Rehabilitation of National Route R56 Section 8 from Matatiele (KM 130.15) to the KZN border (KM168.71)

J35193

Baseline Aquatic Biodiversity Assessment

June 2016

Declaration of Independence

I, **BYRON BESTER**, in my capacity as a specialist consultant, hereby declare that I -

- Act as an independent consultant;
- Do not have any financial interest in the undertaking of the activity, other than remuneration for the work performed in terms of the National Environmental Management Act, 1998 (Act 107 of 1998);
- Have and will not have vested interest in the proposed activity proceeding;
- Have no, and will not engage in, conflicting interests in the undertaking of the activity;
- Undertake to disclose, to the competent authority, any material information that has or may have the potential to influence the decision of the competent authority or the objectivity of any report, plan or document required in terms of the National Environmental Management Act, 1998 (Act 107 of 1998);
- Will provide the competent authority with access to all information at my disposal regarding the application, whether such information is favourable to the applicant or not;
- As a registered member of the South African Council for Natural Scientific Professions, will undertake my profession in accordance with the Code of Conduct of the Council, as well as any other societies to which I am a member;
- Based on information provided to me by the project proponent and in addition to information obtained during the course of this study, have presented the results and conclusion within the associated document to the best of my professional ability; and
- Undertake to have my work peer reviewed on a regular basis by a competent specialist in the field of study for which I am registered.

10 / 06 / 2016

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Date

Executive Summary

The South African National Roads Agency SOC Ltd (SANRAL) had previously earmarked Section 8 of the R56 National Route, which extends from the intersection of East Road in the town of Matatiele in the Eastern Cape to the KwaZulu-Natal border near the town of Kokstad, for rehabilitation and reseal. Of the numerous alternative approaches proposed to SANRAL, the road upgrade was concluded to comprise of offsetting the centreline of the existing road by 7 m and widening the shoulder of the existing road by 3 m on each side. While this approach required demolition and reconstruction of under-capacitated bridges and culvert systems along the route, the Mzimvubu River Bridge (Km 155) was not to be altered or modified.

The GIBB (Pty) Ltd, as independent environmental scientists and biodiversity specialists, was appointed by SANRAL to undertake a specialist aquatic assessment (including EcoStatus assessment) of six selected watercourse crossings along the proposed road reserve design. This report presents the findings obtained following an assessment of the aquatic ecosystems associated with the proposed R56 Road Upgrade, the field survey for which was conducted between the 11th of April 2016 and the 15th of April 2016.

Based on results obtained during the April 2016 field survey, it was determined that the unnamed tributaries of the Mzimvubu River each represented an integrated EcoStatus of largely modified (Ecological Category D; i.e. Site T3MZIM-ALING and Site T3MZIMSTRYD;) and largely-to-seriously modified (Ecological Category D/E; i.e. Site T3MZIM-EDNDL) conditions. These conditions were attributed to an absence of fish along each of the assessed watercourses, which may have been facilitated by the fragmented habitat created by established farm dams within the study area, and a poor habitat availability for macroinvertebrate colonisation, which was likely to a result of the inherent nature of the suspected channelled valley-bottom wetland systems (i.e. Site T3MZIM-EDNDL and Site T3MZIM-ALING). With respect to the larger perennial systems, it was determined that the integrated EcoStatus was representative of moderately modified (Ecological Category C) at Site T3MZIM-STRYD and largely modified (Ecological Category D) conditions at Site T3MZIM-DSR56 conditions. However, it was suspected that the instream biological integrity was skewed at the time of the current survey as a result of the low water levels and subsequent lack of niche habitat, especially along the main stem Mzimvubu River. Nevertheless, these systems were still believed to support the established aquatic communities within the study area and to effectively provide refugia habitat during periods low rainfall (or drought), as observed at the time of the survey.

Given the presence of wetland-related features and classification of many of the associated watercourses as wetlands (according to Nel et al., 2011), as well as the limitations of EcoStatus determination in wetland systems, it is strongly recommended that a wetland assessment (including determination of Present Ecological State according to the Wet-Health approach) be conducted by a recognised wetland specialist. The study should not be limited to those sites assessed during the current assessment, but should consider the extent of all wetland features associated with the proposed R56 road reserve design, as well as an assessment of potential mitigation measures, so as to limit potential impacts of the proposed study on associated wetlands.

Matatiele R56 Road Rehabilitation (Section 8): Aquatic Assessment

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List of Acronyms

CSIR	Council for Scientific and Industrial Research
DEA	Department of Environmental Affairs
DWS	Department of Water and Sanitation
EC	Ecological Category
EIS	Ecological Importance and Sensitivity
ETS	Ecosystem Threat Status
EPL	Ecosystem Protection Level
FAII	Fish Assemblage Integrity Index
FEPA	Freshwater Ecosystem Priority Area
FRAI	Fish Response Assessment Index
GPS	Global Positioning System
IHAS	Invertebrate Habitat Assessment System, Version 2.2
IHI-96-2	Index for Habitat Integrity, Version 2
IUCN	International Union for Conservation of Nature
MAP	Mean Annual Precipitation
MIRAI	Macro-Invertebrate Response Assessment Index
NBA	National Biodiversity Assessment
NFEPA	National Freshwater Ecosystem Priority Areas project
NEMBA	National Environmental Management: Biodiversity Act
PES	Present Ecological State
REMP	River EcoStatus Monitoring Programme
RHP	River Health Programme
SAIAB	South African Institute of Aquatic Biodiversity
SANBI	South African National Biodiversity Institute
SANParks	South African National Parks
SASS5	South African Scoring System, Version 5
ToPS	Threatened and Protected Species Regulation (as described in NEMBA)
WMA	Water Management Area
WRC	Water Research Commission
WWF	Worldwide Fund for Nature

1 Introduction

Biodiversity within inland water ecosystems in southern Africa is both highly diverse and of great regional importance to local livelihoods and economies, as these valuable natural resources (including any associated biota) provide a broad array of goods and services e.g. a source of water for domestic, industrial and agricultural purposes, as well as integral roles in the power generation and waste disposal industries (Dudgeon et al., 2006; Darwall et al., 2009). However, the fact that these freshwater systems may well be the most endangered ecosystem in the world presents a potential risk toward any of the 126,000 described species that depend upon freshwater habitats for any critical part of their life cycle (Dudgeon et al., 2006).

The major global threats identified within these species-rich systems include (i) ecosystem destruction, (ii) habitat alteration, (iii) changes in water chemistry and (iv) direct additions and/or losses of aquatic biota (Malmqvist & Rundle, 2002). The magnitude of the threat to, and loss of, biodiversity in these vulnerable ecosystems is an indicator of the extent to which current practices are unsustainable. Hence, the importance of implementing conservation and management strategies that protect all elements of freshwater biodiversity, which in turn would also help to guarantee water availability in the future (Dudgeon et al., 2006).

The fact that South Africa is a water-scarce country makes these aquatic ecosystems even more susceptible to anthropogenic activities and their associated impacts. Consequently, the state (quality and quantity) of the county’s water resources are fully dependant on good land management practices within catchments. The fate of our natural water resources therefore lies on an integrated approach to natural resource management, in order to achieve ecological and socio-economic sustainability.

1.1 Project Description

The South African National Roads Agency SOC Ltd (SANRAL) had previously earmarked Section 8 of the R56 National Route, which extends from the intersection of East Road in the town of Matatiele in the Eastern Cape to the KwaZulu-Natal border near the town of Kokstad, for rehabilitation and reseal. Of the numerous alternative approaches proposed to SANRAL, the road upgrade was concluded to comprise of offsetting the centreline of the existing road by 7 m and widening the shoulder of the existing road by 3 m on each side. While this approach required demolition and reconstruction of under-capacitated bridges and culvert systems along the route, the Mzimvubu River Bridge (Km 155) was not to be altered or modified.

GIBB (Pty) Ltd, as independent environmental scientists and biodiversity specialists, was appointed by SANRAL to undertake a specialist aquatic assessment (including EcoStatus assessment) of six selected watercourse crossings along the proposed road reserve design. It should be noted that this study represented the initial site-based investigation for the project and as such, each of the associated watercourse crossings were not yet differentiated (i.e. wetland versus riverine system) and the applicability of the EcoStatus approach was still to be determined.

1.2 Terms of Reference

The terms of reference for the current study were as follows:

- Perform a specialist aquatic assessment (including EcoStatus) of the six (6) selected watercourse crossings (i.e. IDB 0528, IDC 0649, IDB 0529, IDC 0653, IDB 0532 and IDC 0661) associated with the proposed R56 National Route rehabilitation.
  - Determine the Present Ecological Status (PES; or Ecological Category) of the associated watercourses, where possible; and
  - Assess the Ecological Importance and Sensitivity (EIS) associated with each of the selected watercourses.

This report presents the findings obtained following an assessment of the aquatic ecosystems associated with the proposed R56 Road Upgrade, the field survey for which was conducted from the 11th to 15th of April 2016.

1.3 Approach to Study

To enable an adequate description and the determination of the Present Ecological State (or Ecological Category) and Ecological Importance and Sensitivities associated with the aquatic ecosystem, the following indicators were evaluated as part of the study:

- Stressor Indicators:
  - *In situ* water quality (Temperature, pH, Electrical Conductivity, and Dissolved Oxygen);
- Habitat Indicators:
  - Adapted Invertebrate Habitat Assessment System (IHAS, Version 2.2);
  - Index for Habitat Integrity (IH, Version 2);
- Response Indicators:
  - Aquatic macroinvertebrates with the use of the South African Scoring System (SASS, Version 5) rapid bio-assessment protocol and the Macro-Invertebrate Response Assessment Index (MIRAI);
  - Ichthyofauna with the use of the Fish Response Assessment Index (FRAI); and
  - Determination of the integrated EcoStatus.

For a detailed description of the methodology employed during the present assessment please refer to Appendix A.

1.4 Assumptions and Limitations

In order to obtain a comprehensive understanding of the dynamics of the biota present within a watercourse (e.g. migratory pathways, seasonal prevalence, etc.), studies should include investigations conducted during different seasons, over a number of years and extensive sampling efforts. Given the time constraints of the present study, such long-term research could not be conducted. Instead, conclusions provided within the present report are based on data collected during a single sampling event, a literature review, and professional experience.

It should be noted that the present study cannot be regarded as an adequate assessment of any of the associated wetland systems, as the EcoStatus approach applied within this study are specifically designed for the determination of ecological health and integrity of associated riverine systems (or watercourses with notable riverine elements). Accordingly, if a wetland health and functionality assessment is required, a wetland specialist should be consulted and site-based investigations should be conducted.

2 Description of the Environment

2.1 Location

The associated section of the R56 National Route earmarked for rehabilitation is situated within the Matatiele Local Municipality, which occurs within the Alfred Nzo District Municipality in north-eastern portion of the Eastern Cape (QDGC 3028BD and 3029AC). As mentioned above, the proposed road reserve design is located between the major towns of Matatiele and Kokstad, and separated by the small town of Cedarville into two focus areas, namely Focus Area A and Focus Area B (Figure 1, 2,-3). The area is dominated by pockets of relatively natural grassland (see Section 2.2.2) and the surrounding land use activities comprise of varying agricultural activities, including the crop cultivation (e.g. maize) and livestock farming (e.g. cattle, horses, etc.).

2.2 Biophysical Description

2.2.1 Climate

The study area is located within the South Eastern Uplands ecoregion (Level 2 Ecoregion 16.04 and 16.08) between altitudes ranging from 1400 – 1600 m above mean sea level (a.m.s.l.). Relative to the country's average mean annual precipitation (MAP) of 490 mm, these ecoregions experience moderate-to-high rainfall of 600-700 mm that falls predominantly during early- to mid-summer (Kleynhans et al., 2007; WWF-SA, 2016). Mean daily maximum temperatures recorded within these sub-humid to humid areas in February range from 22 to 26°C, while mean daily minimum temperatures in July range from 4 to 6°C (Kleynhans et al., 2007).

2.2.2 Regional Geology

According to the relevant 1: 250 000 geological map (i.e. 3028 Kokstad), compiled by the Council for Geoscience, the dominant geology surrounding the 'Cedarville Flats' area is comprised of Tarkastad, which is classified within the Beaufort group and the Kaoo Supergroup. It should be noted that the presence of intrusive dolerite dykes within this geology often creates geological knickpoints and determine the topography of the area (including the longitudinal profile of the associated watercourses. Also, the low lying areas of the 'Cedarville Flats' are largely dominated by alluvium deposits, which facilitates the formation of a number of wetland-type habitats.

2.2.3 Regional Vegetation

According to Mucina & Rutherford (2006), four different vegetation units are associated with the study area, predominantly terrestrial East Griqualand Grassland and Mabela Sandy Grassland, as well as the Eastern Temperate Freshwater Wetlands and Highveld Alluvial Vegetation associated with saturated portions of the 'Cedarville Flats.' These areas are generally characterised by undulating, grassland covered hills and/or valleys with patches of bush clumps in lower lying areas. Consequently, any associated riparian vegetation was likely to be absent of indigenous trees and largely dominated by grasses and a few sedges.

With the exception of the well-drained dominant soils types (e.g. Hutton, Clovelly and Oatsdale forms) associated with the East Griqualand Grassland areas (i.e. adjacent to main towns of Matatiele and Cederville), each of the other dominant soil types were regarded as poorly-draining (or impermeable) soils types (e.g. Champagne, Rensberg, Katspruit forms; Mucina & Rutherford, 2006). Consequently, many of the associated watercourses were likely to be saturated for extended periods and characteristic of numerous wetland-related features.

2.2.4 Associated Watercourses

The present study area is located within the Mzimvubu sub-management area of the Mzimvubu to Keiskamma water management area, which is predominantly comprised of the main stem Mzimvubu River that extends over 200 km from its source in the Maloti-Drakensberg watershed to the Port St Johns estuary. There are four major perennial tributaries that join the Mzimvubu River, including the Kinira, Tina, Tsitsa and Umzintlava rivers, which together form the Tsitsa Falls near Shawbury Mission approximately 120 km from the river's origin (Department of Water Affairs and Forestry, 2004; Environmental and Rural Solutions, 2011).

With the exception of livestock farming, subsistence agriculture and several irrigation developments, economic activity within the water management area is largely restricted to industrial development in the East London area, which is known for its automotive and textile industries. As such, the Mzimvubu River has been identified as the largest undeveloped water resource in the country, as no noteworthy dams had been constructed within its catchment prior to 2004 (Department of Water Affairs and Forestry, 2004). However, it should be noted that the Mzimvubu Water Project was commissioned by the government through the Department of Water and Sanitation (DWS) in 2012, which aims to alleviate water supply through the construction of two large storage dams (namely Ntabelanga and Lalini dams), a tunnel and powerhouse for generating hydropower, as well as bulk water infrastructure for domestic and agricultural uses.

More specifically, while a minor western portion of the proposed road upgrade near Matatiele drains into the Kinira River via the Botsola River (Quaternary Catchments T33A), each of the remaining watercourses associated with the eastern portion of the proposed road upgrade drain into the Mzimvubu River (Quaternary Catchment T31F). The associated minor watercourses observed within the study area were identified as non-perennial drainage lines potentially associated with various wetland systems, while the main stem Mzimvubu River and adjoining major tributary (hereafter referred to as Con Amore Stream) were defined to exhibit a perennial nature (Figure 4, 5). While the geomorphic zonation of many of the minor tributaries within the area were 'unclassified,' the two prominent reaches within the study area, namely the Mzimvubu River and the Con Amore Stream, were classified as 'lowland river' and 'upper foothills,' respectively.

Furthermore, it should be noted that a number channelled valley-bottom wetlands, seepages, and floodplain wetlands were observed to be directly associated with the watercourse under study, as indicated by the extent of wetland habitat delineated through the NFEPA project (Figure 4, 5; Nel et al., 2011).

2.3 Bioregional Context

The proposed R56 Road Reserve Design was directly associated with two different global freshwater ecoregions, namely the Drakensberg-Maloti Highlands (minor portion of the proposed route to the west) and the Southern Temperate Highveld (predominant portion of proposed route to the east):

2.3.1 Drakensberg – Maloti Highlands

The Drakensberg – Maloti Highlands freshwater ecoregion is highly valued in southern Africa for the excellent water quality and the high water supply yield of the associated rivers (Day, 2016). The ecoregion encompasses the whole of Lesotho (excluding the westernmost lowland areas), with the whole of the ecoregion lying above 1,850m (Darwall et al., 2009; Day, 2016).

This region contains rare examples of Afromontane and Afro-alpine rivers, as well as high altitude wetlands with limited occurrences in the Eastern Cape and largely absent elsewhere across southern Africa (Lesotho Government, 2000; Wetlands International, 2003, cited in Day, 2016). The only freshwater fish species endemic to the ecoregion, namely *Pseudobarbus quathlambae* (Maloti Minnow), is regionally regarded as Endangered and known from only six high-altitude tributaries of the Orange River (Darwall et al., 2009; Day, 2016).

2.3.2 Southern Temperate Highveld

The Southern Temperate Highveld global freshwater ecoregion is delimited by the South African interior plateaux sub-region of the Highveld aquatic ecoregion, of which the main habitat type (in terms of watercourse) is Savannah-Dry Forest Rivers (Darwall et al., 2009). Aquatic biota within this bio-region have mixed tropical and temperate affinities, sharing many species between the Limpopo and Zambezi systems (Skelton, 1990; Skelton et al., 1995; Darwall et al., 2009).

It should be noted that the level of biological and ecological investigation within this ecoregion was noted to be high, while the threats to this ecosystem integrity are also relatively well known, which have broadly been attributed to surface water abstraction and impacts associated with the human development and/or 'footprint' (Scott, 2013). Consequently, this global freshwater ecoregion has been defined largely by the temperate upland rivers and seasonal pans present throughout the area, and is considered to be bio-regionally outstanding with a conservation status of Endangered (Nel et al., 2004; Darwall et al., 2009).

Table 1 provides a summary of the relevant location-specific environmental attributes associated with the study area.

Table 1: Summary of site characteristics and attributes of the associated study area

Map Reference	3028BD 3029AC
Political Region	Eastern Cape
Level 1 Ecoregion	South Eastern Uplands
Level 2 Ecoregion	16.04 16.08
Freshwater Ecoregion	Southern Temperate Highveld Drakensberg-Maloti Highlands
Geomorphic Province	Southeastern Coastal Hinterland
Vegetation Type	Mabela Sandy Grassland East Griqualand Grassland Eastern Temperate Freshwater Wetlands Highveld Alluvial Vegetation
Water Management Area	12. Mzimvubu to Kieskamma
Secondary Catchment	T3
Quaternary Catchment	T31F T33A
Watercourse/s	Mzimvubu River and tributaries Unnamed tributary of Botsola River
Slope Class	F – Lowland River (Mzimvubu Section) D – Upper Foothills (Con Amore Section) Unclassified (Unnamed tributaries)
Flow Seasonality	Perennial (Mzimvubu & major tributaries) Non-perennial (Minor tributaries)

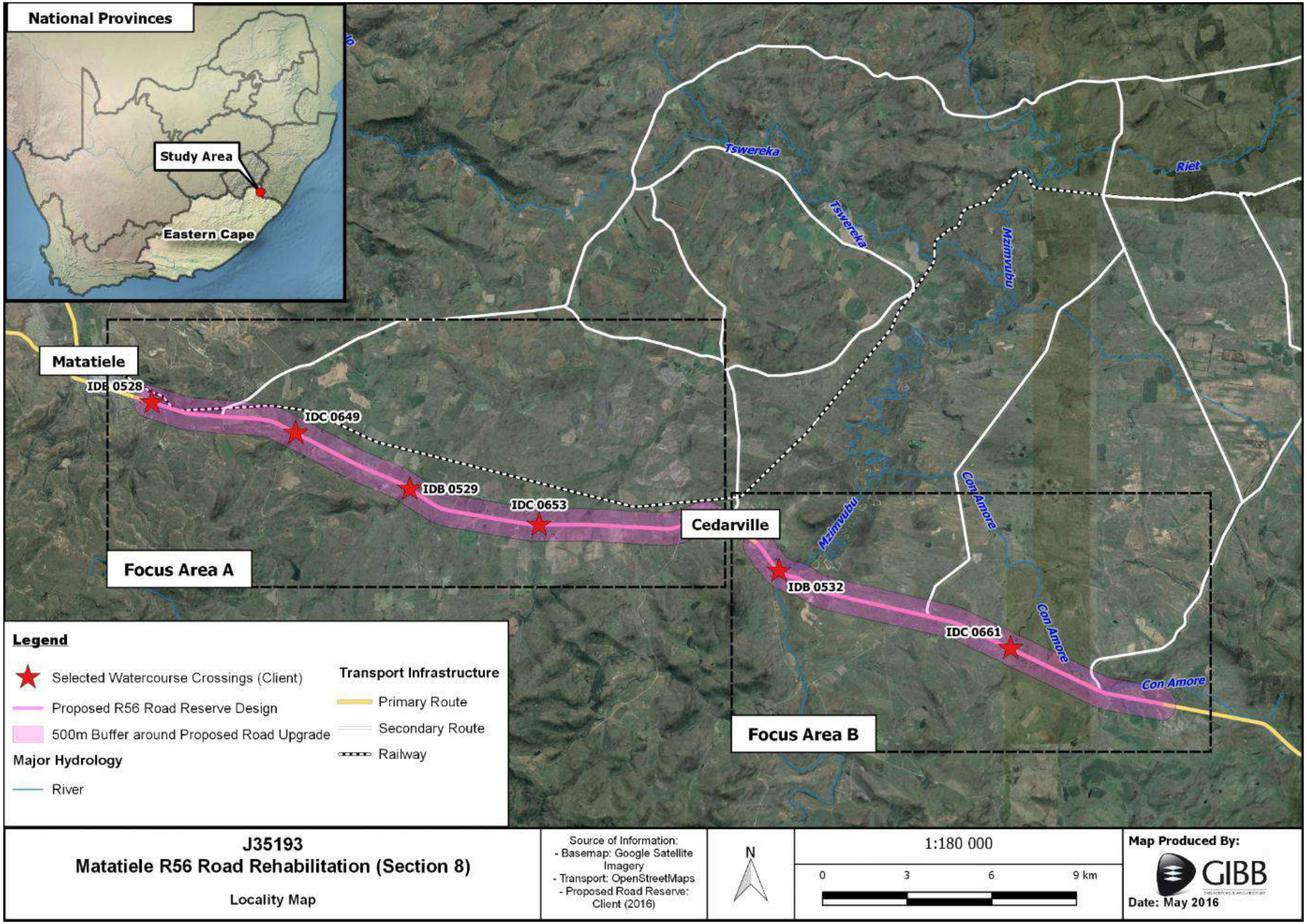

Figure 1: Aerial overview of the study area showing two focus areas for the proposed R56 Road Upgrade

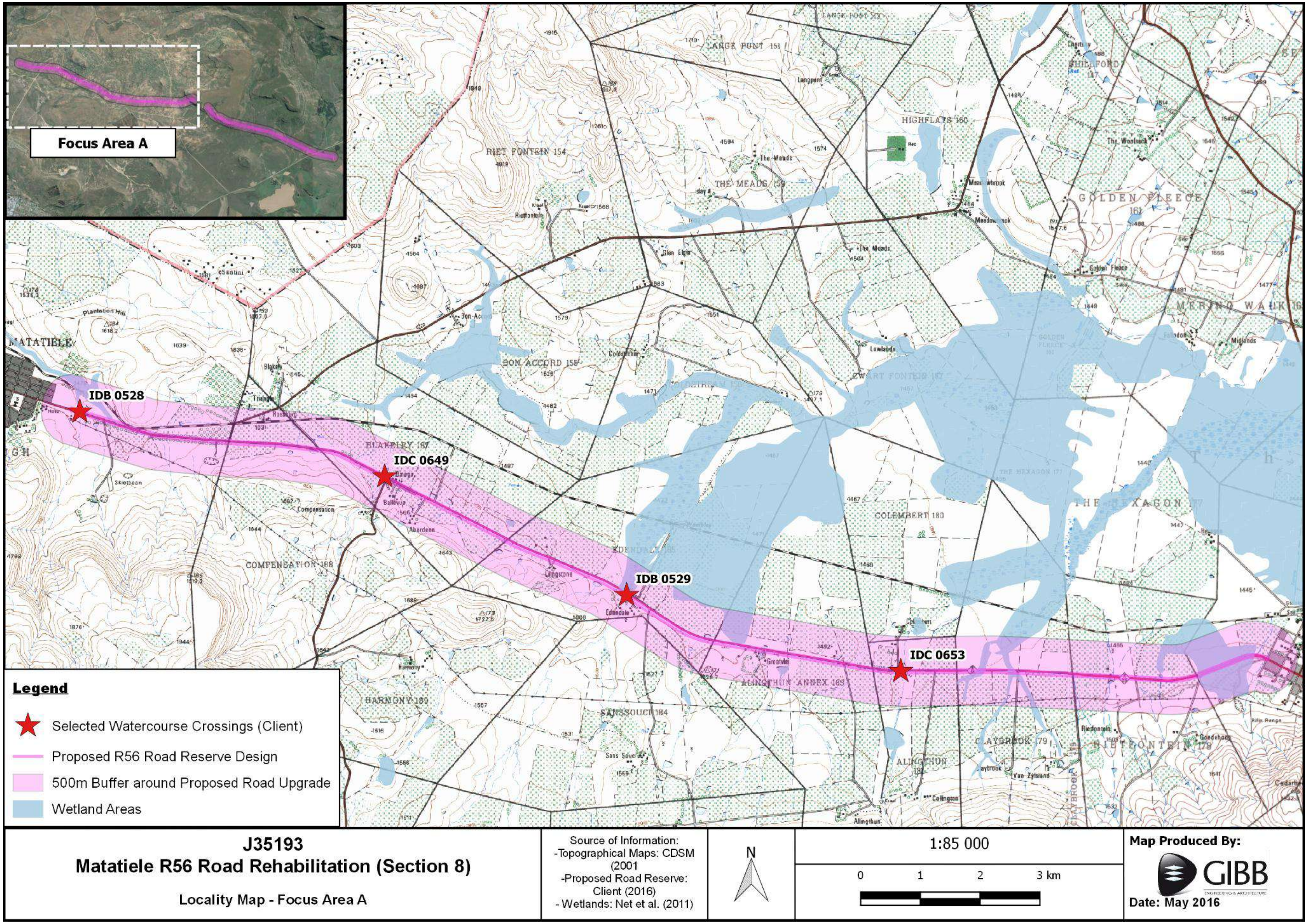

Figure 2: Topographical map of proposed R56 Road Upgrade within Focus Area A (west of Cedarville), showing four of the six selected watercourse crossings

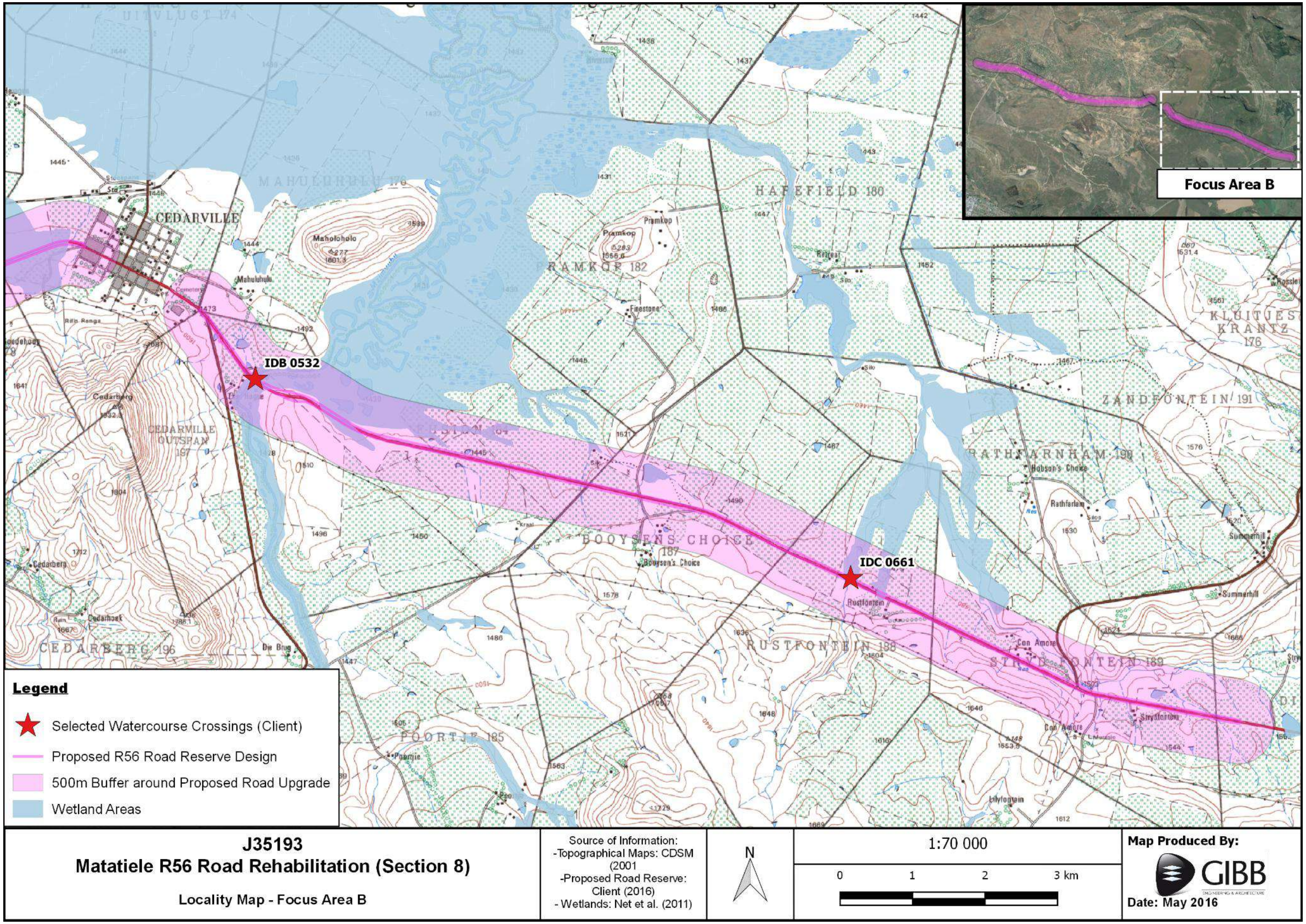

Figure 3: Topographical map of proposed R56 Road Upgrade within Focus Area B (east of Cedarville), showing two of the six selected watercourse crossings

2.4 National Biodiversity Assessment

The 2011 National Biodiversity Assessment (NBA) was a three-year study led by the South African National Biodiversity Institute (SANBI), in partnership with the Department of Environmental Affairs (DEA) and a wide participation from stakeholders, scientists and biodiversity management experts. In terms of the National Environmental Management: Biodiversity Act (NEMBA, Act 10 of 2004), SANBI is required to monitor and report regularly on the status of the country's biodiversity, which is essentially fulfilled in the purpose and aim of the regularly occurring NBA (i.e. approx. every seven years). The NBA endeavours to capture the challenges and opportunities embedded within the country's rich natural heritage at three separate levels (i.e. genes, species and ecosystems) and across each of the major environments (i.e. terrestrial, freshwater, estuarine and marine environments). Subsequently, the NBA informs the revision and updating of key national biodiversity policies and strategies, including the National Biodiversity Strategy and Action Plan, the National Biodiversity Framework, and the National Protected Area Expansion Strategy (Driver et al., 2012).

In an effort to support a landscape management approach to conserving biodiversity and to facilitate the identification of developing trends over time, two major headline indicators were carried through from the previous NBA (i.e. 2004 study) and assessed within the most recent 2011 study. These are referred to as: (i) Ecosystem Threat Status (ETS), which is a measure of a specific ecosystem's intactness, modifications and/or ability to provide ecosystem services; and (ii) Ecosystem Protection Level (EPL), which describes the extent (or state) of a specific ecosystem's protection, in relation to their location within declared protected areas (i.e. as defined within the Protected Areas Act; Driver et al., 2012).

With respect to the freshwater component of the NBA study, aquatic ecosystems across the country were classified into 1,014 distinct ecosystem types, comprising of 223 river ecosystem types and 791 wetland ecosystem types (Nel & Driver, 2012; WWF-SA, 2016), of which two river (i.e. South Eastern Uplands) and two wetland (i.e. Sub-Escarpment Grassland groups) ecosystem types were identified to be directly associated with the proposed R56 road reserve design (Table 2). With respect to the associated river ecosystem types, the assessed sections of the Mzimvubu River (i.e. 16_P_L; Endangered) and the Con Amore Stream (i.e. 16_P_U; Vulnerable) were classified as threatened, which suggested that less than 35% and less than 60% of the length of these defined river ecosystem types represented good conditions (i.e. A or B ecological category), respectively. In addition, it should be noted that less than 1% and 10% of the total length of these riverine ecosystem types were in a good condition and situated within formally protected areas (Nel & Driver, 2012; Table 2). Consequently, the minimum biodiversity protection target of 20%, as defined in the cross-sectoral policy document, was not attained to and these systems were likely to remain deteriorate further.

In contrast, neither the associated channelled valley-bottom systems associated with the larger tributaries of the Mzimvubu (including Con Amore Stream) and Kinira rivers, nor the floodplain wetlands associated with the main stem Mzimvubu River and adjoining tributaries were deemed to be threatened at the time of this study (Table 2; Nel & Driver, 2012a). Also, as above, each of these wetland ecosystem types were defined to be largely unprotected within formally recognised areas. However, the associated wetlands were recognised as FEPA wetlands and as a result, improving protection and conservation of these systems should be a priority (see Section 2.5).

2.5 National Freshwater Ecosystem Priority Areas

The National Freshwater Ecosystem Priority Areas (NFEPA) project represents a multi-partner project between the Council for Scientific and Industrial Research (CSIR), South African National Biodiversity Institute (SANBI), Water Research Commission (WRC), Department of Water Affairs (DWA; now Department of Water and Sanitation, or DWS), Department of Environmental Affairs (DEA), Worldwide Fund for Nature (WWF), South African Institute of Aquatic Biodiversity (SAIAB) and South African National Parks (SANParks). More specifically, the NFEPA project aims to:

1. Identify Freshwater Ecosystem Priority Areas (hereafter referred to as 'FEPAs') to meet national biodiversity goals for freshwater ecosystems; and
2. Develop a basis for enabling effective implementation of measures to protect FEPAs, including free-flowing rivers.

The first aim uses systematic biodiversity planning to identify priorities for conserving South Africa's freshwater biodiversity within the context of equitable social and economic development. The second aim is comprised of two separate components: the *(i)* national component aimed to align DWA (or DWS) and DEA policy mechanisms and tools for managing and conserving freshwater ecosystems, while the *(ii)* sub-national component is aimed to use three case study areas to demonstrate how NFEPA products should be implemented to influence land and water resource decision-making processes. The project further aimed to maximize synergies and alignment with other national level initiatives, including the National Biodiversity Assessment (NBA) and the Cross-Sector Policy Objectives for Inland Water Conservation(Driver et al., 2011).

Based on current outputs of the NFEPA project (Nel et al., 2011; Figure 5), each of the three sub-quaternary catchments that the proposed road reserve design traverses are regarded as being of national importance. The predominant central portion of the proposed road reserve is defined as a FEPA catchment as a result of the associated river ecosystem types (i.e. South Eastern Uplands), wetland ecosystem types (i.e. Sub-Escarpment Grassland groups) and the presence of a threatened freshwater fish species, namely *Enteromius (Barbus)* cf. *anoplus* (Chubbyhead Barb; Table 2). These catchments help to achieve national biodiversity targets, as the ecological condition of the associated systems are currently regarded to be in a good condition (A or B ecological category) and as such, these catchments and adjacent areas should be managed in a way that maintains their ecological condition, so as to conserve freshwater ecosystems and protect water resources for sustainable human use. Also, the catchments associated with the outer portions of the proposed road reserve, to the east and west of the small town of Cedarville, are defined to be Upstream Management Areas. These areas need to be managed to prevent degradation of downstream river FEPAs and Fish Support Areas, especially with regards to surrounding anthropogenic activities (Nel *et al*., 2011).

Furthermore, it should also be noted that the sub-quaternary catchment directly associated with the main stem Mzimvubu River, as well as numerous upstream and downstream catchments along the Mzimvubu River were regarded as important Fish Migration Corridors (Nel *et al*., 2011). Fish migration corridors usually provide links between certain habitats necessary for migration of threatened fish species, especially main stem and tributary habitat. Accordingly, these catchments were recognised as Fish Support Areas and should be managed to support conservation of the associated threatened or near threatened fish populations.

Table 2: Freshwater ecosystem types directly associated with the proposed R56 road reserve design route, showing National Biodiversity Assessment (NBA) indicators and National Freshwater Ecosystem Priority Areas (NFEPA) categories (Nel et al., 2011; Nel & Driver, 2012)

Type of Watercourse	Freshwater Ecosystem Type	NBA Indicators		NFEPA Category
		ETS*	EPL**	
River	South Eastern Uplands-Perennial/Seasonal-Lowland River **(16_P_F)**	EN	NP	• FEPA
	South Eastern Uplands-Perennial/Seasonal-Upper Foothill **(16_P_U)**	VU	PP	• Upstream Management Area
Wetland	Sub-Escarpment Grassland: Group 6-Channelled Valley-bottom	LT	PP	• Wetland FEPAs • Non-FEPA Wetlands
	Sub-Escarpment Grassland: Group 6-Floodplain	LT	NP	• Wetland FEPAs

* **Ecosystem Threat Status: EN** – Endangered (21-35% of ecosystem extent intact), **VU** – Vulnerable (36-60% of ecosystem extent intact), and **LT** – Least Threatened (61-100% of ecosystem extent intact). **** Ecosystem Protection Level: NP** – Not Protected (<1% of extent protected), **PP** – Poorly Protected1 (<10% of extent protected).

2.6 Selection of Sampling Sites

Given that the sampling sites identified on a national and provincial level for the River EcoStatus Monitoring Programme (REMP; previously the River Health Programme, or RHP) were expected to provide suitable biotopes for application of standard biomonitoring approaches, potential sampling sites were preliminarily identified based on the relative proximity to each of the selected watercourse crossings. However, this selection process proved to be unsuccessful due to the relatively extensive distance of these previously identified sites from the study area and the lack of sampling sites within each of the watercourses associated with the six selected road crossings requiring ecological characterisation. Consequently, the in-field selection of the sampling sites was based upon available habitat (i.e. sufficient water level and sampling habitat), as well as relative proximity to the pre-selected crossings within the study area.

Protocol used for the naming each of the selected sampling sites assessed during the present assessment was based on standardised naming protocols of the REMP, allowing for unique identification of sites relative to other studies that may have been carried out within the area (Dallas, 2005). Co-ordinates of the sampling sites utilised during this investigation were determined using a Garmin global positioning device (GPS) and are listed in Table 3 and presented graphically in Figure 4. Photographs of the sites sampled are provided in Appendix B.

Table 3: Location and description of each of the selected sampling sites assessed during the survey

Name	Co-Ordinates	Altitude (a.m.s.l.)	Description
T3KINI-USMAT	30°20'35.59" S 28° 49'31.56" E	1,482 m	Located along an unnamed non-perennial tributary of the Botsola (Kinira) River, directly north of the eastern residential suburbs of Matatiele and downstream of bridge crossing ***IDB 0528***.
T3MZIM-CMPSN	30°21'48.17" S 28° 52'33.21" E	1,536 m	Located along an unnamed non-perennial tributary of the Mzimvubu River, directly upstream of culvert system ***IDC 0649*** and within a small vegetated gorge on parent farm Compensation 188.
T3MZIM-EDNDL	30°22'16.28" S 28° 55'33.01" E	1,480 m	Located along an unnamed non-perennial tributary of the Mzimvubu River, upstream of a large dam situated on parent farm Edendale 185 and downstream of bridge crossing ***IDB 0529***.
T3MZIM-ALING	30°23'14.07" S 28°58'14.65" E	1,478 m	Located along an unnamed non-perennial tributary of the Mzimvubu River, upstream of a series of dams situated on parent farm Alingthun 181 and downstream of culvert system ***IDC 0653.***
T3MZIM-DSR56	30°24'14.50" S 29° 03'28.08" E	1,441 m	Located along the main stem of the Mzimvubu River (and associated floodplain wetlands), downstream of the major bridge crossing ***IDB 0532*** and adjacent to parent farm Hague No.17189.
T3MZIM-RSTFN	30°25'35.83" S 29° 08'39.85" E	1,489 m	Located along an unnamed tributary of the Con Amore Stream, directly downstream of culvert system ***IDC 0661*** and upstream of a series of dams situated on parent farm Rustfontein 188.
T3MZIM-STRYD	30°26'02.65" S 29° 10'15.26" E	1,488 m	Located along the perennial stream referred to as the Con Amore Stream (for the purposes of this report), directly downstream of a small, low-water crossing on parent farm Strydfontein 189.

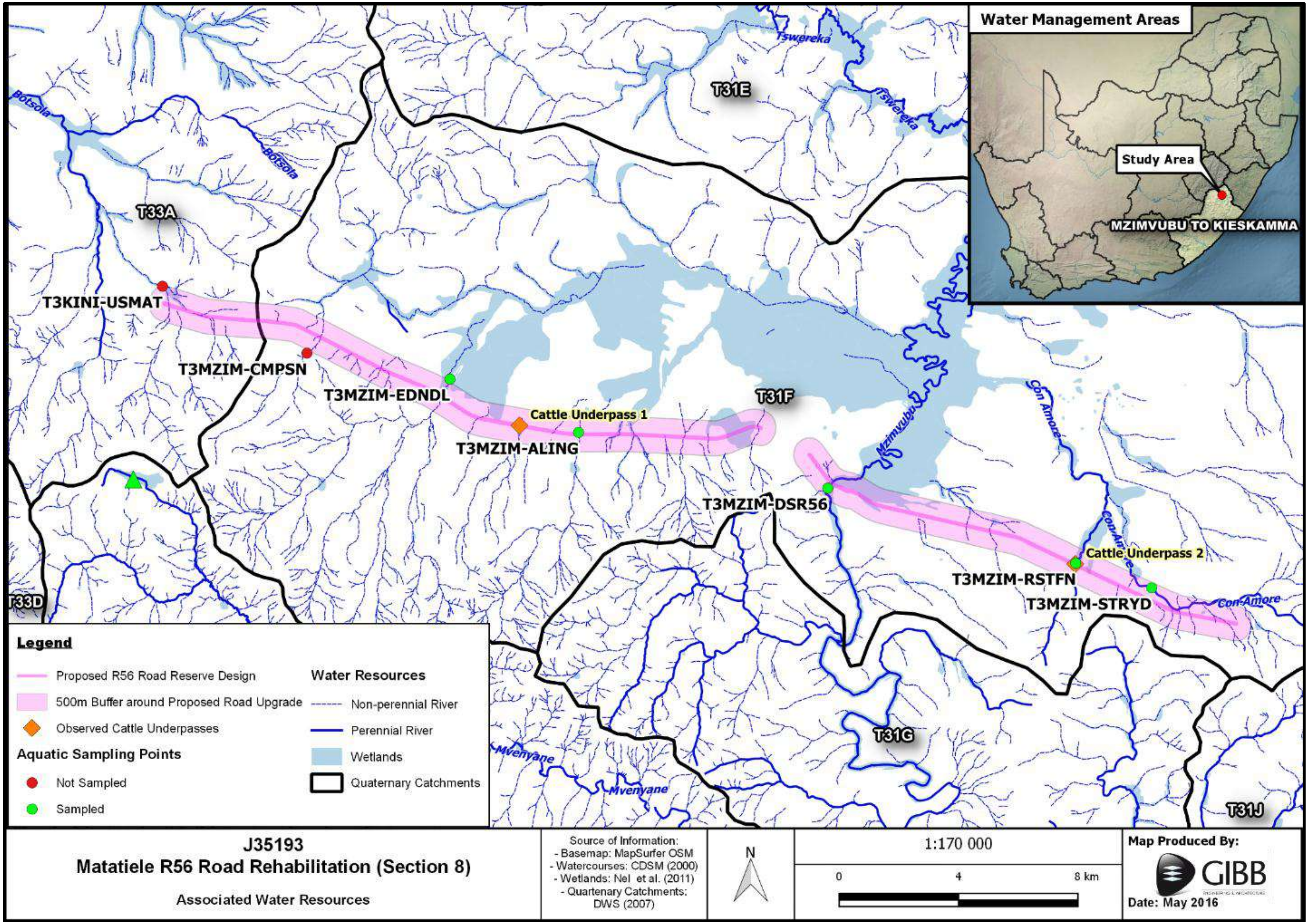

Figure 4: Water resources associated with the proposed R56 Road Reserve Design showing each of the selected aquatic sampling points

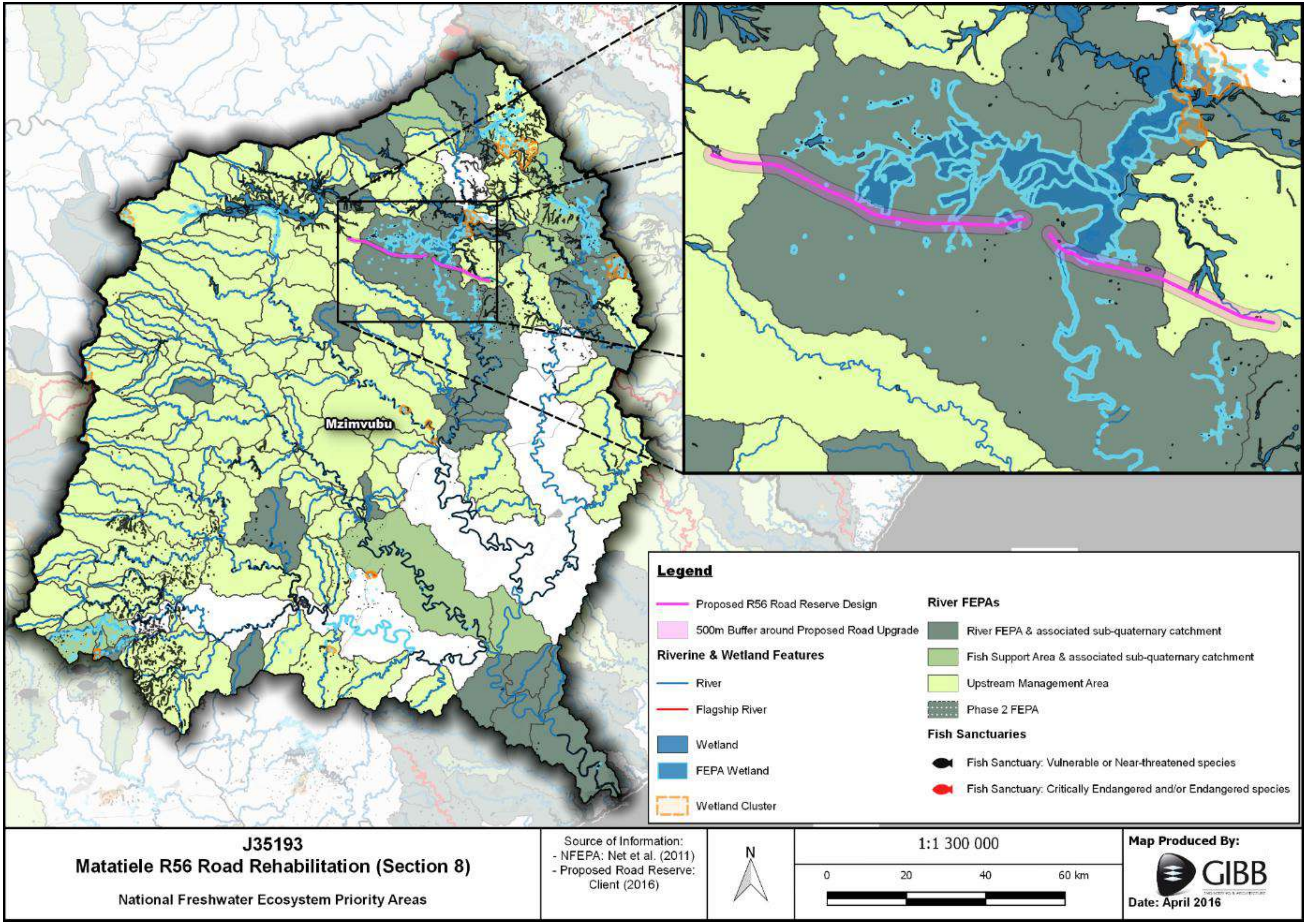

Figure 5: Freshwater Ecosystem Priority Areas status of the Mzimvubu sub-management area

3 Results

3.1 *In Situ* Water Quality

The assessment of water quality variables is important for the interpretation of results obtained during biological investigations, as aquatic organisms are influenced by the environment in which they live. Table 4 provides the *in situ* water quality data obtained at each site assessed during the field survey.

Table 4: *In situ* water quality variables recorded at each of the sites assessed during the April 2016 field survey

Site	Time	Temp. (°C)	pH	Electrical Conductivity (µS/cm)	Dissolved oxygen	
					(mg/ℓ)	(% sat)
T3KINI-USMAT	Site Dry					
T3MZIM-CMPSN	Site Dry					
T3MZIM-EDNDL	11h15	22.1	7.94	292.0	4.99	57.1
T3MZIM-ALING	16h30	24.8	8.48	145.4	8.22	88.8
T3MZIM-DSR56	10h20	19.4	7.68	148.5	7.78	84.4
T3MZIM-RSTFN	08h15	13.9	6.82	166.4	4.83	47.7
T3MZIM-STRYD	16h30	21.4	7.83	325.0	5.65	65.2

Based on the *in situ* water quality variables obtained at each of the sites assessed at the time of the survey, no major limiting factors toward the colonisation and/or inhabitation of these watercourses by indigenous aquatic biota was expected (Table 4). Nevertheless, the temperatures recorded were still observed to vary within acceptable ranges throughout the day (i.e. irradiated by the sunlight) and corresponded to changes in flow and depth observed at each of the assessed sites at the time of the survey.

Similarly, the majority of the pH levels recorded deviated marginally within the expected natural ranges of most South African aquatic systems, while electrical conductivity was observed to be relatively low at each of the assessed sites despite the characteristic wetland nature of many of the assessed systems, which was expected to accumulate dissolved solutes originating from upstream and adjacent surface runoff (Department of Water Affairs And Forestry, 1996). Furthermore, these values were suspected of being marginally elevated as a result of the low water levels observed at the time of the survey, which was likely to concentrate the solutes present within these systems, especially at sites T3MZIM-EDNDL and T3MZIM-STRYD.

Unfortunately, there is very limited information available on salinity tolerances of freshwater organisms in South Africa. Available research only indicates that changes in the distribution patterns of individual species or communities can be attributed to changes in salinities. Based on results from this research, a number of generalisations can be made and should be noted, especially within a catchment vulnerable to erosion and agricultural runoff (Dallas & Day, 2004):

- It is often the rate of change rather that the final salinity that is most critical;
- Juvenile stages are often more sensitive to increased salinity concentrations;
- Salinity may act as an antagonist or synergist in relation to a variety of toxicants; and
- The responses of freshwater organisms to alterations in salinity are likely to be related to the evolutionary origins on the taxon of which they are part.

Dissolved oxygen concentrations of 80%-120% saturation were considered to protect all life stages of the vast majority of aquatic organisms that are endemic or adapted to inhabiting aerobic warm water habitats (DWAF, 1996). Consequently, with the exception of the acceptable values recorded at Site T3MZIM-ALING and Site T3MZIM-DSR56, the dissolved oxygen concentrations were observed to be marginally reduced, which was expected to deter a few organisms with a requirement for well-oxygenated habitats.

3.2 Aquatic and Riparian Habitat

Assessment of aquatic habitat within the study area was based largely on a qualitative assessment at various sampling points within the associated watercourses under study, the Index for Habitat Integrity (or IHI) is a rapid, field-based, visual assessment of modifications to a number of pre-selected biophysical drivers (i.e. semi-quantitative) for the determination of the Present Ecological State (PES, or Ecological Category) of associated instream and riparian habitats, while the use of the Invertebrate Habitat Assessment System (or IHAS) presented an indication of the representivity of "ideal" habitat for supporting diverse aquatic macroinvertebrates at each of the assessed sites.

3.2.1 Index for Habitat Integrity

In addition to the application of the IHAS approach for aquatic habitat characterisation, the ecological condition of the instream and riparian habitats was determined through the application of version 2 of the Index for Habitat Integrity (IHI-96-2; Dr. C. J. Kleynhans, *pers. comm.*, 2015). While the recently upgraded IHI-96-2 replaces the relatively comprehensive and expensive IHI assessment model developed by Kleynhans (1996), it is important to note that the IHI-96-2 does not replace the IHI model developed by Kleynhans et al. (2008a), which should preferably be applied where sufficient data is available (i.e. intermediate and comprehensive Reserve Determinations). Consequently, the IHI-96-2 model should be applied in cases where a relatively large number of river reaches need to be assessed, budget and time provisions are limited, and/or detailed available information is lacking (i.e. rapid Reserve Determinations and for RHP purposes).

For the purposes of the present study, watercourse assessment segments were typically delineated by major riverine homogeneities (e.g. adjoining tributaries) and/or potential instream barriers (e.g. dams, weirs, etc.). Consequently, each of the selected sampling points constituted a separate habitat assessment unit and any associated impacts within this unit is described below in Table 6.

Table 5: Index for Habitat Integrity (IHI) values obtained for associated watercourses under study

Site	Component	IHI (%)	Ecological Category	Major Impacts
T3KINI-USMAT	Instream Habitat	**N/R**	**-**	- No instream habitat assessment could be undertaken at the time of the survey, as site was observed to be **dry.** - A *large* infestation of alien vegetation (i.e. *Eucalyptus* sp., *Salix* sp., *Melia azedarach*, and *Populus* cf. *canescens*) was evident. - *Severe* erosion was most likely attributed to the irregular high-energy spates and the adjacent land management practices (e.g. limited grazing areas, urbanisation, etc.).
	Riparian Habitat	48.1	D	
T3MZIM-CMPSN	Instream Habitat	**N/R**	**-**	- No instream habitat assessment could be undertaken at the time of the survey, as site was observed to be **dry.** - A *serious* infestation of alien vegetation (i.e. *Gleditsia triacanthos*, *Acacia mearnsii*, and *Populus* cf. *canescens)* was evident. - *Moderate* vegetation removal was suspected due to agricultural activities (e.g. homestead construction, livestock grazing area, etc.).
	Riparian Habitat	62.3	C	
T3MZIM-EDNDL	Instream Habitat	63.6	C	- *Largely* modified flows and changes to the channel morphology (i.e. bank erosion) was present due to the establishment of a number of impoundments. - *Largely* modified physico-chemical changes were suspected as a result of the adjacent agricultural practices (i.e. maize crops, cattle rearing, etc.). - *Large* infestation of alien vegetation (i.e. *Robinia pseudoacacia, Salix sp., Gnnamomum comphora, Eucalyptus sp.,* and *Capreasus sp.*) was evident.
	Riparian Habitat	40.7	D/E	
T3MZIM-ALING	Instream Habitat	70.4	C	- *Largely* modified flows, *large* changes to the channel morphology and *large* alterations to the physico-chemical conditions were suspected due to the adjacent agricultural practices (i.e. maize crops, cattle rearing, etc.). - *Severe* fragmentation of the associated riparian elements was facilitated by the construction of the upstream and downstream impoundments.
	Riparian Habitat	43.2	D	
T3MZIM-DSR56	Instream Habitat	70.2	C	- An infestation of alien fish species along the associated section was suspected to *largely* impact upon the indigenous fauna within the system. - Minor water abstraction from irrigation pumps and construction of a stone weir was expected to *moderately* modify the system's flow. - The well-established *Salix sp.* lining the profile of the system was expected to demonstrate a *large* alien vegetation infestation.
	Riparian Habitat	57.4	D	

				- Adjacent livestock rearing activities (i.e. grazing, trampling, drinking, etc.) was suspected to facilitate the *largely* evident bank erosion.
T3MZIM-RSTFN	Instream Habitat	74.8	**C**	- The flow, bed and channel morphology of the associated system was suspected to be *moderately* modified. - An elevated upstream runoff was suspected to facilitate the *large* erosive processes evident along the system.
	Riparian Habitat	66.5	**C**	
T3MZIM-STRYD	Instream Habitat	70.0	**C**	- The bed/substrate of the associated section was suspected to be *largely* modified through sedimentation from adjacent livestock grazing areas. - *Moderate* alien vegetation encroachment (i.e. *Salix sp.*) was evident along the associated section of the stream. - The livestock grazing the adjacent areas and drinking from the steam facilitated a *large* physico-chemical deterioration and *moderate* bank erosion.
	Riparian Habitat	81.6	**B/C**	

Instream Habitat

With the exception of the dry sites that were not assessed at the time of the survey (i.e. Site T3KINI-USMAT and Site T3MZIM-CMPSN), instream habitat conditions associated with each of the selected sampling sites were determined to be moderately modified (Ecological Category C), as the majority of impacts observed were deemed to be small to moderate in magnitude (Table 6). The water quality conditions were suspected to be moderately impacted within the study area (i.e. Site T3MZIM-EDNDL and Site T3MZIM-STRYD) were attributed to the surrounding agricultural activities including crop propagation (i.e. maize) and livestock rearing (cattle, horses, etc.), which was also expected to facilitate a marginally elevated surface runoff and potential sedimentation (i.e. Site T3MZIM-STRYD). Also, the establishment of a number of farm dams along each of the associated watercourses was expected to fragment the longitudinal nature of riverine habitat (i.e. Site T3MZIM-EDNDL and Site T3MZIM-ALING), which affects *in situ* conditions (e.g. sediment transport), the movement (or migration) of aquatic biota, and most notably the historical flow regime.

Lastly, the observed presence of alien invasive fish species *Lepomis macrochirus* (Bluegill Sunfish) and *Micropterus* cf. *punctulatus* (Spotted Bass) along the main stem Mzimvubu River (i.e. Site T3MZIM-DSR56) was likely to affect the prevalence of indigenous aquatic organisms (e.g. competition, predator-prey interactions, hybridisation, etc.). Consequently, the ecological integrity of assemblages collected along this section was suspected to be compromised.

Riparian Habitat

The riparian habitat integrity was determined to range widely between each of the delineated assessment units with conditions varying from minor modifications in integrity (Ecological Categories B/C) to largely-to-seriously modified conditions (Ecological Category D/E). In light of the largely grassland-dominated riparian (or wetland) areas, large-to-severe alien vegetation encroachment and notable bank erosion (facilitated by livestock overgrazing adjacent upland areas) were observed to be the most prominent impacts upon the associated riparian habitats. This was demonstrated by the largely limited occurrence of indigenous riparian species identified within the study area (i.e. *Leucosidea sericea* African Ouhout, *Searsia* cf. *pyroides* Common Wild Current and various grassland species), which were overshadowed by numerous alien floral species including *Eucalyptus sp*. (Gum species), *Opuntia* sp. (Prickly Pear species), *Salix sp.* (Willow species), *Cupreasus sp.* (Cypress species), *Melia azedarach* (Syringa), *Populus* cf. *canescens* (Poplar species), *Acacia mearnsii* (Black Wattle), *Acacia dealbata* (Silver Wattle), *Gleditsia triacanthos* (Honey Locust), *Robinia pseudoacacia* (Black Locust), and *Gnnamomum camphora* (Camphor Tree).

According to WWF-SA (2016), alien invasive vegetation can drastically reduce available water resources (i.e. up to 4% decrease in relation to indigenous species) and cause subsequent impacts upon stream flows, siltation within impoundments, and degradation of water quality conditions (WWF-SA, 2016). Consequently, clearing alien invasive vegetation and ensuring rehabilitative management of riparian elements within areas with limited water availability is vitally important to restoring and maintaining the associated aquatic ecosystems.

3.2.2 Invertebrate Habitat Assessment System

The Invertebrate Habitat Assessment System (IHAS, Version 2.2), developed by McMillan (1998), has routinely been used in conjunction with the SASS approach as a measure of variability in the quantity and quality of representative aquatic macroinvertebrate biotopes available during sampling. However, according to a study conducted within the Mpumalanga and Western Cape regions, the IHAS method does not produce reliable scores at assessed sampling sites, as its performance appears to vary between geomorphologic zones and biotope groups (Ollis et al., 2006). While no final conclusion can be made regarding the accuracy of the index until further testing has been conducted, these potential limitations and/or shortfalls should be noted. Nevertheless, due to the value of basic instream habitat assessment data and its suitability for comparison of available macroinvertebrate habitats between various sampling sites, an adapted IHAS approach was maintained during the interim period, excluding assessment of the 'surrounding physical stream condition' (see Appendix A). Results are thus presented relative to an "ideal" diversity of aquatic macroinvertebrate sampling habitat and need to be interpreted with caution taking into consideration the abovementioned variability (Table 6).

Table 6: Adapted IHAS values obtained within the study area during the April 2016 field survey

Site	Adapted IHAS Value (%)	Description
T3KINI-USMAT	Site Dry	
T3MZIM-CMPSN	Site Dry	
T3MZIM-EDNDL	22	**Poor**
T3MZIM-ALING	24	**Poor**
T3MZIM-DSR56	58	**Adequate / Fair**
T3MZIM-RSTFN	40	**Poor**
T3MZIM-STRYD	38	**Poor**

Due to the onset of drought conditions within the area prior to the field survey, the water levels were observed to be relatively low within many of the assessed watercourses and as a result, in the associated watercourses that were inundated, the hydraulic diversity observed to be limited and a large portion of any occurring marginal vegetation remained unavailable for colonisation. In fact, the only sampling sites observed to provide a sufficiently productive vegetation biotope was observed at Site T3MZIM-STRYD, which yielded a particularly high diversity of macroinvertebrate families with an affinity for colonising available vegetation (see Section 3.3). Lastly, while the presence of the stones habitat (in-current and/or out-of-current) along the Con Amore Stream and adjoining tributary (i.e. at Site T3MZIM-RSTFN and Site T3-MZIM-STRYD) marginally improved the habitat availability, these niche habitats were observed to be largely smothered by algae and/or sedimentation, respectively.

With the exception of Site T3MZIM-DSR56, which exhibited adequate-to-fair aquatic macroinvertebrate habitat availability at the time of the survey, each of the remaining sites assessed were defined to exhibit poor habitat availability for macroinvertebrate assemblages (Table 5). While these conditions were inherently characteristic of the nature of channelled valley-bottom wetlands and expected within selected systems, especially at Site T3MZIM-EDNDL and Site T3MZIM-ALING, further assessment of the associated watercourses should be conducted by a wetland specialist to confirm nature of these watercourse.

3.3 Aquatic Macroinvertebrates

According to the known distribution ranges of select groups of extant and probably extant aquatic macroinvertebrates, at least nine families of Odonata (Dragonflies and Damselflies; estimated 49 species), six families of molluscs (i.e. Gastropoda and Pelecypoda; estimated 12 indigenous species) and a single family of crabs (i.e. Potamanautidae; estimated 2 indigenous species) exhibited a potential to occur within the study area (Darwall et al., 2009). This was further supported by the potential occurrence of approximately 73 different aquatic macroinvertebrate families which have known or potential distribution ranges correlating with the study area (Mrs. C. Thirion, *pers. comm*., 2016).

Of these families, a total of 39 different aquatic macroinvertebrate taxa were collected within the study area, ranging from 12 families within an impounded tributary of the Mzimvubu River (i.e. Site T3MZIM-ALING) to 26 taxa along the assessed section of the well-vegetated Con Amore Stream (i.e. Site T3MZIM-STRYD). Accordingly, the corresponding SASS5 scores ranged from a low 44 at Site T3MZIM-ALING to a moderate 128 at Site T3MZIM-DSR56, while the Average Score Per Taxon (ASPT) values ranged from 3.67 to 5.33 at the same respective sampling sites (Table 7; Appendix C). Furthermore, several taxa regarded as moderately to highly sensitive to water quality impairment were sampled, including Hydrachnellae (Water Mites), Leptophlebiidae (Prongill Mayflies), Tricorythidae (Stout Crawler), Aeshnidae (Hawker and Emperor Dragonflies), Lestidae (Emerald Damselflies/Spreadwings), Platycnemidae (Stream Damselflies), Ecnomidae (Caseless Caddisflies) and Elmidae (Riffle Beetles).

While the greatest macroinvertebrate diversity was observed within the larger perennial systems, namely the Mzimvubu River (i.e. Site T3MZIM-DSR56) and the Con Amore Stream (i.e. Site T3MZIM-STRYD), the diversity observed within each of the other assessed sites was considered to be representative of the aforementioned nature of the assessed watercourse (see Section 3.2.1). It was expected that these larger systems, as well as the numerous farm dams established within the study area, functioned as refugia for macroinvertebrate communities during drier periods, and aid in rapid colonisation of non-perennial watercourses following inundation.

Table 7: SASS5 data obtained from within the study area at the time of the April 2016 field survey

Site	SASS5 Score	Number of Taxa	ASPT*
T3KINI-USMAT	Site Dry		
T3MZIM-CMPSN	Site Dry		
T3MZIM-EDNDL	54	13	4.15
T3MZIM-ALING	44	12	3.67
T3MZIM-DSR56	128	24	5.33
T3MZIM-RSTFN	58	15	3.87
T3MZIM-STRYD	121	26	4.65

* Average Score Per Taxon

Comparatively, the biomonitoring assessments conducted by the regional office of the Department of Water Affairs at the REMP Site T3MZIM-SPRIN, which is located approximately 8 km south of Site T3MZIM-DSR56 along the main stem Mzimvubu River, collected 28 different taxa during September 2008 (SASS5 Score: 161, ASPT Value: 5.75) and 25 different taxa during August 2010 (SASS5 Score: 144, ASPT Value: 5.80; Mbikwana et al., 2010). While some degree of spatial and/or temporal (or seasonal) variation was to be expected between each of observed macroinvertebrate assemblages, the assemblages observed during the current survey showed a high degree of correlation, especially in consideration of the limited vegetation biotope available at the time of the survey.

3.3.1 Species of Conservation Concern

Only one species of Odonata exhibited any potential for occurrence within the study area (Darwall et al., 2009), while no aquatic macroinvertebrate species of commercial or economic values were listed on NEMBA Threatened and Protected Species (ToPS) regulations:

- *Syncordulia gracilis* (listed as Vulnerable) – This endemic damselfly species inhabits clear, fast-flowing, hard-bottomed rivers within treeless river valleys and has been known to be sensitive to invasive alien trees encroaching into riparian/instream habitats, as well as the associated impacts originating from surrounding agricultural activities (Samways, 2010). While the population previously believed to occur within KwaZulu-Natal is possibly extinct, two known populations are believed to be widely dispersed across the Western and Eastern Cape (Samways, 2006, 2010). Given the habitat preferences of the species and the highly relevant major threats**, the probability of occurrence of this species within the study area was highly unlikely**.

Since no species from the Cordulidae family were collected at the time of the current field survey (Appendix C), no evidence was available to indicate its potential presence at the time of the survey. However, further investigation would be necessary to definitively determine whether this species occurs within the study area.

3.3.2 Invasive Alien Species

Only two alien species from the Order of Gastropoda exhibited any potential for occurrence within the study area (Darwall et al., 2009), of which only *Psuedosuccinea (Lymnaea) columella* was listed on the NEMBA Alien and Invasive Species regulations:

- *Physa acuta* (Acute Bladder Snail) – This alien freshwater snail was accidentally introduced prior to 1956, probably in association with aquatic plants imported through the aquarium trade and/or through the activities of water birds (de Moor & Bruton, 1988; Day & de Moor, 2002). This species is believed to have originated from North America and has become globally invasive in water-bodies on four different continents (Appleton, 2003). Due to its superior and adaptable reproductive capacity, its ability to migrate upstream and its ability to quickly recolonize a water-body, it was previously considered the second most widespread alien invasive freshwater snail species in the country. It has been recorded in all types of water-bodies, but their largest prevalence was recovered in dams and rivers around the major ports and urban centres of South Africa (de Moor & Bruton, 1988; de Kock & Wolmarans, 2007). Although the occurrence of this particular species could not be definitively confirmed, the collection of freshwater snails from the Physidae family at the time of the field survey **suggested potential confirmation of occurrence of this species within the study area**, as only two alien species are classified within the Physidae family within South Africa and the other species has not been recorded within the area (Dana & Appleton, 2007).
- *Psuedosuccinea (Lymnaea) columella* (Reticulate Pond Snail) – A widespread freshwater snail originally from North America and believed to have been accidently introduced prior to 1944 through potential trade and import of aquarium plants (de Moor & Bruton, 1988; de Kock et al., 1989). The invasion of this species has also previously been noted to facilitate the further spread of fascioliasis, which is a disease that affects cattle, as this gastropod successfully acts as an intermediate host of *Fasciola hepatica* (de Moor & Bruton, 1988). Although the occurrence of this particular species could not be definitively confirmed, the collection of freshwater snails from the Lymnaeidae family at the time of the field survey **suggests that the probability of occurrence of this species within the study area was regarded as moderate-to-high**.

3.3.3 Present Ecological State

Although Chutter (1998) originally developed the SASS protocol as an indicator of water quality, it has since become clear that the SASS approach gives an indication of more than mere water quality, but also a general indication of the current state of the macroinvertebrate community. While SASS does not have a particularly strong cause-effect basis for interpretation, as it was developed for application in the broad synoptic assessment required for the River Health Programme (RHP), the aim of the MIRAI is to provide a habitat-based cause-and-effect foundation to interpret the deviation of the aquatic macroinvertebrate community (assemblage) from the reference condition (Thirion, 2008). This does not preclude the calculation of SASS scores, but encourages the application of MIRAI assessment, even for River Health Programme purposes, as the preferred approach. Accordingly, the SASS5 data obtained during the present assessment was used in the Macro-Invertebrate Response Assessment Index (MIRAI; Thirion, 2008) to determine the Present Ecological State (PES), or Ecological Category of the associated macroinvertebrate assemblage. It should be mentioned that due to the fact that the

Present Ecological State (or Ecological Category) limits along the A to F continuum are notional and artificially-defined, some sites exhibit attributes associated with two closely related categories and as a result, ecological boundary categories were defined (Robertson et al., 2004).

Table 8: Results obtained following the application of the Marcoinvertebrate Response Assessment Index (MIRAI) at selected sampling sites at the time of the April 2016 field survey

Site	MIRAI Value	Ecological Category	Description
T3KINI-USMAT	Site Dry		
T3MZIM-CMPSN	Site Dry		
T3MZIM-EDNDL	37.38	**E**	Seriously modified
T3MZIM-ALING	35.01	**E**	Seriously modified
T3MZIM-DSR56	54.02	**D**	Largely modified
T3MZIM-RSTFN	33.35	**E**	Seriously modified
T3MZIM-STRYD	54.75	**D**	Largely modified

In relation to perceived reference conditions (Mrs. C. Thirion, *pers. comm.,* 2016; Appendix C), it was determined that the ecological condition of the macroinvertebrate assemblages collected within the study area exhibited largely modified conditions (i.e. Ecological Category D) along the main stem Mzimvubu River (i.e. Site T3MZIM-DSR56) and the Con Amore Stream (i.e. Site T3MZIM-STRYD) and seriously modified conditions (i.e. Ecological Category E) along the each of the associated tributaries of the Mzimvubu River (Table 8). Further interrogation of the MIRAI index suggested that the primary drivers at each of the assessed site was related to both flow modification and deteriorated water quality despite the low water levels and the poor habitat availability (see Section 3.2.1).

Unfortunately, the only data related to the health (or ecological condition) of the associated watercourses available at the time of writing was the abovementioned biomonitoring assessments by Mbikwana et al., 2010. However, no MIRAI-determined ecological integrity was available for comparison, as the fair-to-good river health class determined for the downstream site (i.e. T3MZIM-SPRIN) was based solely upon the ASPT value, which is a highly variable measure of integrity. It should be noted that the MIRAI approach by (Thirion, 2008) is currently the preferred method by the Department of Water and Sanitation, and for comparative reasons, future assessments should apply this index, especially for monitoring purposes.

3.4 Ichthyofauna

Based on distribution ranges of extant and possibly extant species, approximately twelve indigenous species of fish were likely to occur within the greater Mzimvubu catchment area, including a number of species with an affinity for coastal and/or estuarine conditions (Darwall et al.,2009; Appendix D). Following a review of available collection records of fish species occurring within the watercourses associated with the study area (including records from SAIAB, the Albany Museum, as well as DWS), a total of only two indigenous fish species were expected to occur within the area under study (Table 9). However, due the extensive migratory abilities of *Anguilla marmorata*, *Glossogobius giuris* and

Pseudomyxus capensis, these species could not be excluded despite the lack of any confirmed collection records within the catchment area.

Table 9: Fish species expected to occur within the associated watercourses

Scientific Name	Common Name	Expected Occurrence	Observed/Confirmed
Indigenous species			
Anguilla marmorata	Giant Mottled Eel		
Anguilla mossambica	Longfin Eel	**X**	
Enteromius (Barbus) cf. *anoplus*	Chubbyhead Barb	**X**	**X**
Glossogobius giuris	Tank Goby		
Pseudomyxus (Myxus) capensis	Freshwater Mullet		
Extralimital species			
Tilapia sparrmanii	Banded Tilapia	-	
Alien species			
Cyprinus carpio	Common Carp	-	
Lepomis macrochirus	Bluegill Sunfish	-	**X**
Micropterus sp.	Bass species	-	**X**
Oncorhynchus mykiss	Rainbow Trout	-	
Perca fluviatilis	Perch	-	

In addition, a total of six non-native fish species (i.e. one extralimital indigenous species and five alien species) were noted to have been previously recorded within the upstream and adjacent tributaries of the associated watercourses (Table 9). It should be noted that following recent taxonomic studies, the taxonomy of several species expected and/or confirmed to occur within the study area have changed. Accordingly, taxonomy presented within this report was valid at the time of writing and where recent changes were made, previous names are provided in parentheses following the revised names.

3.4.1 Fish Assemblage and Catch Record

Due to the relative ease of access at each of the selected sampling sites at the time of survey, the electro-narcosis method of sampling (between 15-30 minutes effort per site) was deemed to be sufficient for collecting a representative ichthyofaunal assemblage. Given the low water levels observed at the time of the survey, catch numbers were expected to be relatively low due to a limited extent of available habitat (i.e. water column, marginal vegetation, substrate cover, etc.) and a limited ability for established fish species to migrate and move between river reaches and/or catchments (e.g. *Anguilla* sp.).

Of each of the five sampling sites assessed, only the major perennial systems were observed to support fish communities at the time of the survey, namely the Mzimvubu River (i.e. Site T3MZIM-DSR56) and the Con Amore Stream (i.e. Site T3MZIM-STRYD). A total number of only 11 individual specimens were collected, comprising three different species including the indigenous *Enteromius (Barbus)* cf. *anoplus* (Chubbyhead Barb) and two alien species (Figure 6). Given that the main stem section of the assessed watercourses (i.e. Mzimvubu River) was dominated by alien species and the adjoining tributaries (including the Con Amore Stream) observed to support fish were dominated by indigenous species, it was suspected that selected tributaries function as refuge for indigenous species within the area. However, follow-up sampling surveys would be necessary to confirm this suspicion with any degree of confidence. A brief description of the indigenous fish species collected is presented below, while further information regarding the observed alien fish species is provided in Section 3.4.3.

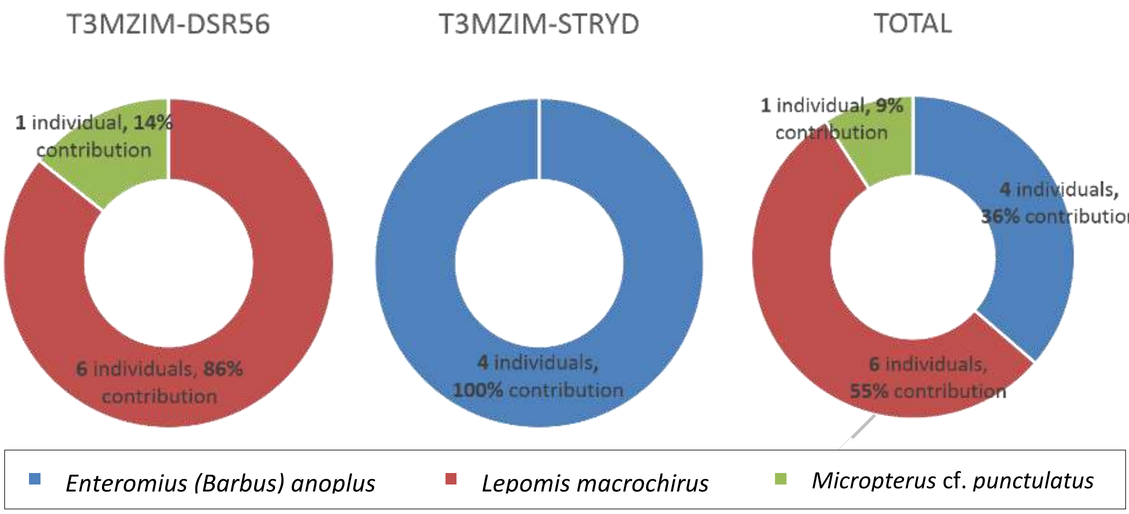

Figure 6: Catch record and percentage contribution of each of the fish species collected

Enteromius cf. *anoplus* (Chubbyhead Barb; Figure 7) prefers cool waters in a wide variety of habitats (including small streams, large rivers and lakes) and feeds on insects, zooplankton, seeds, green algae and diatoms. This fish species breeds during summer after major rainfall events and reaches sexually maturity in one year (Skelton, 2001). Given its preference for slow flowing systems (i.e. slow-shallow and slow-deep velocity-depth classes), as well as its affinity toward marginal and aquatic vegetation for cover, this species is well-suited to channelled wetland systems (Kleynhans, 2008). Also, it should be noted that this species is considered a species complex and requires further genetic studies, which will most likely yield several new potential species (Darwall et al., 2009).

Figure 7: Example of *Enteromius* cf. *anoplus* (Chubbyhead Barb)

3.4.2 Species of Conservation Concern

Each of the expected fish species that were likely to occur within the study area (Table 9) were classified as on a regional scale by the IUCN as Least Concern and as a result, no species of conservation concern were determined to potentially occur within the associated watercourses (Darwall et al., 2009). However, one species of potential occurrence is listed on the applicable NEMBA Threatened or Protected Species (ToPS) regulations:

- *Pseudomyxus capensis* (Freshwater Mullet; listed as Vulnerable) – This endemic species occurs within east coastal rivers and estuaries from the Bree River to Kosi Bay, but it may occur further than 100 km inland. Breeding occurs at sea throughout the year, juveniles then enter the estuaries and move into rivers between mid-winter or early spring each year, males and females inhabit freshwater systems for up to 4 and 7 years, respectively (Skelton, 2001). Species exhibits a preference for slow-deep velocity-depth classes and has a low tolerance to elevated flows, which is expected to limit its upstream migration at times of flood or minor spates (Kleynhans, 2008). In consideration of the large distance between the study area and the Port St John's estuary, **the probability of occurrence of this species within the study area was highly unlikely**.

3.4.3 Invasive Alien Species

Based on collection records, there was evidence of the potential occurrence of one extralimital indigenous species and five alien exotic species, of which two were confirmed at the time of the current survey:

<u>Extralimital indigenous species:</u>

- *Tilapia sparrmanii* (Banded Tilapia) – an indigenous species with a wide natural distribution record, which extends from the Orange River and KwaZulu-Natal south coast (i.e. Tugela River) northwards to the upper reaches of the southern tributary of the Zaire, Lake Malawi and the Zambezi River (Skelton, 2001). However, it was stocked as an alternative to *Lepomis macrochirus* (Bluegill Sunfish) as a forage fish for bass in the eastern and southern Cape rivers prior to 1945, where it has become widely established (de Moor & Bruton, 1988) This species prefers stagnant water with submerged and/or protruding marginal vegetation, feeding upon various sources including algae, soft plants, small invertebrates and even small fish (Skelton, 2001). According to Ellender & Weyl (2014), this species' non-native extralimital range was noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011). Consequently, **the probability of occurrence of this species within the study area was regarded as moderate**, as only unverified records were reported in adjacent systems.

<u>Exotic alien species</u>

- *Cyprinus carpio* (Common Carp; listed as Category 3 in NEMBA Regulations) – this species was introduced into South Africa from Europe in the 18th century as an ornamental fish species, but has since become a desirable angling species and a cheap source of protein with considerable aquaculture potential. Widely regarded as a pest species, *C. carpio* is held responsible for the introduction of numerous fish parasites and for major habitat alterations and increased turbidity levels in watercourses and water bodies directly as a result of their destructive feeding behaviour (de Moor & Bruton, 1988). According to Ellender & Weyl (2014), this species' non-native range was noted to be established with a defined invasion category of E (Blackburn et al., 2011). Although not confirmed to occur within the study area at the time of the survey, **the probability of occurrence of this species within the study area was regarded as moderate-to-high,** as verified records have been noted within adjacent and downstream systems.

- *Lepomis macrochirus* (Bluegill Sunfish, Figure 8; listed as Category 3 in NEMBA Regulations) – this species was introduced from the USA in 1938 as a forage fish for bass, as well as for angling purposes. Breeding populations are widely established in the eastern, southern and south-western Cape with a major detrimental impact observed upon indigenous species, as it tends to overpopulate waters with dwarfed individuals, as well as preys upon the young individuals and competes for food (de Moor & Bruton, 1988). Species prefers quiet, well-vegetated waters in both rivers and dams (Skelton, 2001). According to Ellender & Weyl (2014), this species' non-native range was noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011). **This species was confirmed to occur along the main stem Mzimvubu River at the time of the field survey**, as well as unverified records of observation located further upstream.

Figure 8: *Lepomis macrochirus* (Bluegill Sunfish) collected at Site T3MZIM-DSR56 at the time of the field survey

- *Micropterus dolomieu* (Smallmouth Bass; listed as Category 3 in NEMBA Regulations) – species imported from the USA in 1937 for sport fishing. It is an aggressive invader with devastating effects on a number of rare endemic species in various parts of the country (de Moor & Bruton, 1988). Favours flowing waters and exhibits an affinity toward loose rocky substrates (Skelton, 2001). While no specimens were collected at the time of the present study, **the probability of occurrence of this species within the study area was regarded moderate**, as unverified records were reported further upstream of the study area. According to Ellender & Weyl (2014), this species' non-native range was noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011).
- *Micropterus punctulatus* (Spotted Bass, Figure 9; listed as Category 3 in NEMBA Regulations) – this species was first imported into South Africa from Ohio, USA, by the Cape Piscatorial Society and the Natal Provincial Administration in October 1939 for the purposes of sport angling in South Africa. According to historic records however, the species failed to establish in most of the localities into which it was introduced (de Moor & Bruton, 1988). However, while established populations of this species in South Africa are known from various localities in KwaZulu-Natal and the Eastern Cape, it is likely that the morphological similarities of this species to *M. salmoides* (Largemouth Bass) has in many instances lead to misidentification. According to unconfirmed collection records, **this species was tentatively confirmed to occur along the main stem Mzimvubu River at the time of the field survey**, pending confirmation

of identification as a result of the morphological similarities with *Micropterus salmoides*. According to Ellender & Weyl (2014), this species' non-native range was noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011).

Figure 9: Suspected *Micropterus* cf. *punctulatus* (Spotted Bass) collected at Site T3MZIM-DSR56 at the time of the field survey

- *Micropterus salmoides* (Largemouth Bass; listed as Category 3 in NEMBA Regulations) - species formally from the freshwaters of the lower great lakes in North America, *Micropterus salmoides* (Largemouth Bass) was originally imported into South Africa from the United Kingdom in 1928 for the purpose of sport fishing and aquaculture. The first recorded invasions of *M. salmoides* into the natural systems within South Africa were recorded in 1936 in the Cape Province, with the species being first recorded within the Vaal system in 1956. Due to the predatory nature of this species, *M. salmoides* has had a negative impact on numerous indigenous fish species, particularly in river systems where no large indigenous predators occurred previously (de Moor & Bruton, 1988). While no specimens were collected at the time of the present study, **the probability of occurrence of this species within the study area was regarded moderate-to-high**, pending confirmation of identification of collected sample (see above). According to Ellender & Weyl (2014), this species' non-native range was also noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011).
- *Oncorhynchus mykiss* (Rainbow Trout; listed as Prohibited in NEMBA Regulations) – from an angling and aquaculture perspective, the species was successfully imported from England as early as 1897. However, species has been implicated as a potential threat to indigenous species (de Moor & Bruton, 1988) It requires clear, well-aerated waters to be able to survive (especially for breeding purposes) and as a result, the introductions were largely limited to cooler, upland waters (Skelton, 2001). In light of the unverified records reported upstream and confirmed collection records observed downstream of the study area, the probability of **occurrence of this species within the study area was regarded low-to-moderate**, as the species was expected to remain within the upper systems. According to Ellender & Weyl (2014), this species' non-native range was noted to be widespread and established with a defined invasion category of E (Blackburn et al., 2011).
- *Perca fluviatilis* (Perch; listed as Category 3 in NEMBA Regulations) – species introduced from Europe for angling purposes in 1915, but most introductions were unsuccessful. In areas where species was able to successfully establish, no apparent threats toward other fish was evident (de Moor & Bruton, 1988). Species is a cold-water species favouring lakes and dams or slow-flowing rivers (Skelton, 2001). According to Ellender & Weyl (2014), this species' non-native

range was noted to be localised and established with a defined invasion category of C3 (Blackburn et al., 2011). Given the location of the confirmed collection records from 1979/87 within the upper reaches of an adjoining tributary of the Mzimvubu River, **the probability of occurrence of this species within the study area was regarded as low.**

3.4.4 External Parasites

Two parasites were inadvertently collected and/or observed during the collection of the representative fish assemblages, including:

- *Lernaea* cf. *cyprinacea* (Anchor Worm; Figure 10) – *Lernaea cyprinacea* is a parasitic copepod of various families of freshwater fish with a cosmopolitan distribution. Adult females attach to the exposed body surfaces of host fish where they cause acute haemorrhage and ulcers at the area of penetration, with fatalities of host fish occurring as a result of blood loss and secondary infections (Putz and Bowen, 1964; cited in Robinson & Avenant-Oldewage, 1996). This species is not considered to be endemic to Africa, with its origins considered to be from Eurasia. Nevertheless, it was first recorded in Africa from Lake Victoria on *Oreochromis variabilis* and *O. esculenta* (Robinson & Avenant-Oldewage, 1996), and were thought to have invaded North Africa via the Nile River, and from there infested the rivers and lakes of Central Africa. According to Robinson and Avenant-Oldewage (1996), it is likely that the occurrence of this species in South Africa can be ascribed to accidental introduction as a result of the import of tropical fish from Europe and/or Asia, with a strong preference being shown by the species for indigenous members of the Cyprinidae and Cichlidae families. As such, the *Lernaea* sp. remains unconfirmed as the distinguishing characteristics located around the head could not be assessed while impregnated within the tissue of the host, nevertheless two instances of attachment to the Centrarchidae family was noted.

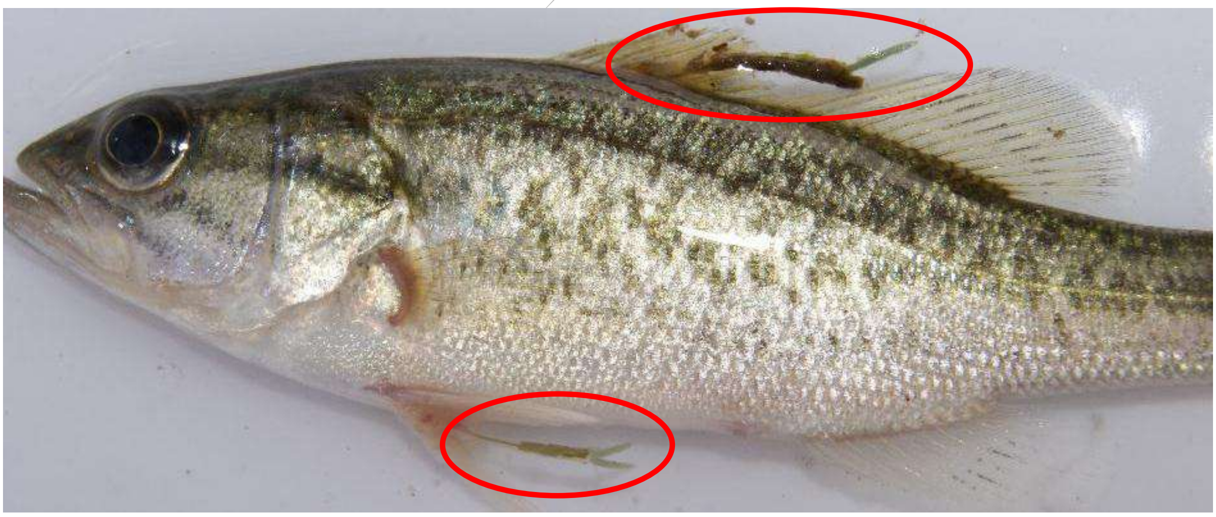

Figure 10: Two individuals of *Lernaea* cf. *cyprinacea* (red circles) observed to be attached to *Micropterus* cf. *punctulatus* collected during the present study

- Tremetode (Flatworms; Figure 11) – over 50 different species of this free-living flatworm occur in a variety of freshwater fish throughout Africa. Each species exhibits a heteroxenous (with multiple host) life cycle with species specific intermediate and definitive host selection, target tissue for infection and associated impact upon the host/s. However, most trematode species are largely harmless toward fish (usually a second intermediate host), so as to ensure progression into the definitive host (usually a piscivorous bird). The free-swimming cercariae (usually the fourth stage of the life cycle) invades the tissue of the second intermediate host and forms metacercariae (sometimes referred to as 'black spot'), which is encysted by the hosts immune reaction (Paperna, 1996).

Figure 11: Evidence of encysted metacercariae (red circles) of an undetermined Tremetode species infecting *Enteromius* cf. *anoplus*

3.4.5 Present Ecological State

Assessment of Present Ecological State (PES, or Ecological Category) of the fish assemblages collected at each of the assessed sampling sites was conducted by means of the Fish Response Assessment Index (FRAI), which is an integration of ecological requirements of fish species in an assemblage and their derived or observed responses to modified habitat conditions (Kleynhans, 2008). Since the assessment presented below in Table 10 relies only on a single sampling event along an accessible portion of the associated watercourse, a low confidence in these results exists and further studies are recommended over various spatial and temporal periods to determine potential changes to ecological condition.

Table 10: Results obtained following the application of the Fish Response Assessment Index (FRAI) at selected sampling sites at the time of the April 2016 field survey

Site	FRAI value	Ecological Category	Description
T3KINI-USMAT	Site Dry		
T3MZIM-CMPSN	Site Dry		
T3MZIM-EDNDL	15.1	**F**	Critically modified
T3MZIM-ALING	14.6	**F**	Critically modified
T3MZIM-DSR56	12.9	**F**	Critically modified
T3MZIM-RSTFN	19.7	**E/F**	Seriously-to-critically modified
T3MZIM-STRYD	63.8	**C**	Moderately modified

Based on results obtained for each of the fish assemblages collected along the main stem Mzimvubu River and adjoining non-perennial tributaries (i.e. Site T3MZIM-EDNDL, Site T3MZIM-ALING and Site T3MZIM-DSR56), each of the assessed section of the associated watercourse were determined to in a critically modified state (Ecological Category F) due to the total absence of any indigenous species.

With the exception of the main stem Umzimvubu section, each of these watercourses was observed to be largely fragmented by numerous farm dams, which was expected to limit the upstream migration (or movement) of species into the assessed reaches and as a result, occurrence of expected species was limited. Furthermore, the confirmed presence of two alien fish species along the main stem Mzimvubu River was further expected to limit the occurrence of indigenous species, as the indigenous *Enteromius* cf. *anoplus* was likely to be preyed upon or outcompeted.

Similarly, the absence of fish along the tributary of the Con Amore Stream (i.e. T3MZIM-RSTFN) suggested that the system was representative of a seriously-to-critically modified state despite the relatively natural state of the associated habitat (see Section 3.2.2). While it is acknowledges that a relatively large downstream impoundment exists (which is expected to present a notable movement barrier to potential fish species) it is suspected that the wetland nature of the associated section of the system was not likely to support large fish populations, if any. Lastly, the confirmed presence of *Enteromius* cf. *anoplus* within the Con Amore Stream (i.e. T3MZIM-STRYD) at the time of the survey substantially improved the defined ecological condition observed across the study area, as the fish assemblage collected was determined to be representative of a moderately modified (Ecological Category C) system.

3.5 Integrated EcoStatus Determination

The EcoStatus is defined as: *The totality of the features and characteristics of the river and its riparian areas that bear upon its ability to support an appropriate natural flora and fauna and its capacity to provide a variety of goods and services"* (Iversen et al., 2000). In essence, the EcoStatus represents an ecologically integrated state representing the drivers (hydrology, geomorphology, physico-chemical) and responses (fish, aquatic invertebrates and riparian vegetation) (Kleynhans & Louw, 2008). The reader is referred to Appendix A for the approach utilised during the present study. The Instream Biological Integrity, as well as the integrated EcoStatus, for the associated section was determined below (Table 11).

Following integration of the defined ecological conditions obtained for the instream biological integrity (i.e. combination of MIRAI from aquatic invertebrates and FRAI from fish) and the riparian indicators (i.e. vegetation-related metrics of the IHI for riparian habitat), it was determined that the unnamed tributaries of the Mzimvubu River each represented an integrated EcoStatus of largely modified (Ecological Category D; i.e. Site T3MZIM-ALING and Site T3MZIMSTRYD;) and largely-to-seriously (Ecological Category D/E; i.e. Site T3MZIM-EDNDL) modified conditions. These conditions were attributed to an absence of fish along each of the assessed sections of the associated watercourses, which may have been facilitated by the fragmented habitat created by established farm dams within the study area, and a poor habitat availability for macroinvertebrate colonisation, which was likely to a result of the inherent nature of the suspected channelled valley-bottom wetland.

On the other hand, each of the larger perennial systems were determined to represent an integrated EcoStatus of moderately modified (Ecological Category C; i.e. Site T3MZIM-STRYD) to largely modified (Ecological Category D; i.e. Site T3MZIM-DSR56) conditions. However, it was suspected that the instream biological integrity was skewed at the time of the current survey as a result of the low water levels and subsequent lack of niche habitat, especially along the main stem Mzimvubu River. Nevertheless, these systems were still believed to support the established aquatic communities within the study area and to effectively provide refugia habitat during periods low rainfall (or drought), as observed at the time of the survey.

Table 11: Summary of results obtained for delineated assessment units assessed at the time of the April 2016 field survey through the use of the ECOSTATUS4 (Version 1.02; Kleynhans & Louw, 2008)

Site/Reach	Response Indices					
	Aquatic Macroinvertebrate EC (MIRAI)	Fish EC (FRAI)	INSTREAM EC	Riparian Vegetation EC (IHI)	ECOSTATUS	
					Score (%)	Category
T3KINI-USMAT	Site Dry					
T3MZIM-CMPSN	Site Dry					
T3MZIM-EDNDL	E	F	E	D	40.14	D/E
T3MZIM-ALING	E	F	E	C	42.77	D
T3MZIM-DSR56	D	F	D	C	53.51	D
T3MZIM-RSTFN	E	E/F	E	B	52.19	D
T3MZIM-STRYD	D	C	D	C	62.45	C

3.6 Ecological Importance and Ecological Sensitivity

Ecological importance refers to biophysical aspects in the sub-quaternary reach that relates to its capacity to function sustainably. In contrast, ecological sensitivity considers the attributes of the sub-quaternary reach that relates to the sensitivity of biophysical components to general environmental changes such as flow, physico-chemical and geomorphic modifications. Essentially, the ecological importance and the ecological sensitivity of the relevant reaches are assessed to obtain an indication of its vulnerability to environmental modification within the context of the Present Ecological State (PES, or Ecological Category). This would relate to the ability of the sub-quaternary reach to endure, resist and recover from various forms of human use (Department of Water and Sanitation, 2014).

Although conducted at a desktop level, the assessment of ecological importance and ecological sensitivity by Department of Water and Sanitation (2014) for the associated reaches of the Mzimvubu River and adjoining non-perennial tributaries (Sub-Quaternary Reach T31G-05071) and the Con Amore Stream and adjoining tributary (Sub-Quaternary Reach T31F-05134), as well as the unnamed tributary of the Botsola River (Sub-Quaternary Reach T33A-04991) does provide context with regards to the ecological sustainability of the associated reach. Additional site-based information collected during the course of the present study was used to supplement the desktop approach to provide a more accurate depiction of the ecological importance and sensitivity of the individual watercourses under study. Table 12 provides the results obtained for the determination of the ecological importance and ecological sensitivity of each of the assessed watercourses, and provides reasoning therefore.

Table 12: Ecological Importance and Sensitivity derived for sites assessed during April 2016 field survey

Site	Ecological Importance	Ecological Sensitivity
T3KINI-USMAT*	**Low** No species of conservation concern were expected occur. Invertebrate representivity and rarity was noted to be *high*, however available biotopes were not expected to support these assemblages.	**Low-to-Moderate** Flow dependent biotope were largely absent within the associated section of the watercourse. Invertebrate and fish sensitivity to physico-chemical deterioration was noted to be high and moderate, respectively.
T3MZIM-CMPSN*	**Low-to-Moderate** No species of conservation concern were expected occur. Representivity and rarity for invertebrates and fish were noted to be *very high* and *very low*, respectively.	**Moderate-to-High** Flow dependent biotopes present along associated section of the watercourse and *very highly* sensitive invertebrate taxa are expected to occur.
T3MZIM-EDNDL	**Low-to-Moderate** Invertebrate representivity and rarity was noted to be *very high*, however available biotopes were not expected to support these assemblages.	**Low** High prevalence of alien riparian vegetation associated with adjacent areas of the watercourse. Available biotopes limited and not expected to support sensitive taxa.
T3MZIM-ALING	**Low** Invertebrate representivity and rarity was noted to be *very high*, however available biotopes were not expected to support these assemblages.	**Low** High prevalence of alien riparian vegetation associated with adjacent areas of the watercourse Available biotopes limited to vegetation and GSM, which was not expected to support sensitive taxa.
T3MZIM-DSR56	**Moderate** Absence of indigenous fish species and prevalence of alien fish species confirmed. Invertebrate rarity and representivity was *high* and *very high* with a good probabilities of occurrence once water levels increase.	**Low-to-Moderate** A few flow dependent biotopes present along the watercourse with *very highly* sensitive invertebrate and *moderately* sensitive fish taxa expected to occur.
T3MZIM-RSTFN	**Moderate-to-High** High prevalence of natural vegetation associated with adjacent areas of the watercourse. Invertebrate representivity and rarity considered *high* and *very high*.	**Moderate** Invertebrate taxa with *very high* sensitivities to changes in velocity and physico-chemical conditions are expected. However, available habitat is likely to be largely limited.
T3MZIM-STRYD	**Moderate** High prevalence of natural vegetation associated with adjacent areas of the watercourse. However, adjacent land use dominated by agricultural activities.	**Moderate-to High** Size of stream channel, morphology and geomorphic habitat is regarded as *highly* sensitive to modifications.

* Solely desktop-based (DWS, 2014), as no field assessment was conducted at these sites.

4 Conclusion and Recommendations

Based on results obtained during the April 2016 field survey, it was determined that the unnamed tributaries of the Mzimvubu River each represented an integrated EcoStatus of largely modified (Ecological Category D; i.e. Site T3MZIM-ALING and Site T3MZIMSTRYD;) and largely-to-seriously modified (Ecological Category D/E; i.e. Site T3MZIM-EDNDL) conditions. These conditions were attributed to an absence of fish along each of the assessed watercourses, which may have been facilitated by the fragmented habitat created by established farm dams within the study area, and a poor habitat availability for macroinvertebrate colonisation, which was likely to a result of the inherent nature of the suspected channelled valley-bottom wetland systems (i.e. Site T3MZIM-EDNDL and Site T3MZIM-ALING). With respect to the larger perennial systems, it was determined that the integrated EcoStatus was representative of moderately modified (Ecological Category C) at Site T3MZIM-STRYD and largely modified (Ecological Category D) conditions at Site T3MZIM-DSR56 conditions. However, it was suspected that the instream biological integrity was skewed at the time of the current survey as a result of the low water levels and subsequent lack of niche habitat, especially along the main stem Mzimvubu River. Nevertheless, these systems were still believed to support the established aquatic communities within the study area and to effectively provide refugia habitat during periods low rainfall (or drought), as observed at the time of the survey.

4.1 Recommendations

Given the presence of wetland-related features and classification of many of the associated watercourses as wetlands (according to Nel et al., 2011), as well as the limitations of EcoStatus determination in wetland systems, it is strongly recommended that a wetland assessment (including determination of Present Ecological State according to the Wet-Health approach) be conducted by a recognised wetland specialist. The study should not be limited to those sites assessed during the current assessment, but should consider the extent of all wetland features associated with the proposed R56 road reserve design, as well as an assessment of potential mitigation measures, so as to limit potential impacts of the proposed study on associated wetlands.

In addition, given the demolition and reconstruction of the majority of the affected watercourse crossings (i.e. bridge and culvert systems) along the proposed rehabilitation route, it is recommended the financial provisions also be set aside for the upgrade of various cattle underpasses, included those observed at the time of the survey (shown in Figure 4). As a result of the cursory interviews conducted with some of the local farm owners, it emphasized that these underpasses ensure safe passage for labourers, farm equipment, livestock as well as users of the regional route, as the high risks associated with crossing the busy road is all but avoided in the provision of these underpasses.

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Appendix A: Methodology

Water Quality Parameters

Selected *in situ* water quality variables were measured at each of the selected sampling sites using water quality meters manufactured by Extech Instruments, namely an ExStik EC500 Combination Meter and an ExStik DO600 Dissolved Oxygen Meter. Temperature, pH, electrical conductivity and dissolved oxygen were recorded prior to sampling, while the time of day at which the measurements were assessed was also noted for interpretation purposes.

Invertebrate Habitat Assessment System (IHAS), Version 2.2

Assessment of the available habitat for aquatic macroinvertebrate colonization at each of the sampling sites is vital for the correct interpretation of results obtained following biological assessments. It should be noted that the available methods for determining habitat quality are not specific to rapid biomonitoring assessments and are inherently too variable in their approach to achieve consistency amongst users.

Nevertheless, the Invertebrate Habitat Assessment System (IHAS) has routinely been used in conjunction with the South African Scoring System (SASS) as a measure of the variability of aquatic macroinvertebrate biotopes available at the time of the survey (McMillan, 1998). The scoring system was traditionally split into two sections, namely the sampling habitat (comprising 55% of the total score) and the general stream characteristics (comprising 45% of the total score), which were summed together to provide a percentage and then categorized according to the values in Table 13.

Table 13: Adapted IHAS Scores and associated description of available aquatic macroinvertebrate habitat

IHAS Score (%)	Description
>75	**Excellent**
65-74	**Good**
55-64	**Adequate / Fair**
<55	**Poor**

However, the lack of reliability and evidence of notable variability within the application of the IHAS method has prompted further field validation and testing, which implies a cautious interpretation of results obtained until these studies have been conducted (Ollis et al., 2006). In the interim and for the purpose of this assessment, the IHAS method was adapted by excluding the assessment of the *general stream characteristics*, which resulted in the calculation of a percentage score out of 55 that was then categorised by the aforementioned Table 13. Consequently, the assessment index describes the quantity, quality and diversity of available macroinvertebrate habitat relative to an "ideal" diversity of available habitat.

Index of Habitat Integrity, Version 2 (IHI-96-2)

The IHI (Version 2, Kleynhans, *pers. comm.*, 2015) aims to assess the number and severity of anthropogenic perturbations along a river/stream/wetland and the potential inflictions of damage toward the habitat integrity of the system (Dallas, 2005). Various abiotic (e.g. water abstraction, weirs, dams, pollution, dumping of rubble, etc.) and biotic (e.g. presence of alien plants and aquatic animals, etc.) factors are assessed, which represent some of the most important and easily quantifiable, anthropogenic impacts upon the system (Table 14).

In accordance with the original IHI approach (Kleynhans, 1996), the instream and riparian components were each analysed separately to yield two separate ecological conditions (i.e. Instream and Riparian components). However, it should be noted that the data for the riparian area is primarily interpreted in terms of the potential impact upon the instream component and as a result, may be skewed by a potentially deteriorated instream condition.

Table 14: Descriptions of criteria used to assess habitat integrity (Kleynhans, 1996; cited in Dallas, 2005)

Criterion	Relevance
Water abstraction	Direct impact upon habitat type, abundance and size. Also impacted in flow, bed, channel and water quality characteristics. Riparian vegetation may be influenced by a decrease in the supply of water.
Flow modification	Consequence of abstraction or regulation by impoundments. Changes in the temporal and spatial characteristics of flow can have an impact on habitat attributes such as an increase in duration of low flow season, resulting in low availability of certain habitat types or water at the start of the breeding, flowering or growing season.
Bed modification	Regarded as the result of increased input of sediment from the catchment or a decrease in the ability of the river to transport sediment. Indirect indications of sedimentation are stream bank and catchment erosion. Purposeful alteration of the stream bed, e.g. the removal of rapids for navigation is also included.
Channel modification	May be the result of a change in flow, which may alter channel characteristics causing a change in marginal instream and riparian habitat. Purposeful channel modification to improve drainage is also included
Water quality modification	Originates from point and diffuse sources. Measured directly, or agricultural activities, human settlements and industrial activities may indicate the likelihood of modification. Aggravated by a decrease in the volume of water during low or no flow conditions.
Inundation	Destruction of riffle, rapid and riparian zone habitat. Obstruction to the movement of aquatic fauna and influences water quality and the movement of sediments.
Alien/Exotic macrophytes	Alteration of habitat by obstruction of flow and may influence water quality. Dependent upon the species involved and scale of infestation.
Alien/Exotic aquatic fauna	The disturbance of the stream bottom during feeding may influence the water quality and increase turbidity. Dependent upon the species involved and their abundance
Solid waste disposal	A direct anthropogenic impact which may alter habitat structurally. Also a general indication of the misuse and mismanagement of the river.
Vegetation removal	Impairment of the buffer the vegetation forms to the movement of sediment and other catchment runoff products into the river. Refers to physical removal for farming, firewood and overgrazing.
Exotic vegetation encroachment	Excludes natural vegetation due to vigorous growth, causing bank instability and decreasing the buffering function of the riparian zone. Allochtonous organic matter input will also be changed. Riparian zone habitat diversity is also reduced
Bank erosion	Decrease in bank stability will cause sedimentation and possible collapse of the river bank resulting in a loss or modification of both instream and riparian habitats. Increased erosion can be the result of natural vegetation removal, overgrazing or exotic vegetation encroachment.

In accordance with the magnitude of the impact created by the abovementioned criterion, the assessment of the severity of the modifications was based on six descriptive categories ranging between a rating of 0 (no impact), 1 to 5 (small impact), 6 to 10 (moderate impact), 11 to 15 (large impact), 16 to 20 (serious impact) and 21 to 25 (critical impact; Table 15). Based on available knowledge of the site and/or adjacent catchment, a confidence level (high, medium, low) was assigned to each of the scored metrics.

Table 15: Descriptive of scoring guidelines for the assessment of modifications to habitat integrity (Kleynhans, 1996; cited in Dallas, 2005)

Impact Category	Description	Score
None	No discernible impact or the factor is located in such a way that it has no impact on habitat quality diversity, size and variability.	0
Small	The modification is limited to a very few localities and the impact on habitat quality, diversity, size and variability is also very small.	1 - 5
Moderate	The modification is present at a small number of localities and the impact on habitat quality, diversity, size and variability is also limited.	6 - 10
Large	The modification is generally present with a clearly detrimental impact on quality habitat quality, diversity, size and variability. Large areas are, however, not influenced	11 - 15
Serious	The modification is frequently present and the habitat quality, diversity, size and variability almost the whole of the defined section are affected. Only small areas are not influenced.	16 - 20
Critical	The modification is present overall with a high intensity; the habitat quality, diversity, size and variability in almost the whole of the defined section are detrimentally influenced.	21 - 25

Given the subjective nature of the scoring procedure utilised within the general approach to habitat integrity assessment (including IHI-96-2; see Appendix A), the most recent version of the IHI application (Kleynhans *et al*., 2008) and the Model Photo Guides (Graham & Louw, 2008) were used to calibrate the severity of the scoring system. It should be noted that the assessment was limited to observed and/or suspected impacts present within the immediate vicinity of the delineated assessment units, as determined through the use of aerial photography (e.g. Google Earth) and observations made at each of the assessed sampling points during the field survey. However, in cases where major upstream impacts (e.g. construction of a dam, major water abstraction, etc.) were confirmed, potential impacts within relevant sections were considered and accounted for within the application of the method.

Each of the allocated scores are then moderated by a weighting system (Table 16), which is based on the relative threat of the impact to the habitat integrity of the riverine system. The total score for each impact is equal to the assigned score multiplied by the weight of that impact. The estimated impacts (assigned score / maximum score [25] X allocated weighting) of all criteria are then summed together, expressed as a percentage and then subtracted from 100 to determine the Present Ecological State score (PES; or Ecological Category) for the instream and riparian components, respectively.

However, in cases where selected instream component criteria (i.e. water abstraction, flow, bed and channel modification, water quality and inundation) and/or any of the riparian component criteria exceeded ratings of large, serious or critical, an additional negative weight was applied. The aim of this is to accommodate the possible cumulative effect (and integrated) negative effects of such impacts (Kemper, 1999). The following rules were applied in this respect:

- Impact = Large, lower the integrity status by 33% of the weight for each criterion with such a rating.
- Impact = Serious, lower the integrity status by 67% of the weight for each criterion with such a rating.
- Impact = Critical, lower the integrity status by 100% of the weight for each criterion with such a rating.

Table 16: Criteria and weightings used to assess habitat integrity (Kleynhans, 1996; cited in Dallas, 2005)

Instream Criteria	Weight	Riparian Zone Criteria	Weight
Water abstraction	14	Indigenous vegetation removal	13
Flow modification	13	Exotic vegetation encroachment	12
Bed modification	13	Bank erosion	14
Channel modification	13	Channel modification	12
Water quality modification	14	Water abstraction	13
Inundation	10	Inundation	11
Alien/Exotic macrophytes	9	Flow modification	12
Alien/Exotic aquatic fauna	8	Water quality	13
Solid waste disposal	6		
TOTAL	**100**	**TOTAL**	**100**

Subsequently, the negative weights were added for both the instream and riparian facets of the assessment and the total additional negative weight subtracted from the provisionally determined integrity to arrive at a final habitat integrity estimate (Kemper, 1999). The eventual total scores for the instream and riparian zone components are then used to place the habitat integrity in a specific habitat integrity ecological category (Table 17).

Table 17: Ecological Categories for the habitat integrity scores (Kleynhans, 1999a; cited in Dallas, 2005)

Ecological Category	Description	Score (% of Total)
A	Unmodified, natural.	90 - 100
B	Largely natural with few modifications. A small change in natural habitats and biota may have taken place but the ecosystem functions are essentially unchanged.	80 - 89
C	Moderately modified. A loss and change of natural habitat and biota have occurred but the basic ecosystem functions are still predominantly unchanged.	60-79
D	Largely modified. A large loss of natural habitat, biota and basic ecosystem functions has occurred.	40-59
E	The loss of natural habitat, biota and basic ecosystem functions is extensive.	20-39
F	Modifications have reached a critical level and there has been an almost complete loss of natural habitat and biota. In the worst instances the basic ecosystem functions have been destroyed and the changes are irreversible.	0 - 19

South African Scoring System, Version 5 (SASS5)

While there are a number of indicator organisms that are used within these assessment indices, there is a general consensus that benthic macroinvertebrates are amongst the most sensitive components of the aquatic ecosystem. This was further supported by their largely non-mobile (or limited mobility) within reaches of associated watercourses, which also allows for the spatial analysis of disturbances potentially present within the adjacent catchment area. However, it should also be noted that their heterogeneous distribution within the water resource is a major limitation, as this results in spatial and temporal variability within the collected macroinvertebrate assemblages (Dallas & Day, 2004).

SASS5 is essentially a biological assessment index which determines the health of a river based on the aquatic macroinvertebrates collected on-site, whereby each taxon is allocated a score based on its perceived sensitivity/tolerance to environmental perturbations (Dallas, 1997). However, the method relies on a standardised sampling technique using a handheld net (300 mm x 300 mm, 1000 micron mesh size) within each of the various habitats available for standardised sampling times and/or areas. Niche habitats (or biotopes) sampled during SASS5 application include:

- Stones (both in-current and out-of-current);
- Vegetation (both aquatic and marginal); and
- Gravel, sand and mud.

Once collection is complete, aquatic macroinvertebrates are identified to family level and a number of assemblage-specific parameters are calculated including the total SASS5 score, the number of taxa collected, and the Average Score per Taxa i.e. SASS score divided by the total number of taxa identified (Thirion et al., 1995; Davies & Day, 1998; Dickens & Graham, 2002; Gerber & Gabriel, 2002). The SASS bio-assessment index has been proven to be an effective and efficient means to assess water quality impairment and general river health (Dallas, 1997; Chutter, 1998).

Macroinvertebrate Response Assessment Index (MIRAI)

In order to determine the Present Ecological State (PES; or Ecological Category) of the aquatic macroinvertebrates collected/observed, the SASS5 data is used as a basic input (i.e. prevalence and abundance) into the MIRAI. This biological index integrates the ecological requirements of the macroinvertebrate taxa in a community (or assemblage) and their response to flow modification, habitat change, water quality impairment and/or seasonality (Thirion, 2008). The presence and abundance of the aquatic macroinvertebrates collected are compared to a derived list of families/taxa expected to be present under natural, un-impacted (or reference) conditions. Consequently, the three (or four) metric groups utilised during the application of the MIRAI were combined within the model to derive the ecological condition of the site in terms of aquatic macroinvertebrates (Table 18).

Table 18: Allocation protocol for the determination of the Present Ecological State for aquatic macroinvertebrates following application of the MIRAI

MIRAI (%)	Ecological Category	Description
90-100	A	Unmodified and natural. Community structures and functions comparable to the best situation to be expected. Optimum community structure for stream size and habitat quality.
80-89	B	Largely natural with few modifications. A small change in community structure may have taken place but ecosystem functions are essentially unchanged.

60-79	C	Moderately modified. Community structure and function less than the reference condition. Community composition lower than expected due to loss of some sensitive forms. Basic ecosystem functions are still predominantly unchanged.
40-59	D	Largely modified. Fewer species present then expected due to loss of most intolerant forms. An extensive loss of basic ecosystem function has occurred.
20-39	E	Seriously modified. Few species present due to loss of most intolerant forms. An extensive loss of basic ecosystem function has occurred.
0-19	F	Critically modified. Few species present. Only tolerant species present, if any.

Fish Response Assessment Index (FRAI)

Fish were collected by means of electro-narcosis (or electro-fishing), whereby an anode and a cathode are immersed in the water to temporarily stun fish in the near vicinity. A photographic record of fish collected was taken. Each of the collected fish specimens were identified in the field, a photograph was taken of each species representative and/or specimens with a notable macroscopic abnormality and released back into the river, where possible.

Assessment of the Present Ecological State (PES; or Ecological Category) of the fish assemblage of the watercourses associated with the study area was conducted by means of the FRAI (Kleynhans, 2008). This procedure is an integration of ecological requirements of fish species in an assemblage and their derived (or observed) responses to modified habitat conditions. In the case of the present assessment, the observed response was determined by means of fish sampling, as well as a consideration of species requirements and driver changes (Kleynhans, 2008). The expected fish species assemblage within the study area was derived from (Kleynhans et al., 2008b) and aquatic habitat sampled.

Although the FRAI uses essentially the same information as the Fish Assemblage Integrity Index (FAII), it does not follow the same procedure. The FAII was developed for application in the broad synoptic assessment required for the River Health Programme, and subsequently does not offer a particularly strong cause-and-effect basis. The purpose of the FRAI, on the other hand, is to provide a habitat-based cause-and-effect underpinning to interpret the deviation of the fish assemblage from the perceived reference condition(Kleynhans, 2008).

The FRAI is based on the assessment of metrics within metric groups. These metrics are assessed in terms of:

- Habitat changes that are observed or derived;
- The impact of such habitat changes on species with particular preferences and tolerances; and
- The relationship between the drivers used in the FRAI and the various fish response metric groups, as are indicated in Figure 12 Table 19 provides the steps and procedures required for the calculation of the FRAI.

Interpretation of the FRAI score follows a descriptive procedure in which the FRAI score is classified into a particular PES (or Ecological Category) based on the integrity classes of (Kleynhans, 1999b). Each category describes the generally expected conditions for a specific range of FRAI scores (Table 20).

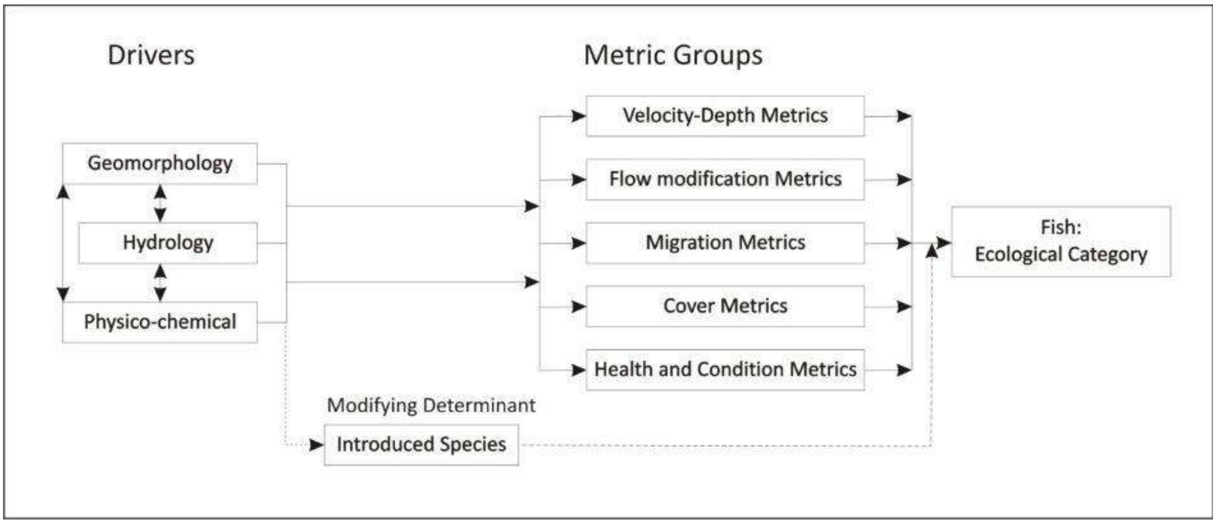

Figure 12: Relationship between drivers and fish metric groups

Table 19: Main steps and procedures followed in calculating the Fish Response Assessment Index

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

Table 20: Allocation protocol for the determination of the Present Ecological State (or Ecological Category) of the sampled/observed fish assemblage following application of the FRAI

FRAI (%)	Ecological Category	Description
90-100	A	Unmodified and natural. Community structures and functions comparable to the best situation to be expected. Optimum community structure for stream size and habitat quality.
80-89	B	Largely natural with few modifications. A small change in community structure may have taken place but ecosystem functions are essentially unchanged.
60-79	C	Moderately modified. Community structure and function less than the reference condition. Community composition lower than expected due to loss of some sensitive forms. Basic ecosystem functions are still predominantly unchanged.
40-59	D	Largely modified. Fewer species present then expected due to loss of most intolerant forms. An extensive loss of basic ecosystem function has occurred.
20-39	E	Seriously modified. Few species present due to loss of most intolerant forms. An extensive loss of basic ecosystem function has occurred.
0-19	F	Critically modified. Few species present. Only tolerant species present, if any.

EcoStatus4 1.02 Model

For the purpose of the present assessment, the latest ECOSTATUS4 1.02 model was used, which is an upgraded and refined version of the original ECOSTATUS4 model (Kleynhans & Louw,2008). The results obtained from the fish and aquatic macroinvertebrate response indices (i.e. FRAI and MIRAI) are to be integrated within the model to determine an Instream Ecological Category, whereas the riparian elements from the IHI-96-2 model can be used as a surrogate for the Riparian Ecological Category in the following manner (Dr. C.J. Kleynhans, *pers. comm.*, 2015):

*Riparian Vegetation EC = 100-((IHI 'Natural vegetation removal')+(IHI 'Exotic Vegetation Encroachment'))/50*100)*

Species of Conservation Concern

According to Driver et al., (2012), individual species "are the building blocks of ecosystems" and as a result, the conservation of a particular species (e.g. umbrella species, keystone species, or threatened species) is expected to facilitate the preservation of established communities and in turn, functioning ecosystems, as well as the provision of ecosystem services (Nel & Driver, 2012). However, due to the limited conservation resources available, it is important to identify and support the most effective research areas and/or implementation strategies. Hence, various conservation assessments (or Red List assessments) are irregularly conducted and/or legislated Threatened and Protected Species (ToPS) Regulations of NEMBA (Act 10 of 2004) are compiled to facilitate the allocation of available resources (Driver et al., 2012).

For the purposes of this assessment, the most recent regional conservation assessment of freshwater-dependent taxonomic groups (i.e. fishes, molluscs, dragonflies, crabs, and vascular plants) by Darwall et al. (2009a) will be used as a base indication of a particular species' vulnerability to extinction. These indications will also be supported by the corresponding lists of threatened species formulated within the NBA and NFEPA studies (Nel et al., 2011b; Nel & Driver, 2012). Lastly, the originally gazetted ToPS list, published in 2007 and subject to subsequent amendments, will be used to support and/or identify other aquatic species with some potential commercial or economic value.

Invasive Alien Species

Although the terminology used within this arena of research varies widely, a basic definition of an *alien species* can be defined as 'a non-native species introduction into a geographical region beyond its natural distribution.' Furthermore, for an alien species to become *invasive*, the introduction, establishment and spread of this non-native species essentially threatens ecosystems, habitats and/or other species (Driver et al., 2012). In order to determine the degree of invasion within a particular area, there are a number of stages (transport, introduction, establishment and spread) that the species needs to pass through and various barriers (geography, captivity or cultivation, survival, reproduction, and dispersal) that the species needs to overcome (de Moor & Bruton, 1988; Blackburn et al., 2011). Given that alien species have been introduced and indigenous species have been translocated for a variety of reasons including sport fishing, aquaculture, biocontrol, increasing range of rare species, through the aquarium trade, for enhancing fisheries or by accident, it is important to assess and investigate the potential impacts that the presence of these organisms have upon the natural biota within the associated ecosystem (de Moor & Bruton, 1988; Jones et al., 2013).

For the purposes of this assessment, the initial 'Atlas of alien and translocated indigenous aquatic animal in Southern Africa' by de Moor & Bruton (1988) will be used as an indication of the potential presence of non-native species within the study area. Following the determination of potential species of occurrence, each of the species' invasion category will be listed according to the NEMBA Alien and Invasive Regulations, which was officially gazetted during 2014, and the review paper by Ellender & Weyl (2014) for fish species.

Appendix B: Photographs of Sampling Sites

T3KINI-USMAT – Upstream perspective of unnamed non-perennial tributary of Kinira River (Downstream of bridge crossing IDB 0528 along R56 regional route)

T3MZIM-CMPSN – Upstream perspective of unnamed non-perennial tributary of Mzimvubu River (Upstream of culvert system IDC 0649 along R56 regional route)

T3MZIM-EDNDL – Upstream perspective of unnamed tributary of Mzimvubu River
(Downstream of bridge crossing IDB 0529 along R56 regional route)

T3MZIM-ALING – Downstream perspective of unnamed non-perennial tributary of Mzimvubu River
(Directly downstream of culvert system IDC 0653 along R56 regional route)

T3MZIM-DSR56 – Upstream perspective of main stem Mzimvubu River
(Directly downstream of bridge crossing IDB 0532 along R56 regional route)

T3MZIM-RSTFN – Downstream perspective of unnamed perennial tributary of Con Amore Stream
(Directly downstream of culvert system IDC0661 along R56 regional route)

T3MZIM-STRYD – Upstream perspective of Con Amore Stream

Appendix C: Aquatic Macroinvertebrate Data

Abundances:
1 = 1 individual
A = 2 – 10 individuals
B = 11 – 100 individuals
C = 101 – 1000 individuals
D = >1000 individuals

Reference Frequency:
1 = low probability of collection/observation
2 = low-to-moderate probability of collection/observation
3 = moderate probability of collection/observation
4 = moderate-to-high probability of collection/observation
5 = high probability of collection/observation

Taxon	Reference Abundance	Reference Frequency	T3KINI-USMAT	T3MZIM-CMPSN	T3MZIM-EDNDL	T3MZIM-ALING	T3MZIM-DSR56	T3MZIM-RSTFN	T3MZIM-STRYD
PORIFERA (Sponge)*	P	1	Site Dry – No sampling conducted.	Site Dry – No sampling conducted			P		
COELENTERATA (Cnidaria)*	A	1							
TURBELLARIA (Flatworms)	A	4							
ANNELIDA									
Oligochaeta (Earthworms)	A	4			A	A	B	A	B
Hirudinea (Leeches)	A	2					A		
CRUSTACEA									
Potamonautidae (Crabs)	A	5				1	A	1	A
Atyidae (Freshwater Shrimps)	A	3							
HYDRACARINA (Mites)	A	3			A	A		A	
PLECOPTERA (Stoneflies)									
Perlidae	A	5							
EPHEMEROPTERA (Mayflies)									
Baetidae 1sp					A	A		A	A
Baetidae 2spp									
Baetidae >2spp	B	5					B		
Caenidae (Squaregills/Cainflies)	B	5					A		A
Heptageniidae (Flatheaded Mayflies)	A	4							

Leptophlebiidae (Prongills)	**B**	**5**					B		
Oligoneuridae (Brushlegged Mayflies)*	**A**	**1**							
Polymitarcyidae (Pale Burrowers)	**A**	**2**							
Prosopistomatidae (Water Spec)	**A**	**3**							
Trichorythidae (Stout Crawlers)	**B**	**5**					A		
ODONATA (Dragonflies & Damselflies)									
Chlorocyphidae (Jewels)	**A**	**2**							
Chlorolestidae (Sylphs)*	**A**	**1**							
Coenagrionidae (Sprites& Blues)	**A**	**4**			A		A	1	B
Lestidae (Emerald Damselflies/Spreadwings)	**A**	**1**							A
Platycnemidae (Stream Damselflies)	**A**	**1**							1
Protoneuridae (Threadwings)*	**A**	**1**							
Aeshnidae (Hawkers & Emperors)	**A**	**3**							B
Corduliidae (Cruisers)	**A**	**1**							
Gomphidae (Clubtails)	**A**	**5**					A		A
Libellulidae (Darters/Skimmers)	**A**	**3**							A
LEPIDOPTERA (Aquatic Caterpillars/Moths)									
Pyralidae*	**A**	**1**							
HEMIPTERA (Bugs)									
Belostomatidae (Giant Water Bugs)	**A**	**2**				1			A
Corixidae (Water Boatmen)	**B**	**4**			B	C	B	C	C
Gerridae (Pond Skaters/Water Striders)	**A**	**3**			A	A	B	B	B
Hydrometridae (Water Measurer)*	**A**	**1**							
Naucoridae (Creeping Water Bugs)	**A**	**3**							A
Nepidae (Water Scorpions)	**A**	**2**							
Notonectidae (Backswimmers)	**A**	**2**			A			B	B
Pleidae (Pygmy Backswimmers)	**A**	**1**					1	A	B
Veliidae (Ripple Bugs)	**A**	**4**					1		
TRICHOPTERA (Caddisflies)									
Dipseudopsidae*	**A**	**1**							
Ecnomidae	**A**	**1**			A		A		

Hydropsychidae 1sp							1		
Hydropsychidae 2spp									
Hydropsychidae >2spp	**B**	**4**							
Philopotamidae	**A**	**2**							
Polycentropodidae*	**A**	**1**							
Psychomyiidae*	**A**	**1**							
Hydroptilidae	**A**	**2**							
Lepidostomatidae*	**A**	**1**							
Leptoceridae	**B**	**4**							
COLEOPTERA (Beetles)									
Dytiscidae (Diving Beetles)	**A**	**3**			B	B	A	B	B
Elmidae (Riffle Beetles)	**A**	**4**					1		
Gyrinidae (Whirligig Beetles)	**A**	**5**				B	B	1	B
Haliplidae (Crawling Water Beetles)	**A**	**1**							
Helodidae (Marsh Beetles)	**A**	**1**							
Hydraenidae (Minute Moss Beetles)	**A**	**1**							
Hydrophilidae (Water Scavenger Beetles)	**A**	**3**			B			1	A
Psephenidae (Water Pennies)	**A**	**1**							
DIPTERA (Flies)									
Athericidae (Snipe Flies)	**A**	**3**							
Ceratopogonidae (Biting Midges)	**A**	**4**					A		A
Chironomidae (Midges)	**B**	**5**			A	B	B	B	B
Culicidae (Mosquitoes)	**A**	**3**			A	A		B	
Dixidae (Dixid Midges)*	**A**	**1**							
Empididae (Dance Flies)*	**A**	**1**							
Ephydridae (Shore Flies)*	**A**	**1**							
Muscidae (House Flies/Stable Flies)*	**A**	**1**							
Simuliidae (Blackflies)	**B**	**5**					C	A	
Tabanidae (Horse Flies)	**A**	**3**			A				
Tipulidae (Crane Flies)	**A**	**3**							
GASTROPODA (Snails)									

Ancylidae (Limpets)	**A**	**4**					1		1
Bulinae	**A**	**5**							A
Lymnaeidae (Pond Snails)	**A**	**3**							1
Physidae (Pouch Snails)	**-**	**-**							A
Planorbinae (Orb Snails)	**A**	**3**							
Thiaridae*	**A**	**1**							
PELECYPODA (Bivalves)									
Corbiculidae (Clams)	**A**	**2**					1		A
Sphaeridae (Pill Clams)	**A**	**2**							
Unionidae (Perly Mussels)*	**A**	**1**							
SASS5 Score *(Reference Value = 180)*			-	-	54	44	128	58	121
Number of Taxa *(Reference Value = 73)*			-	-	13	12	24	15	26
Average Score Per Taxon (ASPT) *(Reference Value = 6.50)*			-	-	4.15	3.67	5.33	3.87	4.65

* **'Taxon'** (in Red) – unconfirmed suspicion of occurrence within the study area, 'Taxon' (in Black) – confirmed record of occurrence within the ecoregion, slope class and/or altitude range.

Appendix D: Ichthyofaunal Data

Scientific Name	Common Name	Regional Conservation Status (Darwall et al., 2009)*	NEMBA (2007) Listing**	Endemic to Southern African Region (Darwall et al. 2009)	Expected under Natural Conditions	T3KINI-USMAT	T3MZIM-CMPSN	T3MZIM-EDNDL	T3MZIM-ALING	T3MZIM-DSR56	T3MZIM-RSTFN	T3MZIM-STRYD
Family Anguillidae						Site Dry – No sampling conducted	Site Dry – No sampling conducted					
Anguilla bicolor bicolor	Shortfin Eel	LC	-	-				-	-	-	-	-
Anguilla marmorata	Giant Mottled Eel	LC	-	-				-	-	-	-	-
Anguilla mossambica	Longfin Eel	LC	-	-	**X**			-	-	-	-	-
Family Centrarchidae												
Lepomis macrochirus	Bluegill Sunfish	-	-	-	**Alien**			-	-	6	-	-
Micropterus cf. *dolomieu*	Smallmouth Bass	-	-	-	**Alien**			-	-	-	-	-
Micropterus cf. *punctulatus*	Spotted Bass	-	-	-	**Alien**			-	-	1	-	-
Micropterus salmoides	Largemouth Bass	-	-	-	**Alien**			-	-	-	-	-
Family Cichlidae												
Tilapia sparrmanii	Banded Tilapia	LC	-	-	**Extralimital**			-	-	-	-	-
Family Clupeidae												
Gilchristella aestuaria	Estuarine Round-Herring	LC	-	Endemic				-	-	-	-	-
Family Cyprinidae												
Cyprinus carpio	Common Carp	-	-	-	**Alien**							
Enteromius (Barbus) anoplus	Chubbyhead Barb	LC	-	Endemic	**X**			-	-	-	-	4
Pseudobarbus quathlambae	Maloti Minnow	EN	-	Endemic				-	-	-	-	-

Family Eleotrididae												
Eleotris fusca	Dusky Sleeper	LC	-	-				-	-	-	-	-
Family Gobiidae												
Awaous aeneofuscus	Freshwater Goby	LC	-	-				-	-	-	-	-
Glossogobius callidus	River Goby	LC	-	Endemic				-	-	-	-	-
Glossogobius giuris	Tank Goby	LC	-	-				-	-	-	-	-
Redigobius dewaali	Checked Goby	LC	-	Endemic				-	-	-	-	-
Family Muglidae												
Pseudomyxus (Myxus) capensis	Freshwater Mullet	LC	VU	Endemic				-	-	-	-	-
Family Percidae												
Perca fluviatilis	Perch	-	-	-	**Alien**			-	-	-	-	-
Family Salmonidae												
Oncorhynchus mykiss	Rainbow Trout	-	-	-	**Alien**			-	-	-	-	-
Number of Species					**10**	**-**	**-**	**0**	**0**	**2**	**0**	**1**
Total Catch (Number of Individuals)					**-**	**-**	**-**	**0**	**0**	**7**	**0**	**4**

* **IUCN Conservation Categories:** LC – Least Concern, NT – Near Threatened, EN – Endangered

** **NEMBA Threatened and Protected Species (ToPS) Categories:** VU – Vulnerable, P - Protected

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FORM IP180_B

CLIENT	:	South African National Road Agency Limited (SANRAL)
PROJECT NAME	:	Matatiele R56 Road Upgrade **PROJECT No.** : J35193
TITLE OF DOCUMENT	:	Matatiele R56 Road Rehabilitation (Section 8) – Aquatic Assessment
ELECTRONIC LOCATION	:	\\durban-5\projects\3230 - Environmental\J35193 - Matatiele R56 Upgrade\Reports\Aquatic\J35193_R56 Road Upgrade (Matatiele)_ Aquatic Assessment_FINAL_2016.06.10.docx

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DATE **10 / 06 / 2016**	SIGNATURE	SIGNATURE	SIGNATURE

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