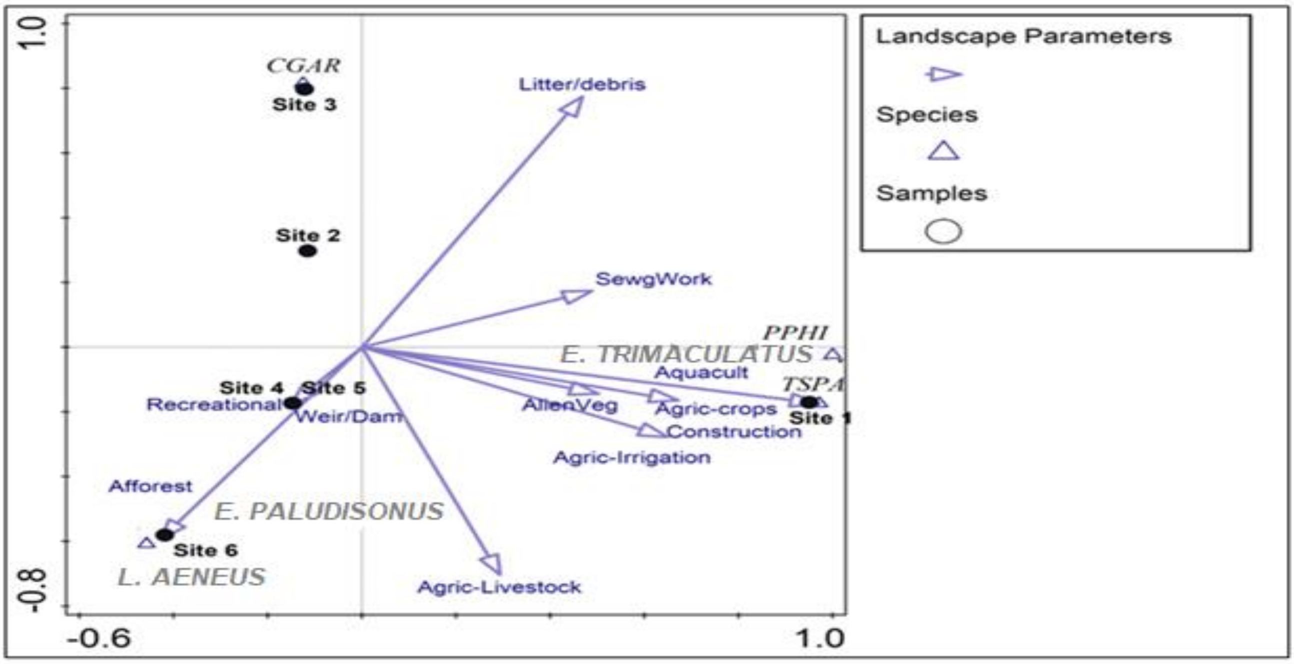

Figure 4. 2: PCA triplot for landscape parameters on fish species at selected sites in the Mooi River during 2018 sampling survey. Full names of species abbreviations are presented in Table 3.5. The triplot explains 85% of the variance on the first axis, 8% on the second axis, 5% on the third axis and 2% on the fourth axis.

Table 4. 12: PCA statistical results for landscape parameters and fish species

Summary table				
Statistics	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Eigen values	0.8529	0.0773	0.0484	0.0200
Explained Variation	**85**	**8**	5	2
Pseudo-canonical correlation (suppl.)	0	0	0	0

4.6.2 Instream impact effects on fish communities

Figure 4.3 and Table 4.13 below represent the statistical analysis of soil properties as cumulative variables for fish species across six sampling sites. The triplot in Figure 4.3

explains 85% of the variance on the first axis and 8% on the second axis, 5% on the third axis and 2% on the fourth axis. The summary of variation is presented in Table 4.13.

According to Figure 4.3, the spatial variation was present at Sites 3 and 2 with an abundance in CGAR; however, the decrease in other species was attributed to in-stream effects identified such as gabions, causeways, and bridge and side channels. Spatial variations were also indicated at Sites 4, 5 and 6 that grouped together. This was potentially due to the abundance of *L. AENEUS* and *E. PALUDINOSUS,* while the in-stream effects responsible for this grouping were canalisation, fence, channel and dams. Spatially, Site 1 was the only sampling point that grouped separately due to the abundance of TSPA and PPHI. There seemed to be a decline in *CGAR, L. AENEUS and E. PALUDINOSUS* which attributed to the multitude of in-stream effects.

According to statistical analysis, certain species are available at certain sites, while absent at other sites. The absence of *CGAR* species at Site 2 may be attributed to a variation in soil properties with more activities such as causeway, dams, bridge, side channel, gabions and roads which may be responsible for migration of fish species to a more suitable habitat at Site 3. Similarly, the absence of *E. PALUDINOSUS and L. AENEUS* at Sites 4 and 5 shows that fish species migrated to Site 6 due to instream variables. Site 1 did not have dominant in-stream variables; therefore, there are limited instream variables.

Construction of dams in rivers has over the years caused various impact on the freshwater environment, moreover the alteration of flow from one stream to another (Schmutz & Moog, 2018). The damming of rivers changes the temperature of water leading to conditions that are unfavourable to special species, leading to loss of native species and community (Macura *et al.,* 2019). Similarly, gabions are constructed to control erosions, however they may redirect flows which affect the downstream rivers, increasing erosion and sediments depositions on adjacent stream (Zhang et al., 2016). The fish species react very sensitively to changes on shape and material of riverbed. Site 2, 4 and 5 shows the present of dams and gabions, there was also notable absent of species such as *CGAR, E. PALUDINOSUS and L. AENEUS*.

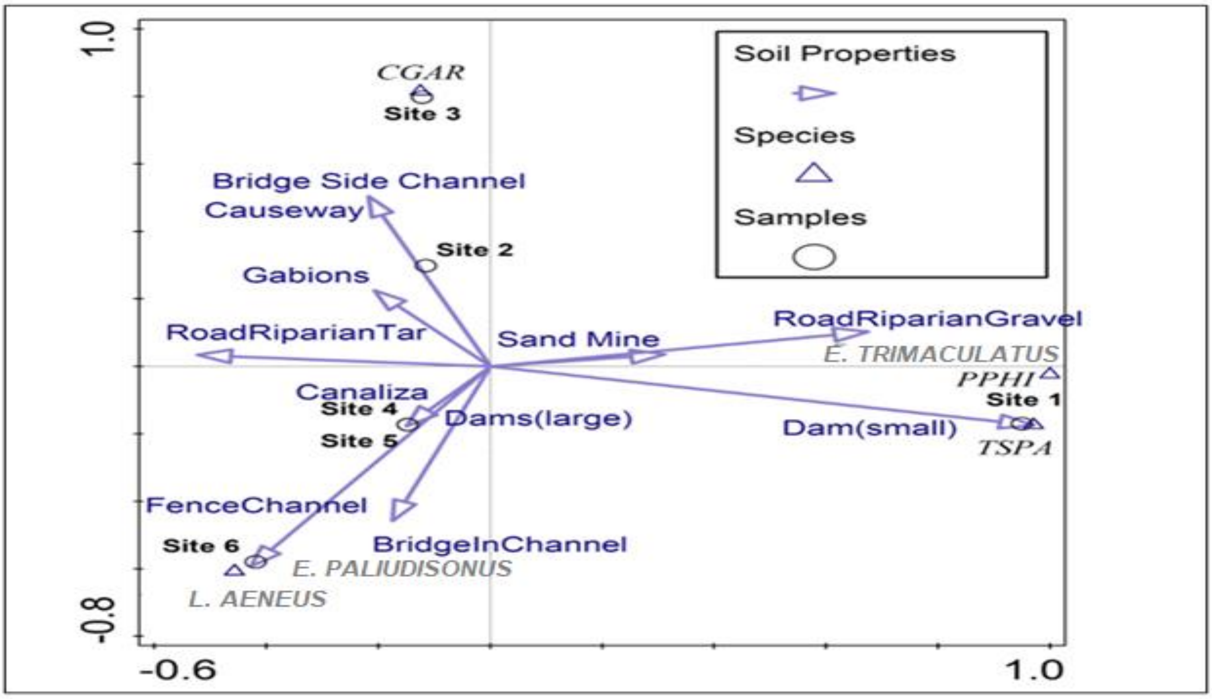

Figure 4. 3: PCA triplot for cumulative instream impacts variables for fish species in the Mooi River during 2018 survey. Full names of species abbreviations are presented in Table 3.5. The triplot explains 85% of the variance on the first axis, 8% on the second axis, 5% on the third axis and 2% on the fourth axis.

Table 4. 13: PCA statistical results for cumulative soil properties and fish species

Summary table				
Statistics	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Eigen values	0.8529	0.0773	0.0484	0.0200
Explained Variation	**85**	**8**	5	2
Pseudo-canonical correlation (suppl.)	0	0	0	0

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study aimed to evaluate the ecological status of the Mooi River in the North West province by assessing fish diversity and habitat integrity. The research method was based on a qualitative and quantitative analysis for catchment conditions, in-channel and bank modification conditions, habitat integrity and FRAI. It can be concluded that there are various anthropogenic factors which impact on the catchment and require to be monitored for the protection and conservation of the aquatic ecosystem. The results indicated that most anthropogenic activities occurred within the riparian zone with a medium to high level of confidence. There were limited in-channel and bank modifications across all six sampling sites and the channels and banks were not impacted, except for available large dam structures (Boskop Dam) which had altered extensively with the reduced flows due to the impoundment. The in-stream riparian zone habitat integrity results showed a limited to medium modified status while the riparian zone showed a medium to largely modified habitat. In conclusion, the number of species caught and habitat modifications resulted in an overall fish ecological status which was largely modified.

The PCA analysis together with the unconstrained ordination were used to determine the temporal and spatial variation of species attributed to cumulative landscape and soil properties variables. The results of PCA showed spatial variation on Sites 1, 2 and 3 as well as Sites 4, 5 and 6 due to landscape and soil properties present. There were three distinct grouping of sites: the first was Site 1 which featured *PPHI and TSPA,* the second group was Sites 2 and 3 which featured *CGAR* and the third group was Sites 4, 5 and 6 which featured L. AENEUS and *E. PALUDINOSUS*. This shows that the in-stream variables and soil properties vary across sites, creating more favourable conditions to describe each site as distinctive and suitable for certain fish species.

5.2 Recommendations

- The study was done on selected sites and quaternary catchments within the Mooi River catchment. A broad study of fish and habitat representing ecological state needs to be done across all quaternary catchments at the Mooi River.
- The PES across all sampling sites is moderately to largely modified; therefore, there is a great need to continuously monitor the catchment and alert the community and stakeholders about possible activities that may trigger a critical modified environment. The RQOs should also be developed across all quaternary catchments to enable a holistic River Health Monitoring Programme to be done.
- There seems to be a gradual increase in land-use activities within the catchment as the river flows downstream. The Department of Human Settlement Water and Sanitation (DHSWS) needs to work closely with responsible authorities and stakeholders to minimise the impact of land-use activities which is gradually altering the environment from its natural conditions.
- The availability of alien invasive species such as reeds, *Salix babylonica* woodland community (Weeping willow) and *Rubus* (Bramble) needs to be given attention. Invasive plants outcompete and displace native plants that many native fish species depend on for food and cover.
- Programmes like adopt a river or working for water should be implemented with the aim of removing alien invasive species, reforestation and river clean-up. These programmes can be initiated in line with the responsible authorities such as the Municipalities, the Department of Water and Sanitation (DWA), the Department of Environmental Affairs (DEA), Catchment Management Forums as well as the communities. These programmes should also include community awareness programmes in order to limit the water pollution and encourage cleaning up of the rivers.

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Appendices

Appendix A: River Health Programme-Field Data Sheet

RIVER HEALTH PROGRAMME: FIELD-DATA SHEETS

Assessor Name(s)			
Organisation		Date	/ /

NB: An explanation of the terminology used in the field-data sheets is given in the associated River Health Programme - Site Characterisation field-manual.

SECTION A: SITE INFORMATION (to be filled in before or during initial visit to site)

1. GENERAL SITE INFORMATION

Site information - assessed at the site							
RHP Site Code			Project Site Number				
River			Tributary of				
Latitude and longitude co-ordinates: Degrees-minutes-seconds or Decimal degrees or Degrees & decimal minutes							
S ° ' . "	S . °	S ° . '	Cape datum Clarke 1880				
E 0 ° ' . "	E 0 . °	E 0 ° . '	WGS-84 datum HBH94				
Site Description							
Map Reference (1: 50 000)		Site Length (m)		Altitude (m)			
Longitudinal Zone	Source zone	Mountain headwater stream	Mountain stream	Transitional	Upper foothill	Lower foothill	Lowland river
	Rejuvenated cascades (gorge)	Rejuvenated foothill	Upland floodplain	Other:			
Hydrological Type: "natural"	Perennial	Seasonal	Ephemeral				
Hydrological Type: "present-day"	Perennial	Seasonal	Ephemeral				
Associated Systems:	Wetland	Estuary	Other:	Distance:			
Additional Comments:							

Desktop / spatial information – data used for classifying a site and subsequent querying of data									
Political Region		Water Management Area							
Ecoregion I		Ecoregion II							
Secondary Catchment		Quaternary Catchment							
Water Chemistry Management Region									
Vegetation Type		Geological Type							
Contour Range (m): From:	to:								
Source Distance (km)		Stream Order							
Rainfall Region	Summer	Winter	Aseasonal	Other:					
DWAF Gauging Station	Yes	No	Code:		Distance Upstream		Or Downstream		

2. LOCATION DETAILS

Sketch a map of the site showing the following details: scale, north, access to site, roads, bridges/crossings, gauges/ instream barriers, buildings, flow direction. Record the following:

Location and Landowner Detail:			Contact No.:		
			Notify Owner?	yes	no
Permit Required?	yes	no	Details:		
Key Needed?	yes	no	Details:		
Farm Name:			Farm Reg. Code:		
Comments:					

SECTION B. CATCHMENT CONDITION AND LAND-USE (to be checked on each visit to site)

Assessor Name(s)			
Organisation			
Date	/ /	Time	

1. PHOTOGRAPHIC RECORD

		Photograph Number	Comments
Photographs	Upstream		
	Downstream		
	Bank to bank		
	Specific features		

2. CONDITION OF LOCAL CATCHMENT - Rate extent (land-use) or impact on a scale of 0 to 4: 0–none; 1–limited; 2–moderate; 3–extensive; 4-entire. Indicate level of confidence: High (H), medium (M) or low (L).

Land-use	**Within riparian zone**	**Beyond riparian zone**	**Potential impact on River Health**	**Level of confidence (H,M,L)**	**Comments (e.g. distance upstream/downstream, time since disturbance, etc.)**
Afforestation - general					
Afforestation - felled area					
Agriculture - crops					
Agriculture - livestock					
Agriculture - irrigation					
Alien vegetation infestation					
Aquaculture					
Construction					
Roads					
Impoundment (weir/dam)					
Industrial Development					
Urban Development					
Rural Development					
Informal settlement					
Recreational					
Sewage Treatment Works					
Nature Conservation				N/A	
Wilderness Area				N/A	
Litter/debris					
Disturbance by wildlife					
Other:					

3. CHANNEL CONDITION (In-channel and bank modifications) - Rate impacts on a scale of 0 to 4: 0–none; 1–limited; 2–moderate; 3–extensive; 4-entire

In-channel and bank modifications	Upstream		Downstream		Comments
	Impact score	Distance	Impact score	Distance	
Bridge – elevated; in channel supports					
Bridge – elevated; side channel supports					
Causeways / low-flow bridges					
Bulldozing					
Canalisation – concrete / gabion					
Canalisation – earth / natural					
Gabions / reinforced bank					
Fences – in channel					
Gravel, cobble and/or sand extraction					
Roads in riparian zone - tar					
Roads in riparian zone - gravel					
Dams (large)					
Dams (small) / weir					
Other:					

4. INDEX OF HABITAT INTEGRITY - Rate impacts on a scale of 0 to 25: 0 - none, 1 to 5 - limited, 6 to 10 - moderate, 11 to 15 - extensive, 16 to 20 - extreme, 21 to 25 - critical (see manual for explanation). Indicate level of confidence: High (H), medium (M) or low (L).

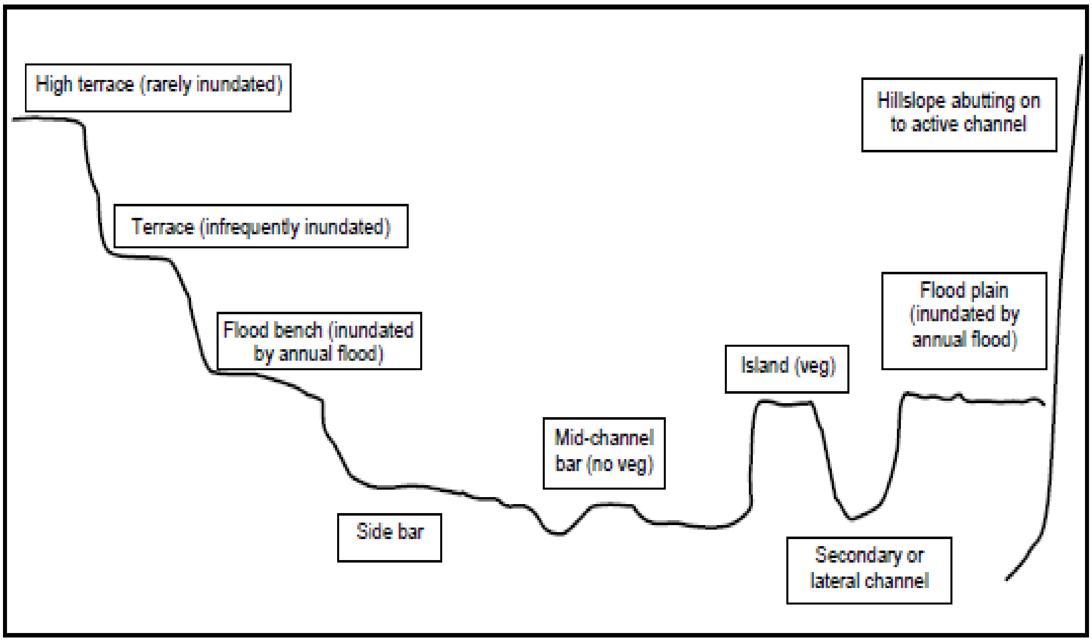
CRITERION	Score	Level of confidence (H,M,L)	Comment
INSTREAM			
Water abstraction (presence of pumps, irrigation etc.)			
Extent of inundation			
Water quality (clarity, odour, presence of macrophytes etc.)			
Flow modifications			
Bed modification (bulldozing of bed)			
Channel modification			
Presence of exotic macrophytes			
Presence of exotic fauna (e.g. fish)			
Presence of solid waste			
RIPARIAN ZONE			
Water abstraction (presence of pumps, irrigation etc.)			
Extent of inundation			
Water quality (clarity, odour, presence of macrophytes etc.)			
Flow modifications			
Channel modification			
Decrease of indigenous vegetation from the riparian zone			
Exotic vegetation encroachment			
Bank erosion			

5. CHANNEL MORPHOLOGY

Channel type: tick channel type indicating dominant type(s)				
Bedrock				
Mixed bedrock and alluvial - dominant type(s)	sand	gravel	cobble	boulder
Alluvial with dominant type(s)	sand	gravel	cobble	boulder

Indicate the cross-sectional features present on the left and/or right banks (see diagram below) – **Note** Left Bank is when looking downstream.

Cross Sectional Feature	Left Bank	Right Bank
High terrace (rarely inundated)		
Terrace (infrequently inundated)		
Flood bench (inundated by annual flood)		
Side bar		
Mid-channel bar (no vegetation)		
Island (vegetation)		
Secondary or lateral channel		
Flood plain (inundated by annual flood)		
Hillslope abutting onto active channel		



SECTION C: FIELD-BASED DATA FOR EACH SITE VISIT

1. GENERAL SITE VISIT INFORMATION

Assessor Name(s)			
Organisation			
Date	/ /	Time	

Water level at time of sampling -tick appropriate category

Dry	Isolated pools	Low flow	Moderate flow	High flow	Flood

Velocity and discharge estimates - optional

Horizontal distance (m)						
Velocity (ms^{-1})						
Depth (m)						
Water surface width (m):		Discharge (m^3s^{-1}):				

Significant rainfall in the last week? - i.e. likely to have raised the water level

Yes	No	Comment:

Canopy Cover -tick appropriate category

Open	Partially Open	Closed	Comment:

Impact on stream habitat - Rate impacts on a scale of 0 to 3: 0 – no impact; 1- limited impact; 2 – extensive impact; 3 – channel blocked

	Score	Source: local / upstream
Coarse woody debris		
Other:		

Water chemistry data – Recording of the *in situ* measurements is also included in the SASS5 data-sheet – please complete here if doing the full RHP assessment. Instruments should be positioned in the clearly-flowing points on the river where possible.

Instruments in fast flow?	Yes	No	If no, where:	
Samples collected?	Yes	No	Date sent for analysis?	
Water filtered?	Yes	No	Volume filtered (mL):	
Samples frozen?	Yes	No	Other preservation?	
Name of institution to which samples were sent:				

Variable	Value	Units
pH		
Conductivity		
Temperature		
Dissolved Oxygen (mgL^{-1})		
Percentage O_2 Saturation		

Water turbidity - tick appropriate category

Clear	Discoloured	Opaque	Silty	Comment:
Turbidity (if measured (NTUs)				
Secchi Depth (m)				

-

2. STREAM DIMENSIONS - estimate widths and heights by ticking the appropriate categories; estimate average depth of dominant deep and shallow water biotopes.

(m)	< 1	1-2	2-5	5-10	10-20	20-50	50-100	>100
Macro-channel width								
Active-channel width								
Water surface width								

Bank height – Active channel			
(m)	**< 1**	**1-3**	**>3**
Left Bank			
Right Bank			

Dominant physical biotope	Average Depth (m)	Specify physical biotope type
Deep-water (>0.5m) physical biotope (e.g. pool)		
Shallow-water (<0.5m) physical biotope (e.g. riffle)		

3. SUBSTRATUM COMPOSITION - Estimate abundance of each material using the scale: 0 – absent; 1 – rare; 2 – sparse; 3 – common; 4 - abundant; 5 - entire

Material	Size class (mm)	Bed	Bank
Bedrock			
Boulder	> 256		
Cobble	100 – 256		
Pebble	16 – 100		
Gravel	2 – 16		
Sand	0.06 – 2		
Silt / mud / clay	< 0.06		

Degree of embeddedness of substratum (%)
0-25
26-50
51-75
76-100

4. INVERTEBRATE BIOTOPES (present at a site compared to those actually sampled)

Summarised river make up: ('pool'=pool only; 'run' only; 'riffle/rapid' only; '2mix'=2 types, '3mix'=3 types)				
pool	run	Riffle/rapid	2 mix	3 mix

Rate abundance of each SASS and specific biotope present at a site using the scale: 0 – absent; 1 – rare; 2 – sparse; 3 – common; 4 - abundant; 5 – entire. Add additional specific biotopes if necessary.

		Specific Biotope					
SASS Biotope	Rating		Rating		Rating		Rating
Stones in current		Riffle		Run		Boulder rapid	
		Chute		Cascade		Bedrock	
Stones out of current		Backwater		Slackwater		Pool	
		Bedrock					
Marginal vegetation in current		Grasses		Reeds		Shrubs	
		Sedges					
Marginal vegetation out of current		Grasses		Reeds		Shrubs	
		Sedges					
Aquatic vegetation		Sedges		Moss		Filamentous algae	
Gravel		Backwater		Slackwater		In channel	
Sand		Backwater		Slackwater		In channel	
Silt/mud/clay		Backwater		Slackwater		In channel	

7. FISH

FISH HABITAT SEGMENT:

5 km sector:	

FISH HABITAT – Velocity-Depth classes and cover present at site

Estimate abundance of each velocity-depth class and cover type using the scale: 0 – absent; 1 – rare; 2 – sparse; 3 – common; 4 - abundant; 5 – very abundant

SLOW DEEP:	Slow shallow:	Fast deep:	**FAST SHALLOW:**
Overhanging vegetation:	Overhanging vegetation:	Overhanging vegetation:	Overhanging vegetation:
Undercut banks & root wads:	Undercut banks & root wads:	Undercut banks & root wads:	Undercut banks & root wads:
Substrate:	Substrate:	Substrate:	Substrate:
Aquatic macrophytes:	Aquatic macrophytes:	Aquatic macrophytes:	Aquatic macrophytes:
Water Column:	Water Column:	Water Column:	Water Column:
Remarks:	Remarks:	Remarks:	Remarks:

VELOCITY-DEPTH CLASSES SAMPLED AND EFFORT – indicate which velocity-depth classes were sampled. Where the mosaic of velocity-depth classes makes it difficult or impossible to sample classes separately (e.g. combinations of fast-deep and fast-shallow classes), the dominant velocity-depth class should be used as the unit of reference for sampling effort, but the presence of other velocity-depth classes should also be indicated.

Sampling effort	**Slow deep (SD)**	**Slow shallow (SS)**	**Fast deep (FD)**	**Fast shallow (FS)**
Dominant velocity-depth class				
Electro shocker (min)				
Small seine (mesh size, length, depth, efforts)				
Large seine (mesh size, length, depth, efforts)				
Cast net (dimensions, efforts)				
Gill nets (mesh size, length, time)				

Remarks:

FISH CAUGHT - indicate whether velocity-depth classes were combined

Habitat:	
Sampling method:	
Species	**Number (J=juvenile, A=abnormality)**

8. RIPARIAN VEGETATION – The relevant method and data-sheets are under development. They will be inserted when validated and ready for general use.

Appendix B: Scoring procedure and weighted criterion for Index of Habitat Integrity

Table A: in stream and Riparian zone index integrity scoring procedure.

Impact Class	Description	Score
None	None in proximity of site, no discernible impact	0
Small	Limited to a few localities, minimal impact	1-5
Moderate	Modifications are present at small numbers and impact on habitat are fairly limited	6-10
Large	Significance impact to habitat with larger areas not affected	11-15
Serious	Modifications are widespread, impact significant, small areas unaffected.	16-20
Critical	The area is predominantly modified with significance impact in all areas	21-25

Table B: in stream and riparian zone weighting criterion (Wgt)

in stream Criteria	Wgt	Riparian zone criteria	Wgt
Water Abstractions	14	Water Abstraction	13
Extent of inundation	10	Extent of inundation	11
Water quality	14	Water quality (Clarity, odour, macrophytes)	13
Flow modification	7	Flow modification	7
Bed modification	13	Channel modification	12
Channel modification	13	Decreased indigenous vegetation	13
Exotic macrophytes	9	Exotic vegetation encroachment	12
Exotic fauna (E.g. Fish)	8	Bank erosion	14
Solid waste disposal	6		
Total	100	Total	100

Appendix C: Ethical Approval Letter

Private Bag X1290, Potchefstroom
South Africa 2520
46712
Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za
46712
Senate Committee for Research Ethics
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on the review by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)**, the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Fish and habitat present ecological state in the Mooi River catchment in the North West Province
Study Leader/Supervisor: Dr CW Malherbe
Student: MFM Tele

Ethics number: N W U - 0 1 6 5 4 - 2 0 - A 9
Institution Study Number Year Status

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single **Risk Category:** No Risk

Commencement date: 01/02/2020
Expiry date: 01/04/2021

General conditions:
The following general terms and conditions apply:

- *The commencement date indicates the date when the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,

Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)

File reference: 9.1.5.4.2 Page 1 of 1

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