THE USE OF FISH AND MACROINVERTEBRATE ASSEMBLAGES AND ICHTHYOPARASITES AS INDICATORS OF THE HEALTH STATUS OF NWANEDI AND LUPHEPHE RIVERS IN LIMPOPO PROVINCE, SOUTH AFRICA

By

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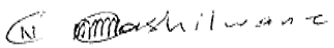
DEDICATION

In loving memory of my mom

DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Zoology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.



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Mashilwane CN (Mr) **Date**

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ABSTRACT

Nwanedi and Luphephe rivers originates from the Soutpansberg mountains and flows downstream through three ecoregions, namely, Soutpansberg 2.01 Ecoregion at an altitude of 1100 m, Limpopo Plain 1.02 Ecoregion in the middle of the catchment, and Limpopo Plain 1.01 Ecoregion where the Nwanedi River meets the Limpopo River. However, due to increasing human interactions and agricultural developments in the lower reaches, there is a need to determine the present ecological state (PES) of this system. The aim of the study was to determine the PES of the Nwanedi and Luphephe rivers based on fish and macroinvertebrate assemblages and the composition of ichthyoparasites from *Pseudocrenilabrus philander* (Weber, 1897), a fish species that commonly occurs in the middle and upper catchment of the system. The findings were then compared to a previous survey conducted by Angliss et al. (2007) to determine if the state of these rivers has changed over time.

This study was conducted at 10 sites traversing the length of the rivers and across the aforementioned ecoregions, within the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), Limpopo Plain 1.02 Ecoregion (sites 6 and 7) and Limpopo Plain 1.01 Ecoregion (sites 8, 9 and 10). Field surveys were undertaken during summer (October 2021) and winter (May 2022). Water quality measurements were taken at each site using a handheld multiparameter instrument and water samples collected to analyse nutrients and metal content at an accredited laboratory. Fish were sampled using electroshocking and a cast net. Specimens caught were identified and recorded and thereafter returned live to the environment. These data were used to categorise the respective ecoregions using the Fish Response Assessment Index (FRAI) protocol. Macroinvertebrates were sampled using the South African Scoring System Version 5 (SASS5) protocol and using the Macroinvertebrate Response Assessment Index (MIRAI) method the PES for each ecoregion was determined. Specimens of *P. philander* were examined for ichthyoparasites and infestation indices calculated to determine if there was a change in parasite load and diversity between the sites caught.

Water constituents measured were compared against the acceptable target water quality range (TWQR) prescribed by DWAF (1996a) for aquatic ecosystems. The concentration of NO_3 was high in the lower reaches of the river where the aquatic vegetation was denser. All physico-chemical parameters, nutrients and metals content

were within the TWQR guidelines across the sites and ecoregions. Fish assemblages' results revealed Soutpansberg 2.01 Ecoregion to have a high abundance and species richness, with numbers decreasing with altitude. A canonical correspondence analysis (CCA) revealed most fish species in the Soutpansberg 2.01 Ecoregion to be strongly correlated with high oxygen concentrations with only one species in the Limpopo Plain 1.01 strongly associated with water conductivity, total dissolved solids and salinity. The FRAI results indicated the PES of the Nwanedi and Luphephe rivers to be in good or close to natural conditions based on an assigned ecological category (EC) of class A/B calculated for the Soutpansberg 2.01 Ecoregion and fair with an ECs of classes C and D determined for the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions, respectively. When compared to the findings by Angliss et al. (2007) with scores of C, C and D designated for the respective ecoregions, the ecological state reported in this study for the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions would indicate that system conditions have deteriorated over time.

With regard to macroinvertebrates a decline in water quality at sites 1, 2, 3 and 7 based on SASS scores and Average Score per Taxon (ASPT) was reported. The ASPT scores reported for sites 4 and 5 indicated natural water quality conditions while those reported for sites 6 and 9 indicated a slight deterioration in water quality. Conversely, the MIRAI results categorised the PES of the Nwanedi and Luphephe rivers as fair with an EC of class D. When compared to the work undertaken by Angliss et al. (2007) the invertebrate communities in these rivers have deteriorated from an EC of class C/D to that of class D. Ichthyoparasites results revealed the Soutpansberg 2.01 Ecoregion had good water quality based on the high prevalence of *Cichlidogyrus philander* Douëllou, 1993, a gill monogenean specific to *P. philander*, and poor water quality for Limpopo Plain 1.02 Ecoregion due to the absence of this parasite observed from *P. philander* collected in this section of the river.

In conclusion, water quality was reported to be good in the Soutpansberg 2.01 Ecoregion with conditions deteriorating slightly in the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions. The water quality results were supported by the FRAI, MIRAI and parasite indices findings. The PES of the pooled data for each ecoregion revealed conditions to have deteriorated when compared to the historic work done by Angliss et al. (2007). For comparative purposes future studies should consider doing more frequent surveys using the methods elucidated above when establishing the PES.

RESEARCH OUTPUTS
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LIST OF ABBREVIATIONS AND ACRONOMY

ASPT	Average Score Per Taxon
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity (for respective chapter)
EC	Ecological Category (for respective chapter)
FRAI	Fish Response Assessment Index
GSM	Gravel, Sand and Mud biotopes
MA	Mean Abundance
MI	Mean Intensity
MIRAI	Macroinvertebrate Response Assessment Index
P	Prevalence
PES	Present Ecological State
pH	Potential of Hydrogen
RHP	River Health Programme
SASS	South African Scoring System
SAWQG	South African Water Quality Guidelines
SIC	Stones in Current
SOC	Stones out of Current
TDS	Total Dissolved Solids
TWQR	Target Water Quality Range
VEG	Vegetation biotope

CHAPTER 1
GENERAL INTRODUCTION

1.1 INTRODUCTION

Freshwater ecosystems, which include lakes, ponds, rivers, streams, springs, and wetlands, are environmental hotspots that support most biological forms on earth (Dallas and Day 2004; Dudgeon et al. 2006; Carpenter et al. 2011). Freshwater ecosystems are important to humans as they provide a source of water and food. However, many freshwater ecosystems are among the most altered and impacted systems on earth due to the high demand and over-extraction of water for anthropogenic purposes, which has resulted in changes in river flow, water quality and environmental habitats to occur (Davies and Day 1998). These alterations have consequently impacted the physical, chemical and biological features of aquatic ecosystems, with sensitive species being negatively affected by these changes (Dallas and Day 2004).

Water quality is affected by the discharge of anthropogenic pollutants (Castello et al. 2013) from point and diffuse sources. Point sources are sources that are localised and fixed whereby pollution such as sewage and industrial effluent are discharged directly into the aquatic environment (Chapman 2021). Conversely, diffuse sources are land-based sources of pollution with no specific or localised point of discharge, such as pesticides and fertilizers from agricultural runoff (Chapman 2021). Consequently, the impacts associated with the abovementioned factors have led to monitoring water usage and water quality as a means to ensure that the supply of this finite resource is sustainable in South Africa (Dallas and Day 2004).

With South Africa being a semi-arid country, the monitoring and management of freshwater ecosystems is critical for the conservation and preservation of aquatic environments (Musingafi and Tom 2014). Water contamination by anthropogenic pollutants negatively impacts the structure and distribution of fauna in aquatic ecosystems resulting in the loss of biodiversity and a degradation of ecosystem services (Roux et al. 2008). In South Africa, monitoring water quality has traditionally been done by measuring the physical and chemical parameters of water (Dallas and Day 2004).

1.2 WATER QUALITY ANALYSIS

Measuring physical and chemical properties in water provides knowledge of the water constituents; however, using this approach only considers and records the water conditions at the time of sampling (Davies and Day 1998). As a result, different approaches of monitoring the ecological state of aquatic conditions are constantly being researched pending the application and question asked. One such technique is biological monitoring whereby aquatic organisms such as fish, macroinvertebrates and ichthyoparasites are used as bioindicators to determine aquatic conditions.

1.3 BIOLOGICAL MONITORING

Biomonitoring is the use of biological organisms to monitor the health of aquatic systems and has been shown to be an effective and alternative means to using traditional monitoring methods (Holt and Miller 2010). In biomonitoring assessment, ecological indicators which involve biological assemblages of species or taxa, based on their presence or condition, can provide information on the integrity of an ecosystem (Holt and Miller 2010). Consequently, the variation between ecological indicators can serve as a representative of pressures, and both natural and anthropogenic impacts acting on ecosystems at various temporal and spatial scales (Zhang et al. 2021). Additionally, ecological indicators are crucial since they help understand the integrity of an ecosystem in order to guide management, direct conservation matters and maintain sustainable livelihood (Ponti et al. 2009).

Moreover, in biomonitoring, the use of indicator species or taxa and/or measuring their responses to external stimuli can be used to examine and indicate how external factors such as pollution from domestic, industrial and agricultural effluent affects water conditions and, in turn, the health of the organism over time (Palmer et al. 2004). Thus, an indicator species or taxa serves as an early warning of community or ecosystem degradation. For example, some indicator organisms such as fish (e.g., trout) and macroinvertebrates (e.g., Ephemeroptera and Plecoptera larvae), require clean water with high dissolved oxygen concentrations. Thus, a decline in the number of the abovementioned organisms is an indication that the water and/or habitat conditions have deteriorated (Fierro et al. 2017).

Since organisms in aquatic systems have varying sensitivities and resistance to different levels of pollutants and as a consequence respond differently to toxins, the use of biological indicators can substantially improve the assessment of freshwater ecosystems (Roux et al. 1993). Furthermore, physical and chemical conditions of water alone do not provide an overall and long-term assessment of an ecosystem's health (Roux et al. 1993). Moreover, the presence or absence of sensitive species, or change in community composition over time, can signal a change in water chemistry that may not be identified by physico-chemical measurements (Palmer et al. 2004). Therefore, fish and macroinvertebrate assemblages, and ichthyoparasites have proven to be effective at monitoring and determining the health status of aquatic systems.

1.3.1 FISH ASSEMBLAGES AS BIOINDICATORS

Fish assemblages refer to the diversity and abundance of fish species occupying the same habitat within marine and freshwater systems and play a major role in maintaining the integrity of aquatic food webs (Wootton 1991). In freshwater systems fish assemblages can be affected by changes in water flow and water quality due to habitat modification, anthropogenic stressors and the introduction of alien fish species (Dudgeon et al. 2006). Since most fish are affected to some degree by changes in environmental conditions, a knowledge of fish assemblages as indicators of biological integrity and water quality can be used to categorise the ecological state of aquatic systems based on the presence and abundance of fish species within a given habitat. In South Africa, Kleynhans (2007) developed the Fish Response Assessment Index (FRAI) for monitoring and assessing the status of aquatic systems. The FRAI incorporates the environmental intolerances and preferences of fish assemblages to determine the response of such species to environmental drivers (Kleynhans 2007).

The FRAI provides a habitat-based cause and effect model that compares the fish assemblages observed against the number of fish species expected in the area under investigation (Kleynhans 2007). Habitat descriptions such as flow velocity, river depth, physico-chemical properties, the introduction of alien species, and vegetative cover are among the metrics evaluated when using the FRAI. Although, the FRAI index adopts the same ranking and weighting mechanisms as other indices for ecological state determinations the FRAI, however, is based on the increase or

decrease of fish communities when compared to the reference species. These are species expected to occur under natural or pristine conditions for a given system (Kleynhans and Louw 2007). Thus, the FRAI categorises and rates the state of a system based on the presence and absence of certain fish species and various environmental metrics by assigning percentage scores that are then translated into different ecological categories (Kleynhans 2007).

1.3.2 MACROINVERTEBRATE ASSEMBLAGES AS A BIOINDICATORS

Macroinvertebrate assemblages are diverse and ecologically significant group of aquatic organisms that consists of invertebrates of macroscopic size that live continuously or during certain stages of their life cycle in the aquatic environment (Benetti et al. 2012). This group of organisms play an important role and has been used intensively to monitor the health status of aquatic systems (Ormerod and Edwards 1987; Miserendino 2001; Masese et al. 2009; Sharma and Chowdhary 2011; Sharifinia et al. 2016; Niba and Sakwe 2018; Raphahlelo et al. 2022) as they are a source of food for most fishes (Wallace and Webster 1996). Hence, a decline in the abundance and composition of the macroinvertebrates can directly affect fish survival and, in turn, can be an indication of poor water quality or habitat loss. Macroinvertebrate assemblages in rivers are assessed by applying the South African Scoring System (SASS) community index developed by Chutter (1994) that was later modified into SASS5 by Dickens and Graham (2002). The SASS5 technique is a rapid bio-assessment index developed for South African river conditions in which invertebrate families are used to assess the health of rivers based on their sensitivity scores in response to water quality conditions and habitat integrity (Gerber and Gabriel 2002).

In addition to using the SASS5 technique, Thirion (2007) refers to the inclusion and use of the Macroinvertebrate Response Assessment Index (MIRAI), described in the EcoClassification and EcoStatus manual created by Kleynhans et al. (2005), as a means to determine the ecological category (EC) and status of invertebrate species within a system of interest. The MIRAI protocol incorporates the results obtained from SASS5 by considering and evaluating habitat conditions in relation to the ecological requirements of invertebrate species within an assemblage sampled (Thirion 2007). Although SASS5 provides an indication of water quality and the present ecological

state (PES) of invertebrate populations, the MIRAI is intended to provide a habitat-based cause-and-effect framework for evaluating the divergence of the aquatic invertebrate community assemblage from the natural or close to natural reference condition (existing reference macroinvertebrate data) (Thirion 2007).

The MIRAI approach is the chosen method to be used as part of the former River Health Program (RHP), currently known as the River Eco-status Monitoring Programme (REMP). The REMP is a component of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) that assesses the ecosystem status of rivers of South Africa based on drivers such as hydrology, geomorphology, and physicochemical processes and the response of biological indicators such as fish, aquatic invertebrates, and riparian vegetation (Mangadze et al. 2019; Riddell et al. 2019). The MIRAI employs ratings based on four distinct metric classes: flow alteration, habitat modification, water quality modification, system connectedness, and seasonality. These metric groupings assess the invertebrate assemblage's divergence from the reference or expected conditions in an assessment site (Thirion 2007). Thus, the MIRAI protocol adopts similar ranking and weighting mechanisms as FRAI for ecological state determination.

1.3.3 ICHTHYOPARASITES AS A BIOINDICATORS

Fish in freshwater systems act as a host to a vast variety of ecto- and endoparasites. The occurrence of parasitic infestations can be influenced by several factors such as a change in water quality (Galli et al. 2001). In recent years, there has been increased interest in using ichthyoparasites as biological indicators of aquatic health (Blanar et al. 2009). According to Sures (2006), an increase of parasite infections can be influenced by a deterioration in water quality from pollutants whereby the host's immune and physiological response are negatively affected and made susceptible to infection. Conversely, parasitism, e.g., ectoparasites, can also be reduced by pollution if the parasites are more sensitive to a particular pollutant than the host.

Moreover, instead of merely monitoring physical and chemical constituents of water, the presence or absence of parasites can be employed as a means to assess environmental conditions and system health (Sures 2001; Madanire-Moyo et al. 2012;

Gilbert et al. 2017). Blanar et al. (2009) stated that parasites appear to be potential indicators of environmental pollution in aquatic systems due to the way in which they respond to water changes. This is supported by the hypothesis that ectoparasites are more prevalent in an unpolluted water body and the inverse being true for endoparasites (Watson et al. 2012). Ecological indices suggested by Bush et al. (1997), which include percentage prevalence (P%), mean abundance (MA) and mean intensity (MI), are used to describe parasite loads. Host selection should be based on the occurrence and distribution of the host across the sites to be sampled so as to allow for comparisons between sites.

1.4 PROBLEM STATEMENT

With South Africa being a semi-arid country, the monitoring and management of freshwater systems and their resources is critical (Dallas and Rivers-Moore 2014). In addition to analysing water quality the use of fish, macroinvertebrates and ichthyoparasites have been used in recent years as bioindicators to assess the ecological state of rivers. For example, information on fish assemblages and the application of the FRAI developed by Kleynhans (2007) provides a means to categorise the ecological state of freshwater systems. Similarly, knowledge of macroinvertebrates assemblages, a source of food for most fishes (Maroneze et al. 2011), in combination with the MIRAI are used to categorise the status of freshwater bodies. Conversely, the diversity and abundance of ichthyoparasites have proven to be suitable bioindicators of aquatic conditions (Sures 2001).

In South Africa, the PES of the Nwanedi and Luphephe rivers, that originates in the Soutpansberg mountains and flows through the Nwanedi Nature Reserve and Limpopo Plain is unknown. Although, there have been studies done by Mokgalong (1981) and Angliss et al. (2007) on aquatic biota in Nwanedi and Luphephe rivers, the findings of these studies are not readily available as they have not been published in peer-reviewed journals. Conversely, studies conducted by Madanire-Moyo et al. (2010), Madanire-Moyo et al. (2012), Smit et al. (2012), Mbokane et al. (2015), and Mbokane et al. (2019) have primarily focused on the health and parasite loads of fish from the Nwanedi-Luphephe Dam.

Moreover, Walter et al. (2023) isolated and described the monogenean parasites of *Pseudocrenilabrus philander* (Weber, 1897) from the Nwanedi River within the Nwanedi Nature Reserve. The study, however, did not incorporate water parameters. Besides field the guide by Skelton (2001), there is no peer reviewed literature on fish and/or macroinvertebrate assemblages and ichthyoparasites composition as bioindicators in and from the Nwanedi and Luphephe rivers. The knowledge gap as to the PES of these rivers is to the detriment of conserving these systems as they form part of the nature reserve and as a consequence this study, through the application of the FRAI, MIRAI and ichthyoparasite findings, aims to provide a baseline for future studies, monitoring and for conservation purposes.

1.5 PURPOSE OF THE STUDY

The present ecological state of the Nwanedi and Luphephe rivers has not been established recently. Following previous work whereby Angliss et al. (2007) used the FRAI and MIRAI to assess the ecological state of these rivers, albeit unpublished, this study will evaluate and categorise the PES of these systems by establishing the water quality, fish and macroinvertebrate assemblages, including ichthyoparasite composition and the findings compared to those by Angliss et al. (2007) so as to determine if there has been a change to the ecological status overtime. Information of this nature can be used in future studies for the purpose of monitoring, conserving and managing the ecology of these rivers.

1.5.1 AIM

The aim of the study was to determine the ecological status of the Nwanedi and Luphephe rivers based on fish and macroinvertebrate assemblages and the composition of ichthyoparasites from selected fish species.

1.5.2 OBJECTIVES

The objectives of the study were to:

i) determine the water quality at selected sites by measuring the physico-chemical parameters and analysing for nutrients and aqueous metal concentrations,
ii) establish the ecological state of these rivers based on the diversity and abundance of fish collected using different fishing gear at these sites to categorise each of the ecoregions using the FRAI protocol,
iii) establish the ecological state of these rivers based on macroinvertebrate assemblages at each site using the SASS5 and MIRAI methods,
iv) establish the diversity, P%, MA and MI of parasites from selected fish species so as to determine if fish parasites can be used as bioindicators of water quality,
v) compare the FRAI and MIRAI findings from this study with those of Angliss et al. (2007).

1.5.3 RESEARCH QUESTIONS

i) How does water quality, fish and macroinvertebrates assemblages differ between sampling sites and surveys and how do the results thereof affect the categorisation of the Nwanedi and Luphephe rivers based on the FRAI and MIRAI?
ii) Will the diversity and abundance of parasites from the selected fish species vary spatially and temporally due to differences in water quality, habitat, fish, and macroinvertebrate assemblages?
iii) Has the ecological state of these rivers changed when compared to the classifications provided by Angliss et al. (2007)?

1.6 DISSERTATION OUTLINE

Chapter 1: General introduction – introduces the title and literature review of the study. It outlines the problem statement, purpose of the study, aim and objectives, and research questions.

Chapter 2: Study area – outlines the study area of the Nwanedi and Luphephe rivers. It describes topology and geomorphology, land activities of the catchment area,

selected sites, respective methodologies for each chapter, and data analyses/interpretation.

Chapter 3: Water quality – discusses water quality constituents from different sites based on physico-chemical parameters, nutrients and metals in water samples.

Chapter 4: Fish assemblage – focuses on fish assemblage as biological monitoring tool and incorporates the results into FRAI.

Chapter 5: Macroinvertebrate assemblage – includes background of aquatic macroinvertebrates. This chapter discusses SASS5 results using MIRAI.

Chapter 6: Ichthyoparasites – contains background information on fish parasites as biological indicators of the ecosystem health and discusses results based on infestation indices of parasites (P%, MA and MI).

Chapter 7: Includes the general conclusions and recommendations of the study.

CHAPTER 2

MATERIALS AND METHODS

2.1 THE STUDY AREA

The Nwanedi River catchment is located in the Limpopo province in the far north-eastern corner of South Africa (see Figure 2.1). The river originates in the Soutpansberg mountains at an altitude of 1100 m and flows in a north-easterly direction where it is joined by the Luphephe River to form the Nwanedi-Luphephe Dam inside the Nwanedi Nature Reserve (Angliss et al. 2007). Immediately downstream of the impoundment the Nwanedi River continues to flow beyond the boundary of the nature reserve to meander across the Limpopo plain where it finally meets and drains into the Limpopo River.

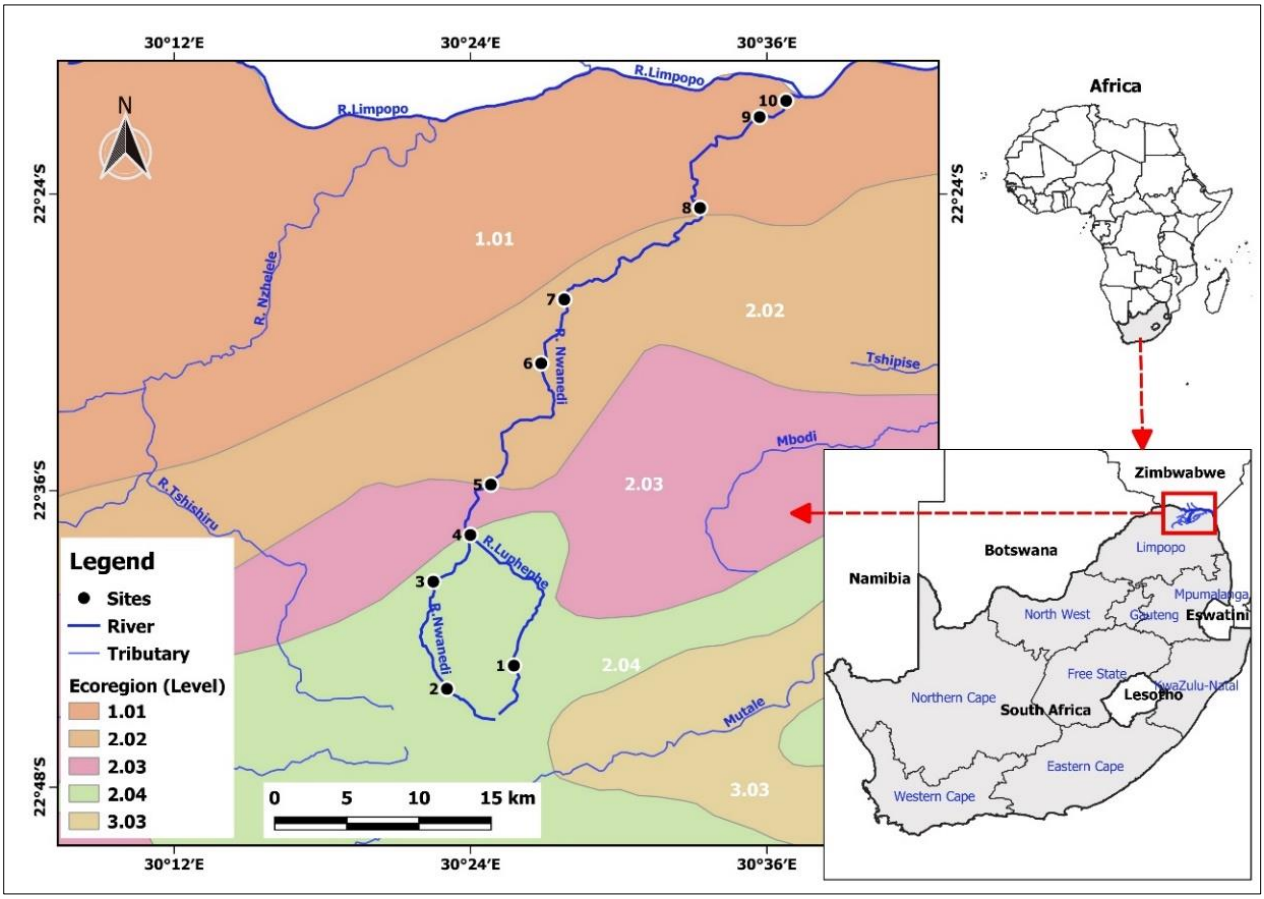

Figure 2.1: A map of South Africa showing the general locality of Nwanedi and Luphephe rivers and the position of ten sampling sites.

The Limpopo River is one of the largest rivers in South Africa and forms the South Africa's northern boundary as it flows in an eastward direction towards and across Mozambique and into the Indian Ocean. The river and its tributaries supports agriculture, wildlife, and humans living within the Limpopo River Basin. The Nwanedi and Luphephe rivers fall within the Limpopo Water Management Area (WMA) comprising three ecoregions (see Figure 2.2) each having specific attributes. These are the Soutpansberg 2.01 Ecoregion, the Limpopo Plain 1.02 Ecoregion and the Limpopo Plain 1.01 Ecoregion (Angliss et al. 2007). The map above (Figure 2.1) was generated using latest shapefiles to indicate the position of the study area within the Limpopo province. Whereas the old map (Figure 2.2) adapted from Angliss et al. (2007) was used for this study for comparison purposes.

2.1.1 TOPOLOGY AND GEOMORPHOLOGY

The topography of the region is influenced by low and erratic rainfall that occurs during the summer months between September and April. The mean annual precipitation of the area varies between 450 and 650 mm (Ashton et al. 2001). The intermittent periods of precipitation result in high flow during the rainy season and low flow during the dry season (Ashton et al. 2001). The topography at the upper reaches of the Nwanedi and Luphephe rivers is mountainous with the formation of vast flood plains occurring in the lower reaches distinguishable by rolling grasslands, sparse bushveld shrubbery and large riverine trees e.g., *Vachellia* spp. interspersed by patches of Mopane *Colophospermum* sp.

Consequently, the geomorphology in the upper foothills of the Soutpansberg 2.01 Ecoregion, is characterised by the underlying geology comprising metamorphic and igneous rock. Along this ecoregion, the river channel is moderately steep with varying channel features such as mixed bedrock, cobbles, plain-bed, riffles and rapids (Rowntree and Wadeson 2000). As the river descends into the lower foothills of the Limpopo Plain 1.02 Ecoregion, the terrain becomes shallower giving way to mixed bed alluvial channels that are dominated by sand and gravel. As the river flows down a gentle gradient into the lowland, the Limpopo Plain 1.01 Ecoregion is characterised by a distinct flood plain channel having increasing silt content (Rowntree and Wadeson 2000).

2.1.2 LAND USE ACTIVITIES WITHIN THE CATCHMENT

The Nwanedi River catchment forms part of a nature conservation area that includes the Nwanedi-Luphephe Dam. In the Soutpansberg 2.01 Ecoregion, the upper catchment is used to provide water for livestock and small-scale agriculture, while in the nature reserve a safe haven is provided for terrestrial and aquatic life. On entering the Limpopo 1.02 Plain Ecoregion, the Nwanedi River flows into the Cross Dam which forms part of the lower reaches. The dam was constructed to supply water for the irrigation of agricultural crops, game ranching, livestock farming and for neighbouring rural settlements (Angliss et al. 2007).

2.2 SELECTED SAMPLING SITES

Following the historic data by Kleynhans et al. (2005) and Angliss et al. (2007) ten sites with nine being along the Nwanedi River and one in the Luphephe River were selected (Figure 2.2).

The study was conducted during two surveys, the first being in summer (October 2021) and the second in winter (May 2022). At each site the water quality along with fish and macroinvertebrate assemblages, and the ichthyoparasites of the selected fish species were determined. This was done to establish changes in water quality and to determine if the state of the rivers has changed by comparing this study's findings with those by Angliss et al. (2007) (for more detail please see sections 2.4 to 2.7). Sampling sites were selected based on the objectives of this study as stipulated in Chapter 1 and methodologies in Chapter 2.

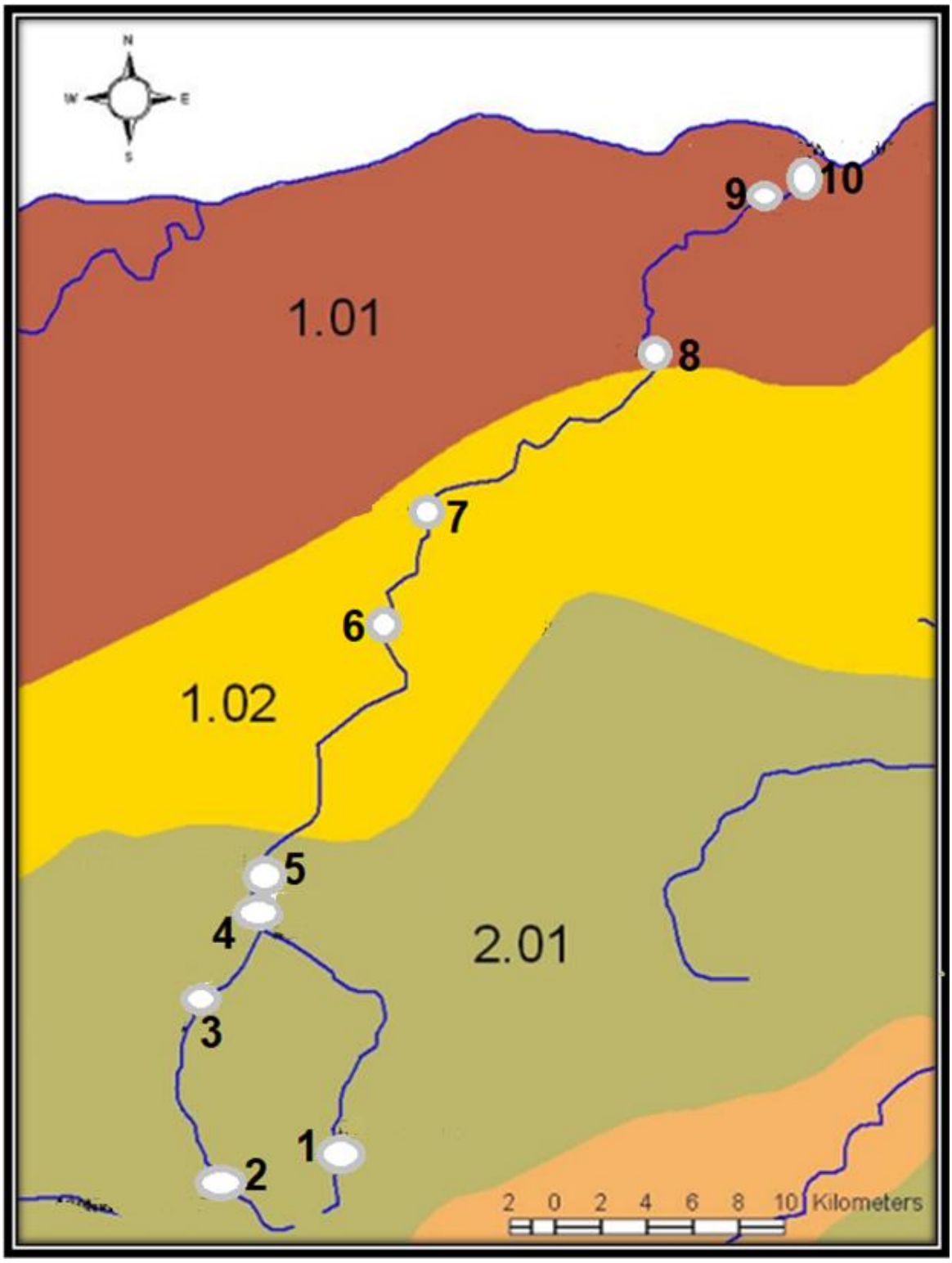

Figure 2.2: Map of the study area showing the locations of the 10 sampling sites in the Nwanedi and Luphephe rivers and the different ecoregions indicated here as: Soutpansberg 2.01 Ecoregion comprising sites 1 to 5; the Limpopo Plain 1.02 Ecoregion comprising sites 6 and 7 and the Limpopo Plain 1.01 Ecoregion comprising sites 8 to 10 [adapted and modified from Angliss et al. (2007)].

2.3 DESCRIPTION OF SAMPLING SITES SELECTED

2.3.1 SOUTPANSBERG 2.01 ECOREGION (SITES 1 – 5)

This region includes the upper reaches and headwaters of the Nwanedi (Site 1) and Luphephe (Site 2) rivers, the Nwanedi Nature Reserve (sites 3 and 4) is nestled in the foot heels of the Soutpansberg with Site 5 situated outside and in close proximity to the reserve.

Site 1

Site 1 (22°71’79”S: 030°42’93”E) is located in the Guyuni area, north of Thohoyandou close to the source of the Luphephe River. From the source the Luphephe River runs into the Luphephe Dam, where it joins with the Nwanedi Dam, which in turn are located in the Nwanedi Game Reserve. Near the source, the stream has a width of between 2 to 3 meters with biotopes that include overhanging foliage, some undercut banks, sand and submerged stones present (Figure 2.3). The riparian canopy along this reach of the river reduces the light intensity that reaches the water’s surface which, in turn, leads to low productivity. The underlying substrate in this region is dominated by bedrock and is characterised by gentle rapids, shallow pools and fast flowing turbulent waters (Figure 2.3). Communities living on small holdings in the region utilise the river for washing clothes, irrigating crops and for maintaining livestock. At the time when undertaking this research many human and animal trails were observed crossing the stream. Given the few village settlements present in this section of the river the impact from anthropogenic sources was considered to be low.

Figure 2.3: Site 1 situated near the source of the Luphephe River. Image taken during summer survey.

Site 2

Site 2 (22°73’36”S: 030°38’41”E) lies in the Soutpansberg mountains near the source of the Nwanedi River. This section of the river is between 2 to 5 metres wide where it flows downstream into the nature reserve and is joined by the Luphephe River to form the Nwanedi-Luphephe Dam. This section of the river is characterised by the presence of big and medium sized boulders, bedrock, sandy riffles, water lilies, reed beds and sparse overhanging vegetation (Figure 2.4). The presence of plastic bags and what appeared to be stains from washing clothes on large protruding rocks were observed. An indication that residents in the immediate vicinity use this site for bathing, laundry, and for the washing of vehicles. The latter activity being observed whilst sampling at this site.

Figure 2.4: Site 2 situated near the source of the Nwanedi River. Image taken during summer survey.

Site 3

Site 3 (22°66'13"S: 030°37'49"E) is located approximately one kilometre below the Nwanedi River waterfall in a valley that is flanked by steep-sides. The rivers' width varied between 6 and 8 m during the time of sampling, with several distinguishing biotopes that included rapids, marginal vegetation (e.g., common reeds) in and out of current and with the bottom substrate being covered by sand (Figure 2.5). Deep isolated pools and large riverine trees (e.g., *Vachellia* spp. and *Colophospermum* sp.), sandy riffles, large boulders, and patches of bedrock feature at this section of the river. Located within the nature reserve, and mainly void of human activities, this site looked pristine with visible signs of habitat modification caused by flooding events during high flow periods.

Figure 2.5: Site 3 situated after the waterfall of the Nwanedi River. Image taken during summer survey.

Site 4

Site 4 (22°62’98.0”S: 030°39’99.0”E) is located within the Nwanedi Nature Reserve, at the confluence of the Nwanedi and Luphephe rivers. The site is situated immediately downstream and below the wall of the Nwanedi-Luphephe Dam where the river is 2 to 3 m wide. At this site there are undercut banks, root wads, and a dense riparian canopy (Figure 2.6). The bottom substrate in this section comprises tiny boulders, silt, clay, sand, and mud. Immediately downstream where the rivers converge there is a small low lying concrete bridge that crosses the river. This portion of the river is covered by thick forest canopy that provides shade to the waters below.

Figure 2.6: Site 4 situated at the confluence of the Nwanedi and Luphephe rivers. Image taken during summer survey.

Site 5

This site (22°61’38”S: 030°39’99”E) is located outside the Nwanedi Nature Reserve downstream of the Nwanedi-Luphephe Dam where the valley opens out into a large flood plain opposite the Gaandrik farm (Angliss et al. 2007). At this site, the river is 2 to 3 m wide and flanked by steep banks with sand and boulders that vary in size giving rise to rapids (Figure 2.7). Riparian and marginal vegetation and undercut banks are present. The water in the stream is shallow (about 40 cm in places) having a moderate flow and comprising different velocity depth classes. There are gravel roads, foot paths, animal trails and small rural farms in close proximity to the riverbank.

Figure 2.7: Site 5 located middle stream of the Nwanedi River. Image taken during summer survey.

2.3.2 LIMPOPO PLAIN 1.02 ECOREGION (SITES 6 AND 7)

This ecoregion and succeeding ecoregion lies in the river catchment where the river opens into a large flood plain and where land use activities in the surrounding areas is characterised by subsistent farming and rural settlements.

Site 6

This site (22°51’41”S: 030°44’77”E) is located immediately downstream of the Cross Dam in the valley where the watercourse has a wider width of 3 to 6 m (Figure 2.8) and where the water is somewhat brackish from the outflow from the Cross Dam. Large riverine trees surround the area, with undercut banks and marginal vegetation, both in and out of current, present. Riffles generated by boulders both in and out of the current were present. A low-lying bridge for vehicles to traverse this portion of the river leads to the Cross Dam wall. Upstream of the bridge the river flow appears to be

moderate and the water depth deep. Downstream of the road the river flow is shallower and faster, with deep pools visible at some parts of the reach. The bottom substrate is bedrock with small patches of phragmites reeds present below the low-lying bridge. Construction of the dam and the low-lying bridge has resulted in creating a diverse habitat when compared to the previous sites.

Figure 2.8: Site 6 situated middle stream of the Nwanedi River. Image taken during summer survey.

Site 7

This site (22°47’14”S: 030°46’33”E) is downstream of the bridge where the Tshipise/Pafuri tar road crosses the Nwanedi River. All along this site there are steep sloping banks, a series of rapids, medium-sized boulders, and extensive vegetation on either bank with little submerged vegetation present (Figure 2.9). Water conditions at this site are turbid (Angliss et al. 2007) with isolated pools observed under the bridge. The surrounding area comprises cultivated lands and grazing pastures for livestock. There is a natural geological formation known as the Tshipise Fault that

runs below or in close proximity to this site which may be the cause for high levels of electrical conductivity (EC), total dissolved solids (TDS) and salinity reported in this and sites downstream by Angliss et al. (2007).

Figure 2.9: Site 7 situated in the middle stream of the Nwanedi River. Image taken during summer survey.

2.3.3 LIMPOPO PLAIN 1.01 ECOREGION (SITES 8, 9 AND 10)

This ecoregion lies in the lower reaches of the Nwanedi River catchment before draining into the Limpopo River. Similarly, as done by Angliss et al. (2007), a decision was taken not to sample fish and macroinvertebrate at sites 8 and 10 due to elevated EC conditions and because of dangerous wildlife present at the latter site. Additionally, the weir and military road have been washed away leaving the area unsuitable for the rapid survey methods being used in this study. Only water quality parameters were measured at these sites.

Site 8

This site (22°40’93”S: 030°55’49”E) is downstream of the road bridge leading to the Adelaide police station. This reach has an average width of approximately 5 m. The water in this section is shallow with places void of boulders and rocks. The substrate was primarily sand and mud providing poor conditions for sampling fish and macroinvertebrates with little habitat variability including marginal vegetation observed in and out of current (Figure 2.10). The surrounding area is largely dominated by extensive cultivated fields and livestock farming, that has contributed to the erosion of the riverbank in places.

Figure 2.10: Site 8 is situated downstream of the bridge that leads to Adelaide Police Station of the Nwanedi River. Image taken during summer survey.

Side 9

This site (22°34’81”S: 030°59’48”E) lies downstream of the Popallin Ranch Dam inside the Popallin Ranch. The water in this reach is slow flowing with some deep

pools, marginal vegetation (in current and out of current), reeds, and few rapids in some sections. There are no riverine trees which leads to full sun exposure, which might affect the productivity of this site (Figure 2.11). The bottom substrate is dominated by sand, mud and gravel with stones-in-current being covered with thick layer of filamentous algae leading to little or few suitable habitats.

Figure 2.11: Site 9 situated downstream of the Nwanedi River. Image taken during summer survey.

Side 10

This site is situated at the lower reaches of the Nwanedi River (22°33’72”S: 030°61’32”E) where it drains into the Limpopo River. This section of the river is approximately 8 m wide (Figure 2.12) with the surrounding area dominated by large riverine trees such as African baobab *Adansonia digitata*, and Marula tree *Sclerocarya birrea* and *Vachellia* spp. The features presents at this site are slow-deep pools, reeds and small-medium stones that cover the riverbed. Consequently, no fish and

macroinvertebrate surveys were conducted at this site due to the presence of deep pools and the possible occurrence of crocodiles.

Figure 2.12: Site 10 situated in the downstream where the Nwanedi River flows into the Limpopo River. Image taken during summer survey.

2.4 WATER QUALITY PARAMETERS

2.4.1 WATER QUALITY MEASUREMENTS

Water quality parameters (Chapter 3) were recorded at three different points per each site using a handheld multiparameter instrument (YSI model 54 Combo meter) (Figure 2.13). Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS) and salinity were measured at each site.

Figure 2.13: YSI meter used to measure physico-chemical parameters.

2.4.2 WATER SAMPLES

Subsurface water samples at each site were collected at three points, each 10 meters apart, for chemical analysis. The samples were collected in 500 ml polypropylene bottles that were acid-treated using 5% HCl and stored immediately at -20˚C. The frozen samples were sent to a South African National Accreditation System (SANAS) water laboratory (WATERLAB (PTY) LTD) in Pretoria for chemical analysis. The samples were analysed for nutrients i.e. ammonia (NH_3), nitrate (NO_3), nitrite (NO_2) sulphate (SO_4) and orthophosphate as phosphorus (P) as well as aqueous metal concentrations of aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pd), manganese (Mn), mercury (Hg), and zinc (Zn). These parameters were selected for their importance in aquatic ecosystem as well as their toxicity potential. Water quality parameters were evaluated by comparing the results to the Target Water Quality Range (TWQR) values for aquatic ecosystems as prescribed by DWAF (1996a) for aquatic systems in the South African Water Quality Guideline (SAWQG) manual.

2.5 FISH SURVEYS

2.5.1 FISH RESPONSE ASSESSMENT INDEX

Fish were collected during each survey following the Fish Response Assessment Index (FRAI) protocol developed by Kleynhans (2007). Sampling was conducted during periods of low (beginning of summer, October 2021) to moderate (beginning of winter, May 2022) river flow. During sampling, the river was divided into three ecoregions with fish sampled at each ecoregion. Each river ecoregion was divided into a distinct velocity-depth class which include the following sections: fast-deep, fast-shallow, slow-shallow, and slow-deep (Kleynhans 2007). All sections at each sampling site were sampled for fish using a portable electro-shocking apparatus and generator (RYOBI RG2700 4-STROKE GENERATOR 240V 50Hz 8.3A 2.5KVA 12L). A circular dip-net and electrodes mounted on an A-frame were used to survey fish (Figure 2.14).

Figure 2.14: Collecting fishes by means of electroshocking and circular dip-net.

Fish surveys in each site was conducted by two participants with the first participant being in charge of handling the electrodes, while the second held a circular dip-net 2 to 3 m downstream of the electrodes. The electroshocking was done by beginning downstream and slowly moving upstream in a zig zag pattern. A period of 30 minutes was used to sample fish at each site. Captured fish were placed in buckets containing river water so as to be identified later using identification keys provided in the field guide by Skelton (2001) (Figure 2.15). Additional literature by Skelton (2016) was used to confirm species names. Fish were counted to establish which species were abundant to be used for parasite survey and all other fish caught returned live to the habitat sampled. Fish that could not be identified on site were first euthanised and then placed in 10% formalin for later identification.

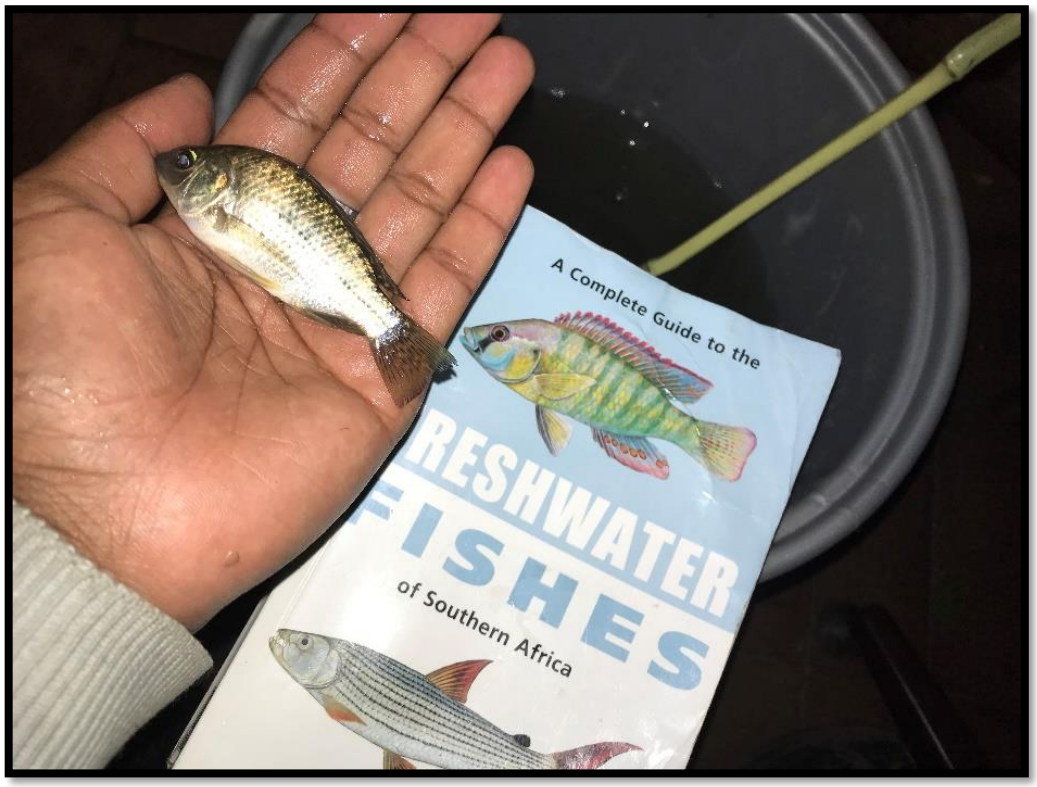

Figure 2.15: Fish identification using classification keys provided by Skelton (2001).

The fish data collected at each ecoregion was incorporated into the FRAI database developed by Kleynhans (2007). The FRAI programme is an excel

spreadsheet that can be downloaded free from the internet (see the link provided in 2.6.4). The metrics in FRAI weight and assigned each ecoregion a specific EC so as to compare the present ecological state against natural or close to natural expected conditions. Calculated FRAI scores expressed as a percentage and an EC of either A (100%: Unmodified), B (80-99%: Largely natural), C (60-79%: Moderately modified), D (40-59%: Largely modified), E (20-39%: Seriously modified) or F (0-19%: Critically modified) were assigned following Kleynhans et al. (2007). The reference (expected) list for the fish community and the frequency of occurrence (FROC) data (see Table 2.1) were adopted from the historic by Kleynhans et al. (2007) survey in both rivers across all three ecoregions. Reference fish species refers to fish species expected to occur within the catchment based on literature such as Kleynhans et al. (2007). Frequency of occurrence refers to how often a species occurs within the ecoregion. The observed and reference fish species were used to complete and populate the FRAI spreadsheets so as to allow one to compare if there is an increase or decrease of species between the ecoregions.

Table 2.1: Reference fish species expected within the three ecoregions of the Nwanedi River catchment [Adapted from Kleynhans et al. (2007)].

Reference (expected) species (Site 1 – 10)	**Species code**	**Soutpansberg 2.01 Ecoregion (sites 1 – 5)**	**Limpopo Plain 1.02 Ecoregion (sites 6 – 7)**	**Limpopo Plain 1.01 Ecoregion (sites 8 – 10)**
		Reference Frequency of Occurrence (FROC)		
Awaous aeneofuscus (Peters, 1852)	AAEN	-	-	-
Anguilla bengalensis labiata Peters, 1852	ALAB	1	2	2
Anguilla mossambica Peters, 1852	AMOS	2	3	3
Amphilius uranoscopus (Pfeffer, 1889)	AURA	3	-	-
Clarias gariepinus (Burchell, 1822)	CGAR	-	2	5
Chiloglanis paratus Crass, 1960	CPAR	1	3	3
Chiloglanis pretoriae Van Der Horst, 1931	CPRE	4	-	-
Clarias theodorae Weber, 1897	CTHE	2	-	-
Enteromius afrohamiltoni (Crass, 1960)	BAFR	-	-	3

Table 2.1 Continued…

Reference (expected) species (Site 1 – 10)	Species code	Soutpansberg 2.01 Ecoregion (sites 1 – 5)	Limpopo Plain 1.02 Ecoregion (sites 6 – 7)	Limpopo Plain 1.01 Ecoregion (sites 8 – 10)
		Reference Frequency of Occurrence (FROC)		
Brycinus imberi (Peters, 1852)	BIMB	-	-	3
Enteromius annectens (Gilchrist & Thompson, 1917)	BANN	-	-	3
Enteromius eutaenia (Boulenger, 1904)	BEUT	3	-	-
Enteromius lineomaculatus (Boulenger, 1903)	BLIN	3	2	-
Enteromius paludinosus (Peters, 1852)	BPAU	3	3	-
Enteromius radiatus (Peters, 1853)	BRAD	-	3	3
Enteromius toppini (Boulenger, 1916)	BTOP	-	3	3
Enteromius trimaculatus (Peters, 1852)	BTRI	3	4	3
Enteromius unitaeniatus (Günther, 1866)	BUNI	2	3	3
Enteromius viviparus (Weber, 1897)	BVIV	3	4	3
Hydrocynus vittatus Castelnau, 1861	HVIT	-	-	3
Labeo cylindricus Peters, 1852	LCYL	4	5	3
Labeobarbus marequensis (Smith, 1841)	LMAR	3	5	3
Labeo molybdinus Du Plessis, 1963	LMOL	3	3	3
Labeo rosae Steindachner, 1894	LROS	-	-	3
Micralestes acutidens (Peters, 1852)	MACU	4	5	-
Mesobola brevianalis (Boulenger, 1908)	MBRE	-	3	4
M*arcusenius macrolepidotus* (Peters, 1852)	MMAC	1	2	3
Oreochromis mossambicus (Peters, 1852)	OMOS	2	4	-
Pseudocrenilabrus philander (Weber, 1897)	PPHI	4	5	-
Petrocephalus wesselsi Kramer & Van Der Bank, 2000	PCAT	2	2	-
Schilbe intermedius Rüppell, 1832	SINT	1	2	-
Synodontis zambezensis Peters, 1852	SZAM	-	-	-
Tilapia rendalli (Boulenger, 1896)	TREN	-	-	-
Tilapia sparrmanii Smith, 1840	TSPA	3	-	-
TOTAL	**34**	**23**	**22**	**28**

FROC: 1 = Present at very few sites; 2 = Present at few sites (>10-25%); 3 = Present at about >25-50% of sites; 4 = Present at most sites (>50-75%); 5 = Present at all sites.

2.6 MACROINVERTEBRATE SURVEYS

2.6.1 SOUTH AFRICAN SCORING SYSTEM

Aquatic macroinvertebrate assessment (Chapter 5) was conducted using the South African Scoring System version 5 (SASS5) bio-assessment protocol (Dickens and Graham 2002). Aquatic macroinvertebrate samples were collected at each site using a 30 x 30 cm SASS net having 1 mm mesh size (Figure 2.16). Macroinvertebrates were sampled against the current (i.e., from downstream to upstream) with a net placed at the bottom of the stream to collect invertebrates. The kick sampling method was used to sample invertebrates. Biotopes sampled were stones (in current; SIC, and out of current; SOC), sediments (gravel, sand, and mud; GSM), and aquatic and marginal vegetation (VEG). Stones-in-current and bedrock were kicked for 2 to 5 minutes to free benthic macroinvertebrates prior collecting, whereas, to collect macroinvertebrates from SOCt, small boulders were picked and visualised for a period of 1 minute. The GSM biotopes were stirred by shuffling one's feet on or along the underlying substrate for a period of 1 minute whereby the invertebrates disturbed were then collected. Aquatic vegetation covering an area of 1 m^2 was swept with a SASS net and similarly marginal vegetation 2 m^2 both in and out of current. Macroinvertebrates were gently removed from the net by washing the sides of the net with river water and from the debris using a jeweller forceps and/or Pasteur pipettes. Macroinvertebrates sampled from each biotope were placed into white sorting trays 30 cm x 40 cm in size and covered with river water.

Figure 2.16: Collecting macroinvertebrates using a SASS net.

The specimens were viewed and identified using a magnifying glass to family level following morphological features as indicated in the Aquatic Invertebrates of South African Rivers' guide by Gerber and Gabriel (2002). This was done for a period of 15 minutes whereby the macroinvertebrates per biotope sampled were scored and categorised based on their tolerance level towards pollution. For example, tolerant families having quality values (QV) of 1 to 5, moderately tolerant families QV from 6 to 10, and families sensitive to pollution having QV ranging from 11 to 15 (Gerber and Gabriel 2002). The higher the score the more sensitive the family. However, the scoring and categorising exercise was stopped after 5 minutes if no new taxa were observed. Once identified and scored the macroinvertebrates were returned live to the environment sampled.

2.6.2 INTERPRETATION OF SASS5 DATA

The SASS score was calculated by adding all the sensitivity/tolerance scores of taxa per site. To determine the average score per taxon (ASPT), the SASS score value was divided by the number of taxa (Gerber and Gabriel 2002). The SASS scores and ASPT values were interpreted following guidelines by Chutter (1995) (Table 2.3).

Table 2.3: Interpretation of the SASS5 results (Chutter 1995).

SASS Score	ASPT	INTERPRETATION
>100	>6	Water quality natural, habitat diversity high
<100	>6	Water quality natural, habitat diversity reduced
>100	<6	Borderline between water quality natural and some deterioration
50-100	<6	Some deterioration in water quality
<50	Variable	Major deterioration in water quality

Key: ASPT = average score per taxon.

2.6.3 MACROINVERTEBRATE RESPONSE ASSESSMENT INDEX

The invertebrates' results from the SASS5 protocol were analysed using the Macroinvertebrate Response Assessment Index (MIRAI) software in a excel spreadsheet (Kleynhans et al. 2005). The MIRAI database is a free software in excel spreadsheet that can be downloaded from the internet (see the link in 2.6.4). The MIRAI protocol considers the abundance and frequency of occurrence of the invertebrate' communities under reference and present conditions (Thirion 2007). The weighing and ranking matrix in MIRAI is based on the increase or decrease of macroinvertebrates from the reference (expected) assemblage in terms of the following modifications: flow, habitat, water quality, and system connectivity and seasonality (see examples in Appendix B Table 2B to 7B for detailed procedure). The MIRAI results were interpreted following the same criteria as done for FRAI (see Table 2.2). The MIRAI results were also expressed as either A (100%:

Unmodified), B (80-99%: Largely natural), C (60-79%: Moderately modified), D (40-59%: Largely modified), E (20-39%: Seriously modified) or F (0-19%: Critically modified) as described in Kleynhans et al. (2005).

2.6.4 FRAI AND MIRAI ANALYSES

To determine the health status for each ecoregion of a river system, fish and macroinvertebrate abundances' data were incorporated into FRAI and MIRAI model and results were presented into a specific ecological category (EC) based on the scores (%) obtained (Table 2.2). FRAI and MIRAI analyses were conducted using excel spreadsheets (see Appendix A and B for procedures undertaken) that comprised built in macros (Kleynhans et al. 2005). The FRAI and MIRAI excel spreadsheets used in this study can be downloaded following the sources: https://www.dws.gov.za/iwqs/rhp/eco/EcoStatus/ModuleD_FRAI/FRAI.xls and https://www.dws.gov.za/iwqs/rhp/eco/EcoStatus/ModuleE_MIRAI/MIRAI_template_boundary_classes.xlsx, respectively.

Table 2.2: Generic ecological categories applied in the Nwanedi River Catchment [Modified from Kleynhans et al. (2005)].

ECOLOGICAL CATEGORY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS	SCORE (% OF TOTAL)
A	Unmodified, or approximates natural conditions closely	90 - 100
B	Largely natural with few modifications. A change in community characteristics may have taken place but richness in biota and presence of intolerant biota indicate little modification.	80 - 89
C	Moderately modified. A lower than expected biota richness and presence of most intolerant biota. Some impairment of health may be evident at the lower end of this scale.	60 - 79
D	Largely modified. A clearly lower than expected biota richness and absence or much lowered presence of intolerant and moderately intolerant biota. Impairment of health may become more evident at the lower end of this class.	40 - 59

Table 2.2 Continued…

ECOLOGICAL CATEGORY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS	SCORE (% OF TOTAL)
E	Seriously modified. A strikingly lower than expected biota richness and general absence of intolerant and moderately intolerant biota. Impairment of health may become very evident.	20 - 39
F	Critically/Extremely modified. An extremely lowered biota richness and an absence of intolerant and moderately intolerant biota. Only tolerant biota may be present with a complete loss of biota at the lower end of the class. Impairment of health generally very evident.	0 - 19

2.7 ICHTHYOPARASITE SURVEYS

2.7.1 DESCRIPTION AND HANDLING PROCEDURES OF HOST

As indicated in Chapter 1, for a fish species to be considered suitable for parasite surveys the species of interest should commonly occur at most sites selected for the study and be widely distributed within these rivers. After having conducted the first survey, the southern mouthbrooder, *Pseudocrenilabrus philander* (Weber, 1897) was identified and chosen to be suitable for conducting the parasite survey. *Pseudocrenilabrus philander* (Figure 2.17) is a small haplochromine cichlid found in southern and central Africa. This fish is a freshwater species with a wide environmental tolerance and occupies a variety of biotopes including areas that are slightly brackish (Skelton 2001). *Pseudocrenilabrus philander* usually prefers standing water with vegetated zones. As with most fish, this species is known to harbour several species of parasites with a specific gill monogenean known as *Cichlidogyrus philander* Douëllou, 1993 that was first recorded in Lake Kariba in Zimbabwe and later in South Africa by Le Roux and Avenant-Oldewage (2010) and Walter et al. (2023).

Figure 2.17: A specimen of southern mouthbrooder *Pseudocrenilabrus philander*.

Specimens of *P. philander* were collected from three sites in the Soutpansberg 2.01 Ecoregion and one site in the Limpopo Plain 1.02 Ecoregion during the high flow (summer 2021: Only Site 3) and low flow (winter 2022: sites 3, 4, 5 and 6) to allow comparison across sites. Thus, due to the logistical issues and time constrains parasite surveys were conducted only at Site 3 during the first survey with fish specimens collected at sites 3, 4, 5 and 6 during the second survey examined for parasites (see Appendix C: Table 1C and 2C). Specimens of *P. philander* were transferred from the river to a makeshift field laboratory using containers containing well aerated river water for the parasite examination (Figure 2.18).

Figure 2.18: Tanks comprising river water that is aerated using portable air pumps to house fish.

2.7.2 EXAMINATION OF HOST FOR PARASITES

In the field laboratory, the body's surface, mouth cavity and fins of the fish were visually examined for any noticeable ectoparasites. Mucous smears were taken from the body surface (Attia et al. 2021) and fins of a host using a microscope slide and examined for ectoparasites using a stereo microscope (Leica EZ4). Measurements of the host including weight, standard and total lengths were recorded. Specimens were sacrificed by severing the spinal cord directly behind the head region using a sharp pair of scissors. A pithing needle was used to pith the brain of individuals sacrificed. A euthanising agent could not be used to anesthetise the fish since ectoparasites are known to detach from the host surface once subjected to any euthanising treatment e.g. clove oil (Jones et al. 2015).

In addition to morphometric data, the sex of each fish was identified based on sexual reproductive organs (testes in males and ovaries in females) after having been sacrificed and dissected using a scalpel blade. Fins, gills, eyes, and visceral organs were removed and put in different Petri dishes containing distilled water for gills, eyes, and fins and saline for visceral organs. Fins and gills of the fish were examined for ectoparasites (Figure 2.19) with the gastrointestinal tract and other

internal organs examined for the presence of endoparasites using a stereo microscope. All carcasses were packaged in bio-hazard bags/containers and delivered to authorities in charge of proper disposals of biological material at the University of Limpopo.

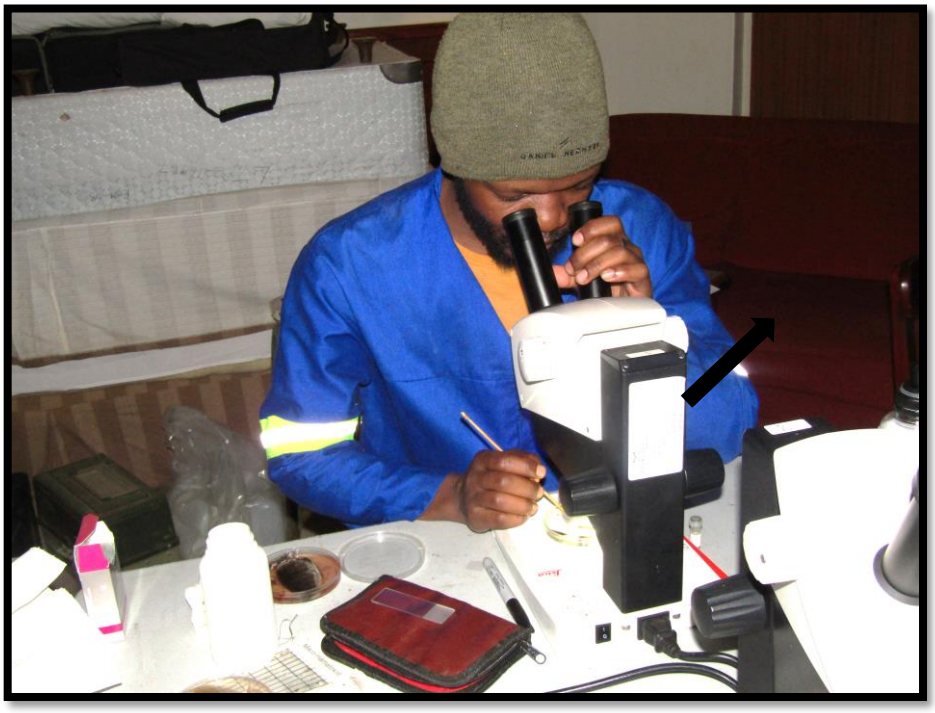

Figure 2.19: Examining host specimens for parasites.

2.7.3 PARASITE FIXATION, PRESERVATION AND IDENTIFICATION

Monogeneans from the gills were mounted onto a microscope slide using glycerine ammonium picrate (GAP) solution (Malmberg 1970; Truter et al. 2016). Cestodes and nematodes from the outer layer of the intestine and intestinal wall were relaxed in saline solution, fixed, and preserved in 70% and 96% ethanol for morphological examination and molecular characterisation, respectively. Identification of parasites was done using a compound microscope (Olympus BX50) and available literature specific for each parasite species.

2.8 STATISTICAL ANALYSIS

i) The mean and standard deviation of physico-chemical parameters were calculated, and Shapiro-Wilk test was used to test for normality. However, due to water quality data being non-parametric the Friedman test was used to test for significant differences between sites and surveys with the significance level ascertained at $p \leq 0.05$. All analyses were performed using Statistical Package for Social Scientists (IBM SPSS Statistics 29).

ii) To determine differences in fish diversity the Shannon Weiner Diversity Index (H') was performed (Shams et al. 2013) variation among sites and ecoregions using the following equation (Shannon and Weaver 1949).

$$H' = - \Sigma \, Pi \ln Pi$$

where H' is the diversity index, Pi is the relative abundance (s/N), s is the number of individuals for each species, N is total number of individuals.

iii) To integrate the response of fish assemblages to physico-chemical parameters a Canonical Correspondence Analyses (CCA) was conducted. The CCA analysis is a multivariate approach used to analyse the relationship between the biotic communities and their responses to environmental variables. Data were log (x+1) transformed and the Monte Carlo permutation tests applied to test for significance (Heino 2000). The CCA tests were done using Canoco Version 4.5.

iv) The prevalence (P%), mean intensity (MI), and mean abundance (MA) of collected parasites were calculated according to Bush et al. (1997).

- Prevalence (%) = number of hosts by a particular parasite species divided by the total number of hosts examined and multiplied by a hundred to be expressed as a percentage.
- Mean Abundance (MA) = number of parasites of a particular species divided by the number of hosts examined.
- Mean Intensity (MI) = total number of parasites collected of a particular species divided by the number of hosts by that parasite.

2.9 ETHICAL CLEARANCE AND SAMPLING PERMIT

Ethical clearance to conduct research at Nwanedi and Luphephe rivers was obtained from the Animal Research and Ethics Committee (AREC) of the University of Limpopo with project number: AREC/04/2022: PG. Surveys were carried out with approval from the Limpopo Department of Economic Development, Environmental and Tourism (LEDET) with permit number: ZA/LP/109375.

CHAPTER 3

WATER QUALITY

3.1 INTRODUCTION

South Africa is a water scarce country, and as a consequence, many of the country's freshwater systems are threatened and are of conservation concern (Dallas and Rivers-Moore 2014). Due to an increase in the country's population and the discharge of industrial and mining effluent, many inland waters in South Africa are frequently being exposed to contaminants that, in turn, negatively affect the state, quantity and quality of water that is available for domestic and agricultural purposes. As a consequence, the frequent monitoring of water quality as well as the conservation of water bodies are of socio-economic importance (Peart and Govender 2001).

In South Africa, standard guidelines known as the South African Water Quality Guidelines (SAWQGs) have been developed by the Department of Water and Sanitation, previously known as the Department of Water Affairs and Forestry, to gauge and monitor the suitability of most water constituents for aquatic ecosystems (DWAF 1996a). Additionally, the Target Water Quality Range (TWQR) provide a means to compare and maintain the health and integrity of aquatic ecosystems (DWAF 1996a). Therefore, to specify the standard concentration ranges and water quality criteria for a specific constituent, the TWQR can be used as a tool to monitor, inform, and manage water quality.

Water quality refers to the physical, chemical and biological characteristics of water based on the standards for which it is to be used e.g., domestic, agriculture and industrial (Chapman 2021). Generally, water quality in aquatic systems is influenced by land use activities around the catchment areas (Chapman 2021). Additionally, many freshwater ecosystems have undergone increased habitat modifications that has led to a reduction in water quality and river flow caused by the high demand and over-extraction of water (Davies and Day 1998). These modifications have consequently impacted the physical, chemical and biological features of aquatic systems, with aquatic species that are sensitive to changes in environmental conditions being negatively affected by poor water quality and

habitat loss (Dallas and Day 2004). A report by the Department of Water Affairs and Forestry (DWAF 1996a) has indicated that water quality can be influenced by aqueous constituents that are either dissolved or suspended.

Water quality constituents can be divided into categories, namely, system variables, nutrients, non-toxic constituents and/or toxic constituents, based on the effects that the constituents may have on aquatic biota (DWAF 1996a). System variables such as temperature, dissolved oxygen (DO) and water pH are physico-chemical properties that regulates the essential ecosystem processes such as migration and spawning. Nutrients are essential to the growth and survival of aquatic organisms (Davies and Day 1998) and are generally not toxic but can stimulate eutrophic conditions if present at high concentrations (e.g., nitrogen compounds such as ammonia, ammonium, nitrite, nitrate and orthophosphate). Non-toxic inorganic constituents, may cause toxic conditions at high concentrations, but are generally considered “system characteristics” due to their natural concentrations being determined by localised geochemical, physical and hydrological processes (DWAF 1996a). These constituents include electrical conductivity (EC), total dissolved solids (TDS) and salinity. Lastly, there are elements known as toxic constituents (e.g., aluminium, copper, iron, manganese and zinc) that are assimilated in small concentrations by aquatic organisms but if present at high concentrations can adversely affect the health of an organism.

As described in Chapter 2, the Nwanedi and Luphephe rivers originates in the Soutpansberg mountains and descends through three ecoregions namely, Soutpansberg 2.01, Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions. In the Soutpansberg 2.01 Ecoregion, the two rivers emerges from their sources respectively and flows down the gradient through the Nwanedi Nature Reserve and Limpopo Plain ecoregions that constitute the lower reaches of the Nwanedi River catchment. In the lower reaches of the Nwanedi River the river supports land use activities such as the production of agricultural crops and game farming. Thus, the purpose of this chapter was to establish the spatial changes in water quality at selected sites within the different ecoregions by measuring the physico-chemical parameters, nutrients and aqueous metal concentrations.

3.2 MATERIALS AND METHODS

3.2.1 WATER QUALITY PARAMETERS

Methods and Materials used to collect, measure physico-chemical parameters and the analyses of nutrients and heavy metal content, as well as data analyses are described in Chapter 2.

3.3 RESULTS

3.3.1 PHYSICO-CHEMICAL PARAMETERS

Overall water temperatures were shown to increase with a decrease in elevation, with the highest temperature of 27.43°C recorded at Site 10 and a low of 13.40°C at Site 1 in summer. In the Soutpansberg 2.01 Ecoregion, comprising sites 1 – 5, mean values recorded within this region of the river ranged from 13.53°C to 19.10°C during summer and between 14.13°C to 19.43°C in winter (see Table 3.1). Dissolved oxygen concentrations were reported to be high to saturated during summer with mean values ranging from 7.67 mg/L to 8.63 mg/L and 8.42 mg/L to 9.33 mg/L in winter. The pH values recorded at this ecoregion ranged from 5.86 to 7.85 during summer and 5.52 to 7.94 in winter with the upper reaches of the river being acidic and the lower reaches alkaline. The water in this ecoregion was found to be soft, with EC increasing from Site 1 to Site 4. The reported EC in summer ranged from 61.60 µs/cm to 110.60 µs/cm and from 18.33 µs/cm to 61.33 µs/cm during winter. Total dissolved solids levels were high in summer (50.92 – 81.25 mg/L) with relatively lower values in winter (14.88 – 47.23 mg/L). The highest salinity concentration with the mean value of 0.06 ‰ was recorded at sites 4 and 5 during summer while the lowest mean value of 0.01 ‰ was reported at sites 1 and 2 in winter.

In the Limpopo Plain 1.02 Ecoregion (comprising sites 6 and 7), the water temperature increased moderately during both seasons with the highest temperature of 22.90°C recorded at Site 7 in summer and a low of 19.20°C recorded at Site 7 in winter. Mean values recorded at both sites and seasons ranged from 19.90°C to 22.83°C during summer and between 19.90°C to 19.20°C in winter. The presence of rapids at sites 6 and 7 facilitated DO readings to be above saturation with mean values of 8.27 mg/L to 7.73 mg/L reported in summer and 9.04 mg/L to 8.85 mg/L in winter. The pH level remained alkaline in summer ranging from 8.05 to 8.09 becoming less

alkaline in winter (7.68 – 8.03). Electrical conductivity and TDS levels were moderately higher in summer and slightly lower in winter at both sites (see Table 3.1). Salinity concentrations were higher at Site 7 with mean value of 0.36 ‰ recorded during the summer survey and lower (0.15 ‰) at Site 6 in winter.

In the Limpopo Plain 1.01 Ecoregion (comprising sites 8 – 10) mean water temperatures were slightly warmer in summer (22.00 – 27.43°C) and low in winter (20.60 – 17.37°C) as compared to temperatures in ecoregions 2.01 and 1.02, respectively. Dissolved oxygen levels recorded for summer (7.00 – 7.97 mg/L) and winter (8.47 – 9.33 mg/L) were within the TWQR range of 80 – 120% saturation for all sections. The recorded pH was slightly alkaline at both sampling sites during both seasons with the highest pH recorded during summer (pH 8.94) at Site 10 and the lowest in winter (pH 8.01) at Site 8. Water was hard in this ecoregion, when compared to Soutpansberg 2.01 and Limpopo Plain 1.02 ecoregions, with higher EC and TDS recorded during both seasons (see Table 3.1). The highest EC was recorded in summer (2091.00 µs/cm) at Site 10. Across all sites the mean EC values ranged between 1249.00 – 2091.00 µs/cm during summer and 797.33 – 1010.70 µs/cm in winter, apart from Site 10 where a slight decrease in EC (991.33 µs/cm) was reported in winter. Total dissolved solids concentrations of between 858.00 mg/L and 1297.70 mg/L were recorded during the summer survey and 565.50 mg/L to 756.17 mg/L during the winter survey. The salinity levels recorded at this ecoregion varied from 0.66 ‰ to 1.01 ‰ during summer and 0.43 ‰ to 0.58 ‰ in winter.

In general, the physico-chemical parameters were shown to vary between sites and ecoregions for each survey and fell within the TWQR limits (DWAF 1996a) for aquatic ecosystem. The Friedman test indicated a significant difference ($p<0.05$) for temperature, DO, EC, TDS, and salinity when comparing between sites and surveys.

Table 3.1: The mean ± standard deviation of physico-chemical parameters recorded from 10 sampling sites in Nwanedi (*n* = 9) and Luphephe (*n* = 1:Site 1) rivers during surveys conducted in summer (S) 2021 and winter (W) 2022.

	Sampling sites																				Water Quality Guidelines (TWQR)
	Site 1		Site 2		Site3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10		
Water quality parameters	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	
Temperature (ºC)	13.53 ±0.12	14.13 ±0.06	14.77 ±0.11	16.23 ±0.06	18.23 ±0.06	17.50 ±0.00	19.10 ±0.00	19.43 ±0.47	18.33 ±0.06	16.60 ±0.00	19.9± 0.10	19.90 ±0.00	22.83 ±0.06	19.20 ±0.00	22.00 ±0.00	20.60 ±0.00	22.10 ±0.10	19.40 ±0.00	27.43 ±0.06	17.37 ±0.23	Should not vary by > 10%[1]
Dissolved Oxygen (mg/L)	8.63± 0.31	8.79± 0.15	8.43± 0.25	8.82± 0.22	7.67± 0.42	9.21± 0.02	8.10± 0.10	8.42± 0.06	8.27± 0.15	9.33± 0.03	8.27± 0.23	9.04± 0.06	7.73± 0.32	8.85± 0.02	7.60± 0.36	8.47± 0.00	7.00± 0.20	9.00± 0.05	7.97± 0.15	9.33± 0.07	–
Dissolved Oxygen (%)	91.67 ±5.03	96.27 ±1.26	92.33 ±2.52	100.63±2.12	86.00 ±4.36	103.07±0.06	92.33 ±1.53	97.47 ±0.67	94.00 ±3.00	101.07±0.31	95.33 ±1.53	103.83±0.75	91.00 ±7.55	100.73±0.71	90.33 ±4.04	98.57 ±0.12	84.00 ±1.00	101.90±0.66	104.67±1.53	100.47±0.95	80 – 120% of Saturation [1]
pH*	5.86-7.04	5.85-6.03	6.10-6.34	5.52-5.63	7.24-7.64	7.29-7.56	7.01-7.04	7.07-7.17	7.36-7.85	7.82-7.94	8.05-8.11	8.03-8.31	8.09-8.11	7.46-7.68	8.28-8.38	8.01-8.19	8.25-8.38	8.11-8.22	8.86-8.94	8.10-8.31	Should not vary by > 5%[1]
Electrical Conductivity (µs/cm)	61.6± 1.31	18.33 ±0.12	72.20 ±0.35	19.53 ±0.40	101.6 ±0.17	42.33 ±0.06	110.60±0.52	61.33 ±3.49	108.67±0.91	60.53 ±0.35	510.67±1.15	277.37±0.15	713.67±4.04	416.73±0.15	1249.00±1.00	797.33±0.58	1812.00±2.65	1010.70±8.39	2091.00±5.20	991.33±6.43	No criteria available
Total Dissolved Solids (mg/L)	50.92 ±0.75	14.95 ±0.00	58.08 ±0.38	14.88 ±0.74	75.62 ±0.38	32.28 ±0.38	81.25 ±0.00	45.07 ±3.34	80.82 ±0.75	47.23 ±0.38	366.19±3.80	199.54±0.01	485.50±3.90	304.63±0.38	858.00±0.00	565.50±0.00	1245.70±4.04	738.83±3.75	1297.70±4.04	756.17±7.51	Should not vary > 15% from cycle[1]
Salinity (‰)	0.04± 0.00	0.01± 0.00	0.04± 0.00	0.01± 0.00	0.05± 0.00	0.02± 0.00	0.06± 0.00	0.03± 0.00	0.06± 0.00	0.03± 0.00	0.27± 0.01	0.15± 0.00	0.36± 0.01	0.23± 0.00	0.66± 0.00	0.43± 0.00	0.97± 0.01	0.56± 0.00	1.01± 0.00	0.58± 0.01	0.5 ‰ or < 0.05 ‰[1]

Key: 1 = DWAF 1996a – South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems and where TWQR indicates Targeted Water Quality Range;
* pH is given as a range

3.3.2 NUTRIENTS – NITROGEN COMPOUNDS AND ORTHOPHOSPHATE

In the Soutpansberg 2.01 Ecoregion (sites 1 – 5), ammonia (NH_3) was below detection in summer (<0.1 mg/L) increasing to 0.1 mg/L at most sampling sites in winter. Nitrate (NO_3) was not detected in summer for sites 1, 2 and 5, indicating oligotrophic conditions, but ranged from 0.1 mg/L – 0.7 mg/L with the highest concentration of 0.7 mg/L recorded at Site 3 (Table 3.2) during both surveys. Nitrite (NO_2) and sulphate (SO_4) were below detection (<0.05 mg/L and <0.2 mg/L, respectively) across all sites and for both surveys. Orthophosphate concentrations were below detection (<0.1 mg/L) during both seasons at most sampling sites except during winter at Site 4 and Site 5 with 0.1 mg/L and 0.2 mg/L detected respectively.

In the Limpopo Plain 1.02 Ecoregion (sites 6 and 7), ammonia levels were below detection (<0.1 mg/L) in the summer increasing to 0.1 mg/L in winter across all sites. With NO_3 levels below detection (<0.1 mg/L) and water condition oligotrophic for both surveys at Site 6, NO_3 concentrations increased to 0.6 and 0.9 mg/L with conditions becoming mesotrophic at Site 7 for winter and summer respectively. Nitrite and orthophosphate concentrations were below detection at <0.05 mg/L and <0.1 mg/L, respectively. However, during the winter survey, orthophosphate concentrations increased to 0.1 mg/L at Site 7 (Table 3.2). Sulphate concentrations were relatively high, given that the highest concentrations of 38 mg/L and 26 mg/L were only recorded during the summer survey at Site 7 and Site 6, respectively.

In the Limpopo Plain (1.01 Ecoregion comprising sites 8 – 10) most nutrients were above detection level, except NO_2 (<0.05 mg/L) and orthophosphate (<0.1 mg/L) at all sites during both surveys whereas ammonia was below detection (<0.1 mg/L) only at Site 9 during summer, but above detection level (0.1 mg/L) at all sampling sites during both seasons. Sulphate levels were fairly high at all sites, given that, the highest concentrations were recorded during the summer survey, followed by the lowest concentrations recorded for winter survey (Table 3.2).

Given that nitrite concentrations were below the detection level in all ten sites this nutrient will not be discussed further.

Table 3.2: Nutrient concentrations in the water column recorded from the ten sampling sites in Nwanedi (*n* = 9) and Luphephe (*n* = 1: Site 1) rivers during surveys conducted in summer (S) and winter (W).

Nutrients (mg/L)	Sampling sites																				Water Quality Guidelines
	Site 1		Site 2		Site3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10		
	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	
Ammonia	< 0.1	0.1	< 0.1	0.1	< 0.1	0.1	< 0.1	0.1	<0.1	0.1	<0.1	0.1	<0.1	0.1	0.1	0.1	<0.1	0.1	0.1	0.1	Not more than 7
Nitrate	< 0.1	0.4	<0.1	0.2	0.7	0.7	0.1	0.3	<0.1	0.2	<0.1	<0.1	0.9	0.6	1.9	0.7	0.9	0.6	0.5	0.9	< 0.5[1] Oligotrophic, 0.5 – 2.5[1] Mesotrophic
Nitrite	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	< 0.5[1] Oligotrophic, 0.5 – 2.5[1] Mesotrophic
Orthophosphate	< 0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.2	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	Not change by > 15%[1]
Sulphate	< 2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	26.0	10.0	38.0	10.0	75.0	30.0	122.0	28.0	65.0	47.0	-

Key: 1 – DWAF 1996a – South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems. 2 – Cells indicated with a "<" represents values that are below detection.

3.3.3 TOXIC CONSTITUENTS IN SOLUTION

In the Soutpansberg 2.01 Ecoregion (sites 1 – 5), most of the metals selected and analysed were below detection level except for Cu and Mn. Copper concentrations were only detected from site 2 to 5 (0.123 mg/L to 0.126 mg/L) during the winter survey (Table 3.3). Manganese concentrations at Site 4 were recorded to be 0.143 mg/L during the summer survey, while at Site 5 concentrations were reported to be 0.045 mg/L and 0.027 mg/L in the summer and winter surveys, respectively.

In the Limpopo Plain 1.02 Ecoregion (sites 6 – 7) Al, Cu, Mn and Hg were above detection level. Aluminium concentrations were only recorded at Site 6 (0.216 mg/L) and 7 (0.134 mg/L) during the summer survey. Copper concentrations of 0.125 mg/L and 0.126 mg/L, were recorded at Site 6 and 7 respectively during the winter survey. Manganese concentrations were detected in summer at both sites with the highest concentration of 0.060 mg/L reported at Site 6 and a low of 0.028 mg/L at Site 7 (Table 3.3).

In the Limpopo Plain 1.01 Ecoregion (sites 8 – 10), only concentrations of Al, As, Cu, Mn and Hg were detected at most sites. Aluminium and As were recorded at Site 8 and 9 in summer. Aqueous concentrations of Al detected at sites 8 and 9 were 0.171 mg/L and 0.154 mg/L, respectively. Conversely a constant concentration of 0.001 mg/L was reported for arsenic at both sites. Copper and Mn were the most abundant metals recorded between sites within this region for both surveys. Sites 8, 9 and 10 had insignificant concentration of Cu and Mn with highest values of 0.128 mg/L at Site 8 and 0.078 at Site 9 during winter and summer, respectively (Table 3.3). A mean concentration of 0.002 mg/L for Hg was only detected at Site 9 during the summer survey.

Since toxic metals (viz. Cd, Cr, Pb, Zn) were below the detection level at all ten sites these metals will not be discussed further in this study.

Table 3.3: Metals in the water column recorded from sampling sites in Nwanedi (*n* = 9) and Luphephe (*n* = 1: Site 1) rivers during surveys conducted in summer (S) and winter (W).

	Sampling site/Season																				
Metals (mg/L)	**Site 1**		**Site 2**		**Site3**		**Site 4**		**Site 5**		**Site 6**		**Site 7**		**Site 8**		**Site 9**		**Site 10**		Water Quality Guidelines
	S	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	
Al	<0.100	<0.100	<0.100	<0.001	<0.100	<0.001	<0.100	<0.001	<0.100	<0.001	0.216	<0.001	0.134	<0.001	0.171	<0.001	0.154	<0.001	<0.001	<0.001	7 pH < 6.5 & 10 pH > 6.5
As	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	7
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.15
Cr	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	(IV) 7, (III) 12
Cu	<0.010	0.126	<0.010	0.124	<0.010	0.123	<0.010	0.126	<0.010	0.126	<0.010	0.125	<0.010	0.126	0.126	0.128	<0.010	0.124	0.125	0.126	0.3 (Soft < 60)
Pb	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.2
Mn	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.143	<0.025	0.045	0.027	0.060	0.050	0.031	0.028	0.031	0.032	0.078	0.052	<0.025	0.045	180
Hg	<0.0001	<0.001	<0.0001	<0.001	<0.0001	<0.001	<0.0001	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.04
Zn	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	2

Key: 1 – DWAF 1996a – South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems. 2 – Cells indicated with a “<” represents values that are below detection.

3.4 DISCUSSION

3.4.1 PHYSICO-CHEMICAL PARAMETERS

Temperature

Temperature is a system variable that determine the transfer of heat within water molecules. In surface waters, temperature can affect biological processes of aquatic organisms, for example metabolism, growth and reproduction. Additionally, the distribution and survival of aquatic organisms depend on temperature (Chapman 2021). The variation in temperature is subjected to climatic conditions such as air circulation, humidity, cloud cover and time of the day. Similarly, geographical features such as altitude, latitude, seasons, water flow and depth can affect water temperatures (Chapman 2021). In South Africa, the temperature of inland waters ranges from 5 – 30°C and most organisms have a specific temperature through which their general wellbeing is enhanced (DWAF 1996a; Dallas and Day 2004). Temperature affects solubility and concentration of many physical and chemical variables in water (Dallas and Day 2004). Moreover, the oxygen consumption and decomposition of organic matter increases with increasing levels of respiration of aquatic organisms in warm waters (Bartram and Balance 1996).

The water temperature recorded from all ten sites showed little variation between summer and winter readings for a given site. Water temperatures were reported to increase with decreasing elevation with most water temperatures reported to be higher in summer with the exception of Site 1 and 2 where water temperatures were higher in winter. This was thought to be attributed to environmental conditions such as cloud cover and rainfall when sampling and that these sites were located at higher altitudes within the Soutpansberg mountains. For example, due to a change in elevation, a minimum of 13.53°C was recorded at Site 1 increasing to a maximum of 27.43°C at Site 10, which is in the Limpopo Plain ecoregions. The rainfall at the upper sites could have caused the temperature to be lower during the sampling period in summer. Moreover, lower water temperatures at the Soutpansberg 2.01 Ecoregion can be attributed to differences in the structural aspects of the river, such as an increase in vegetation cover, water volume, deeper channels, channel structure and turbidity that, in turn, reduces the amount of solar energy that heats the water and affects its thermal regime (Dallas and Day 2004). The recorded temperatures fell within limits of not more than 2°C, or by > 10% (DWAF 1996a). A significant difference

($p<0.05$) was detected/reported for summer/winter temperatures between the sites. Therefore, the observed temperatures in the Nwanedi and Luphephe rivers could have had an impact in the distribution of fish and macroinvertebrates as these organisms have different temperature requirements for their optimal survival.

Dissolved oxygen

Dissolved oxygen (DO) is the amount of oxygen dissolved or incorporated in water. Solubility of oxygen in water depends on atmospheric pressure, water temperature and salinity of the water body (Dallas and Day 2004). Dissolved oxygen is one of the most important abiotic factors that is essential for the survival of many aquatic organisms including fish, macroinvertebrates and plants (Chapman 2021). In inland waters, DO enters the water through the direct diffusion of oxygen gas from the atmosphere from turbulences caused when water flows over rapids and/or from the photosynthetic respiration of aquatic plants and phytoplankton (Jallet et al. 2016). Factors that affects the saturation of DO concentration in water are high temperature, oxidisable organic matter and high concentrations of suspended materials (DWAF 1996a). DWAF (1996a) stated that a decline in DO concentrations adversely affect the health of most aquatic organisms with most fish dying in DO concentrations below 2 mg/L.

In addition, reduction of DO concentrations in aquatic systems may result in the loss and death of species which have a low environmental tolerance. In southern Africa, most aquatic organisms are suitably adapted to DO concentrations of 80 – 120% saturation (DWAF 1996a). Since oxygen dissolves better in cool water than warm water (Dallas and Day 2004; DWAF 1996a), DO concentrations in this study fell within the targeted quality range of 80 – 120%. The DO readings were significantly different ($p<0.05$) between sites and seasons with DO concentrations decreasing from upstream to downstream and with DO levels in summer being lower than those recorded during the winter survey. Although, river temperatures were higher in the Limpopo Plain 1.02 and 1.01 ecoregions, DO concentrations remained high in these regions due to the presence of fast flowing rapids and wind action as this region of the river are broader and more susceptible to water turnover/mixing caused by winds blowing over surface waters.

Water pH

Water pH refers to the concentration of charged particles (hydrogen ions) in the water ranging from acidic to alkaline. Davies and Day (1998) state that most freshwater ecosystems have a pH range of 6 to 8. Water pH plays a crucial role in regulating the reproduction and growth of most aquatic organisms. The pH recorded at the Soutpansberg 2.01 Ecoregion were slightly acidic to alkaline and throughout the study. The pH at sites 1 and 2 was acidic and different from the pH of 7.00 and 6.80 recorded by Angliss et al. (2007). This acidification at the upper reaches could be attributed to natural aspects such as acid rain and soil that is acidic. Additionally, leaching from certain types of vegetation in the catchment could also cause the pH to decrease (Dallas and Day 2004). At the lower reaches of the Soutpansberg ecoregion (Site 4), the pH became slightly alkaline and matched with the pH of 7.2 to 8.0 as recorded by Madanire-Moyo et al. (2010), Madanire-Moyo et al. (2012) and Mbokane et al. (2015). This might be due to the increased levels of suspended ions in the water, which act as a buffer within the distal part of the Soutpansberg 2.01 Ecoregion as compared to the upper reaches and to the previous study by Mbokane et al. (2015). In the Limpopo Plain 1.02 and 1.01 ecoregions, outside and in close proximity to the reserve, the pH was alkaline (range 8.05 – 8.94) along all sampling sites for both summer and winter. Although, there is no pH related impacts such as industries in this ecoregion, there reported pH could be due to the presence of photosynthetic organisms (e.g., algae) which may be the cause of alkalinity because water pH increases when carbon dioxide is removed (Dallas and Day 2004). The pH levels in the entire catchment fell within the permissible TWQR value of not varying by more than 5%.

Electrical conductivity

Electrical conductivity (EC) is defined as the capability of water to conduct electrical current. This depends on the availability of major ions that can dissolve in water (e.g., SO_4, NO_3, K, C and Mg). In the current study, the EC in water was found to increase with decreasing elevation with higher levels recorded during the summer survey with lower levels recorded during the winter survey. The EC levels were lower in the Soutpansberg 2.01 Ecoregion and increased in the intermediate to the lower reaches

of the Nwanedi River in the Limpopo Plain 1.02 and 1.01 Ecoregion, respectively. The low levels of EC at the Soutpansberg 2.01 Ecoregion (sites 1 – 5) could be due to lower anthropogenic factors impacting water quality and the EC in the upper catchment of these rivers. Additionally, the EC levels recorded at Site 4 matched with the conductivity levels of 7.5 -10.5 mS/m recorded by Madanire-Moyo et al. (2010); Madanire-Moyo et al. (2012) and Mbokane et al. (2015) when conducting research in Nwanedi-Luphephe Dam. Consequently, high levels of EC recorded at the Limpopo Plain 1.02 and 1.01 ecoregions (sites 6 – 10) could be attributed to agricultural runoff which contributes to siltation and inorganic salts. Thus, there was a significant difference ($p<0.05$) for EC concentrations recorded between the sampling sites.

Total dissolved solids

Total dissolved solids (TDS) refers to the water body's natural constituents, which comprise charged inorganic and organic substances as well as minerals, salts, cations, and anions (DWAF 1996a). Total dissolved solids concentrations in natural systems vary because of different rates of mineral dissolution in rocks, soils, and the decomposing of plant matter (DWAF 1996a). The TDS levels in all inland waters should not vary by more than 15% from the normal cycles of the water body under unimpacted conditions at any time of the year (DWAF 1996a). Moreover, no baseline TDS values have been published to allow for comparison or to determine whether the percentage change deviate from the normal. However, the TDS levels in all sampling sites were found to increase with the decrease in elevation with the highest levels reported in summer and lowest levels reported in winter. The lower TDS concentrations were recorded in the Soutpansberg 2.01 Ecoregion, whereas intermediate TDS levels were reported in the Limpopo Plain 1.02 Ecoregion, with higher TDS levels reported downstream of the Nwanedi River in the Limpopo Plain 1.01 Ecoregion. The high levels of TDS reported in the Limpopo Plain ecoregions (sites 6 – 10) might be attributed to the presence of dissolved major ions in water from surface runoff from cultivated lands. Other factors that facilitated the high levels of TDS might be due to domestic or livestock effluent downstream near the banks of the Nwanedi River. In aquatic ecosystems, high levels of TDS could affect the growth of organisms and lead to death in some cases (DWAF 1996a). The observed TDS between sites and seasons varied significantly ($p<0.05$).

Salinity

Salinity refers to the amount of salt dissolved in a body of water (Davies and Day 1998). Salinity concentrations were lower at the Soutpansberg ecoregion 2.01 and higher as the rivers drains into the Limpopo Plain (1.02 and 1.01) ecoregions, respectively. The possible reason for higher salinity in the bottom ecoregion (sites 8 – 10) could have been due to the accumulation of siltation and inorganic salts from irrigation water and runoff from domestic and cultivated lands from the surrounding area. The lower water velocity in the lower reaches could have resulted in higher salinity levels as ions have enough time to settle and ionise. Additionally, elevated evaporation in summer could have resulted in high salinity levels as dissolved ions and salts become too concentrated. The high levels of salinity can affect the physiological and metabolic processes of aquatic organisms (Noyes et al. 2009). Salinity levels were statistically significant ($p<0.05$) between sites and seasons. The TWQR limit (<0.05 ‰ or 0.5 ‰) at sites 8 to 10 in the Limpopo Plain 1.01 Ecoregion was exceeded.

3.4.2 NUTRIENTS – NITROGEN COMPOUNDS AND ORTHOPHOSPHATE

Ammonia

Inorganic nitrogen levels in reduced form (NH_3) occurred at a constant concentration of 0.1 mg/L and categorised the rivers as oligotrophic systems in all three ecoregions. In the Soutpansberg 2.01 Ecoregion and Limpopo Plain 1.02 Ecoregion, NH_3 occurred only in winter at all sites, whereas, in the Limpopo Plain 1.01 Ecoregion, downstream of the Nwanedi River, NH_3 occurred at all sites for both seasons, except at Site 9 in summer where levels were below detection (Table 3.2). A possible reason for the small amount of NH_3 in the upper reaches could be due to free NH_3 which is present in the air, soil and water. Consequently, the NH_3 concentration at the lower reaches, could be due to decomposing organic matter and/or be a result of soil erosion, which is also connected to clay minerals (DWAF 1996a). Additionally, NH_3 could have occurred as a metabolic waste product from fish (Dallas and Day 2004). Moreover, the occurrence and quantity of NH_3 in the lower reaches of the river may be influenced by factors such as the presence of other chemical species or constituents, their relative quantities, as well as other water

parameters like pH, temperature and DO content (Dallas and Day 2004). Across all sites for both surveys the concentration of NH_3 remained within the permissible TWQR value of < 7 mg/L.

Nitrate

Nitrate is the end product of the aerobic stabilisation of organic nitrogen which occurs in abundance in aquatic environments (Dallas and Day 2004). In nature, NO_3 and NO_2 co-exist. However, it has been reported that NO_3 is more abundant in inland water bodies because it undergoes chemical reduction before being converted to atmospheric nitrogen (N^2) by nitrobacteria (Davies and Day 1998). Similar to NO_2, sewage discharge and agricultural runoff have a significant impact on NO_3 concentrations, which in turn encourages algal growth (Dallas and Day 2004). There are no TWQR guidelines for NO_3 in aquatic environments, however, NO_3 ranges of 0 to 6 mg/L are prescribed for residential use (DWAF 1996b) and 300 mg/L for aquaculture (DWAF 1996c). Nitrate is the least harmful of the inorganic nitrogen molecules, but it may be toxic at concentrations of 10 mg/L and higher, which is indicative of water contamination. Nitrate concentrations in the Soutpansberg 2.01 Ecoregion were largely detected in winter, with aqueous levels ranging from 0.1 mg/L to 0.7 mg/L. The possible source of NO_3 in this ecoregion could be from leaching into the soil and irrigation water containing nitrogen fertilizer (Craswell 2021). In the Limpopo Plain 1.02 Ecoregion, NO_3 was only recorded at Site 7 during both surveys. In the Limpopo Plain 1.01 Ecoregion, NO_3 was recorded in all sites. This could be the result of anthropogenic activities, agricultural and domestic runoff from local settlements in the middle and lower parts of the Nwanedi River. In the upper reaches NO_3 concentrations were reported to be within the permissible TWQR limit of < 0.5 mg/L, indicative of an oligotrophic system (DWAF 1996a) increasing from 0.5 mg/L to 2.5 mg/L, which is indicative of mesotrophic conditions (DWAF 1996a), in the intermediate and lower reaches of the Nwanedi River.

Orthophosphate

Orthophosphate in this study was measured as phosphorus (P). According to DWAF (1996a) P exist in nature as inorganic phosphate (PO_4), polyphosphates,

metaphosphates, pyrophosphates or orthophosphate. Phosphorus may enter the inland waters as a result of rock weathering, leaching of PO_4 salts or decomposition of organic matter (DWAF 1996a). Orthophosphate concentrations in the catchment were only recorded above detection in winter at Site 5 (0.2 mg/L) and Site 7 (0.1 mg/L). This could be due to the breakdown of organic matter and through domestic and agricultural practices (Dallas and Day 2004). There are some settlements and cultivated lands near Site 7 along the Nwanedi River, which could facilitate the introduction of orthophosphate. Based on the levels of orthophosphate detected and following the criteria by DWAF (1996a) the Nwanedi and Luphephe rivers can be categorised as oligotrophic systems.

Sulphate

Sulphate (SO_4) as sulphur is the essential macronutrient needed in small amounts by animals for proteins and enzymes synthesis. Sulphur in aquatic systems occur in the form of SO_4 (Dallas and Day 2004). Sulphate concentrations where below the detection level in the Soutpansberg 2.01 Ecoregion as the catchment area is void of any visible signs of industrial activities e.g., power plants that use coal to produce electricity. In the Limpopo Plain 1.02 Ecoregion (sites 6 and 7) and Limpopo Plain 1.01 Ecoregion (sites 8 – 10) the SO_4 concentrations were detected to be above detection and ranged from 10 mg/L to 38 mg/L and 30 mg/L to 122 mg/L, respectively. The possible cause for these higher SO_4 concentrations could be due to the availability of agricultural runoff and domestic activities in these regions. There are currently no guidelines for SO_4 concentrations for aquatic ecosystems.

3.4.3 TOXIC METALS IN SOLUTION

Aluminium

Aluminium (Al) is the most abundant element in the earth's crust and its solubility in water is pH dependent. Aluminium is insoluble in neutral pH, but soluble under acidic (pH < 6.5) and alkaline (pH > 6.5) conditions. Thus, when abundant, Al becomes highly toxic to aquatic organisms when the pH is below 6.5 but less toxic when the pH is above 6.5 (DWAF 1996a) with high levels of soluble Al being toxic to variety of aquatic organisms. However, Al toxicity depends on the species and life

stages of organisms, calcium concentrations in solution and water pH. Aluminium is a non-essential metal for living organisms and its biochemical mechanisms of toxicity in organisms is poorly understood (Dallas and Day 2004). Although Al is naturally present in aquatic environments (e.g., produced from organic molecules), acid mine drainage can cause concentrations to rise (Dallas and Day 2004). In this study, Al concentrations in the upper reaches (i.e., Soutpansberg 2.01 Ecoregion) of the Nwanedi and Luphephe rivers were lower (<0.100 mg/L) during both seasons. Whereas, at sites 6 to 8 in the Limpopo Plain 1.02 and 1.01 ecoregion, Al concentrations were only higher during the summer survey. Although DWAF (1996a) indicated that elevated concentrations of Al are normally associated with acid mine drainage or acid rain, however, it is not clear what could have been the source of Al in sites 6 to 8. The possible reason for the higher Al concentrations at these ecoregions might have been due to underlying geology and not analysed in this study. Aluminium concentrations were within the permissible TWQR of aquatic ecosystem (DWAF 1996a:Table 3.3).

Arsenic

Arsenic (As) is a metalloid that is toxic to aquatic life both, marine and freshwater, and is also known to cause cancer in humans (DWAF 1996a). Although the element As is insoluble, many of its compounds are soluble in water. Depending on the pH and redox potential of the water, As can exist in several oxidation states, including III. IV, V, and -III. Arsenic (III) and (V) are the two most common forms, both of which form stable compounds with carbon, resulting in numerous organo-arsenical compounds. Elemental As also readily combines with many metals to form toxic arsenide salts (DWAF 1996a). Arsenic concentrations were below detection at all sampling sites, except in the Limpopo Plain 1.01 Ecoregion (sites 8 and 9), where As was found to be constant at 0.001 mg/L. This could be due to As compounds such as runoff fertilisers and pesticides from cultivated lands in this ecoregion (Foata et al. 2009). The reported concentration of As was within the TWQR of aquatic ecosystem of not more than 10 mg/L.

Copper

Copper (Cu) is a widely used metal found in the rocks and minerals in the earth's crust and is commonly found as an impurity in mineral ores. Copper occurs readily in water bodies through the weathering of rocks and dissolution of Cu minerals (DWAF 1996a). Copper occurs in three states of oxidation namely, as metallic Cu (0), cuprous Cu (I), and cupric Cu (II). Its toxicity is determined by the solubility and chemical state of this chemical in water. To aquatic organisms, free cupric copper ions (Cu^{2+}) are the most toxic, whereas complex forms are the least toxic (DWAF 1996a). Copper has a high mobility and solubility in acidic water and precipitates in alkaline water thereby becoming non-toxic. Furthermore, as water hardness increases, copper's toxicity is reduced. In all sampling sites, except sites 8 and 10 (Table 3.3), Cu was only recorded during the winter survey with the highest concentration of 0.127 mg/L recorded at Site 8. This might be due to low rainfall to aid with dilution along with accelerated evaporation in winter. Copper concentrations recorded in sites 8 and 10 might be due to an underlying geology, runoff from domestic and agricultural practices. The reported concentrations of Cu were within the normal range of not more than 0.3 mg/L for water hardness of less than 60 mg/L.

Manganese

Manganese (Mn) is a micronutrient that is required by both plants and animals (Dallas and Day 2004). Manganese is a necessary component of NO_3 assimilation and a catalyst in many enzyme systems in animals, plants and bacteria. The occurrence of Mn in several ores makes it the eighth most common metal in nature. Manganese does not naturally exist as a metal in aquatic ecosystems; instead, it is present as a variety of salts and minerals, typically in combination with iron compounds (DWAF 1996a). In the Soutpansberg 2.01 Ecoregion, Mn concentrations were only recorded at Site 4 in summer and Site 5 during both seasons (Table 3.3). In the intermediate and lower reaches of the system, Mn concentrations were detected during summer and winter with the highest concentrations of 0.060 mg/L and 0.078 mg/L, respectively. The possible reason for the occurrence of Mn could be due to weathering of metamorphic and sedimentary rocks in the catchment areas. Manganese levels fell within the permissible TWQR limit of <180 mg/L (DWAF 1996a).

Mercury

Mercury (Hg) is the chemical element that in geological occurrence, is relatively rare, and its concentration is typically very low on earth's crust. In the natural aquatic environment, mercury can be found in the form of three oxidation states, which are in the metal, Hg (I) and Hg (II) form (DWAF 1996a). High amounts of mercury may be found in water that have been contaminated by industrial sources, or close to facilities that use or release Hg or its derivatives (DWAF 1996a). Dissolved mercury in aquatic environments is of concern because of it is highly toxic to aquatic animals and is found to bio-accumulate in the food chain. Mercury concentrations were below detection in the Soutpansberg 2.01 Ecoregion. In the Limpopo Plain 1.02 and 1.01 ecoregions, Hg concentrations were only detected in summer at sites 6 and 7 (0.001 mg/L) and Site 9 (0.002 mg/L). The occurrence of Hg at the Limpopo Plain ecoregions could be due to atmospheric deposits and by human activities. Mercury concentrations were within the recommended TWQR value of not more than 0.04 mg/L (DWAF 1996a).

3.5 CONCLUSION

Most of the water quality constituents were within the TWQR for aquatic ecosystems as prescribed by the DWAF (1996a) guidelines. However, due to elevation, seasonal changes and land activities of the catchment, there were variations in some water quality constituents. These include temperature, pH, non-toxic constituents (EC, TDS and salinity) and the nutrient, NO_3. Water temperatures were significantly lower ($p<0.05$) at sites located within the Soutpansberg ecoregion during both surveys. The water pH was slightly acidic to neutral at the upper reaches of the system (sites 1 and 2) and neutral inside the Nwanedi Nature Reserve becoming slightly alkaline in the Limpopo Plain (1.02 and 1.01) ecoregions. The source for alkalinity could have been due to agricultural activities and the presence of photosynthetic organisms (e.g., algae) that resulted in increased water pH when carbon dioxide is removed from the water. Non-toxic constituents such as EC, TDS and salinity were significant ($p<0.05$) between sites and surveys. These constituents were relatively low in the Soutpansberg 2.01 Ecoregion (sites 1 – 5) and elevated in the Limpopo Plain (1.02 and 1.01) ecoregions. However, these constituents were within the TWQR for aquatic ecosystem (DWAF 1996a). The high levels of these

constituents could have been caused by the siltation and inorganic salts from agricultural runoff. Nitrate concentrations categorised the Soutpansberg 2.01 Ecoregion as being oligotrophic with Site 3 and Limpopo Plain (1.02 and 1.01) ecoregions classified as having mesotrophic conditions. Although the SO_4 concentrations were above detection at the Limpopo Plain (1.02 and 1.01) ecoregions, it was unclear what the source could have been. All toxic metals (Al, As, Cu, Mn, Hg) detected were within acceptable TWQR guidelines (DWAF 1996a) for aquatic ecosystems. In conclusion, water quality constituents in general were within acceptable levels, however, there was variation in some constituents across the sites assessed. As a result, to better assess the ecological status of the Nwanedi and Luphephe rivers, the FRAI, MIRAI and fish parasites, as bioindicators, were used.

CHAPTER 4

FISH ASSEMBLAGE AS BIOINDICATORS

4.1 INTRODUCTION

Fish assemblages are a collection of fish species occupying the same habitat that play a crucial role in maintaining the integrity of aquatic systems (Wootton 1991). Knowledge of fish assemblages can be useful as biological indicators for monitoring ecosystems as fish are influenced by changes in aquatic conditions (Naigaga et al. 2011). In freshwater systems, fish assemblages can vary in composition and diversity depending on influential factors such as a change in water quality, habitat availability, anthropogenic factors and the introduction of alien fish species (Dudgeon et al. 2006). Since most fish are relatively sensitive to environmental changes, a knowledge of fish assemblages can prove useful at understanding the ecological state of aquatic systems based on the presence and abundance of fish species within a given habitat (Dudgeon et al. 2006). Thus, for a particular aquatic system, it is important to conduct research with regard to the composition of fish assemblages at regular intervals as part of a monitoring regime.

Globally, various indices have been developed to assess and monitor the health and integrity of aquatic systems. In South Africa, indices implemented to assess and monitor local water bodies are the Fish Assessment Integrity Index (FAII) by Kleynhans (1999), the Sensitivity Index of Biotic Integrity (SIBI) by Kotzé (2001) and the Fish Response Assessment Index (FRAI) protocol developed by Kleynhans (2007). The FRAI was developed to determine the present ecological state (PES) of river systems in South Africa by incorporating the abundances and frequency of occurrence (FROC) of fish species. Consequently, the criteria assigns scores as a percentage and ecological category (EC) to determine the PES of the river under assessment. The reference (expected) list for the fish community and the FROC data were derived from the historic survey (Angliss et al. 2007). In this chapter the FRAI protocol was used to establish the PES of the Nwanedi and Luphephe rivers based on the diversity and abundance of fish caught at 10 sampling sites located along the main stem of the river within the different ecoregions.

4.2 MATERIALS AND METHODS

As described in Chapter 2, fish were collected using electroshocking and a cast net during a period of high flow in the beginning of summer (October 2021) and during a period of moderate flow in winter (May 2022). To determine the fish species diversity, the Shannon Weiner Diversity Index (H') was performed. The FRAI was implemented to categorise the state of the river for each ecoregion and the river as a whole (see Appendix A: Table 2A to 8A for procedures undertaken).

4.3 RESULTS

4.3.1 FISH ABUNDANCE DISTRIBUTION AND RICHNESS

From the Soutpansberg 2.01 Ecoregion a total of 352 individuals comprising 21 fish species were recorded (Table 4.1). The spatial distribution of fish abundance showed the lowest number of individuals recorded (*n* = 3) at Site 2 and the highest (*n* = 185) from Site 5. From Table 4.1., sites 3 and 4 were shown to have the highest species richness scores of 12 and 13, respectively. The lowest species diversity (H' = 0) was recorded at sites 1 and 2 with the highest at Site 3 (H' = 1.99), followed by Site 4 (H' = 1.96) and Site 5 (H' = 1.45). The expected fish species (Skelton 2001; Kleynhans 2007; Angliss et al. 2007; Skelton 2016) that are least tolerant to a change in water quality recorded in this ecoregion were *Amphilius uranoscopus* (Pfeffer, 1889), *Enteromius eutaenia* (Boulenger, 1904), *Enteromius lineomaculatus* (Boulenger, 1903) and *Chiloglanis pretoriae* van der Horst, 1931.

Limpopo Plain 1.02 Ecoregion showed the spatial distribution of fish species having a total of 188 individuals comprising nine fish species (Table 4.1). Sites 6 had the highest fish abundance (*n* = 153) with a species richness of eight reported while Site 7 had the lowest abundance (*n* = 35) and a species richness of five. The spatial distribution of fish species recorded the highest species diversity of H' = 1.54 at Site 6 with the lowest of H' = 1.44 reported for Site 7. The fish species reported by Skelton (2001), Kleynhans (2007) and Angliss et al. (2007) to be least tolerant to water quality changes recorded in this ecoregion was *E. lineomaculatus* at sites 6 and 7.

For Limpopo Plain 1.01 Ecoregion a total of 121 individuals and a species richness of eight was reported at Site 9 (Table 4.1). A species diversity of H' = 1.51 for this ecoregion was revealed to be the lowest when compared to the middle and upper

ecoregions. In this region fish species caught and are reported by Skelton (2001), Kleynhans (2007) and Angliss et al. (2007) to be moderately tolerant of water quality changes were *Clarias gariepinus* (Burchell, 1822) and *Oreochromis mossambicus* (Peters, 1852).

Table 4.1: Fish species recorded at each sampling site and ecoregion within the Nwanedi and Luphephe rivers for surveys conducted in summer (October 2021) and winter (May 2022).

	Ecoregions/sampling sites															
	Soutpansberg 2.01										Limpopo 1.02				Limpopo 1.01	
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 9	
Sampling survey (seasonal)	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**
Anguilla mossambica	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Amphilius uranoscopus	5	4	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	-	-	-	-	-	-	-	-	-	17	-	-	-	-	49	13
Chiloglanis pretoriae	-	-	-	-	-	-	-	32	53	68	55	17	2	13	-	7
Clarias gariepinus	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Clarias theodorae	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Enteromius annectans	-	-	-	-	-	-	4	-	-	-	2	7	9	-	-	-
Enteromius bifrenatus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Enteromius eutaenia	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
Enteromius lineomaculatus	-	-	-	-	4	-	5	-	-	2	-	5	7	-	-	-
Enteromius trimaculatus	-	-	-	-	5	5	-	-	1	-	1	2	-	-	-	-
Enteromius unitaeniatus	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Labeo cylindricus	-	-	-	-	1	1	-	1	8	15	12	3	2	1	7	-
Labeo molybdinus	-	-	-	-	-	1	1	1	-	1	-	-	-	-	-	-
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-
Labeobarbus marequensis	-	-	-	-	3	4	2	2	-	2	-	-	1	-	3	6
Marcusenius macrolepidotus	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens	-	-	-	-	2	7	3	6	-	-	-	-	-	-	-	-
Oreochromis mossambicus	-	-	-	-	-	-	-	-	-	-	8	11	-	-	3	5
Petrocephalus wesselsi	-	-	-	-	1	-	8	5	4	4	-	-	-	-	-	-
Pseudocrenilabrus philander	-	-	-	-	2	18	13	8	4	5	10	18	-	-	-	-
Tilapia rendalli	-	-	-	-	-	1	-	-	-	-	-	2	-	-	4	19
Tilapia sparrmanii	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Total abundance per season	**5**	**4**	**-**	**3**	**20**	**40**	**37**	**58**	**71**	**114**	**88**	**65**	**21**	**14**	**70**	**51**
Total richness per season	**1**	**1**	**-**	**1**	**9**	**8**	**8**	**10**	**6**	**8**	**6**	**8**	**5**	**2**	**6**	**6**
Total abundance per site	**9**		**3**		**60**		**95**		**185**		**153**		**35**		**121**	

Total richness per site	**1**	**1**	**12**	**13**	**10**	**8**	**5**	**8**
H'	**0**	**0**	**1.99**	**1.94**	**1.45**	**1.56**	**1.44**	**1.51**
Total abundance per ecoregion			**352**			**188**		**121**
TCRPE			**21**			**9**		**8**
H' per ecoregion			**2.03**			**1.64**		**1.51**
TREBA			**20**			**11**		**12**
Total expected species per ecoregion			**23**			**22**		**28**

Keys: "-" = Not recorded; H' = Shannon Weiner Diversity Index; TCRPE = Total current richness per ecoregion; TREBA = Total richness per ecoregion by Angliss et al. (2007).

4.3.2 FISH RESPONSE ASSESSMENT INDEX

The present ecological state of fish communities varied seasonally between the three ecoregions (Table 4.2). The Soutpansberg 2.01 Ecoregion had the highest FRAI scores of 87.5% during summer reducing to 84.6% in winter. The Limpopo Plain 1.02 Ecoregion represented the second highest FRAI scores of 55% during summer that improved to 65.1% in winter. The Limpopo Plain 1.01 Ecoregion had the lowest FRAI score of 44.0% during summer that reduced to 39.1% in winter. Overall, the Soutpansberg 2.01 Ecoregion had the highest FRAI scores during both seasons than the Limpopo Plain ecoregions (Table 4.2).

Table 4.2: The summary of FRAI scores (%) and ecological category (EC) for the three ecoregions in the Nwanedi and Luphephe rivers during summer (October 2021) and winter (May 2022).

Ecoregions	Summer		Winter	
	FRAI score (%)	EC	FRAI Score (%)	EC
Soutpansberg 2.01	87.5	A/B	84.6	B
Limpopo Plain 1.02	55.0	D	65.1	C
Limpopo Plain 1.01	44.0	D	39.1	D/E

Key: EC = Ecological category

To compare the results with the historic work undertaken and following the approach by Angliss et al. (2007), data derived from the two surveys were pooled (Table 4.3). In the Soutpansberg 2.01 Ecoregion, the EC values varied spatially and temporally between current and historic studies, that is, having classes of C/D and C, respectively. The Limpopo Plain 1.02 Ecoregion had an EC of class D, which deteriorated from historic class C. Lastly, the Limpopo Plain 1.01 Ecoregion deteriorated from category class D to E (Table 4.3).

Table 4.3: The summary of FRAI scores (%) and ecological categories (EC) between current study and historic work done by Angliss et al. (2007) for the three ecoregions in the Nwanedi River Catchment.

Ecoregions	FRAI Score (%)	EC	FRAI Score (%)	EC
	Current study		Angliss et al. (2007)	
Soutpansberg 2.01	61.7	C/D	77.2	C
Limpopo Plain 1.02	42.9	D	62.7	C
Limpopo Plain 1.01	27.9	E	42.1	D

Key: EC = Ecological category

4.3.3 CANONICAL CORRESPONDENCE ANALYSIS

When conducting a canonical correspondence analysis (CCA) most fish species were shown to be strongly associated with the environmental variables measured (Figure 4.1). *Enteromius eutaenia* and *E. lineomaculatus* were strongly associated with high DO concentrations at Site 3. Similarly, species namely *Anguilla mossambica* Peters 1852, *Coptodon rendalli* (Boulenger, 1896*), Enteromius unitaeniatus* (Günther, 1866), *Micralestes acutidens* (Peters, 1852) and *Petrocephalus wesselsi* Kramer and Van Der Bank, 2000 collected from Site 4 were closely associated with DO levels. In turn, *Labeo rosae* Steindachner, 1894 from Site 9 were shown to be positively correlated with EC, TDS and salinity and strongly correlated with axis 2 (Table 4.4). Alternatively, *A. uranoscopus* was strongly associated with sites 1 and 2 and the environmental conditions at these sites.

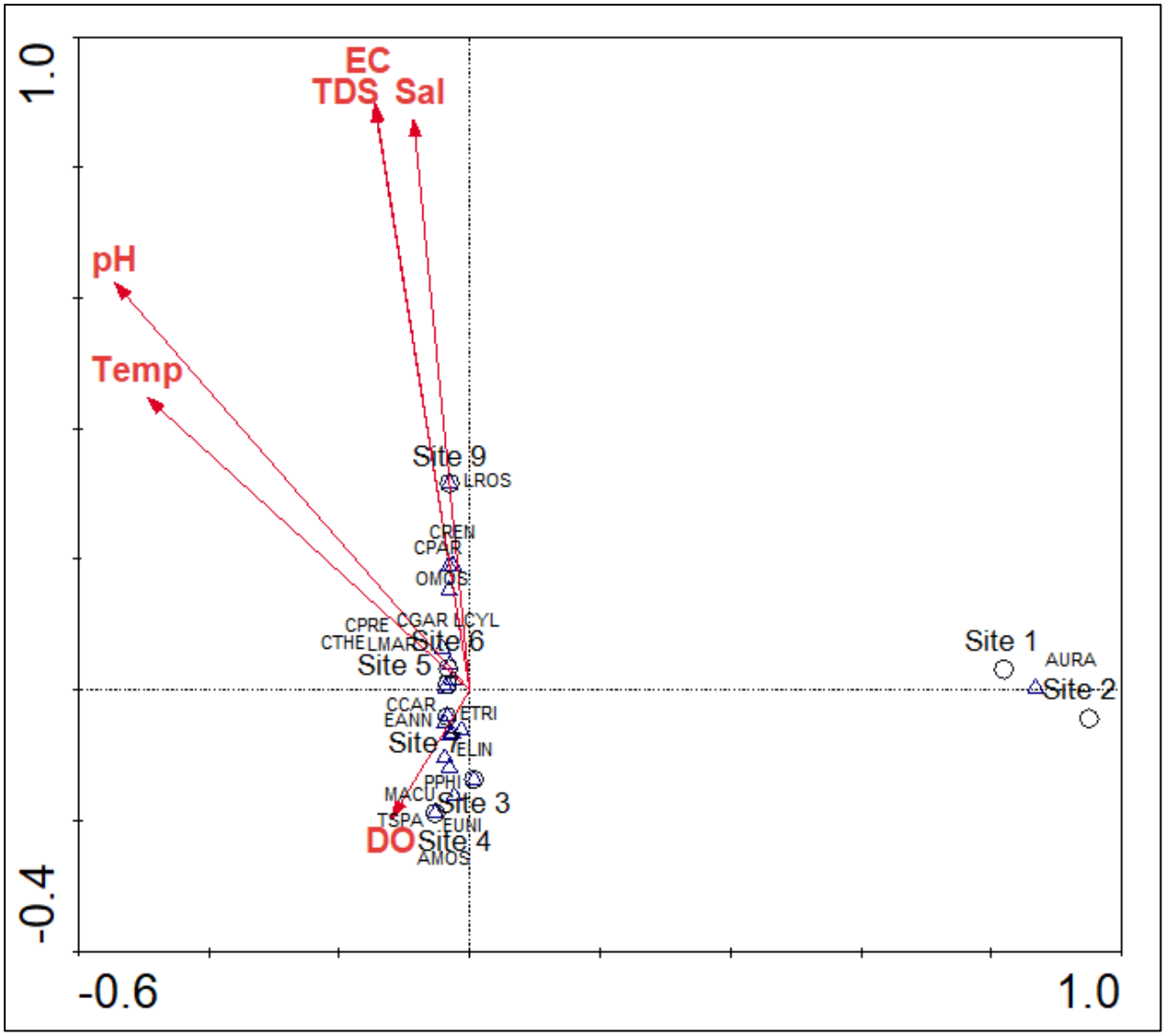

Figure 4.1: CCA plot of the relationship between physico-chemical parameters and fish species that were sampled at eight sites (summer and winter data were combined) within the Nwanedi and Luphephe rivers. Key: temperature (Temp), electrical conductivity (EC), total dissolved solids (TDS) and salinity (Sal).

From the CCA the first axis (ʎ1 = 0.51) displays the relationship between environmental with biological variables and the second axis the residual variation (ʎ2 = 0.21). Axis 1 and 2 accounted for 72.8% of the cumulative variation (see Table 4.4).

Table 4.4: The summary of CCA results showing the association between environmental factors and fish sampled at 8 sites in the Nwanedi and Luphephe rivers.

Statistic	Axis			
	1	2	3	4
Eigenvalues	0.986	0.413	0.266	0.137
Explained variation (Cumulative)	51.3	72.8	86.6	93.8
Pseudo-canonical correlation	0.994	0.996	0.963	0.986
Total variation				2.180

4.4 DISCUSSION

4.4.1 FISH ABUNDANCE DISTRIBUTION AND RICHNESS

The Soutpansberg 2.01 Ecoregion form part of the headwaters of the Nwanedi and Luphephe rivers. This ecoregion represented most of the fish fauna caught during the study in terms of high abundance, species richness and diversity. The upper sites were inhabited by *A. uranoscopus*. The presence of *A*. *uranoscopus* at sites 1 and 2 is an indication of good water quality and habitat conditions. According to Skelton (2001), this species is adapted to mountain streams requiring aerated and good water quality.

In the middle of this ecoregion, which include sites situated in the Nwanedi Nature Reserve, a high species diversity (H' = 1.99) was recorded at Site 3. This could be due to a wide variety of habitats associated with this site. Among 10 species that were recorded, *Labeobarbus marequensis* Smith, 1841*, M. acutidens* and *P. philander* were recorded in high abundance. In the confluence of the Nwanedi and Luphephe rivers, i.e., Site 4, there was a high species richness including the abovementioned species at Site 3 and a single migratory longfish eel *A. mossambica*. According to Vannote et al. (1980), high species richness in headwaters is associated with high oxygen content and the presence of riparian vegetation that provides energy to the watercourse. Leaves from riparian vegetation add shelter to microorganisms that aid as food for most headwater dwelling fish (Uieda and Uieda 2001). This could also be the reason for the high species richness recorded at Site 4 as the area was covered by a thick riparian canopy at the time of sampling.

At Site 5, which is situated just outside the nature reserve before draining into the Limpopo Plain 1.01 Ecoregion, there was a high number of individuals (*n* = 185) recorded collectively for both surveys. The high abundance could have been attributed to the availability of velocity-depth classes and variability in biotopes, which include riffles and rapids. However, the species richness was less than that recorded at Site 4. This could be due to reduction in riparian vegetation as the reach opens into the flood plain. In Site 5, the presence of the snake catfish *Clarias theodorae* Weber, 1897 might be associated with the availability of its preferred food source, e.g., freshwater shrimps as alluded to in Skelton (2001).

In summary, the Soutpansberg 2.01 Ecoregion had the highest abundance, species richness and species diversity (H' = 2.03), which indicates that most of the reference fish species were recorded. The current study recorded 21 species while Angliss et al. (2007) recorded 20 species. The current results indicate the pristine nature of the sites associated with this ecoregion. The construction of the Nwanedi-Luphephe Dam provides a strong refuge for hardy fish while the outflow from the dam improves species richness in the downstream sites, i.e., sites 4 and 5. In addition, the Nwanedi Nature Reserve acts as a hotspot for species richness and diversity due to the absence of human impacts linked to the sites within the reserve. The CCA graph confirms DO being a significant contributor to the distribution of fish fauna in the Soutpansberg 2.01 Ecoregion. This is confirmed by the presence of *E. eutaenia*, *E. lineomaculatus, E. unitaeniatus, M. acutidens* and *P. wesselsi* at sites 3 and 4; species that thrive in waters high in oxygen concentrations.

The Limpopo Plain 1.02 Ecoregion, which include sites 6 and 7, showed a decline in fish abundance, species richness and fish diversity as compared to the Soutpansberg 2.01 Ecoregion. A reason for the decline in fish numbers could be due to fewer sites being surveyed and the dam providing a refuge for fishes downstream. Consequently, openness of the reach into the flood plain resulted in reduction of riparian vegetation, water velocity and increased water temperature that negatively affects the distribution of fish (Murray and Innes 2009). However, these conditions favours high abundance of fish species that are tolerant or moderately intolerant to modified habitat and water quality changes (e.g., *Enteromius annectens* (Gilchrist & Thompson, 1917), *Labeo cylindricus* Peters, 1852).

The substrate at sites 6 and 7 was dominated by boulders of different sizes and the water was fast-shallow and slow-deep. These biotopes are highly favourable for species such as *C. pretoriae* and *E. annectens*. The distribution and diversity of fish species seemingly declining (H' = 1.64) when compared to the Soutpansberg 2.01 Ecoregion. This is due to general reduction of sensitive species that require conditions associated with headwaters descriptive of the Soutpansberg 2.01 Ecoregion. In addition to factors affecting species richness at sites 6 and 7, the impacts of domestic and agriculture from the surrounding area could be negatively influencing the diversity and abundance of fauna in the ecoregion.

Summing up the Limpopo Plain 1.02 Ecoregion, fish abundance, species richness and diversity were lower as compared to the Soutpansberg 2.01 Ecoregion. The current study recorded nine fish species, whereas the study by Angliss et al. (2007) recorded 11 species. Previously recorded species not recorded in the current study were *Enteromius paludinosus* (Peters, 1852), *Enteromius radiatus* (Peters, 1853) and *Enteromius toppini* (Boulenger, 1916). Although, *A. mossambica* was recorded at Site 4 in the Soutpansberg 2.01 Ecoregion there are no records of these abovementioned fish species in the entire catchment. It is therefore unclear to what could have resulted in general absence of these species.

Similarly compared to aforementioned ecoregions the abundance of fish species recorded in the Limpopo Plain 1.01 Ecoregion declined at Site 9. This site was the only accessible site that could be surveyed; a site previously selected and surveyed by Angliss et al. (2007) for this region. An inconsistent number of sampling sites per ecoregion could be the reason for the lower or higher abundance or species richness between the different sections of the river. Although, there were spatial variation in species richness between Limpopo Plain ecoregions, Site 9 showed a decrease in abundance (n = 121). This could be due to the general absence of sensitive species in this ecoregion as previously postulated in Angliss et al. (2007). However, tolerant and moderately sensitive fish species to changes in water quality were recorded in low-moderate abundances. These species were *C. gariepinus*, *O. mossambicus*, *T. rendalli*, *Chiloglanis paratus* Crass, 1960, *L. cylindricus* and *L. rosae.* The possible reason for the reduced abundances of moderately intolerant species at Site 9 could be due to modification in water quality, habitat and water velocity.

Consequently, elevated levels of EC, TDS and salinity recorded at Site 9 could be a cause of lower abundance and species richness reported at this site. The high source of these constituents might be the result of land use activities and the fact that water flow is slower in this region. Additionally, Angliss et al. (2007) suggested that the geological formation of the Tshipise Fault could be the reason for higher EC in the ecoregion. Thus, elevation of the abovementioned constituents could be the cause for a lower species diversity (H' = 1.51) being recorded at this site. Moreover, lower species diversity could have been as a result of the absence of certain migratory species such as *E. trimaculatus, L. marequensis, M. acutidens* in this ecoregion.

Summary of the Limpopo Plain 1.01 Ecoregion: the current study recorded eight different species, whereas the historic study recorded 12 species. The differences might be due to the time/period when the survey was conducted whereby certain fish species were not present at the time of sampling as opposed to those previously recorded by Angliss et al. (2007) such as *S. intermedius, Labeo molybdinus* Du Plessis, 1963, *Mesobola brevianalis* (Boulenger, 1908) and *Hydrocynus vittatus* (Castelnau, 1861). The CCA confirmed *L. rosae* in the Limpopo Plain 1.01 Ecoregion to be strongly associated with high concentrations of EC, TDS and salinity.

4.4.2 FISH RESPONSE ASSESSMENT INDEX

The Soutpansberg 2.01 Ecoregion lies in a good ecological category based on the fish communities recorded during summer and winter. The FRAI score of 87.5% and EC of class A/B were obtained for this ecoregion. Based on Kleynhans et al. (2007) interpretations, the EC of class A/B is an indication of unmodified or approximate to natural conditions. This ecoregion had diversity of habitats that are favourable for intolerant species such as *A. uranoscopus*, *E. lineomaculatus* and *C. pretoriae* recorded during both summer and winter, with *E. eutaenia* only recorded during winter survey. The presence of these species indicates seasonal changes having little impact on fish distribution in the Soutpansberg 2.01 Ecoregion. This might be due to the pristine state of the region based on temporal variations in temperature between summer and winter. The presence of fish species such as *A. uranoscopus* and *C. pretoriae*, that prefer fast flowing waters, indicates unmodified physico-chemical parameters. It is evident that most of the reference fish communities (as

indicated in section 4.3.1) were caught during both seasons and contributed to higher FRAI scores being reported during summer and winter.

The Limpopo Plain 1.02 Ecoregion fell under the EC of class D during summer and improved to EC of class C during winter. The EC of class D in summer was interpreted as being a result of large modifications to the natural habitat, species richness and basic ecosystem functions. The decline in EC values was thought to be attributed to a total of 13 out of 22 expected fish species being reported in this ecoregion (Tables 2.1 and 4.1). In this study only nine fish species at sites 6 and 7 were recorded for both surveys, whereas Angliss et al. (2007) recorded a total of 11 fish species. Fewer number of sites could have also resulted in lower scores for this ecoregion.

The Limpopo Plain 1.02 Ecoregion lies in the flood plain area where the water temperatures are higher, especially at Site 7 where the mean temperature was 22.83°C and 19.20°C during summer and winter, respectively. Increase in temperature during summer could have resulted in modified physico-chemical parameters (e.g., EC and TDS), this might have negatively impacted the distribution of sensitive fish species such as small barbs including *E. lineomaculatus.* The occurrence of this species and *C. pretoriae* were in lower abundance. These species prefer headwaters, and their occurrence downstream could be due to fragmentation caused by the construction of the Cross Dam.

In the Limpopo Plain 1.01 Ecoregion, only one site was surveyed and the FRAI score reported during summer survey correlated with that in the Limpopo Plain 1.02 Ecoregion for the same season. Whereas when comparing seasons for the former ecoregion, the EC deteriorated to class D/E in winter. The PES reported for Site 9 is being transitioned from largely to seriously modified conditions as a result of intensified loss of species richness. In this ecoregion, sensitive species to modified physico-chemical changes have never been documented from the available literature (i.e. Angliss et al. 2007). The absence of migratory fish species to upstream in search of suitable habitats during mating season might have been the reason for the reduction in abundances at Site 9. Thus, only one moderately tolerant and two tolerant fish species against physico-chemical changes were recorded at this site. These were *C. paratus*, *O. mossambicus* and *C. gariepinus* with the latter recorded during winter

only. These species are widely distributed and are not restricted to certain sections of the river as they occur where conditions are suitable for growth and survival.

When comparing the PES of the current research with the historic study done by Angliss et al. (2007) the FRAI pooled for summer and winter results for the Soutpansberg 2.01 Ecoregion were revealed to be lower than that of the historic data. The possible reason for a decline in FRAI scores could be attributed to a decrease in species richness from Soutpansberg to Limpopo Plain and the doubling of the sampling effort when combining the survey data for comparative purposes. The combined seasonal abundance of fish for the three ecoregions yielded an ECs of C/D, D and E (Table 4.3), whereas Angliss et al. (2007) categorised the ecoregions as ECs of C, C and D. Although, the sampling sites were consistent with the historic study there was, however, a slight variation in the abundance and richness of fish species between the studies. A further explanation as to the reason for the deterioration of the PES score. However, most of the reference fish species were recorded in the two studies. These included the occurrence of *A. uranoscopus, A. mossambica, C. theodorae, C. pretoriae* and small barbs in the Soutpansberg 2.01 Ecoregion. Additionally, in the present study, the abundances and frequency of occurrence of fish species in the Limpopo Plain ecoregions were generally lower than those recorded by Angliss et al. (2007) and as a result this might have had an impact on the FRAI scores recorded.

These variations, especially in the Limpopo Plain ecoregions, might have been due to decline in the frequency of occurrence of species caught in the present study compared to when the survey data were pooled. Consequently, the loss or lack of available and suitable habitats might have influenced certain species to move upstream. Additionally, changes in the land uses around the Limpopo Plain ecoregions might have had an impact on the water quality and current FRAI results. These include agricultural activities (e.g., water abstraction) together with agricultural runoff taking place within the surrounding flood plain. When comparing the results recorded in this study with those reported by Angliss et al. (2007), the slight deterioration in pH and the difference in the distribution and abundance of the fish recorded in this study may have been attributed to this system being impacted over time by anthropogenic factors not measured here. Moreover, the alien species in the lower reaches of this system might have been elevated when compared to historic

surveys, for example, the alien carp *Cyprinus carpio* Linnaeus, 1758 recorded at Site 7. This carp modifies habitats and competes with native fishes for space and food resources. Additionally, cast netting was unsuccessful in Site 10 indicating possible modifications in habitats of native species, however, these should be considered with caution as more studies should be undertaken to confirm the status of fish distribution in the lower reaches of the systems given the low number of sampling sites in the Limpopo floodplain ecoregions.

4.5 CONCLUSION

The purpose of this chapter was to establish the ecological state of the Nwanedi and Luphephe rivers based on the diversity and abundance of fish collected using different fishing gear at different sites to categorise each of the ecoregions using the FRAI protocol. The results revealed fish communities in the Soutpansberg 2.01 Ecoregion having high abundance and species richness, with numbers decreasing with altitude. These include highest richness of fish species intolerant to physico-chemical parameters such as *A. uranoscopus, E. eutaenia, E. lineomaculatus* and *C. pretoriae*. When comparing the seasons, the FRAI categorised conditions in the Soutpansberg 2.01 Ecoregion to be natural or proximate to natural conditions, the Limpopo Plain 1.02 as moderately to largely modified and Limpopo Plain 1.01 largely to seriously modified. However, when pooling the data to compare with the previous study done by Angliss et al. (2007), the FRAI results revealed the EC to shift from moderately to seriously modified with a decrease in elevation. Based on the CCA results most of the fish species caught in the present study were positively correlated with the environmental variables. These include species such as *E. eutaenia* and *E. lineomaculatus* at sites 3 and *A. mossambica*, *C. rendalli, E. unitaeniatus, M. acutidens* and *P. wesselsi* at Site 4.

In conclusion, the ecological state of the Nwanedi and Luphephe rivers categorised using the FRAI protocol for both surveys were reported to be in a good or close to natural conditions with EC of class A/B in the Soutpansberg 2.01 Ecoregion and fair to poor with ECs of class C and D in the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions, respectively. However, when compared to the findings by Angliss et al (2007), the ecological state of the Nwanedi River catchment in the

Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions based on fish assemblages appear to have deteriorated.

CHAPTER 5

MACROINVERTEBRATE ASSEMBLAGE AS BIOINDICATORS

5.1 INTRODUCTION

Macroinvertebrate communities as biological indicators have been the focus of much research with aquatic macroinvertebrates having been used successfully worldwide to assess the biological integrity of river systems (Rosenberg and Resh 1992; Barbour et al. 1996; Niba and Sakwe 2018; Raphahlelo et al. 2022). Reasons for using macroinvertebrates as biological indicators are because these organisms are generally sensitive to and effected by environmental and water chemistry changes (Dickens and Grahams 2002; Aschalew and Moog 2015). These changes are driven by the presence and introduction of anthropogenic factors such as pollution and/or changes that impact the habitat. Although water quality primarily affects the distribution of macroinvertebrates within an aquatic environment there are other factors that influence the abundance of invertebrates within a stream system; these include flow regime, habitat structure (e.g., channel form and substrate distribution), system connectivity and seasonality.

In most parts of the world, water quality is determined primarily by the measuring physical and chemical properties of water (Bere et al. 2014). In this chapter, macroinvertebrate assemblages were determined using the refined South African Scoring System Version 5 (SASS5) bio-assessment technique developed to specifically assess the health and ecological integrity of South African river systems (Dickens and Grahams 2002). The technique includes the presence and abundance of different macroinvertebrate taxa across various habitat types to give an indication of river health. In turn, the SASS5 protocol determines the water quality conditions based on the Average Score per Taxon (ASPT), which is the SASS score divided by the number of taxa, for macroinvertebrates collected at sampling site of interest (Chutter 1995; Dickens and Grahams 2002). High ASPT values (>6) indicate natural water quality, whereas low ASPT (<6) is an indication that some deterioration in water quality has occurred. Additionally, to establish the ecological status of the river system,

Thirion (2007) developed a Macroinvertebrate Response Assessment Index (MIRAI) protocol.

The MIRAI technique was described by Thirion (2007) from the Department of Water and Sanitation (DWS), formally the Department of Water Affairs and Forestry (DWAF). The MIRAI technique aims to determine the invertebrate ecological category (EC) by assessing the invertebrate taxa in a community or assemblage and incorporating the invertebrate ecological requirements and their responses to changes in habitat conditions (Thirion 2007). Thus, the MIRAI technique provides a habitat-based cause and effect foundation in order to evaluate the divergence of the invertebrate community (assemblage) from the reference condition, which is the river under natural or pristine conditions, (Thirion 2007). The purpose of this chapter is to categorise the ecological state of the Nwanedi and Luphephe rivers based on the presence and composition of various macroinvertebrate assemblages determined by implementing the SASS5 and MIRAI techniques so as to compare between sites and across ecoregions.

5.2 MATERIALS AND METHODS

5.2.1 MACROINVERTEBRATE SURVEYS

Aquatic macroinvertebrate assessment (see Chapter 2) were conducted using the South African Scoring System version 5 (SASS5) bio-assessment protocol (Dickens and Graham 2002). The specimens collected were identified to family level following identification keys in the Aquatic Invertebrates of South African Rivers guide by Gerber and Gabriel (2002). Each macroinvertebrate taxa has a specific sensitivity score in relation to water quality conditions which are then added to produce the SASS score. In turn the SASS scores per site are then divided by the number of taxa recorded to produce the ASPT (Gerber and Gabriel 2002).

The macroinvertebrate data focused on the occurrence of invertebrate taxa at each site per ecoregion. Thus, the study did not record count data for each family due to time and logistical constraints. However, only estimated abundances for each family and taxa were recorded (see Appendix B: Table 1B). Being a rapid assessment, all macroinvertebrates were returned live at the place where sampled. The study will only discuss the presence or absence of sensitive families where applicable.

5.3 RESULTS

Appendix B: Table 1B shows the presence and absence of aquatic macroinvertebrate families collected from different sites in the Nwanedi and Luphephe rivers during summer and winter. These sites fell within the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), Limpopo Plain 1.02 Ecoregion (sites 6 and 7) and Limpopo Plain 1.01 Ecoregion (Site 9). Aquatic macroinvertebrates collected comprised of 45 families throughout the study period.

5.3.1 SOUTH AFRICAN SCORING SYSTEM

In the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), the SASS scores recorded ranged from 56 to 132 in summer and 56 to 102 in winter (Table 5.1). The SASS scores in sites 1 and 2 were relatively lower and did not vary much between surveys. Site 3, which lies inside the Nwanedi Nature Reserve, had the highest SASS score of 125 during summer decreasing to 75 in winter. Site 4, which is also situated inside the reserve, had SASS scores of 56 and 82 during summer and winter, respectively. The highest SASS score of 132 in summer was recorded at Site 5, which is situated outside the Nwanedi Nature Reserve, on the margin of the Soutpansberg 2.01 Ecoregion. The number of taxa recorded ranged from 10 to 21 and 12 to 13 during summer and winter respectively. The highest number of taxa were recorded at Site 3 during summer and the lowest at Site 4 during the same season. The ASPT ranged between 4.7 and 7.3 across all sites in this ecoregion.

In the Limpopo Plain 1.02 Ecoregion (sites 6 and 7), the SASS scores recorded between the two sites were higher during summer and lower in winter. Site 6 had a SASS score of 143 during summer and reduced to 123 in winter. Site 7 had SASS scores of 111 and 50 during summer and winter, respectively. For both surveys a higher number of taxa was recorded at Site 6 than at Site 7. The ASPT values for sites 6 and 7 ranged from 5.00 to 6.15 for summer and winter (Table 5.1).

In the Limpopo Plain 1.01 Ecoregion (Site 9), the SASS scores recorded during summer (128) and winter (124) were marginally different. The number of taxa were 24 during summer and decreasing to 22 in winter. The ASPT values were 5.33 and 5.64, respectively.

Table 5.1: The SASS score, number of taxa, Average Score per Taxon (ASPT) and mean values obtained at each site during summer (S) and winter (W) in the Nwanedi and Luphephe rivers.

	Soutpansberg 2.01 Ecoregion										Limpopo Plain 1.02 Ecoregion				Limpopo Plain 1.01 Ecoregion	
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 9	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W
SASS score	64	72	86	56	125	75	56	82	132	102	143	123	111	50	128	124
No. of Taxa	12	14	18	12	21	13	10	12	19	14	26	20	19	10	24	22
ASPT	5.33	5.14	4.78	4.67	5.95	5.77	5.60	6.83	6.95	7.29	5.50	6.15	5.84	5.00	5.33	5.64
Mean SASS score	68		71		100		69		117		133		80.5		126	
Mean No. of Taxa	13		15		17		11		16.5		23		14.5		23	
Mean ASPT	5.24		4.73		5.86		6.22		7.12		5.83		5.42		5.49	

Key: S = Summer; W = Winter

5.3.2 MACROINVERTEBRATE RESPONSE ASSESSMENT INDEX

The MIRAI scores for the invertebrate communities varied between seasons and ecoregions (Table 5.2) with MIRAI percentages determined for the Soutpansberg 2.01 Ecoregion being higher than those established for the Limpopo Plain ecoregions during the summer survey. In winter, however, MIRAI scores were recorded to be lower for the Soutpansberg 2.01 and Limpopo Plain 1.02 ecoregions when compared to those reported for the Limpopo Plain 1.01 Ecoregion (see Table 5.2). The Soutpansberg 2.01 Ecoregion had the highest MIRAI score of 56.3% during summer that reduced to 46.6% during winter survey. The Limpopo Plain 1.02 Ecoregion represented the second highest MIRAI score of 50.9% during summer and decreasing to 43.3% in winter. The Limpopo Plain 1.01 Ecoregion had the lowest MIRAI score of 42.6% during summer improving to 50.9% during winter. The MIRAI scores for the three ecoregions were similar with an EC of class D assigned for each survey and ecoregion (Table 5.2). The current study recorded a constant EC of class D while the previous study recorded an EC of class C/D. An indication that a deterioration of invertebrate communities between the two studies has occurred.

Table 5.2: The summary of MIRAI scores (%) and ecological category (EC) for the three ecoregions in the Nwanedi and Luphephe rivers during summer (October 2021) and winter (May 2022) surveys.

Ecoregions	Summer		Winter	
	MIRAI score (%)	EC	MIRAI Score (%)	EC
Soutpansberg 2.01	56.3	D	46.4	D
Limpopo Plain 1.02	50.9	D	43.3	D
Limpopo Plain 1.01	42.6	D	50.9	D

Key: EC = Ecological category

To determine if the conditions in the system have changed over time the data for summer and winter survey were pooled and compared and the class scores for each ecoregion compared with those of Angliss et al. (2007). The outcome of the ecological category (PES) of in the Nwanedi River catchment varied with that of Angliss et al. (2007) with the EC shown to have deteriorated from a class C/D as reported by

Angliss et al. (2007) to a class D as was calculated in the present study (see Table 5.3).

Table 5.3: Comparison of MIRAI and ecological categories (EC) between previous (2007) and present study.

Ecoregions	Angliss et al. (2007)	Present study 2021 - 2022
	Ecological Category (EC)	
Soutpansberg 2.01	C/D	D
Limpopo Plain 1.02	C/D	D
Limpopo Plain 1.01	C/D	D

5.4 DISCUSSION

5.4.1 SOUTH AFRICAN SCORING SYSTEM VERSION 5

In the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), the SASS scores were generally lower for all sites. The SASS scores for sites 1, 2 and 3 fell within the SASS scores range of 50 to 100 with an ASPT of less than 6 reported. According to and following the interpretation of SASS scores by Chutter (1995) the results would indicate that some deterioration in water quality has occurred. However, following the target water quality range (TWQR) by DWAF (1996a) the water quality parameters between these sites were within the range considered suitable for aquatic ecosystems. Davies and Day (1998) stated that most freshwater ecosystems have a pH range of pH 6 to 8, however, this was not the case at sites 1 and 2 where water pH was reported to below 6 (i.e., 5.52 – 5.86) for both surveys. The lowest pH recorded at these sites could be the reason for the low SASS scores associated with this ecoregion. Secondly, the substrate at sites 1 and 2 is dominated by bedrock. The absence of riparian vegetation at Site 2 resulted in a shortage of refuge for some invertebrates such as Leptoceridae (cased caddisflies). Although Site 3, which is situated in a pristine area within the Nwanedi Nature Reserve, had the variety of biotopes in which to conduct SASS, the bottom substrate was dominated by sand. According to Chutter (1998) and Dickens and Graham (2002), in a pristine river condition, a lower or depressed ASPT can be attributed to a sandy substrate. Based

on the interpretations by Chutter (1995), an ASPT score greater than 6, as recorded at sites 4 and 5, could be associated with natural water quality conditions. However, the habitat condition varied between the two sites affecting their SASS scores differently. Site 4 had reduced habitat conditions as there was a shortage of marginal and overhanging vegetation resulting in lower SASS scores. Additionally, the lower number of macroinvertebrate taxa in summer (n = 10) could have been impacted by the presence of the low lying bridge that modified the flow and habitats as there were pools upstream and downstream of this bridge. Consequently, Site 5 had diverse biotopes in which to sample invertebrates that resulted in the high SASS scores reported at this site and, in turn, for the Soutpansberg 2.01 Ecoregion.

In the Limpopo Plain 1.02 Ecoregion (sites 6 and 7) the SASS score between the two sites were all above 100 during summer and winter, except in winter when the score at Site 7 declined to 50. The possible reason for the high SASS scores at these sites might be attributed to the width of the river being broader thereby providing sufficient surface area and habit for macroinvertebrates to inhabit. However, the average scores per taxon for these sites were all below 6. According to Chutter (1995), the reduced ASPT at Site 6 is an indication of a borderline between water quality natural and some deterioration, whereas at Site 7 is an indication of the deterioration in water quality. These interpretations are supported by the water quality results in Chapter 3 where the electrical conductivity (EC) and total dissolved solids (TDS) were elevated as compared to those in the Soutpansberg 2.01 Ecoregion. This could be the reason why there was a loss in abundance of sensitive families to water quality changes such as Heptageniidae and Hydropsychidae. However, the tolerant families to modified physico-chemical parameters recorded in high abundances were Simuliidae, Tabanidae and Thiariidae.

In the Limpopo Plain 1.01 Ecoregion, the mean SASS score of 126 and an ASPT of 5.49 at Site 9 indicated a borderline between water quality under natural conditions and a slight deterioration in water quality (Chutter 1995). Although, the SASS score was high at Site 9, the high abundance of pollution tolerant taxa such as Chironomidae, Corbiculidae, Tabanidae and Thiaridae (Dickens and Graham 2002; Czerniawska-Kusza 2005; Chakona et al. 2008; Bere and Nyamupingidza 2014) contributed to higher SASS scores.

5.4.2 MACROINVERTEBRATE RESPONSE ASSESSMENT INDEX

In the Soutpansberg 2.01 Ecoregion, high MIRAI scores were recorded during summer and winter. Invertebrate communities varied in MIRAI scores of 56.34% in summer and 46.36% during winter (Table 5.5). Following the interpretation of the MIRAI scores by Kleynhans et al. (2005), these values lie under the EC of class D, which is an indication of largely modified river components such as flow modification, habitat and physico-chemical modifications and system connectedness and seasonality. The absence of intolerant and moderately intolerant taxa to modified physico-chemical parameters have negatively impaired the MIRAI scores during the sampling surveys. These include intolerant taxa belonging to the Oligoneuridae and Hydropsychidae and moderately intolerant taxa of the Dixidae, Clorolestidae, Hydrometridae, Cordullidae, Hydrocarinae, Philopotamidae, Psephenidae and Tricorythidae (Chutter 1995; Fry 2021; Raphahlelo et al. 2022). Although, there were high diversity of sampling biotopes in the Soutpansberg 2.01 Ecoregion, the absence of the abovementioned families resulted in the lower MIRAI scores.

Similarly, the Limpopo Plain 1.02 Ecoregion was categorised as being largely modified based on the EC of class D during summer and winter (Table 2.2). The absence of sensitive and moderately tolerant taxa to changes in physico-chemical parameters might have been the result of abovementioned EC value. Thus, the ecological state in terms of invertebrate communities has deteriorated from the natural conditions of the river in this ecoregion. However, the abundance of tolerant families to water quality and habitat changes such as Thiaridae, Turbellaria and Hirudinea might have contributed positively to the obtained MIRAI scores and EC. Additionally, factors such as the absence of canopy and marginal vegetation at Site 7 could have resulted in EC of class D during both surveys. Consequently, anthropogenic and agricultural impacts from the surrounding areas upstream of this reach might have had greater impact on the abundance and frequency of occurrence in this ecoregion as compared to the Soutpansberg 2.01 Ecoregion.

Similarly, the Limpopo Plain 1.01 Ecoregion had the same EC of class D as the Soutpansberg 2.01 and Limpopo Plain 1.02 ecoregions. Despite other factors not examined in this study (e.g., sediments quality and pesticides), high concentrations of water EC, TDS and salinity could have been the cause of less abundance of intolerant taxa to water quality changes. These include families such as Baetidae,

Hydropsychidae, Heptageniidae, Oligoneuridae and Perlidae. Additionally, impacts from agricultural and domestic sources are evident near the catchment area and as such their impact prevails the flow modification and system connectivity (Angliss et al. 2007). Conversely, due to limited number of sites sampled, the results of the Limpopo Plain 1.01 should be viewed with caution.

In comparison with the historic data by Angliss et al. (2007) the ecological categories of the invertebrate communities were slightly different between ecoregions and changed from EC of class C/D to D. Based on the findings in the current study, it would appear that the system has deteriorated based on the MIRAI protocol scores from natural conditions to largely modified with a high absence of intolerant to moderately tolerant families.

5.5 CONCLUSION

The purpose of this chapter which was to categorise the ecological state of the Nwanedi and Luphephe rivers based on the presence of various macroinvertebrate assemblages using SASS5 and MIRAI at each of the selected sites. The SASS5 revealed the spatial distribution in macroinvertebrate communities across sites and ecoregions.

The general SASS scores and ASPT obtained in the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), were generally lower than the scores in the Limpopo Plain ecoregions. Sites 1, 2 and 3 fell within the SASS scores range of 50 to 100 with an ASPT of less than 6 indicating some deterioration in water quality based on the SASS5 interpretations by Chutter (1995). Sites 4 and 5 indicated to have natural water quality and/or conditions based on the ASPT values.

In the Limpopo Plain 1.02 Ecoregion (sites 6 and 7) the SASS scores were all above 100 during summer and winter, except in winter when the score at Site 7 declined to 50. The ASPT for these sites were all below 6 which is an indication that a slight deterioration from natural conditions occurred.

In the Limpopo Plain 1.01 Ecoregion, the mean SASS score and ASPT at Site 9 indicated a slight deterioration in water quality following the SASS5 protocol. In general, the SASS scores in the Nwanedi and Luphephe rivers were lower at the Soutpansberg 2.01 Ecoregion due to a low abundance of taxa that are intolerant of

pollution. However, higher SASS scores were obtained in the Limpopo Plain ecoregions due to a high abundance of families tolerant to pollution such as Simulidae, Tabanidae, Thiaridae and Tipulidae.

Conversely, the MIRAI results indicated Nwanedi and Luphephe rivers as largely modified systems based on the EC of class D that supports the SASS score results due to lesser presence of intolerant families in the entire Nwanedi River catchment. In comparison with work done by Angliss et al. (2007), the invertebrate communities have deteriorated from the EC of class C/D to D.

CHAPTER 6

ICHTHYOPARASITES AS BIOINDICATORS

6.1 INTRODUCTION

Parasite biodiversity and species composition in the aquatic environment can provide perspective on the health status of the surrounding ecosystem. Although, parasites derive almost all their nourishment at the expense of their host and have no desire to eliminate it, large parasite loads can have an impact on the behaviour, physiology, morphology, or reproduction of the host (Marcogliese 2004). Parasites live on or in almost all living organisms (the host) and occur in a majority of ecosystems across all trophic levels (Marcogliese 2005). For example, in aquatic ecosystems, ecto- and endoparasites are found on or in fish and other aquatic organisms, respectively. Parasitic infestations in aquatic systems can be influenced by several factors resulting from environmental stresses such as pollution and/or changes in water quality (Davies and Day 1998). In addition to monitoring water quality parameters, biological indicators such as fish parasites can be used to study and monitor the status of aquatic environments (Galli et al. 2001).

In recent years, there has been an increased interest in using ichthyoparasites as indicators of pollution in aquatic systems (Blanar et al. 2009). Sures (2006) revealed that parasite infestations can be influenced by water pollution, whereby the host defence mechanisms is negatively affected and made more susceptible to infection. Conversely, parasitism can also be reduced by pollution if the parasites are more susceptible to a particular pollutant than the host, especially when the host's defence mechanisms have been compromised (Sures 2006). Additionally, ectoparasites appear to be potential indicators of environmental pollution in aquatic systems due to these parasites being in direct contact with the surrounding medium (Blanar et al. 2009) with the premise that ectoparasites are more prevalent under natural conditions when the water quality is good, (Avenant-Oldewage 2001; Pietrock and Marcogliese 2003; Madanire-Moyo et al. 2012). Conversely, large numbers of endoparasites are to be expected when water quality is poor (Crafford and Avenant-Oldewage 2012). Therefore, in addition to monitoring physical and chemical parameters of water, the

presence or absence of parasites may be employed as means to assess the environmental conditions and the health status of aquatic systems.

In this chapter, the parasite composition from *Pseudocrenilabrus philander* (Weber, 1897) was examined as outlined in Chapter 2. The southern mouthbrooder was considered a suitable host for establishing if parasites associated with this species could be used as bioindicators of water quality in the Nwanedi and Luphephe rivers.

6.2 MATERIALS AND METHODS

6.2.1 PARASITE SURVEY

As indicated in Chapter 2, parasite survey from *P. philander* during summer was only undertaken at Site 4, whereas all other sites (i.e., sites 3, 5 and 6) including Site 4 were surveyed in winter survey. The number of host specimens for each sampling site is indicated under Appendix C: Table 1C and 2C. Collected parasites from *P. philander* were fixed, preserved and identified according to the methods required for a specific parasite group (see Chapter 2).

6.3 RESULTS

6.3.1 ECTO- AND ENDOPARASITES

Among the specimens of *P. philander* examined only specimens from sites 3 and 4 were by one or more parasite group while specimens from Site 5, which is situated outside the reserve within the Soutpansberg 2.01 Ecoregion were not infected with parasites (see Table 6.1). Similarly, specimens from Site 6 located in the Limpopo Plain 1.02 Ecoregion were not infected with parasites. No specimens of *P. philander* were retrieved form the Limpopo Plain 1.01 Ecoregion.

The total number of 169 parasites comprising 160 ecto- and 9 endoparasites were collectively recorded for both surveys (Table 6.1). A total of 144 ectoparasites were recorded at Site 4 in summer and 16 at Site 3 in winter. Endoparasites were more prevalent at Site 4 during summer (n = 7) and less at Site 3 in winter (n = 2). From the 5 and 4 specimens of *P. philander* examined (see Appendix C: Table 1C and 2C) from sites 5 and 6, respectively no parasites were recovered (Table 6.1).

Table 6.1: Total number of parasites from *Pseudocrenilabrus philander* recorded in the Nwanedi and Luphephe rivers during the summer 2021 and winter 2022 surveys.

	COLLECTED FROM	TOTAL NUMBER OF PARASITES COLLECTED		
		Summer	Winter	Total
	Site 3			
Ectoparasite				
Monogenean	Gills	-	16	**16**
Endoparasite				
Cestode larvae	Attached to intestine	-	2	**2**
Nematode	Intestine	-	0	**0**
	Site 4			
Ectoparasite				
Monogenean	Gills	144	0	**0**
Endoparasites				
Cestode larvae	Attached to Intestine	1	0	**0**
Nematode	Intestine	6	0	**0**
	Site 5			
Ecto- and endoparasites		-	0	**0**
	Site 6			
Ecto- and endoparasites		-	0	**0**

Note: "-" No survey conducted

The specific gill monogenean of *P. philander* known as *Cichlidogyrus philander* Douëllou 1993 was identified to species level, endoparasites were only identified to genus level. The identification of *C. philander* was based on the shape of the dorsal and ventral anchors, marginal hooks and male copulatory organ (MCO). The gryporhynchid larvae *Parvitaenia* Burt, 1940 (Figure 6.2) were found encysted in the outer layer of the intestine. Specimens of *Rhabdochona* Railliet, 1916 (Figure 6.3) were collected from the intestine and identified based on the anterior and posterior regions of the nematode.

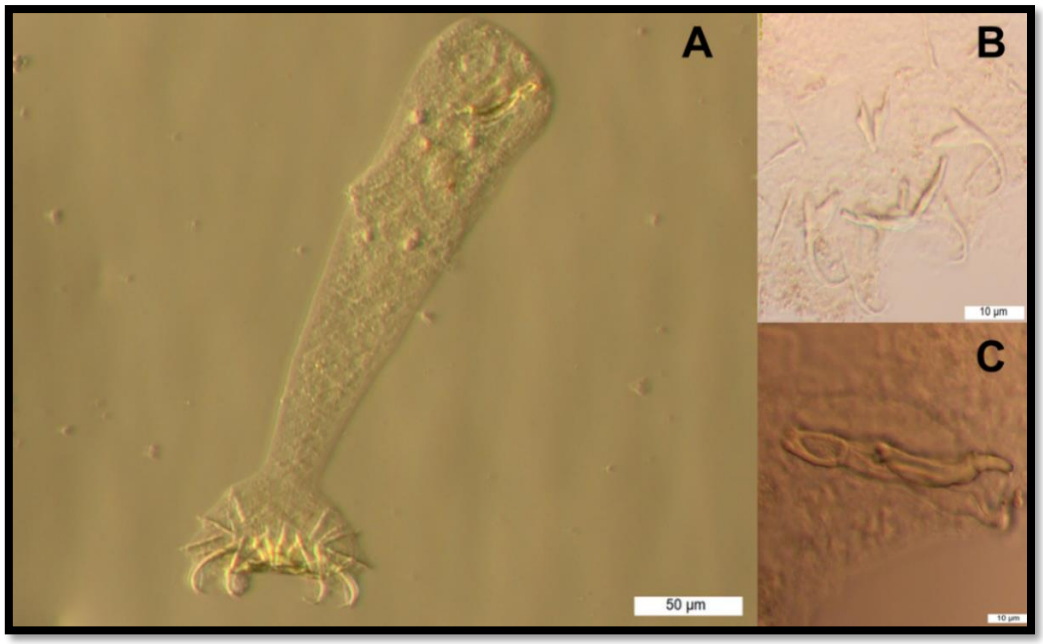

Figure 6.1: (A) Whole mount of the monogenean *Cichlidogyrus philander* from the gills of *Pseudocrenilabrus philander*. **(B)** Opisthaptor (attachment organ) showing the haptoral sclerites of *C. philander*. **(C)** Male copulatory organ (MCO) of *C. philander*.

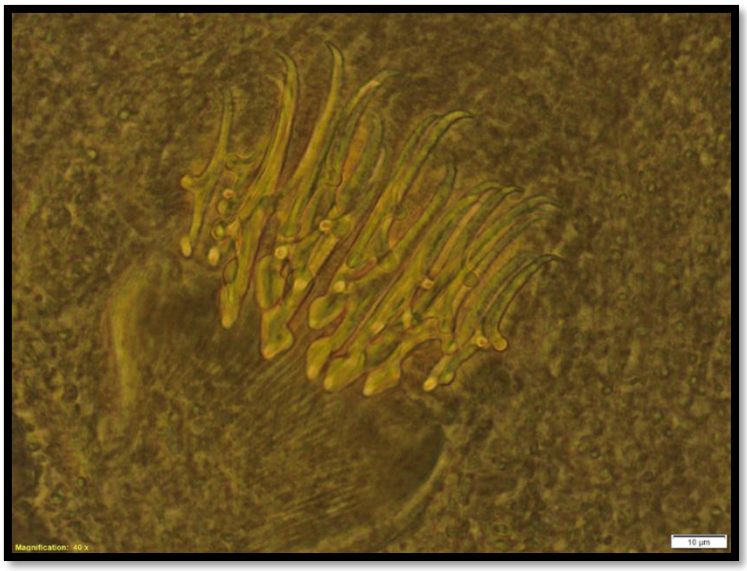

Figure 6.2: Hooks of *Parvitaenia* sp. collected from the outer layer of the intestine of *Pseudocrenilabrus philander*.

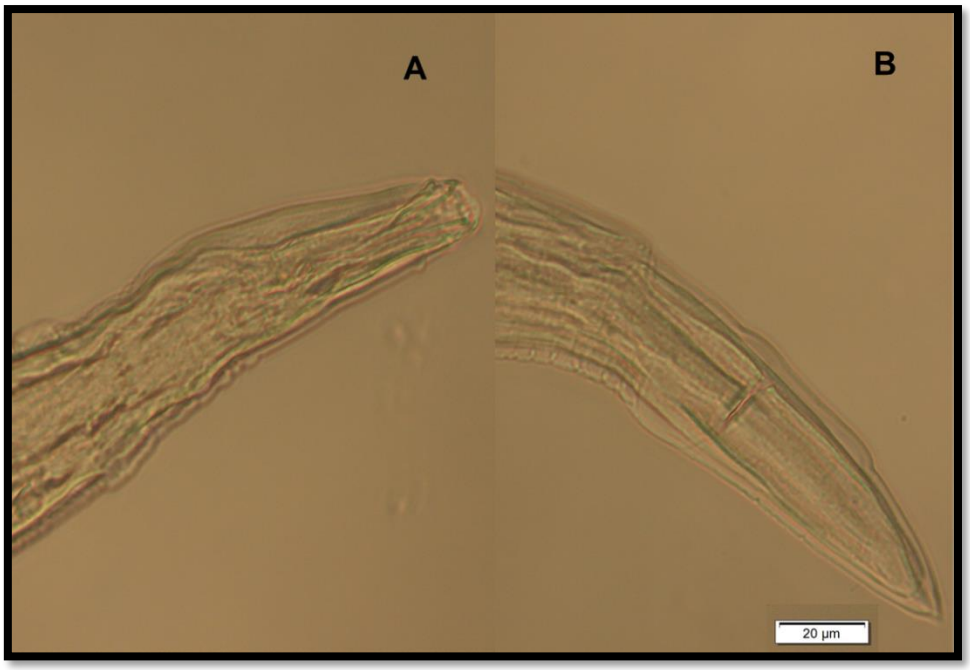

Figure 6.3: Anterior **(A)** and posterior **(B)** regions of the female *Rhabdochona* sp. used for identification purposes.

Cichlidogyrus philander and cestode larvae *Parvitaenia* sp. were recorded from both rivers at sites located within the reserve during the summer survey, whereas *Rhabdochona* sp. was recorded during the summer and winter surveys. *Cichlidogyrus philander* and *Rhabdochona* sp. represent new host and distribution records from Luphephe River (Site 4).

6.3.2 INFESTATION INDICES

The highest MA and MI were recorded for *C. philander* at sites 3 and 4 during winter and summer surveys, respectively (Table 6.2). When comparing between surveys a higher MA and MI for *C. philander* were recorded in summer. *Parvitaenia* sp. was observed to be the second most prevalent parasite during both seasons with the highest prevalence recorded during the winter survey. *Rhabdochona* sp. was only prevalent during the summer survey and was not recorded during the winter survey.

Table 6.2 Prevalence (P%), mean abundance (MA) and mean intensity (MI) of parasites from *Pseudocrenilabrus philander* recorded in the Nwanedi and Luphephe rivers during the summer 2021 and winter 2022 surveys.

	Summer			Winter		
Parasite group and species	**P%**	**MA**	**MI**	**P%**	**MA**	**MI**
		Site 3				
Monogenean *Cichlidogyrus philander*	-	-	-	30	1.6	5.3
Nematode *Rhabdochona* sp.	-	-	-	0	0	0
Cestode *Parvitaenia* sp.	-	-	-	20	0.2	1
		Site 4				
Monogenean *Cichlidogyrus philander*	80	14.4	18	0	0	0
Nematode *Rhabdochona* sp.	10	0.6	6	0	0	0
Cestode *Parvitaenia* sp.	10	0.1	1	0	0	0
		Site 5				
Ecto- and endoparasites	-	-	-	0	0	0
		Site 6				
Ecto- and endoparasites	-	-	-	0	0	0

6.4 DISCUSSION

6.4.1 ECTOPARASITES

This study recorded only one ectoparasite group, the monogenean from the gills of *P. philander*. This monogenean inhabit and move along the gills of the host with the aid of attachment organs known as anchors and hooks while feeding on mucus and gill debris. Most monogeneans are monoecious and have direct life cycles. Some Monogenea reproduce by laying eggs that hatch in water with the larvae attaching to the same or a new host. Studies have indicated that this group of ectoparasite is affected by poor water quality, which in turn may adversely affect its diversity in aquatic systems (Avenant-Oldewage 2001; Pietrock and Marcogliese 2003; Madanire-Moyo et al. 2012).

In the present study, a specific gill monogenean was recovered from the gills of *P. philander* and identified as *Cichlidogyrus philander* Douëllou, 1993. The first records of this parasite was reported from Lake Kariba in Zimbabwe by Douëllou (1993) and later in Padda Dam, Gauteng, South Africa by Le Roux and Avenant-Oldewage (2010). Walter et al. (2017) reported the monogenean *C. philander* from *P. philander* in the Nwanedi River. *Cichlidogyrus philander*, like other monogeneans, uses a specialised posterior haptor to attach on the gills of the host (Douëllou 1993). This haptor include attaching sclerites such as a dorsal bar, a ventral bar, anchors and marginal hooks forming a functional unit. The occurrence of *C. philander* in the current study was limited to sites 3 and 4 situated in the Soutpansberg 2.01 Ecoregion inside the Nwanedi Nature Reserve. The possible reason for the occurrence of *C. philander* at sites in the reserve may be related to the high abundance of the host species and habitats that are suitable and preferable for the *P. philander.* Moreover, water conditions at the abovementioned sites were reported to be good (see Chapter 3) and hence favourable for monogeneans.

6.4.2 ENDOPARASITES

Gryporhynchid larvae have complex life cycles, which commonly infest several African cichlids (Scholz et al. 2018). The adult tapeworm of this larvae infect piscivorous birds such as cormorants and herons (Scholz et al. 2018). Bray (1974) published the first record of metacestodes of gryporhynchids, *Amirthalingamia macracantha* (Joyeux and Baer, 1935), from *Oreochromis niloticus* (Linnaeus, 1758). Khalil and Polling (1997) reported two larval species of gryporhynchids, namely, *A. macracantha* and *Paradilepis delachauxi* (Fuhrmann, 1909). Additionally, Khalil and Polling (1997) reported a previously unidentified species of the genera *Anomotaenia* Cohn, 1900 and a ‘dilepidid’ larvae from tilapias. Most recently, Truter et al. (2016) reported four species of gryporhynchid larvae in South Africa from *P. philander*, these include *Paradilepis scolecina* (Rudolphi, 1819), *Paradilepis maleki* Khalil, 1961 *Neogryporhynchus lasiopeius* Baer and Bona, 1960 and *Valipora campylancristrota* (Wedl, 1855). The present study, however, is the first to record *Parvitaenia* sp. from *P. philander* from Nwanedi and Luphephe rivers.

Nematodes such as *Rhabdochona* sp. use fish as their final host to complete their life cycle and are normally found in the digestive tract. However, some species of

nematodes (e.g., larvae of *Contracaecum*), can be found in the body cavity, internal organs and muscle tissue. Moravec (2010) stated that the genus *Rhabdochona* belong to the order Spirurida, class Nematoda. The genus *Rhabdochona* include a large number of species that parasitise cyprinids worldwide (Moravec 2010). In the present study, this parasite was recorded from *P. philander* (Cichlidae) during the summer survey from Site 3 inside the Nwanedi Nature Reserve. This parasite is a medium-sized nematode having a smooth cuticle a narrow shape in the anterior and posterior ends of both sexes (Kuchboev et al. 2021). The recorded *Rhabdochona* sp. (Figure 6.3 A, B) in this study displayed female morphological features as indicated by the conical tail tip in the posterior region. Given that only premature female *Rhabdochona* sp. were recorded in this study, it was difficult to morphologically identify individuals to species level as the eggs and male copulatory organ (MCO) required to identify these parasites to species level. The possible reason for the occurrence of the *Rhabdochona* sp. in *P. philander* from Site 4 could be due a higher availability of the definitive host and the presence of the intermediate host such as mayfly nymphs of to the order Ephemeroptera (Anderson 1988).

6.4.3 INFESTATION INDICES OF PARASITES

The ectoparasite *C. philander* was recorded to have the highest MA and MI while endoparasites had the lowest MA and MI. When comparing infestation indices at Site 4 between the two surveys, higher MA and MI were recorded in summer compared to winter. This might be due factors with regard to the life cycle of these parasites e.g., breeding period, which was not investigated in this study. Avenant-Oldewage (1994) stated that ectoparasites are exposed to the external environment and as a consequence it is unclear to what extend water quality constituents have an influence on ectoparasites as opposed to endoparasites. Conversely, when looking at water quality parameters, reported in Chapter 3, it is noticeable that the readings recorded at the sites where these parasites and fish were collected fell within the water quality guidelines as suggested by DWAF (1996a). However, no records of *C. philander* were obtained in sites 5 and 6, which are situated outside the nature reserve and in the Limpopo Plain 1.02 Ecoregion, respectively. The possible reason for the absence of this parasite in the abovementioned sites could have been due to poor water quality (e.g., elevated EC and TDS in Site 6, see Chapter 3: Table 3.1) and presence of certain

water constituents from land activities such as domestic and agricultural impacts, which were not investigated in this study.

6.5 CONCLUSION

Ichthyoparasite composition of *P. philander* included the ectoparasite *C. philander* and endoparasites of the genera *Parvitaenia* sp. and *Rhabdochona* sp. The monogenean *C. philander* was the most prevalent whereas *Parvitaenia* sp. and *Rhabdochona* sp. were reported to be lower in abundance and represented a new geographical record from this host at Site 4 inside the Nwanedi Nature Reserve. The objective to use fish parasites as bioindicators of water quality was conducted whereby *C. philander* at sites 3 and 4 inside the Nwanedi Nature Reserve, which is regarded as a pristine environment, were recorded to have a high prevalence whereas at Site 5, which is located outside the reserve but still within the Soutpansberg 2.01 Ecoregion, the ectoparasite was absent. Similarly, *C. philander* was not recorded at Site 6 which is located in the Limpopo Plain 1.02 Ecoregion. The absence of this monogenean could be attributed to poor water conditions that may be impacted by the discharge or pulse release of water from the Cross Dam during winter survey. Land use activities such as agricultural and domestic impacts at sites 5 and 6 may have impacted the occurrence of *C. philander* and as such more studies are needed to monitor the presence or absence of this parasite in relation to water quality. In addition, no definite conclusion can be drawn from a once off survey and relatively small sample size. Moreover, future studies should measure additional water constituents such as pesticides not measured in this study so as to identify the presence thereof and the possible affects these may have on the parasite loads in fish from further downstream.

CHAPTER 7

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The aim of the study was to determine the ecological status of the Nwanedi and Luphephe rivers based on fish and macroinvertebrate assemblages and the composition of ichthyoparasites from a fish species that is widely distributed and abundant in the middle and lower reaches of this system. In this case, *Pseudocrenilabrus philander* (Weber, 1897) was identified and selected. To achieve the aim, water quality data was incorporated with the biomonitoring data derived using the Fish Response Assessment Index (FRAI) and Macroinvertebrate Response Assessment Index (MIRAI) in conjunction with ichthyoparasite infestation indices that included the percentage prevalence (P%), mean abundance (MA) and mean intensity (MI).

Water quality

Most of the water quality constituents were within the target water quality range (TWQR) for aquatic ecosystems prescribed by the Department of Water and Sanitation, previously the Department of Water and Forestry, guidelines (DWAF 1996a). However, due to elevation, seasonal changes and land activities in the lower catchments, there were variations in some water quality variables.

Temperature increased with the decrease in altitude. Water pH was slightly acidic to alkaline with the descending elevation. The source of alkalinity in these ecoregions was inferred to be due to agricultural activities and the presence of photosynthetic organisms (e.g., algae) that increases water pH by removing carbon dioxide from the water column and the composition of the underlying substrate.

Non-toxic constituents such as electrical conductivity, TDS and salinity were relatively low in the Soutpansberg 2.01 Ecoregion but found to be higher in the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions. High levels of these constituents could have been caused by the Tshipise Fault, siltation and the presence of inorganic salts from agricultural runoff.

Nitrate concentrations categorised the Soutpansberg 2.01 Ecoregion to be oligotrophic with the exception of Site 3 with concentrations reported within the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions indicative of mesotrophic conditions. Although the SO_4 concentrations were above detection at the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions, there are no guidelines for this nutrient in aquatic ecosystem. The source of SO_4 could have been due to agricultural runoff and domestic activities observed in the regions.

In conclusion, the water quality constituents were within the acceptable levels, readings thereof varied between sampling sites and surveys. As a consequence, water quality results could not be used in isolation to conclude the health status of the Nwanedi and Luphephe rivers since water readings provided a snapshot of water conditions at the time of sampling. Hence, the need for biomonitoring data.

Fish assemblages as bioindicators

The FRAI results revealed Soutpansberg 2.01 Ecoregion to have a high abundance and species richness, with numbers decreasing with altitude. Species that are sensitive to pollution such as *Amphilius uranoscopus, Enteromius eutaenia, Enteromius lineomaculatus* and *Chiloglanis pretoriae* were recorded. Based on the CCA results most of the fish species caught in the present study were positively correlated with the environmental variables. For example, species reported in the Soutpansberg 2.01 Ecoregion such as *E. eutaenia* and *E. lineomaculatus* reported at sites 3 and *Anguilla mossambica*, *Coptodon rendalli, Enteromius unitaeniatus, Micralestes acutidens* and *Petrocephalus wesselsi* at Site 4 were shown to be strongly correlated with high oxygen concentrations. The FRAI results, in turn, revealed the PES of the Nwanedi and Luphephe rivers to be in a good or close to natural conditions with EC of A/B assigned to the Soutpansberg 2.01 Ecoregion and an EC of C (Fair) to an EC of D (Poor) being assigned to the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions, respectively. However, when compared to the findings by Angliss et al (2007), the ecological state of the Nwanedi River Catchment in the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions revealed the PES to have shifted from fair to poor, respectively in terms of fish communities.

Macroinvertebrate assemblages as bioindicators

The purpose of using South African Scoring System Version 5 (SASS5) and MIRAI at each of the selected sites was to supplement the FRAI and water quality analyses, given that macroinvertebrates are more sensitive to water quality changes. The SASS5 revealed the spatial distribution in macroinvertebrate communities across sites and ecoregions. The general SASS scores and Average Score per Taxon (ASPT) obtained in the Soutpansberg 2.01 Ecoregion (sites 1, 2, 3, 4 and 5), were generally lower than the scores obtained from the sites associated with the Limpopo Pain 1.02 Ecoregion (sites 6 and 7) and Limpopo Plain 1.01 Ecoregion (Site 9).

In conclusion, the upper three sites of the Soutpansberg 2.01 Ecoregion and the sites situated in the Limpopo Plain ecoregions had deterioration in water quality. This is due to the lesser presence of sensitive families with more abundance of tolerant families, especially on the Limpopo Plain ecoregions. These families included but not limited to Chironomidae, Hirudinea, Simuliidae, Tabanidae, Thiaridae (Dickens and Graham 2002; Czerniawska-Kusza 2005; Chakona et al. 2008; Bere and Nyamupingidza 2014). Similarly, the MIRAI results indicated Nwanedi and Luphephe rivers as largely modified based on the ecological category of D. Findings that are supported by the SASS scores given the low presence of pollution intolerant families observed when sampling. The invertebrate communities in the Nwanedi and Luphephe rivers have deteriorated from an EC of C/D to D when compared to the work undertaken by Angliss et al. (2007).

Ichthyoparasites as bioindicators

Ichthyoparasites composition of this study from *Pseudocrenilabrus philander* included the ectoparasite of the species *Cichlidogyrus philander* and endoparasites of the genera *Parvitaenia* sp. and *Rhabdochona* sp. The ectoparasite *C. philander* was more prevalent than endoparasites where *Parvitaenia* sp. and *Rhabdochona* sp. were reported to be lower in abundance and represented a new geological record on this host at Site 4 inside the Nwanedi Nature Reserve. The objective to use fish parasites as bioindicators of water quality was conducted whereby *C. philander* at sites 3 and 4 inside the Nwanedi Nature Reserve, which is regarded as a pristine environment, were recorded to have high prevalence whereas at Site 5, which is

located outside the reserve but still within the Soutpansberg 2.01 Ecoregion, the ectoparasite was absent. Similarly, the *C. philander* was not recorded at Site 6 which is located in the Limpopo Plain 1.02 Ecoregion. The absence of this monogenean was attributed to water conditions that may be impacted by land use activities at these sites and as such more studies need to be undertaken to monitor the presence or absence of *C. philander* in relation to water quality.

Recommendations for future studies

Given the findings of this study future studies should consider looking at pesticides and additional metals in both water samples and sediments. To better understand the drivers that may be affecting the water quality and habitat in this system the study period should also be intensified, for example, water quality assessments should be undertaken on a monthly basis in conjunction with the monitoring of biological indictors (e.g., fish and macroinvertebrates assemblages) undertaken every two to three months given the resources available.

The FRAI approaches should be done in regular intervals and per each season instead of pooling the data together between surveys. This will allow an in depth knowledge of species response to habitat, environmental and seasonal changes. Future studies should investigate as to the reason why the following species: *Enteromius paludinosus*, *Enteromius radiatus* and *Enteromius toppini* were absent in this catchment.

Moreover, in addition to estimating the abundances of macroinvertebrate families or taxa, the total number of individuals should be considered when conducting surveys using MIRAI. This will allow that statistical tests be conducted to establish for significant differences between macroinvertebrates counts and the environmental variables measured.

With regard to parasites as bioindicators, more studies are needed in the Nwanedi River to monitor the suitability of *C. philander* along with its parasites and the effect various water constituents, that include pesticides (Kumar et al. 2021 and Sabra and Mehana 2015) and additional metals not measured in this study, may have on parasite load.

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APPENDICES

APPENDIX A: FISH ASSEMBLAGE AS BIOINDICATORS

The following table displays the abundance of fish species collected at Nwanedi and Luphephe rivers during the summer (October 2021) and winter (May 2022).

Table 1A: Fish collected from the Nwanedi and Luphephe rivers during the summer (October 2021) and winter (May 2022) surveys.

		Ecoregions/Sampling sites																			
		Soutpansberg 2.01										Limpopo 1.02				Limpopo 1.01					
Species scientific name	Species Code	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
		S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Anguilla mossambica	AMOS	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Amphilius uranoscopus	AURA	5	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chiloglanis paratus	CPAR	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	49	13	0	0
Chiloglanis pretoriae	CPRE	0	0	0	0	0	0	0	32	53	68	55	17	2	13	0	0	0	7	0	0
Clarias gariepinus	CGAR	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Clarias theodorae	CTHE	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Cyprinus carpio	CCAR	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Enteromius annectans	BANN	0	0	0	0	0	0	4	0	0	0	2	7	9	0	0	0	0	0	0	0
Enteromius bifrenatus	BBIF	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteromius eutaenia	BEUT	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteromius trimaculatus	BTRI	0	0	0	0	5	5	0	0	1	0	1	2	0	0	0	0	0	0	0	0
Enteromius unitaeniatus	BUNI	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1A Continued…

		Ecoregions/Sampling sites																			
		Soutpansberg 2.01										Limpopo 1.02				Limpopo 1.01					
Species scientific name	Species Code	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
		S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Entoromius lineomaculatus	BLIN	0	0	0	0	4	0	5	0	0	2	5	0	7	0	0	0	0	0	0	0
Labeo cylindricus	LCYL	0	0	0	0	1	1	0	1	8	15	12	3	2	1	0	0	7	0	0	0
Labeo molybdinus	LMOL	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0
Labeo rosae	LROS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
Labeobarbus marequensis	LMAR	0	0	0	0	3	4	2	2	0	2	0	0	1	0	0	0	3	6	0	0
Marcusenius macrolepidotus	MMAC	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micralestes acutidens	MACU	0	0	0	0	2	7	3	6	0	0	0	0	0	0	0	0	0	0	0	0
Oreochromis mossambicus	OMOS	0	0	0	0	0	0	0	0	0	0	8	11	0	0	0	0	3	5	0	0
Petrocephalus wesselsi	PWES	0	0	0	0	1	0	8	5	4	4	0	0	0	0	0	0	0	0	0	0
Pseudocrenilabrus philander	PPHI	0	0	0	0	2	18	13	8	4	5	10	18	0	0	0	0	0	0	0	0
Tilapia rendalli	TREN	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	4	19	0	0
Tilapia sparrmanii	TSPA	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Key: S = Summer, W = Winter

Fish Response Assessment Index – Procedures

The following tables (Table 2A to 9A) displays the Fish Response Assessment Index (FRAI) procedures undertaken during the analyses of the fish communities from the Soutpansberg 2.01 Ecoregion during the summer survey. The same procedures were followed in determination of the present ecological state (PES) of the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions during the summer and winter surveys, respectively.

Table 2A: Fish data the Soutpansberg 2.01 Ecoregion

ECOREGION	SPECIES	ABREV.	NUMBER	COUNTRY	PROVINCE	LOCALITY
2.01	*Amphilius uranoscopus*	Aura	5	ZA	L	site 1
2.01	*Enteromius bifrenatus*	Bbif	1	ZA	L	Site 3
2.01	*Enteromius lineomarculatus*	Blin	4	ZA	L	Site 3
2.01	*Enteromius trimaculatus*	Btri	5	ZA	L	Site 3
2.01	*Labeo cylindricus*	Lcyl	1	ZA	L	Site 3
2.01	*Labeobarbus marequensis*	Lmar	3	ZA	L	Site 3
2.01	*Marcusenius macrolepidotus*	Mmac	1	ZA	L	Site 3
2.01	*Micralestes acutidens*	Macu	2	ZA	L	Site 3
2.01	*Petrocephalus wesselsi*	Pwes	1	ZA	L	Site 3
2.01	*Pseudocrenilabrus philander*	Pphi	2	ZA	L	Site 3
2.01	*Enteromius annectans*	Bann	4	ZA	L	Site 4
2.01	*Enteromius lineomarculatus*	Blin	5	ZA	L	Site 4
2.01	*Enteromius unitaeniatus*	Buni	1	ZA	L	Site 4
2.01	*Labeo molybdinus*	Lmol	2	ZA	L	Site 4
2.01	*Labeobarbus marequensis*	Lmar	2	ZA	L	Site 4
2.01	*Micralestes acutidens*	Macu	3	ZA	L	Site 4
2.01	*Petrocephalus wesselsi*	Pwes	8	ZA	L	Site 4
2.01	*Pseudocrenilabrus philander*	Pphi	3	ZA	L	Site 4
2.01	*Enteromius trimaculatus*	Btri	1	ZA	L	Site 5
2.01	*Chiloglanis pretoriae*	Cpre	32	ZA	L	Site 5
2.01	*Clarias theodorae*	Cthe	1	ZA	L	Site 5
2.01	*Labeo cylindricus*	Lcyl	8	ZA	L	Site 5
2.01	*Petrocephalus wesselsi*	Pwes	4	ZA	L	Site 5
2.01	*Pseudocrenilabrus philander*	Pphi	4	ZA	L	Site 5

Table 3A: Changes in commonness of Velocity-Depth classes

CHANGE IN COMMONNESS OF VELOCITY-DEPTH CLASSES					
VELOCITY-DEPTH CLASSES METRICS **WITH REFERENCE TO FLOW MODIFICATIONS AND CHANGES IN SEDIMENT MOVEMENT, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?**	**COMMONNESS ORDER UNDER REF COND**	**%WEIGHT**	**RATINGS**		
Commonness of FAST-DEEP conditions	2	70	1.0		
Commonness of FAST-SHALLOW conditions	1	100	1.0		
Commonness of SLOW-DEEP conditions	3	50	2.0		
Commonness of SLOW-SHALLOW conditions	3	50	1.0		
Absolute sum	4				
Absolute overall weighed % velocity-depth change			**23.7**		
AUTOMATED					
CHANGES IN COMMONNESS OF SPECIES WITH HIGH TO VERY HIGH PREFERENCE FOR VELOCITY DEPTH CLASSES					
VELOCITY-DEPTH CLASSES METRICS **BASED ON OBSERVED AND DERIVED DATA, AND WITH WITH REFERENCE TO VELOCITY-DEPTH CLASS PREFERENCES, HOW DID THE FOLLOWING CHANGE?**	**RANK**	**%WEIGHT**	**RATINGS**	**REF NUMBER OF SPP WITH PREFERENCE**	**PRESENT NUMBER OF SPP WITH PREFERENCE**
Response of species with high to very high preference for FAST-DEEP conditions	2	87	-2.5	8.0	5.0
Response of species with high to very high preference for FAST-SHALLOW conditions	1	100	-2.5	8.0	5.0
Response of species with high to very high preference for SLOW-DEEPconditions	3	83	-1.0	15.0	11.0
Response of species with high to very high preference for SLOW-SHALLOW conditions	4	67	-0.5	11.0	8.0
Absolute sum	4				
Absolute overall weighed % assemblage change			**2.50**		
ADJUSTED					
VELOCITY-DEPTH CLASSES METRICS **BASED ON OBSERVED AND DERIVED DATA, AND WITH WITH REFERENCE TO VELOCITY-DEPTH CLASS PREFERENCES, HOW DID THE FOLLOWING CHANGE?**	**RANK**	**%WEIGHT**	**RATINGS**	**GENERIC GUIDELINES FOR RATING (0-->5**	
Response of species with high to very high preference for FAST-DEEP conditions	2	87	1		
Response of species with high to very high preference for FAST-SHALLOW conditions	1	100	1		
Response of species with high to very high preference for SLOW-DEEPconditions	3	80	1		
Response of species with high to very high preference for SLOW-SHALLOW conditions	4	74	1		
Absolute sum	4				
Absolute overall weighed % assemblage change			**10.00**		

Table 4A: Change in commonness of cover features.

CHANGE IN COMMONNESS OF FISH COVER FEATURES			
COVER METRICS: CHANGES IN FISH COVER FEATURES IN COMPARISON TO THE REFERENCE CONDITION	COMMONNESS ORDER UNDER REF COND	%WEIGHT	RATINGS
Commonness of overhanging vegetation	2	70	-1.0
Commonness of undercut banks and root wads	3	60	-1.0
Commonness of substrate types that can serve as cover	1	100	0.0
Commonness of instream vegetation	5	20	-1.0
Commonness of sufficient water column depth that can serve as cover	4	40	-2.0
Absolute sum	5		
Absolute overall weighed % velocity-depth change			**15.9**

AUTOMATED					
CHANGE IN COMMONNESS OF SPECIES WITH PREFERENCE FOR SPECIFIC COVER FEATURES					
COVER METRICS: WITH REFERENCE TO CHANGES IN FISH COVER FEATURES, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?	RANK	%WEIGHT	RATINGS	REF NUMBER OF SPP WITH PREFERENCE	PRESENT NUMBER OF SPP WITH PREFERENCE
= Response of species with a very high to high preference for overhanging vegetation	3	68	-1.0	10.0	6.0
Response of species with a very high to high preference for undercut banks and root wads	4	60	-1.5	6.0	2.0
Response of species with a high to very high preference for a particular substrate type	1	100	-2.0	10.0	6.0
Response of species with a high to very high preference for instream vegetation	5	54	-5.0	2.0	0.0
Response of species with a very high to high preference for the water column	2	91	-1.0	6.0	5.0
Absolute sum	5				
Absolute overall % assemblage change			**38.5**		

ADJUSTED			
CHANGE IN COMMONNESS OF SPECIES WITH PREFERENCE FOR SPECIFIC COVER FEATURES			
COVER METRICS: WITH REFERENCE TO CHANGES IN FISH COVER FEATURES, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?	RANK	%WEIGHT	RATINGS
= Response of species with a very high to high preference for overhanging vegetation	3	68	1
Response of species with a very high to high preference for undercut banks and root wads	4	60	-2
Response of species with a high to very high preference for a particular substrate type	1	100	-2
Response of species with a high to very high preference for instream vegetation	5	54	1
Response of species with a very high to high preference for the water column	2	91	1
Absolute sum	5		
Absolute overall % assemblage change			**27.0**

Table 5A: Flow modification

FLOW MODIFICATIONS					
FLOW MODIFICATION METRICS: WHAT IS THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE? (CARRIED OVER FROM DRIVER ASSESSMENT)	**RANK**	**%WEIGHT**	**RATINGS**		
Increase or decrease in low-flow conditions					
Increase or decrease in zero-flow conditions					
Change in seasonality					
Increase or decrease in moderate events					
Increase or decrease in events (high flow, floods)					
Absolute sum	0	0			
Absolute overall weighed % change in flow metrics			**#DIV/0!**		
AUTOMATED					
FLOW MODIFICATION METRICS:					
FLOW DEPENDANCE METRICS: BASED ON OBSERVED AND DERIVED DATA, AND WITH WITH REFERENCE FLOW DEPENDANCE, HOW DID THE FOLLOWING CHANGE?	**RANK**	**%WEIGHT**	**RATINGS**	**REF NUMBER OF SPP WITH PREFERENCE**	**PRESENT NUMBER OF SPP WITH PREFERENCE**
Response of species intolerant of no-flow conditions	1	100	-1.5	4.0	3.0
Response of species moderately intolerant of no-flow conditions	2	75	-1.0	5.0	4.0
Response of species moderately tolerant of no-flow conditions	3	41	-1.5	9.0	4.0
Response of species tolerant of no-flow conditions	4	26	-1.5	4.0	4.0
Absolute sum					
Absolute overall % assemblage change			**26.90**		
ADJUSTED					
FLOW MODIFICATION METRICS:					
FLOW DEPENDANCE METRICS: BASED ON OBSERVED AND DERIVED DATA, AND WITH WITH REFERENCE FLOW DEPENDANCE, HOW DID THE FOLLOWING CHANGE?	**RANK**	**%WEIGHT**	**RATINGS**		
Response of species intolerant of no-flow conditions	1	100	1		
Response of species moderately intolerant of no-flow conditions	2	75	-1		
Response of species moderately tolerant of no-flow conditions	3	41	1		
Response of species tolerant of no-flow conditions	4	26	1		
Absolute sum					
Absolute overall % assemblage change			**13.10**		

Table 6A: Physico-chemical metrics

PHYSICO-CHEMICAL METRICS: WHAT IS THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE? (CARRIED OVER FROM PHYSICO-CHEMICAL DRIVER ASSESSMENT)	RANK	%WEIGHT	RATINGS
pH			
SALTS			
NUTRIENTS			
TEMPERATURE			
TURBIDITY			
OXYGEN			
TOXICS			
Absolute sum	0		
Absolute overall % change in physico-chemical conditions			#DIV/0!

AUTOMATED

IMPACT ON SPECIES WITH DIFFERENT INTOLERANCE LEVELS TO CHANGE IN PHYSICO-CHEMICAL CONDITIONS

PHYSICO-CHEMICAL METRICS: BASED ON OBSERVED AND DERIVED DATA, AND WITH REFERENCE TO INTOLERANCE TO MODIFIED PHYSICO-CHEMICAL CONDITIONS, HOW DID THE FOLLOWING RESPOND IN TERMS OF FISH HEALTH AND CONDITION?	RANK	%WEIGHT	RATINGS	REF NUMBER OF SPP WITH PREFERENCE	PRESENT NUMBER OF SPP WITH PREFERENCE
Response of species intolerant of modified physico-chemical conditions	1	100	-1.5	4.0	3.0
Response of species moderately intolerant of modified physico-chemical conditions	2	68	-2.0	5.0	3.0
Response of species moderately tolerant of modified physico-chemical conditions	3	39	-1.0	7.0	4.0
Response of species tolerant of modified physico-chemical conditions	4	39	-1.5	6.0	5.0
Absolute sum	4				
Absolute overall % impact on assemblage			**31.2**		

ADJUSTED

IMPACT ON SPECIES WITH DIFFERENT INTOLERANCE LEVELS TO CHANGE IN PHYSICO-CHEMICAL CONDITIONS

PHYSICO-CHEMICAL METRICS: BASED ON OBSERVED AND DERIVED DATA, AND WITH REFERENCE TO INTOLERANCE TO MODIFIED PHYSICO-CHEMICAL CONDITIONS, HOW DID THE FOLLOWING RESPOND IN TERMS OF FISH HEALTH AND CONDITION?	RANK	%WEIGHT	RATINGS
Response of species intolerant of modified physico-chemical conditions	1	100	1
Response of species moderately intolerant of modified physico-chemical conditions	2	68	-2
Response of species moderately tolerant of modified physico-chemical conditions	3	39	1
Response of species tolerant of modified physico-chemical conditions	4	39	1
Absolute sum	4		
Absolute overall % impact on assemblage			**18.3**

Table 7A: Migration Metrics

IMPACT ON SPECIES WITH DIFFERENT LEVELS OF MIGRATORY REQUIREMENTS

MIGRATION METRICS: BASED ON OBSERVED AND DERIVED DATA, AND WITH REFERENCE TO CHANGES IN SYSTEM CONNECTIVITY, HOW DID THE FOLLOWING CHANGE?	RANK	%WEIGHT	RATINGS	REF NUMBER OF SPP WITH PREFERENCE	PRESENT NUMBER OF SPP WITH PREFERENCE
Response in terms of distribution/abundance of spp with catchment scale movements	2	80	2.0		
Response in terms of distribution/abundance of spp with requirement for movement between reaches or fish habitat segments	1	100	3.0		
Response in terms of distribution/abundance of spp with requirement for movement within reach or fish habitat segment	3	70	1.5		
Absolute sum	3				
Absolute overall % change in assemblage longitudinal continuity			**45.2**		

Table 8A: Introduced species.

INTRODUCED SPECIES IMPACT			
INTRODUCED SPECIES METRICS: **WITH REFERENCE TO THE TYPES OF INTRODUCED SPECIES, THE CHARACTERISTICS OF THE HABITAT AND THE NATIVE SPECIES, WHAT IS THE FOLLOWING OBSERVED OR EXPECTED TO BE?**	**RANK**	**%WEIGHT**	**RATINGS**
The impact/potential impact of introduced competing/predaceous spp?	0	100	0.0
How widespread (frequency of occurrence) are introduced competing/predaceous spp?	0	100	0.0
The impact/potential impact of introduced habitat modifying spp?	0	100	0.0
How widespread (frequency of occurrence) are habitat modifying spp?	0	100	0.0
Absolute sum	0		
Absolute overall potential % assemblage change			**0.0**

Table 9A: FRAI (%) and EC

AUTOMATED	
FRAI (%)	**87.1**
EC: FRAI	**B**
ADJUSTED	
FRAI (%)	**87.5**
EC: FRAI	**A/B**

APPENDIX B: MACROINVERTEBRATE ASSEMBLAGE AS BIOINDICATORS

The following table displays the abundances of macroinvertebrate families/taxa collected at Nwanedi and Luphephe rivers during the summer (October 2021) and winter (May 2022).

Table 1B: The abundances of macroinvertebrate families/taxa collected at Nwanedi and Luphephe rivers during the sampling surveys.

	Ecoregions/Sampling sites																			
	Soutpansberg 2.01 Ecoregion										Limpopo Plain 1.02 Ecoregion				Limpopo Plain 1.01 ecoregion					
Macroinvertebrate Family/Taxa	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w
Aeshnidae	-	1	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Athericidae	-	-	-	-	1	A	A	-	1	A	A	-	1	A	-	-	1	-	-	-
Atyidae	-	-	-	-	A	A	-	-	B	A	A	A	-	-	-	-	-	-	-	-
Baetidae 1sp	A	A	1	A	1	A	-	-	A	-	-	-	-	1	-	-	-	A	-	-
Baetidae 2sp	-	A	-	-	A	-	A	-	-	-	-	B	-	-	-	-	-	A	-	-
Baetidae >2 sp	-	-	A	-	A	-	-	B	B	B	B	B	B	-	-	-	-	A	-	-
Belostomatidae	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	A	-	-	-
Caenidae	-	-	-	-	-	-	A	-	A	-	-	-	A	-	-	-	C	-	-	-
Calopterygidae ST,T	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Ceratopogonidae	-	-	B	-	A	1	-	-	-	-	A	-	B	-	-	-	1	-	-	-
Chironomidae	1	1	B	B	A	A	-	B	-	-	A	A	A	-	-	-	B	A	-	-

Key: S = Summer and W = Winter. Estimate abundances; 1 = 1, A = 2 - 10, B = 10 – 100 and C = 100 – 1000.

Table 1B Continued…

	Ecoregions/Sampling sites																			
	Soutpansberg 2.01 Ecoregion										Limpopo Plain 1.02 Ecoregion				Limpopo Plain 1.01 ecoregion					
Macroinvertebrate Family/Taxa	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w
Chlorocyphidae	-	1	-	-	A	-	-	1	-	-	A	-	-	-	-	-	-	1	-	-
Chlorolestidae (Synlestidae)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coenagrionidae	-	-	B	B	A	A	-	-	B	-	A	A	-	B	-	-	A	B	-	-
Corbiculidae	-	-	-	-	-	-	1	-	-	-	A	B	A	-	-	-	B	B	-	-
Corixidae	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicidae	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dixidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dytiscidae	A	A	B	A	-	A	-	-	-	-	1	-	-	-	-	-	A	1	-	-
Elmidae	-	-	A	-	A	-	-	A	1	-	-	A	-	-	-	-	A	A	-	-
Gerridae	-	-	A	-	B	-	B	-	A	B	A	-	-	-	-	-	-	-	-	-
Gomphidae	A	B	A	B	A	A	-	A	1	-	-	A	B	1	-	-	A	A	-	-
Gyrinidae	A	A	A	B	B	-	-	-	A	B	-	-	-	-	-	-	-	-	-	-
Heptageniidae	-	-	-	-	-	A	-	A	A	B	-	-	-	-	-	-	-	-	-	-
Hirudinea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-
Hydracarina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	1	-	-
Hydrometridae	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-
Hydropsychidae 1sp		A	A	A	1	1	A	-	-	A	-	-	-	-	-	-	-	A	-	-
Hydropsychidae 2sp	-	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	A	-	-

Key: S = Summer and W = Winter. Estimate abundances; 1 = 1, A = 2 - 10, B = 10 – 100 and C = 100 – 1000.

Table 1B Continued…

	Ecoregions/Sampling sites																			
	Soutpansberg 2.01 Ecoregion										Limpopo Plain 1.02 Ecoregion				Limpopo Plain 1.01 ecoregion					
Macroinvertebrate Family/Taxa	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w	S	w
Hydropsychidae > 2sp	-	-	-	-	-	-	-	-	-	-	B	B	A	-	-	-	B	-	-	-
Leptoceridae	B	B	A	B	1	1	-	-	A	-	-	-	-	-	-	-	-	-	-	-
Leptophlebiidae	-	-	-	-	B	-	A	B	B	-	A	A	A	1	-	-	A	B	-	-
Libellulidae	A	A	1	-	1	-	-	1	-	1	A	-	-	-	-	-	B	A	-	-
Naucoridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	-	-	-
Nepidae	-	-	A	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligochaeta	-	-	-	-	-	-	-	-	-	-	A	A	A	1	-	-	1	A	-	-
Perlidae	-	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	-	-	-
Physidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
Planorbinae	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleidae	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Potamonautidae	-	1	1	1	-	1	1	A	A	A	A	B	A	A	-	-	-	A	-	-
Prosopistomatidae	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Psephenidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simuliidae	-	-	-	A	-	-	-	B	A	-	1	B	B	A	-	-	B	A	-	-
Tabanidae	-	-	-	-	-	-	-	A	-	A	A	A	A	A	-	-	A	A	-	-
Thiaridae	-	-	-	-	-	-	-	-	-	-	C	B	A	B	-	-	C	C	-	-
Tipulidae	-	-	-	-	A	-	-	-	-	-	A	-	-	-	-	-	A	-	-	-
Turbellaria	1	-	-	-	-	-	-	-	A	-	B	A	A	-	-	-	-	-	-	-
Veliidae/Mesoveliidae	-	-	B	B	B	B	A	-	A	A	B	B	-	-	-	-	A	A	-	-

Key: S = Summer and W = Winter. Estimate abundances; 1 = 1, A = 2 - 10, B = 10 – 100 and C = 100 – 1000.

Macroinvertebrate Response Assessment Index – Procedures

The following tables (Table 2B to 7B) displays the Macroinvertebrate Response Assessment Index (MIRAI) procedures undertaken during the analyses of the macroinvertebrate communities from the Soutpansberg 2.01 Ecoregion during the summer survey. The same procedures were followed in determination of the present ecological state (PES) of the Limpopo Plain 1.02 and Limpopo Plain 1.01 ecoregions during the summer and winter surveys, respectively.

Soutpansberg 2.01 Ecoregion: Summer (October 2021)

Table 2B: Macroinvertebrate data

Taxon	Season	Ref abun	Ref freq	Pres Abun	Pres freq	<0.1	0.1-0.3	0.3-0.6	>0.6	BEDROCK	COBBLES	VEG	GSM	WATER	QUALITY
Aeshnidae		A	4	A	2	1	2	2	2	0	3	2	0	0	MODERATE
Athericidae		A	4	A	3	0	1	2	2	1	4	1	1	0	MODERATE
Atyidae		A	5	B	3	2	2	0	0	0	1	4	1	0	MODERATE
Baetidae >2spp				B	4	2	2	2	2	2	2	2	2	1	HIGH
Baetidae 1sp				A	5	2	2	2	2	2	2	2	2	1	LOW
Baetidae 2spp		B	5	A	2	2	2	2	2	2	2	2	2	1	LOW
Belostomatidae		A	3			4	1	0	0	0	0	4	0	1	NONE
Caenidae		A	3	A	3	3	2	1	1	0	2	1	3	0	LOW
Ceratopogonidae		A	4	A	3	2	2	2	4	2	3	2	2	0	LOW
Chironomidae		A	5	A	4	1	3	2	2	2	2	2	2	0	NONE
Chlorocyphidae		A	4	A	2	2	3	1	0	1	4	1	0	0	MODERATE
Chlorolestidae (Synlestidae)		A	3			3	2	1	0	0	1	4	0	0	MODERATE
Coenagrionidae		A	5	B	5	1	2	3	1	0	1	4	1	0	LOW
Corbiculidae				1	2	2	3	1	0	0	2	0	4	0	LOW
Corduliidae		A	3			2	3	1	0	0	2	1	3	0	MODERATE
Corixidae		A	2			2	3	1	0	1	1	1	1	4	NONE
Culicidae		A	2	A	2	3	1	0	0	0	0	0	0	5	NONE
Dixidae		1	2			3	2	2	0	0	0	0	0	5	MODERATE
Dytiscidae/Noteridae		B	3	B	3	4	2	1	0	1	2	3	1	2	LOW
Elmidae		A	3	A	5	0	0	4	2	1	4	1	0	0	MODERATE
Gerridae		A	5	B	5	4	1	0	0	0	0	0	0	5	MODERATE
Gomphidae		B	5	A	5	0	2	3	0	0	1	0	5	0	LOW
Gyrinidae		B	5	B	5	1	2	2	3	0	0	0	0	5	LOW
Heptageniidae		A	4	A	2	1	1	3	2	1	4	1	0	0	HIGH
Hydracarina		A	3			0	2	2	0	1	1	2	3	1	MODERATE
Hydraenidae		1	2			2	2	3	2	0	1	3	1	2	MODERATE
Hydrometridae		A	3	A	2	4	1	0	0	0	0	2	0	4	MODERATE
Hydropsychidae >2spp		B	5			0	1	2	4	2	3	1	0	0	HIGH
Hydropsychidae 1sp				A	4	0	1	2	4	2	3	1	0	0	LOW
Hydropsychidae 2spp				A	2	0	1	2	4	2	3	1	0	0	LOW
Leeches		A	4			2	2	1	1	0	4	1	1	0	NONE
Leptoceridae		A	4	B	5	0	1	3	2	2	2	2	2	0	LOW
Leptophlebiidae		B	5	B	4	3	2	2	1	1	3	2	0	0	MODERATE
Lestidae		A	3			4	1	0	0	0	1	4	1	0	MODERATE
Libellulidae		A	5	A	4	1	2	3	1	1	4	0	1	0	LOW
Nepidae		A	2	A	3	4	1	0	0	0	0	5	0	0	NONE
Notonectidae		A	3			4	1	0	0	0	0	2	0	4	NONE
Oligochaeta		A	5			2	2	2	1	0	1	0	4	0	NONE
Oligoneuridae		A	3			0	0	1	5	2	3	1	1	1	HIGH
Perlidae		A	3	A	2	1	1	1	5	1	4	1	0	0	HIGH
Philopotamidae		A	2			0	1	2	3	1	4	1	1	0	MODERATE
Planorbinae				A	2	3	2	0	0	2	2	3	0	0	NONE
Pleidae				A	2	4	1	0	0	0	0	4	0	1	LOW
Potamonautidae		A	5	A	4	1	1	3	2	0	3	1	1	0	NONE
Psephenidae		A	3			0	1	3	4	1	4	1	0	0	MODERATE
Simuliidae		A	5	A	2	0	2	2	4	2	3	2	0	0	LOW
Tabanidae		A	5			2	3	1	0	0	2	0	3	0	LOW
Tipulidae		A	3			3	4	1	1	1	2	0	3	0	LOW
Tricorythidae		B	5			0	1	1	4	1	4	1	0	0	MODERATE
Turbellaria				A	3	1	2	3	4	1	4	0	0	0	NONE
Veliidae/Mesoveliidae		B	5	B	5	5	1	1	0	0	0	0	0	5	MODERATE

Table 3B: Flow modification metrics

FLOW MODIFICATION METRICS. WITH REFERENCE TO VELOCITY PREFERENCES, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?	RATING	RANKING OF METRICS	% Weight
Presence of taxa with a preference for very fast flowing water	2	2	80
Abundance and/or frequency of occurrence of taxa with a preference for very fast flowing water	2	2	80
Presence of taxa with a preference for moderately fast flowing water	1	3	60
Abundance and/or frequency of occurrence of taxa with a preference for moderately fast flowing water	1	3	60
Presence of taxa with a preference for slow flowing water	3	1	100
Abundance and/or frequency of occurrence of taxa with a preference for slow flowing water	3	1	100
Presence of taxa with a preference for standing water	2	2	80
Abundance and/or frequency of occurrence of taxa with a preference for standing water	2	2	80
Overall % change in flow dependance of assemblage			43

Table 4B: Habitat modification metrics

HABITAT MODIFICATION METRICS. WITH REFERENCE TO INVERTEBRATE HABITAT PREFERENCES, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?	**RATING**	**RANKING OF METRICS**	**%WEIGHT**
Has the occurrence of invertebrates with a preference for bedrock/boulders changed relative to expected?	0	0	0
Has the abundance and/or frequency of occurrence of any of the taxa with a preference for bedrock/boulders changed?	0	0	0
Has the occurrence of invertebrates with a preference for loose cobbles changed relative to expected?	2	2	60
Has the abundance and/or frequency of occurrence of any of the taxa with a preference for loose cobbles changed?	1	2	60
Has the occurrence of invertebrates with a preference for vegetation changed relative to expected?	2	2	60
Has the abundance and/or frequency of occurrence of any of the taxa with a preference for vegetation changed?	1	2	60
Has the occurrence of invertebrates with a preference for sand, gravel or mud changed relative to expected?	4	1	100
Has the abundance of any of the taxa with a preference for sand, gravel or mud changed relative to expected?	3	1	100
Has the occurrence of invertebrates with a preference for the water column or water surface changed relative to expected?	1	4	40
Has the abundance and/or frequency of occurrence of any of the taxa with a preference for the water column/water surface changed?	1	4	40
Overall % change in flow dependance of assemblage			44

Table 5B: Water quality metrics

WATER QUALITY METRICS. WITH REFERENCE TO WATER QUALITY REQUIREMENTS, WHAT ARE THE CHANGES TO THE FOLLOWING OBSERVED OR EXPECTED TO BE?	RATING	RANKING OF METRICS	% WEIGHT
Has the number of taxa with a high requirement for unmodified physico-chemical conditions changed?	3	1	100
Has the abundance and/or frequency of occurrence of the taxa with a high requirement for unmodified physico-chemical	2	2	80
Has the number of taxa with a moderate requirement for unmodified physico-chemical conditions changed?	3	1	100
Hasthe abundance and/or fequency of occurrence of the taxa with a moderate requirement for modified physico-chemical	3	3	40
Has the number of taxa with a low requirement for unmodified physico-chemical conditions changed?	1	3	40
Has the abundance and/or frequency of occurrence of the taxa with a low requirement for unmodified physico-chemical	1	3	40
Has the number of taxa with a very low requirement for unmodified physico-chemical conditions changed?	3	3	40
Has the abundance and/or frequency of occurrence of the taxa with a very low requirement for unmodified physico-chemical	3	1	100
How does the total SASS score differ from expected?	2	2	80
How does the total ASPT score differ from expected?	1	3	40
Overall change to indicators of modified water quality			48

Table 6B: System connectivity and seasonality

WHAT IS THE EXTENT OF THE FOLLOWING	RATINGS	COMMENTS
Weirs and causeways	2.00	
Impoundments		
Changes in seasonality		

Based on observed and derived data, with reference to migration and seasonality, how did the following change?	RATING	RANKING OF METRICS	% Weight
Impact on distribution of migratory taxa		2	
Impact on abundance and/or frequency of occurrence of migratory taxa			
Impact on occurrence of taxa with seasonal distribution	2	1	100
Impact on abundance and/or frequency of occurrence of taxa with seasonal distribution			
Overall % change in flow dependance of assemblage			**40**

Figure 7B: MIRAI (%) and EC

Which of these measures will best indicate the response of invertebrates (*in this system at this site*)

INVERTEBRATE EC: BASED ON WEIGHTS OF METRIC GROUPS

INVERTEBRATE EC METRIC GROUP		METRIC GROUP	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR	COMMENTS
FLOW MODIFICATION	**FM**	23.9	*0.357*	8.53175	1	100	
HABITAT	**H**	23.2	*0.286*	6.64093	2	80	
WATER QUALITY	**WQ**	61.2	*0.214*	13.1056	3	60	
CONNECTIVITY & SEASONALITY	**CS**	60.0	*0.143*	8.57143	4	40	
						280	
INVERTEBRATE EC				36.8497			
INVERTEBRATE EC CATEGORY				**E**			

>89=A; 80-89=B; 60-79=C; 40-59=D; 20-39=E; <20=F

EC=Ecological category

APPENDIX C: ICHTHYOPARASITES AS BIONDICATORS

Table 1C: Parasites survey of *Pseudocrenilabrus philander* from three sites in the Soutpansberg 2.01 Ecoregion during the summer 2021 (Site 4) and winter 2022 (sites 3, 4 and 5) surveys in the Nwanedi and Luphephe rivers.

Fish no.	Season	Year	Sampling site	Mass (g)	TL (cm)	ST (cm)	Sex (M/F)	Recorded parasite(s)	No. of parasites	Collected from
Soutpansberg 2.01 Ecoregion										
1	Summer	2021	4	8.6	8.6	7.1	M	Monogeneans, gryporhynchid larvae	46, 1	Gills, intestine
2	Summer	2021	4	6.1	7.8	5.9	M	Monogeneans	40	Gills
3	Summer	2021	4	2.4	5.7	4.4	M	Monogeneans	4	Gills
4	Summer	2021	4	2.8	5.9	4.8	F	Monogeneans, nematodes	14, 6	Gills, intestines
5	Summer	2021	4	2.2	5.2	4.4	F	-	-	-
6	Summer	2021	4	0.7	3.5	2.8	M	-	-	-
7	Summer	2021	4	1.0	4.2	3.2	M	Monogeneans	4	Gills
8	Summer	2021	4	1.6	4.9	3.9	F	Monogeneans	19	Gills
9	Summer	2021	4	0.6	3.7	2.8	M	Monogeneans	2	Gills
10	Summer	2021	4	1.6	4.0	2.8	M	-	-	-

Table 1C Continued…

Fish no.	Season	Year	Sampling site	Mass (g)	TL (cm)	ST (cm)	Sex (M/F)	Recorded parasite(s)	No. of parasites	Collected from
1	Winter	2022	3	2.0	5.4	4.2	F	Gryporhynchid larvae	6	Intestine
2	Winter	2022	3	1.3	4.7	3.9	M	Monogeneans, gryporhynchid larvae	2, 1	Gills, intestine
3	Winter	2022	3	0.9	4.1	3.1	M	Monogeneans	7	Gills
4	Winter	2022	3	0.8	4.1	3.0	M	-	-	-
5	Winter	2022	3	1.0	4.2	3.2	M	-	-	-
6	Winter	2022	3	2.1	4.8	5.7	F	-	-	-
7	Winter	2022	3	3.6	4.6	3.6	F	-	-	-
8	Winter	2022	3	0.9	4.4	3.4	M	-	-	-
9	Winter	2022	3	0.9	3.5	2.8	M	-	-	-
10	Winter	2022	3	0.8	3.9	3.0	M	-	-	-

Table 1C Continued…

Fish no.	Season	Year	Sampling site	Mass (g)	TL (cm)	ST (cm)	Sex (M/F)	Recorded parasite(s)	No. of parasites	Collected from
1	Winter	2022	4	1.4	5.7	3.6	M	-	-	-
2	Winter	2022	4	1.6	5.1	4.1	M	-	-	-
3	Winter	2022	4	3.8	5.5	5.5	F	-	-	-
4	Winter	2022	4	1.8	5.5	4.2	M	-	-	-
5	Winter	2022	4	1.9	5.2	4.8	M	-	-	-
6	Winter	2022	4	3.6	5.7	4.5	F	-	-	-
7	Winter	2022	4	1.9	5.3	4.2	M	-	-	-
8	Winter	2022	4	3.2	5.0	4.2	F	-	-	-
9	Winter	2022	4	0.6	3.5	2.1	M	-	-	-

Table 1C Continued…

Fish no.	Season	Year	Sampling site	Mass (g)	TL (cm)	ST (cm)	Sex (M/F)	Recorded parasite(s)	No. of parasites	Collected from
1	Winter	2022	5	3.1	6.7	5.1	F	-	-	-
2	Winter	2022	5	1.0	4.6	3.5	M	-	-	-
3	Winter	2022	5	0.7	3.8	3.0	M	-	-	-
4	Winter	2022	5	1.6	5.2	4.0	M	-	-	-
5	Winter	2022	5	0.6	3.5	2.7	M	-	-	-

Note: "-" Not detected

Table 2C: *Pseudocrenilabrus philander* from Site 5 in the Limpopo Plain 1.02 Ecoregion during the winter 2022 survey in the Nwanedi River catchment.

Fish no.	Season	Year	Sampling site	Mass (g)	TL (cm)	ST (cm)	Sex (M/F)	Recorded parasite(s)	No. of parasites	Collected from
Limpopo Plain 1.02 Ecoregion										
1	Winter	2022	6	3.6	6.2	5.2	M	-	-	-
2	Winter	2022	6	1.5	4.9	4.2	M	-	-	-
3	Winter	2022	6	0.8	4.0	3.2	M	-	-	-
4	Winter	2022	6	0.6	4.1	3.9	M	-	-	-

Note: "-" Not detected