EXXARO GROOTEGELUK COMPLEX:

MONITORING PROTOCOL FOR NON-PERENNIAL PANS

FINAL

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Signed:

Date: 22 August 2023

Fiona Eggers

Pr. Sci. Nat. (Ecology) Reg. No. 400606/15

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LIST OF ACRONYMS

Acronym	Explanation
DBI	Dragonfly Biotic Index
DO	Dissolved Oxygen
DWS	Department of Water and Sanitation
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
eDNA	Environmental DNA
GPS	Global Positioning System
Gs	Glenrosa Soil Form
GSM	Gravel Sand and Mud
Hu	Hutton Soil Form
IUCN	International Union for Conservation of Nature
LEDET	Limpopo Department of Economic Development, Environment and Tourism
LOM	Life of Mine
NFEPA	National Freshwater Ecosystem Priority Areas
NWA	National Water Act (No 36 of 1998)
ORP	Oxidation-reduction Potential
PERMANOVA	Permutational Analysis of Variance
pH	Potential of Hydrogen
QDS	Quarter Degree Squares
RDA	Redundancy Analysis
SASS	South African Scoring System
Temp	Temperature
TSS	Total Suspended Solids
WRC	Water Research Commission
WULA	Water Use Licence Application
YSI	Yellow Springs Instrument

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1. INTRODUCTION

The Grootegeluk coal mine operation is an opencast mining operation and is located within the Waterberg coalfield of the Limpopo province and is currently the largest of Exxaro Resources coal mines. The mine is located approximately 20km to the west of the town of Lephalale within the Limpopo Province of South Africa. Portions of land located to the west of the current mining operations have been identified as having suitable coal reserves and thus Exxaro intends on expanding its mining activities into these identified areas. The coal reserves within the proposed expansion areas are relatively shallow, and as such opencast mining is considered the most desirable option to access these coal reserves. The coal reserves are considered to include A-grade export-quality coal and power station coal, with both of Eskom's Medupi and Matimba power stations being beneficiaries of the coal.

Opencast coal mining is an operation which results in the excavation of a large pit, the associated infrastructure, and the overburden dumps. Associated with these activities are a suite of potential risks and impacts on water resources and these include not only the direct loss of freshwater ecosystems due to the mining activities, but also the potential contamination of these freshwater resources.

Currently it is proposed that the next 30-year mining plan extends in a predominantly westerly direction from the current pit. The 30-year life-of-mine (LOM) plan is proposed to extend across the following cadastrals (**Figure 1-1**):

- Daarby 458 LQ
- Leeuwdrift 312 LQ
- Grootegeluk 459 LQ
- Hieromtrent 360 LQ
- Vaalpensloop;
- Turfvlakte 463 LQ.

It should be noted, that even though the LOM plan extends across into the Vaalpensloop and Turfvlakte 463 LQ, these areas currently do not fall within the Water Use Licence Application (WULA) and as such, have been excluded from the monitoring protocol.

The LOM area currently has no mining related infrastructure within its boundaries as it is utilised for game farming purposes. As part of the requirement of the relevant local and national regulatory bodies, such as the Department Water and Sanitation (DWS) and the Limpopo Department of Economic Development, Environment and Tourism (LEDET), all wetlands within the study site that included portions of the afore-mentioned cadastrals[1] were identified and delineated (GroundTruth 2018). The objective of the delineation procedure was to identify the boundary between the wetland systems and adjacent terrestrial areas within the specified study site. The process of wetland delineation identified the extent of these ecosystems based on the following legal definition[2]:

> *"Wetland means land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with*

[1] It should be noted that the study site extent of the 2018 study did not coincide with the afore-mentioned cadastral extents and therefore, a portion of the Leeuwdrift 312 LQ cadastral was not surveyed for wetland habitat and thus also does not form part of this monitoring protocol.
[2] As per the National Water Act (Act No. 36 of 1998)

shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil." [3]

Following on from this, the relevant authorities requested that a monitoring protocol be developed for the identified non-perennial pans that will be retained within the study site boundary (refer to Section 1.1 and **Figure 1-1**). The monitoring protocol aims to provide a framework within which the non-perennial pans can be assessed and evaluated. Monitoring of these systems contributes towards the ability to ascertain whether the adjacent mining activities are having an impact on these remaining systems. The details of the monitoring protocol, including the authority's requirements, and some background information to the proposed strategies, are outlined in this report.

1.1 Authority's requirements

The DWS issued Exxaro (Pty) Ltd. Grootegeluk Complex with a Water Use Licence in terms of the National Water Act (No 36 of 1998) (NWA) for the expansion of their mining activities within the afore-mentioned areas (Licence No. 07/A42J/CICI/6418). The validity of the licence is for thirty (30) years, during which period the licence may be reviewed at intervals not more than five (5) years. In terms of Section 21 (c) and (i) of the NWA, Exxaro have been authorised to alter the sixty-three (63) identified non-perennial pans within the mining rights area. Subsequent to the issuing of the licence, some of the conditions have been amended and/or excluded. According to the amended licence (dated 16/02/2022), the following conditions are considered to be crucial in terms of the proposed monitoring protocol:

Amended condition 1.7 "Ecological class of rivers and wetlands (seasonal pans) that will remain within the catchment & offset (PES of B) must not be lowered. Recommended Ecological Class (REC) for wetland must be set as class B.

5.1 "A comprehensive and appropriate environmental assessment and monitoring programme (including bio-monitoring and eco-toxicology) to determine the impact, change, deterioration and improvement of the aquatic system associated with the activities listed under condition 1.1 and other existing activities as well as compliance to these water use licence conditions must be developed and submitted to the Provincial Head for a written approval before commencement and must be subsequently be implemented.

5.2 "Six (6) monthly monitoring reports for Groundwater and surface water must be submitted to the Provincial Head until otherwise agreed in writing with the Provincial Head.

9.1.1 "The Licensee must develop and submit to the Provincial Head within six (6) months of the issuance of the Licence a bio-monitoring programme that will include the compilation of an initial database from which the scope and frequency of future bio-monitoring can be developed. This initial assessment must lead to the establishment of a reliable site-

[3] It should be noted that the wetlands identified within the study area are non-perennial pans that only contain water during the rainy season. Furthermore, these systems are not sustained by subsurface water inputs and as such are ephemeral in nature, which is further exacerbated by the low rainfall and high evaporative rates for this area. The formation of these systems coincides with depressions in the landscape that are underlain by suitable substrate, e.g., clay layers. The combination of these factors has resulted in the formation of these non-perennial systems within the landscape, which are the only wetlands identified within the mining rights area.

specific long-term bio-monitoring programme. This programme must be able to qualify and quantify the impact on biological systems in the water environment in the area directly affected by activities as well as downstream from these activities.

Based on the afore-mentioned licence requirements and the nature of the non-perennial pans, a suitable and comprehensive monitoring protocol has been provided and recommended in this report. The objectives of the monitoring protocol are outlined in the following section.

1.2 Objectives

The objectives of the non-perennial pan monitoring protocol are the following:

- Develop a technologically advanced, user-friendly, but comprehensive monitoring protocol for the monitoring of the non-perennial pans that will be retained within the post-mining landscape[4] of the mining rights area;
- Establish baseline data for the identified non-perennial pans that will be retained within the post-mining landscape with baseline data including:
    - The condition of the wetlands within the project area based on the GroundTruth (2018) report, which used the WET-Health assessment tool (Macfarlane et al. 2007);
    - Invertebrate sampling and specimen collection for comparison between the seasonal site visits (wet and dry season site visits); and
    - Water quality, turbidity, and Yellow Springs Instrument (YSI) sampling for selected sites.
    - Ecotoxicology sampling of all of the identified non-perennial pans.
- The non-perennial pans which will be lost as a result of the expanding mining activities have been assessed by GroundTruth in 2018, and the assessment of the systems is considered to provide the baseline information of the systems.

[4] It should be noted that the post-mining landscape refers to the non-perennial pans that were identified by GroundTruth in 2018 study site extent but that will not be lost as a result of 0–30-year mining plan (dated February 2020).

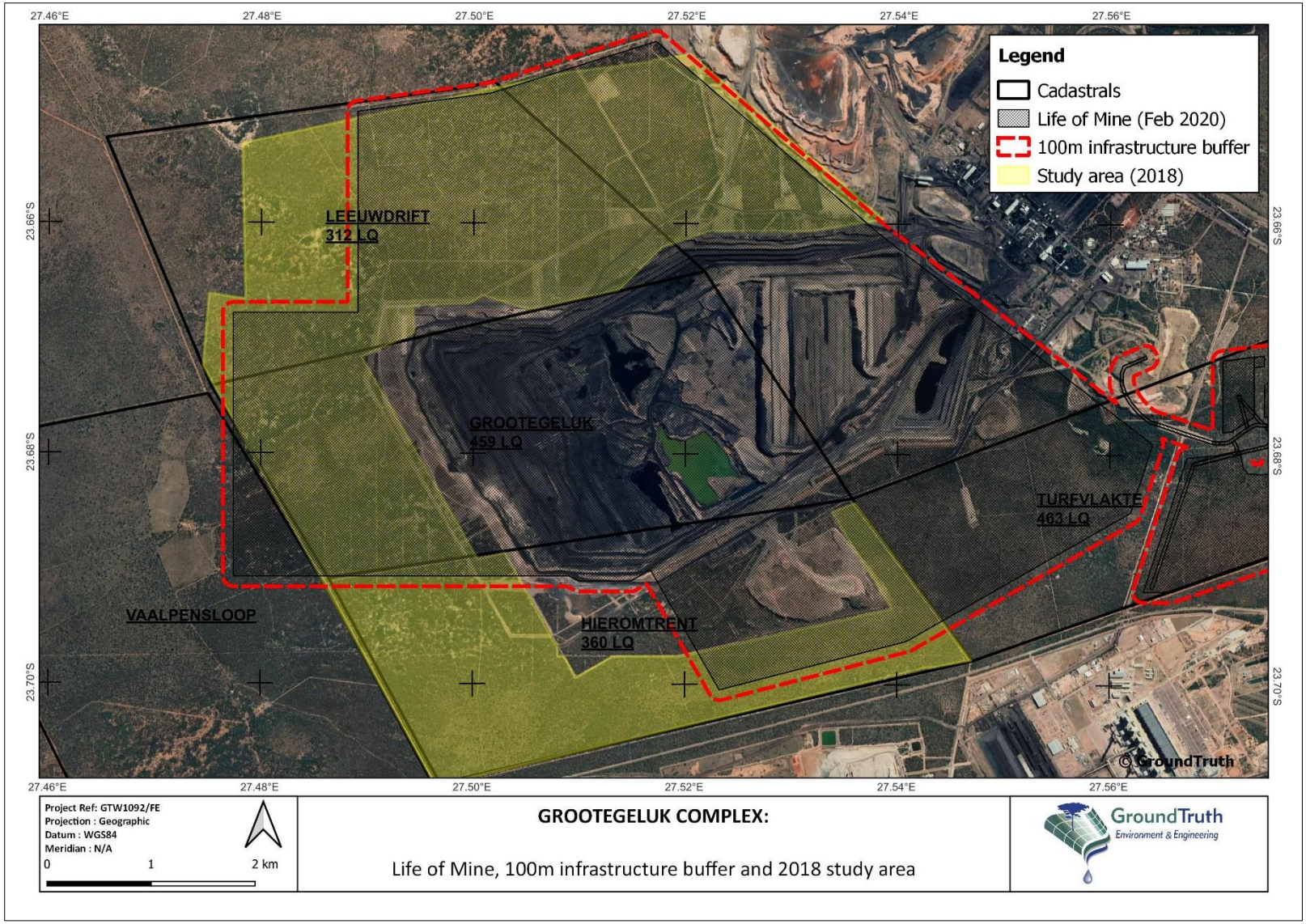

Figure 1-1 Overview of the proposed LOM (February 2020) and the associated 100m buffer

2. BACKGROUND INFORMATION

The following sections provide an overview of aquatic invertebrates, the nature of the non-perennial pans, and sampling methods under wet and dry season conditions including the supporting literature. This background information is considered essential in the understanding of the functioning of the identified non-perennial pans within the landscape, from which a detailed monitoring protocol can be developed. It should be noted that the GroundTruth (2018) report should be consulted for specific information pertaining to the climate of the region, and specific details pertaining to the wetland types.

2.1 General Background Information

Within the originally proposed LOM footprint area in 2018 but limited to all the cadastrals except Vaalpensloop[5] listed under **Section 1**, approximately 63 freshwater ecosystems were identified by GroundTruth in 2018 (**Figure 2-1**). Of the 63 systems, three (3) were classified as artificial systems and comprised of either watering holes or excavated areas with or without additional water inputs. These artificial systems cover an area of approximately 0.62ha, whilst the remaining 7.14ha are natural wetland systems. The following sections provide a description of the various freshwater ecosystems identified within the LOM/study area located within the four (4) cadastrals[6].

[5] Vaalpensloop cadastral did not form part of the GroundTruth wetland studies (2019a and 2019b) and as such, has been excluded from this monitoring protocol.
[6] It should be noted, that portions of the cadastrals were excluded from the 2018 sampling, as it was located beyond the study site boundary, and therefore, any non-perennial pans located within the unsampled areas have not been included in this report.

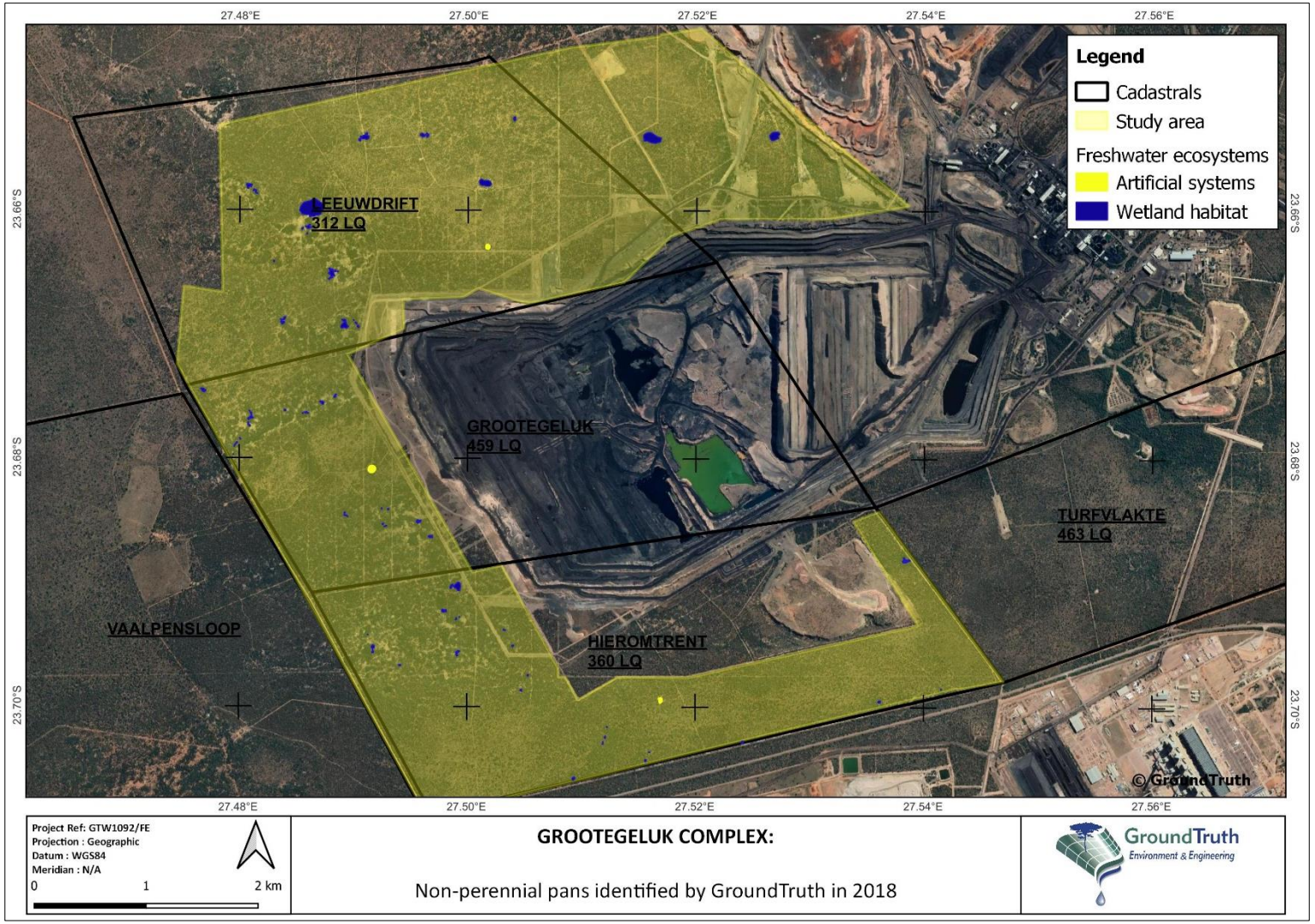

Figure 2-1 Overview of the freshwater ecosystems identified by GroundTruth in 2018 and the associated study area of 2018.

2.1.1 *Artificial systems*

Three (3) artificial wetland systems were identified within the LOM footprint area (GroundTruth 2018). These systems were fundamentally different to the natural systems with some of the characteristics including the following:

- Evidence that the systems had been excavated.
- Substrate of the systems was generally rock or course/gravel-like material and not mud as in the natural systems.
- Evidence of either current or historical water inputs into the systems via a pipe or canal.
- Retention of water throughout the year, whilst the natural systems were dry.
- Water clarity within these systems was often clear in comparison to the natural systems that had increased turbidity.

For additional details pertaining to the artificial systems refer to the GroundTruth (2018) wetland study, which provides a detailed description of these artificial systems.

2.1.2 *Non-perennial pans*

The remaining sixty (60) wetlands within the LOM footprint area are natural systems, i.e., not artificially created and/or sustained. These systems cover an area of approximately 7.14ha, and range in size from 0.0004ha to 2.36ha. However, most of the systems are less than 0.1ha in extent. Of all the systems, there is only one system that is greater than two hectares, namely 2.36ha, which is located within the Leeuwdrift portion of the study area. For the purpose of the monitoring protocol, it is essential to understand the formation and functioning of these non-perennial pans, as conventional methods for monitoring are considered to be inappropriate and would not provide reliable data against which to assess any changes. The following sections provide a detailed overview on the formation of the pans and the driving forces behind these systems. It should be noted that these sections have been adapted from the GroundTruth (2018) wetland study report, and that the report should be consulted should additional information be required.

2.1.2.1 *Formation of non-perennial pans*

The functioning of wetland ecosystems is often considered from a hydrological and biotic perspective, whilst the geomorphic and climatic setting in which these systems are located is often just briefly considered in comparison. With regards to the formation of endorheic[7] depressions/pans, there are currently three suites of controls that contribute towards the formation of such systems, either individually or in combination with one another (Thomas 2011). However, in terms of the non-perennial pans within the LOM footprint area, the process of 'erosional control' is applicable, particularly since these systems are located within a semi-arid region. According to Goudie and Thomas (2011)[8], erosional controls refers to a process known as deflation. Deflation occurs in areas with a low MAP (≤500mm) and overgrazing around localised depressions within the landscape. Erosional controls are greatly influenced by climatic conditions, i.e., rainfall amounts and seasonality, temperature; and biotic factors including the presence and/or absence of herbivores, and the composition and structure of the vegetation within and

[7] Endorheic referring to a "Basin or region from which there is little or no outflow of water (either on the surface as rivers, or underground by flow or diffusion through rock or permeable material (Macfarlane et al. 2007, p.169)".
[8] Information sourced from Ellery (2018) MSc thesis.

surrounding the localised depressions. Furthermore, the underlying geology and local topography of the areas further contribute towards the formation of these systems. The underlying geology needs to be susceptible to weathering/erosion to allow for the formation of pans. The local topography is also considered to be important, in that the nature of the landscape will dictate whether there is transport of sediment into or out of the depression, i.e., the steeper the landscape the greater the opportunity for the mobilisation of sediment into the system. The combination of all the factors results in the deflation of the depressions, i.e., a negative sediment budget, and as such the formation of a depression/pan wetland.

2.1.2.2 *Hydrology of the wetlands*

A review of the national lithology coverage highlights that the greater study area is underlain by shale, which is susceptible to weathering/erosion (Council for Geoscience n.d.). The national soil layer for the greater study area highlights that the predominant soil form in the area is dominated by the Hutton Form (Hu) (Land Type Survey Staff 1972-2006). Based on a study undertaken by Golder in 2017, the predominant soil type found within the proposed LOM footprint area is the Glenrosa Form (Gs). Both these soil forms are poorly structured soils. The Hutton Forms, as described in Red Earth cc (2004), are soils that are very permeable, whilst the Glenrosa Forms are permeable (Red Earth cc 2004). Based on Natural Scientific Services (2011), the predominant soils within the broader landscape are Kalahari sands. These soils in many instances have calcrete and surface limestone layers or are loamy clay soils in low-lying areas. The higher lying areas are often shallow, gravel sandy soils (Natural Scientific Services 2011). Based on Kotze et al. (2018), the soil forms upslope of the local system's catchment can provide an indication of how water is delivered to a wetland system. Catchments characterised by soils containing E horizons e.g., Longlands, the source of water to a wetland would most likely be via lateral inputs, i.e., interflow via the E horizon (Kotze et al. 2018). However, catchments dominated by Hutton forms, which are well drained soils, are generally characterised by the vertical movement of water and as such it is unlikely that lateral flows, i.e., interflow inputs, are a main driving factor of the wetland systems (Kotze et al. 2018). Thus, it can be broadly assumed that system's catchments that are dominated by Longlands would most likely be more sensitive to impacts within the shallow soil horizons, whilst those dominated by Hutton soils would most likely be sensitive to impacts in the deeper soil horizons (Kotze et al. 2018).

It is evident from the review of available soil information that the broader landscape lacks soils characterised by the lateral movement of water (i.e., E horizons), suggesting that the accumulation and retention of water within these depressions is strongly linked to rainfall, surface runoff and the presence of an impermeable layer of clay or bedrock within the depressions, which allows for the retention of water within the landscape. The formation of these pans very closely mimics the aforementioned 'erosional control' process (refer to the above section '*Formation of non-perennial pans*'). As such, these systems are not considered to be groundwater-driven systems, but rather surface water driven, i.e., fed through rainfall events and as such are entirely rainfall dependent. Without the impermeable layer, the infiltration rate of surface water to groundwater would be too rapid for the formation of hydromorphic soils, especially in semi-arid areas. The loss of water in these systems is predominantly associated with evaporative losses. **Figures 2-2** and **2-3** visually depicts the non-perennial pans in their predominant desiccated state, i.e., in the dry season, in comparison to the system during the wet season. It should be noted that the retention of water within these systems is largely dependent on the amount of rainfall received over the rainy season versus the heat units over this period, i.e., the less rain and higher heat units the shorter these systems remain inundated with water.

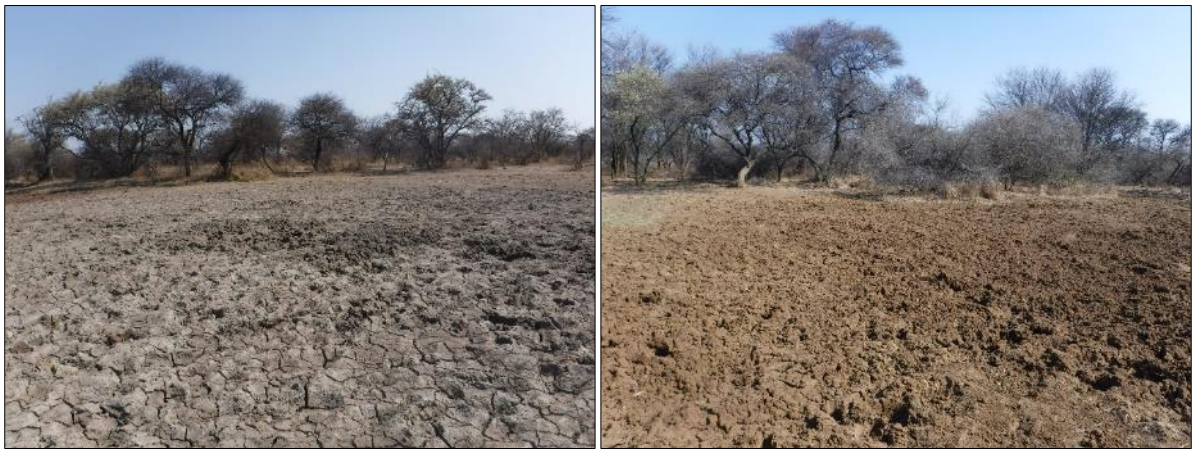

Figure 2-2 Examples of the non-perennial pans during the dry season

Figure 2-3 Examples of the some of the non-perennial pans during the wet season and the variable amount of water within the systems

2.1.2.3 *Visual water quality observations*

In many instances, the water quality and/or turbidity within the larger systems is considered to remain better for longer periods than that of the smaller systems. As the systems start drying up, algal blooms start becoming more prevalent (**Figure 2-4**). These algal blooms can be attributed to the fact that the wildlife utilise these systems, and as such the urine concentrations within these rapidly evaporating systems increases and hence the algal blooms. Therefore, the water quality of these systems is considered to be highly variably over the wet season and tends to deteriorate as the pans dry up.

Figure 2-4 An example of algal blooms within two of the smaller non-perennial pans

2.1.2.4 *Vegetation in and around the wetlands*

Most of the non-perennial pans within the LOM footprint area are surrounded by tall trees with little to no wetland vegetation within the actual systems themselves and are generally characterised by bare, mud-dominated areas. Some of the larger systems that are inundated for longer periods of time, do have seasonal wetland vegetation within the wettest portions of the systems (**Figure 2-5**). The soil profiles within the portions of the systems are generally characterised by seasonal wetness conditions.

Figure 2-5 An example of two of the larger non-perennial pans containing seasonal wetland vegetation within the portions of the systems with sustained wet conditions.

In some instances, the pans are surrounded by dense tree canopies with some of the trees being located within the boundaries of these systems (**Figure 2-6**). The encroachment of trees into the pans has not

compromised the health of the trees, as would be anticipated, as the seasonality and the temporary nature of the water within the systems is too short a period.

Figure 2-6 An example of trees within an inundated non-perennial pan (left) and the actual watermark on the trees following a rainfall event (right)

2.1.2.5 *Aquatic invertebrates*

Southern Africa (bounded by the Cunene in the west and the Zambezi in the east) is predominantly a semi-arid region, with many water bodies (wetlands, vleis, pans and also rivers) being temporary in nature (i.e. they dry out completely in the dry season and become inundated either once or repeatedly for short periods over the wet season) (Day et al. 1999, Brendonck et al. 2008, Rogers 2009; Tuytens et al 2015). Rain fed clay pans, rock pools as well natural depressional wetlands, roadside ditches and farm dams, as opposed to groundwater fed wetlands and vleis, provide ideal temporary aquatic habitats for large branchiopod crustaceans (e.g. fairy shrimp (*Anostraca*), clam shrimp (*Conchostraca*), tadpole shrimp (*Notostraca*)), macroinvertebrates (e.g. dispersing odonata, hemiptera, coleoptera and diptera) and other microinvertebrate fauna such as water fleas (*Cladocera*) and zooplankton (*Copepoda*) (Brendonck et al 2008, Rogers et al 2009, Tuytens et al 2015, Mabidi et al 2016). The large branchiopods in particular are spectacular examples of temporary wetland specialists being largely absent from permanent aquatic habitats owing to their sensitivity to fish predation (Kerfoot and Lynch 1987 as cited in Tuytens et al 2015). As such, large branchiopods can be used for the identification (Day et al 2010) and assessment of the quality and function of temporary wetlands (De Roeck et al. 2007).

While they are found in temporary aquatic habitats worldwide, most families, excepting some of the *anostraca*, are not restricted to specific latitudinal or zoogeographical zones but are however, most abundant in Mediterranean, arid and semi-arid regions with a distinct wet and dry season (Brendonck and Williams 2000, Brendonck et al 2008). Large branchiopods have unique life histories well adapted for life in disturbance driven habitats such as temporary wetlands, which experience intermittent or seasonal drying and inundation with extreme variation of physicochemical conditions. Some of the remarkable life history traits observed in large branchiopods include: rapid maturation, growth and reproduction, drought-resistant dormant egg banks (which remain viable for years in the sediments following drying), bet-hedging strategy whereby only a fraction of eggs in the egg bank hatch with the first inundation,

dispersal by wind, water and large herbivores (Mabidi et al 2016, Brendonck et al 2017) (See also **Appendix 1** for further notes).

Southern Africa is home to sixty-six (66) documented species of large branchiopod (Day et al 1999, Rogers 2013). However, given that these faunas are the still among the least well known in temporary wetland habitats this number is considered a conservative estimate which will likely increase, concurrently with distribution records/ranges, as more studies are conducted in the relatively unexplored Southern African subcontinent.

Regrettably, owing to the reduction in number and quality of temporary wetlands in South Africa and globally as a result of anthropogenic impacts, these obligatory residents are expected to be among the most threatened organisms (De Roecke et al 2007, Mabidi et al 2016). Water quality impacts in the form of diffuse or point source pollution from spills, poorly treated wastewater, chemicals as well as habitat impacts in the form of sedimentation, excavation, and draining and or total destruction form the major risks to these organisms (Mabidi et al 2016). It is for this reason that several large branchiopod species have become endangered and subsequently added to the Red Data list by the International Union for Conservation of Nature (IUCN).

For the reasons documented above, it is imperative that temporary wetlands are correctly identified (in both the wet and dry seasons) and their associated flora and fauna adequately assessed to prevent complete destruction of habitat and loss of species. To this end, a study funded by the Water Research Commission (WRC) was undertaken in 2010, which focused on the assessment of temporary wetlands during dry conditions (Day et al 2010). This document and methods described therein formed the basis of the study presented here of the temporary wetlands[9] in the study area.

2.2 Biomonitoring and non-perennial pans

Biomonitoring was prescribed as the required monitoring method in the conditions of the water use licence (refer to **Section 1.1**). Although biomonitoring is often considered synonymous with the SASS5 rapid sampling method (Dickens and Graham 2002), in this instance due to the lentic and temporary nature of all the pans on site, this method is an unsuitable technique for the monitoring of such systems. Dickens and Graham (2002) indicate that the SASS5 method is designed for the use in wadable, permanent, lotic systems, which the non-perennial pans are not (refer to **Section 2.1.2.2** which provides a description of the hydrology of these systems). The study further reiterates, that the method is not suitable for wetlands or impoundments and that it is not recommended for ephemeral systems, as this requires more testing. Further to this, a 2006 study by Bowd et al, tested the SASS5 technique in wetlands, and found the SASS5 method produced low scores owing to the lack of suitable habitats and species level adaptations to wetland conditions. While Bowd et al. (2006), found that macroinvertebrates were still useful indicators in wetlands, the SASS5 scoring system and full sampling methods were found to be inappropriate for wetland use.

Based on the above-mentioned limitations of SASS5, aquatic macro-invertebrates should be sampled using a standardized technique adapted from Bird (2010) and Day et al. (2010), which is considered to be a modified SASS5 sampling technique and is recommended for the use in the identified non-perennial

[9] Temporary wetlands refers to wetlands where water is not permanently present (Day et al. 2010)

pans (refer to **Section 3.2.1**). This technique has previously been used in these non-perennial pans (GroundTruth 2018 and 2019). The results obtained using this approach, will be compared between the baseline samples, and selected surrounding systems to interpret the condition of the waterbodies over time and prior to these systems being lost in their entirety due to the proposed mining activities.

2.3 New technologies to be investigated

Science is ever evolving, especially with the development of new technologies. With the advancement of technology also comes additional data and as such knowledge on how systems function. eDNA is one such technology, which provides a suite of additional data compared to manually collected information. Although eDNA has not been included in the proposed protocol, a brief overview of this technology has been provided in **Appendix 2**, as it is recommended that this technology be investigated for potential adoption during the duration of the project and monitoring of the pans.

3. LOM FOOTPRINT AREA: APPROACH AND METHODS

This section of the report provides an overview of the approach and methods to be adopted for the baseline and ongoing monitoring of the non-perennial pans, which will be retained within post-mining landscape. The non-perennial pans that will be lost (based on the mining layout of 2020), were assessed as part of the GroundTruth study in 2018, and the loss in functioning and integrity has been derived from this baseline assessment and included the associated impact mitigation strategy, negating further assessment of these systems. However, the monitoring of the systems that will be retained in the landscape is considered to be crucial as these need to be maintained at a REC category of B. Based on **Section 2**, the key considerations for the development of the monitoring protocol for the non-perennial pans include:

- The pans within the LOM area are temporary in nature (refer to **Section 2.1.2**) and as such, the use of the SASS5 assessment technique, as a biomonitoring tool, is considered to be an unsuitable sampling technique within these systems (Dickens and Graham 2002, Bowd et al. 2016).
- Due to the large number of pans that will be retained within the landscape, it is recommended that certain aspects of the monitoring protocol are undertaken on a rotational basis, unless impacts on the system require a full assessment of the pans.
- Although there are many wetlands across the site, these are generally considered to be rather isolated from each other with little connectivity via dendritic drainage networks. As such, should any of the pans be affected by an incident, it is unlikely that this impact would extend beyond the confines of the pan. Therefore, the proposed monitoring is for each of the identified non-perennial pans.

3.1 Wetland selection

As described in GroundTruth (2018) the sixty (60) identified non-perennial pans vary in size from 0.0004ha to 2.36ha, with the majority of the systems being less than 0.1ha in extent. As such, the GroundTruth (2018) report grouped the pans according to size classes. The four (4) size categories adopted were the following:

- Category 1: Pans less than 0.1ha in size (<0.1ha);
- Category 2: Pans greater than 0.1ha but smaller than 0.5ha in size (>0.1ha but <0.5ha);
- Category 3: Pans greater than 0.5ha but smaller than 2ha in size (>0.5 ha but <2ha); and
- Category 4: Pans greater than 2ha in size (>2ha).

Table 3-1 depicts the distribution of the pans across the four size classes. As is evident from the table most of the systems are within Category 1, with fifty (50) systems being less than 0.1ha in size. Thereafter Category 2, which includes all systems that are greater than 0.1ha in extent but smaller than 0.5ha, has eight (8) pans, whilst the last two categories only contain one (1) system each.

Table 3-1 Summary of the number of pans per defined size category

Size Category	Number of pans per category
Category 1: <0.1ha	50
Category 2: >0.1ha but <0.5ha	8
Category 3: >0.5 ha but <2ha	1
Category 4>2ha	1

Based on the LOM footprint area (dated February 2020), of the sixty (60) pans, twenty-seven (27) will be retained within the post-mining landscape[10] (**Figure 3-1**). The systems that will be retained are divided across the size categories as follows:

- Category 1 = 25;
- Category 2 = 1;
- Category 3 = 0; and
- Category 4 = 1.

Baseline monitoring has been prescribed for all the systems, however, due to the substantial number of pans to be monitored, it is recommended that a combination of regular and rotational monitoring of the systems is undertaken thereafter. The baseline monitoring will comprise of, and is described in more detail under Section 3.2:

- Dry season sampling:
    - Landscape review and description;
    - Functional and integrity assessments;
    - Invertebrate sampling;
    - Water chemistry;
- Wet season sampling:
    - Invertebrate sampling;
    - Water chemistry;
    - Ecotoxicology sampling.

[10] It should be noted that the post-mining landscape refers to the landscape over the next 30 years. Thereafter, it is likely that the mining activities may extend into these areas as part of the 30–50-year mining plan.

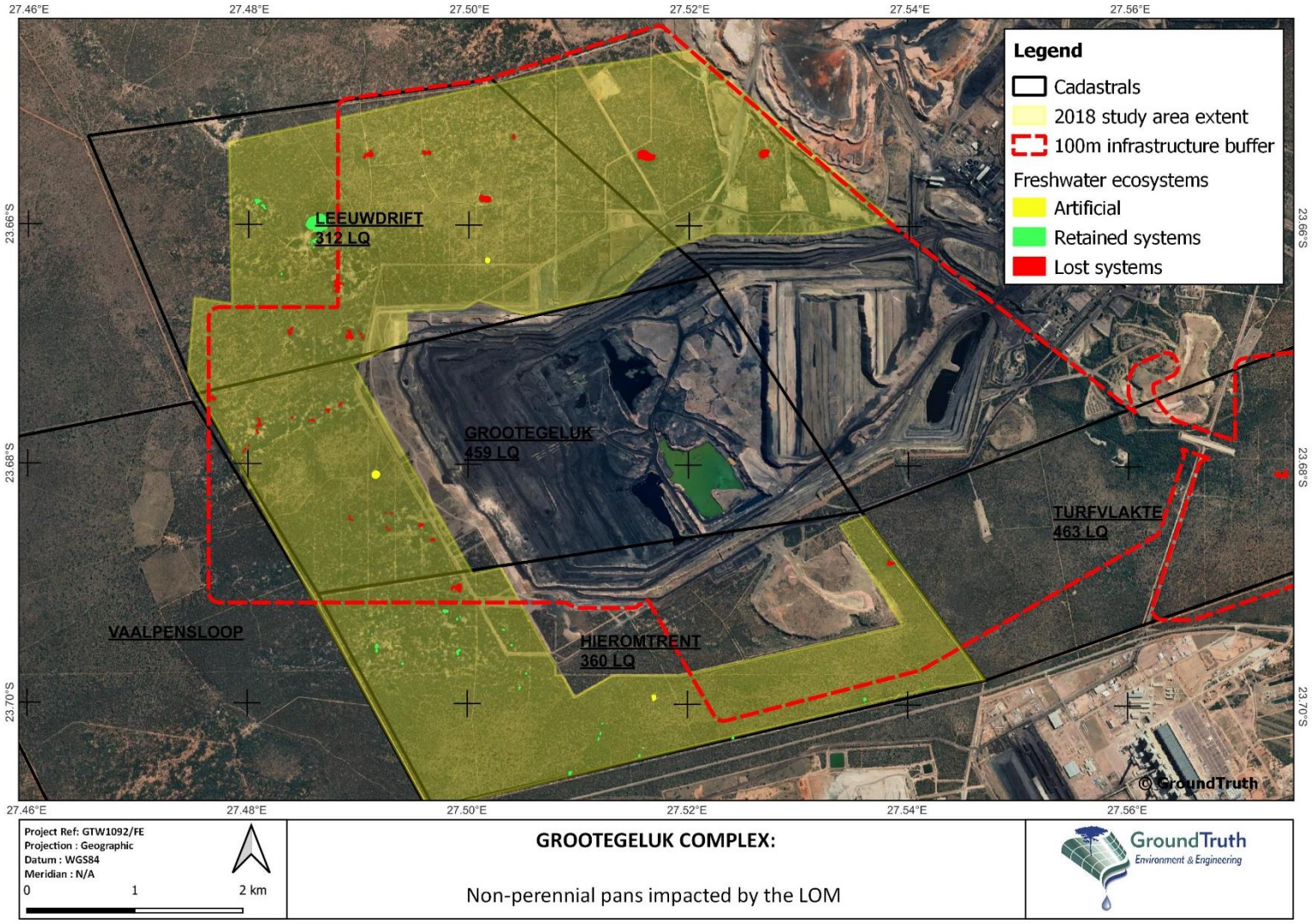

Figure 3-1 Overview of the non-perennial pans that will be lost associated with the expanding mining activities

3.2 Baseline monitoring

The monitoring of the non-perennial pans within the LOM footprint area is anticipated to extend over a minimum period of 30 years and as such a robust, repeatable, and comprehensive monitoring approach should be considered. The proposed approach requires that aquatic macro-invertebrates are sampled using a standardized technique adapted from Bird (2010) and Day et al. (2010) during both the dry and wet seasons for the baseline monitoring. Thereafter, only the wet season sampling method is replicated on an annual basis.

As described above, twenty-seven (27) of the 60 natural non-perennial pans will be monitored, as these are the systems which need to be maintained at a REC category of B. Due to the number of systems that need to be monitored, it is recommended that both routine and rotational monitoring is undertaken, thereby reducing the intensity of the monitoring but allowing for any potential impacts to be detected. Should there be a change in the proposed LOM layout, it is recommended that the monitoring protocol be reviewed. To ensure a comprehensive data set is established at the outset of the monitoring activities, it is recommended that both dry and wet season sampling is undertaken. This is especially important in terms of the invertebrate monitoring, as a single sample during the wet season may result in invertebrate species being overlooked during sampling due to incubation and climatic conditions. By incorporating the dry season sampling it is more likely that a comprehensive invertebrate species list will be created. At the outset of the baseline monitoring activities, it is essential that the seasonality is reviewed, to ensure that the most appropriate approach is adopted as the wet season sampling is highly dependent on the non-perennial pans containing water. The proposed seasonal sampling approaches are described in more detail in the following sections.

3.2.1 *Dry season sampling*

3.2.1.1 *Invertebrate sampling*

At the outset, all identified systems for monitoring (**Figure 3-1**) will be sampled during the dry season. At each location the deepest point of the depression will be identified and visually investigated for signs of the presence of obligate temporary wetland invertebrates. Such signs commonly include the crusts formed from the shells of Conchostraca and Copepoda, which remain behind when they die (**Figure 3-2**). Depressions where crusts are observed will be noted.

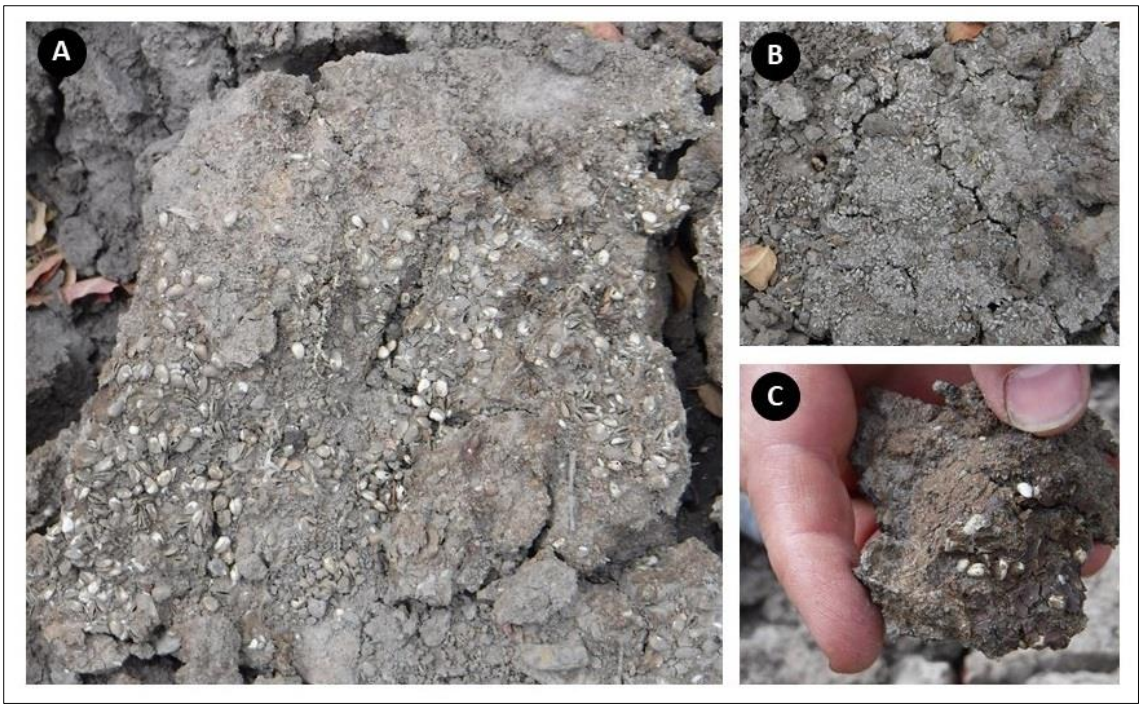

Figure 3-2 Crusts (A) comprising the shells of Copepoda (B) and Conchostraca (C) – both obligate temporary wetland invertebrates - observed on the uppermost sediments of pans at the Grootegeluk complex

Following the visual recording of crusts, photographic records, and GPS extents; sediment samples will be collected from the deepest part of the pans. With some minor exceptions (refer to the GroundTruth 2018 study), the field sampling protocols as described in Day et al. (2010) for the assessment of temporary wetlands during dry conditions will be followed.

At the deepest point in each pan, surface crusts will be hand collected and placed in a labelled sealable plastic bag. Where softer soils prevail, dry soil samples will be collected using a standard soil auger with a closed-bucket head (head diameter of 90mm) from approximately five points randomly spaced within a 20m radius from the estimated deepest part of the pan (**Figure 3-3**). Only the top 5-10cm of soil in each of the five auger samples will be collected, homogenised, and placed together with the surface crusts (if present) into the same sealable plastic bag. Where the use of the auger is prohibited, owing to the hardness of the soil layers, a standard pickaxe will be used to loosen clumps within the top 10-15cm for collection.

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Figure 3-3 An example of the collection of surface crusts and soils from the deepest parts of a temporary pan.

The plastic bags will be sealed and transported to the laboratory where they will be left open for 2 weeks prior to the start of the incubation period. This is done to ensure that sediment is dry at the commencement of the incubation period.

3.2.1.1.1 *Invertebrate incubation*

Soil samples collected in the field at each individual site will be homogenised (but not pooled) and lightly crushed to break up large clumps. Thereafter sub-samples of approximately 50g will be placed in small plastic containers (140 x 200 x 60 mm) where a single replicate will be prepared for each site. Tubs will be filled with distilled water to a height of 45mm which equates to an inundation depth of approximately 25mm. Inundated tubs will be incubated at approximately 20-25°C using a heater fan connected to a Digital STC-1000 thermostat in a controlled environment room equipped with a full spectrum fluorescent light. Lights will be operated on a permanent basis.

A conservative incubation period amounting to a total of 41 days will be applied during which time all tubs will be inspected daily for signs of hatchlings/nauplii and water levels will be topped up if necessary (maintained at 25mm inundation depth).

Frequent photographs will be taken to record changes in water quality and visual characteristics (**Figure 3-4**), while a Yellow Springs Instrument 556 (YSI) handheld probe will be used to record water quality parameters (pH, electrical conductivity, dissolved oxygen and Oxidation Reduction Potential, Total Dissolved Solids). Turbidity should be measured a HANNA Instrument Turbidimeter (HI 98703).

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Figure 3-4 An example of the incubation period setup showing inundated sediment samples in a controlled environment.

3.2.1.1.2 *Identification of invertebrates*

Upon hatching, invertebrates will be counted (as nauplii initially – minute 6-limbed larvae indistinguishable from each other) and where possible identified – though normally identification to Class or Order (Cladocera, Copepoda, Ostracoda, Anostraca, Conchostraca) will only be possible after 5-10 days when taxonomic characteristics begin to appear.

Naked eye identifications will be carried out daily and the number of organisms representing each taxonomic group recorded. Voucher specimens will be collected from each tub upon termination of the experiment and sent to Albany Museum for further identification.

3.2.1.1.3 *Water chemistry*

Water quality parameters of inundated soil samples will be measured with a YSI instrument in the laboratory, once at the beginning of the experiment (one day after inundation) and again at the end of the experiment just prior to termination. These measures will be taken to detect any parameters of potential concern that may be attributed to soils. Mean values for water quality parameters recorded with the YSI, will be calculated from three replicate measurements taken in each tub following inundation.

In addition to water quality, two Hobo TidbiT v2 temperature loggers or similar, will be used to record the following:

- Ambient air temperature in the controlled environment room; and
- Water temperature in the inundated tubs every 30 minutes for the duration of the experiment.

3.2.1.2 *Landscape assessment*

As part of the initial baseline monitoring and on-going monitoring, it is recommended that a visual review of the landscape conditions surrounding the various pans is undertaken. Specific consideration should be given to the area within a 50m-100m radius of the pans. This would include documenting any key features within the landscape based on the current conditions, e.g., road network, surrounding habitat, etc., thereby allowing for any significant changes into the future to be recorded. Significant changes would include any changes in the specified radius that may potentially influence its functionality and integrity e.g. encroachment of mining activities and/or its associated infrastructure such as roads. Further guidance on potential impacts on the systems can be gleaned from the WET-Health Version 2 (Macfarlane et al. 2020) and WET-EcoServices (Kotze et al. 2020) assessment techniques. Should such significant changes be identified, it is recommended that an assessment of the system is undertaken even if it does not fall within in its rotational assessment period.

3.2.2 *Wet season sampling*

The wet season site visit will be undertaken following the initial substantial summer rains, which is most likely to be between the mid and end of summer (approximately January - March). It is recommended that the following sampling is adopted as part of the routine monitoring activities of the systems, especially since any unexpected changes in the invertebrate communities and/or water quality results, may highlight unwarranted changes in the pans integrity.

3.2.2.1 *Wet Season Invertebrate sampling*

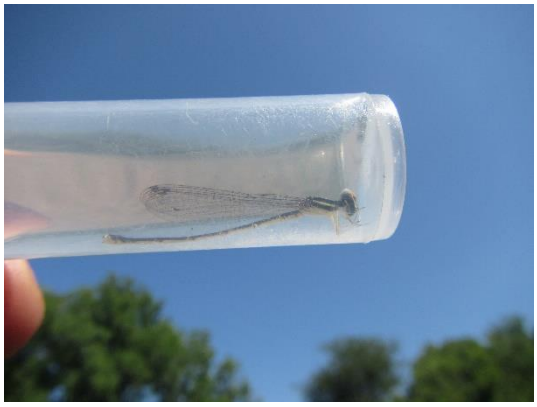
Aquatic macro-invertebrates should be sampled using a standardized technique adapted from Bird (2010) and Day et al. (2010). Each pan must be visually stratified into three distinct biotopes, and each biotope sampled using a D-frame net[11] (**Figure 3-5**). The net is a 500µm mesh and leads to a polycarbonate collecting cylinder that is 50mm in width and 100mm in height. The sampling protocol must be divided into two increments: zooplankton and benthos sampling. Sampling of zooplankton must be undertaken in five 1m replicates, wherein the net is swept through the water column during each replicate. Sampling of the benthos involves kicking for one minute to disturb and suspend the matter and associated fauna, and thereafter sweeping the net through the water column. This kick sampling technique is congruent with the SASS5 technique described in Dickens and Graham (2002) for gravel, sand, and mud (GSM) habitat. To evaluate the efficacy of this sampling protocol, the macro-invertebrate taxa recorded for this assessment must be compared to the taxa recorded during the dry season incubation sampling.

Expected Odonata species must be obtained from OdonataMap, an atlas of African Odonata (http://vmus.adu.org.za). The 2327CB and 2327DA Quarter Degree Squares (QDS) based on the 1:50 000 grid should be used to obtain species lists. During the survey, adult Odonata should be actively collected using a sweep net and identified to species level. The Dragonfly Biotic Index (DBI) should be used to infer the water quality within the assessment area (Samways and Simaika 2016). Furthermore, adult Odonata provide an indication of freshwater ecosystem and surrounding vegetation wellbeing, due to habitat requirements for each species.

[11] The net frame is a flat aluminium bar measuring 25mm wide and 5mm thick and possessing a base length of approximately 305mm and a height of approximately 254mm.

Front-view of D-frame net

Lateral-view of D-frame



Identification of Odonata adult to species level (in this case *Lestes pallidus*)

Active collecting of Odonata adults using a sweep net

Figure 3-5 Photographs illustrating sampling techniques to be utilised to collect aquatic macro-invertebrates within the assessment area

3.2.2.2 *Water chemistry*

During the wet season sampling, surface water from the identified systems will be measured *in situ* for baseline monitoring of a suite of water quality parameters including: potential hydrogen (pH), temperature (Temp), electrical conductivity (EC), dissolved oxygen (DO), total suspended solids (TSS), oxidation-reduction potential (ORP) These *in situ* field-based surface water measurements will then be compared to laboratory measurements taken during the incubation periods of the dry season samples.

While once-off field and lab-based water quality data do not provide information on seasonal variation emanating from dilution, evapo-concentration, as well as biological processes (e.g. photosynthesis, respiration), such data are, however, useful for comparing wet season conditions with those in the laboratory experiments (Day et al. 2010).

3.2.2.3 *Ecotoxicology sampling*

Ecotoxicology is the study of the effects of chemicals on biological organisms at various levels including, population, community, ecosystem, and biosphere. Standard aquatic ecotoxicology assessments include the testing of various aquatic trophic levels and generally consist of:

- Algae (*Selanastrum capriconutum*), representing “primary producers” (SANS 8692:2015).

- Bacteria (*Allivibrio fischeri*), representing the microbial community (SANS 11348-3:2013).
- Invertebrate (*Daphnia magna*), representing "primary consumers/secondary producers" (SANS 6341: 2015).
- Vertebrate (*Poecilia reticulata*), presenting "secondary consumers. (SANS 7346-1: 2013).

There are two levels of assessment that can be undertaken:

1. Screening = A screening toxicity test refers to an undiluted (100% concentration) sample. This is usually performed on a sample from the site in the receiving water bodies, in this instance the non-perennial pans, to determine if any toxicity is present (*pers comm* Biotox Lab).
2. Definitive = A definitive toxicity test refers to the exposure of test organisms to both the 100% concentration as well as a range of dilutions, generally used to determine the risk of a pollution source that may have a toxicity effect on the receiving water body. The range of dilutions are therefore useful if the 100% sample concentration presents acute toxicity and allows for the estimation of a safe dilution factor, to negate acute toxicity effects on the receiving water bodies.

The initial baseline monitoring of the identified non-perennial pans should be at a 'screening' level, thereby providing an overview of the potential degree of toxicity within the systems. Should any of the systems be found to be toxic, then additional sampling of the system would be required however, at the 'definitive' level.

Composite surface water samples will be collected from each of the pans assessed and submitted to a SANS accredited laboratory where ecotoxicology (toxic/non-toxic) tests will be carried out to highlight any risks to specific life forms that would exist in the pans – specifically: *Selenastrum* (algal), *Poecillia* (vertebrate), *Daphnia pulex* (invertebrates) and *Vibrio fischeri* (bacteria) toxicity tests. Samples for ecotoxicology assessments should be collected from the surface water (1L to 2L - depending on the required level of sampling), kept on ice or a suitable cooled container until delivery to a suitable laboratory for analysis. It is important that the samples are delivered to the laboratory as soon as possible, however, no later than 72 hours after collection. Please note samples must not be frozen. The ecotoxicology sampling should be repeated on an annual basis at a 'screening' level, and 'definitive' sampling should only be undertaken if any of the systems are found to be toxic.

3.2.2.4 *Statistical analyses*

An array of statistical analyses should be conducted to evaluate the relationships between the aquatic macro-invertebrate community and selected environmental variables within the assessment area. All statistical analyses must be conducted using R statistical software (Version 3.5.1), via the RStudio Version 1.1.463 interface. Initially, to ascertain whether there is spatial heterogeneity between the pans based on water chemistry parameters, a Permutational Analysis of Variance (PERMANOVA) using 719 re-iterations (maximum possible with the data) should be undertaken using the 'vegan' package (Oksanen et al. 2019). Prior to analyses the data must be 'normalized' using the 'decostand' function, due to the potential disparity between the units of measurement of each parameter.

A modelling-based approach should be undertaken to assess the influence of environmental parameters on the spatial shift, if any, on the macro-invertebrate community using the package 'mvabund' (Wang et al., 2017, 2012), to highlight important variables that influence the macro-invertbrates, and therefore will be used to derive further infield monitoring/observations for the ongoing monitoring .

Water chemistry parameters and pan size (total area, water surface area at time of sampling and the ratio of sampling water area to total area) should be fitted individually to species count data using a generalised linear model with a log-link function. It is assumed that the surface area ratio value provides an indication of water retention time (essentially providing a proxy for hydroperiod). Water chemistry parameters and pan size should be modelled separately, and the data normalized due to the potential disparity in units of measurement. Furthermore, TDS should be omitted from the analyses due to its collinearity with EC. A negative-binomial distribution should be incorporated into the model due to overdispersion (Wang et al., 2017, 2012). To determine which of the environmental variables are significant in influencing the invertebrate community, an analysis of deviation with 999 re-sampling iterations via PIT-trap resampling should be used (Wang et al., 2017, 2012). Univariate p-values should be adjusted for multiple testing, using a step-down resampling procedure (Wang et al., 2017, 2012). In order to visualise the influence of any significant environmental characteristics identified by the aforementioned modelling on the recorded invertebrate species, a constrained ordination plot should be constructed. This can be achieved via a Redundancy Analysis (RDA) using log10(X+1)-transformed count data that was fitted to the identified significant variables.

3.2.2.5 *Wetland functioning and integrity*

All of the systems within the LOM footprint area were assessed in terms of their functioning and integrity by GroundTruth in 2018. A summary of these results should be included in the baseline monitoring report. Due to the nature of the systems – non-perennial pans – the annual assessment of these systems using the WET-EcoServices assessment technique (Kotze et al. 2020) and a Level 2 WET-Health (Macfarlane et al. 2020) assessment, is deemed to be largely academic due to the nature of these assessment techniques. These assessment techniques make use of broad functionality/integrity classes, which are not suited for any subtle changes linked to these systems. As such the monitoring of the invertebrate communities and ecotoxicology is considered to provide more insight into the integrity of these systems over time.

Therefore, it is proposed that for the ongoing monitoring that nine (9) of the twenty-seven (27) systems are assessed on an annual basis i.e., every year nine (9) new systems are assessed. This would result in each system being assessed every three (3) years. However, should any significant changes, e.g., encroachment of the mining activities within the catchment of the pan; be observed during the landscape assessment (refer to Section 3.2.2.2), and an assessment of the pan is not required for that specific year, a detailed assessment of the system/s would have to be undertaken. This is particularly important as Condition 1.7 of the WUL stipulates that the "ecological class of rivers and wetlands (seasonal pans) that will remain within the catchment and the offset (PES of B) must not be lowered. Recommended Ecological Category (REC) for wetland must be set as a category B".

It is recommended that whilst undertaking the assessment of the identified systems that a brief review of the landscape conditions is also undertaken (refer to Section 3.2.1.2) and suitably documented, to ensure that any significant changes in the broader landscape are noted.

3.3 Ongoing monitoring

As described above, the baseline monitoring will be undertaken over both the dry and wet season, to ensure that all the systems are suitably monitored from the outset. However, due to the nature of the systems being temporary/transient in nature, quarterly monitoring of the systems would not provide the authorities with the requested data, as for example, water quality samples can only be

collected in the wet season. Therefore, it is recommended that annual monitoring of the identified non-perennial pans is undertaken during the wet season and following directly on from a substantial rainfall event thereby ensuring that all the systems should have some degree of water within them. It is recommended that sampling/monitoring should take place between December and March; however, due to the variable nature of rainfall events, it is impossible to provide a month in which the sampling/monitoring should take place, but this is rather reliant on the relevant persons at Exxaro informing the appointed accredited service provider of the status of the rainfall events during the wet season. In addition, the annual wet season sampling should be supported by the rotational wetland condition and functioning assessment approach. The rotational assessment approach includes the detailed assessment (functioning and integrity) of nine (9) different wetlands every year. In addition, a brief landscape review of all of the pans should be included to ensure any significant changes have not occurred.

The annual monitoring would follow the same procedure as described in the sections above, however, the results from each of the years will be analysed and compared to the previous cycle/s data to ascertain whether the systems have been impacted upon by the encroaching mining activities. **Table 3-3** provides a summary of the baseline versus ongoing monitoring requirements to be undertaken for the identified non-perennial pans.

Table 3-2 A summary of the monitoring requirements for both the baseline and ongoing monitoring activities for Approach 1

Monitoring event	Monitoring requirements	Dry Season (Jun-Aug)	Wet Season (Dec-Mar)
Baseline	Soil samples	X	
	Invertebrate incubation	X	
	Invertebrate identification	X	
	Water chemistry (laboratory inundated soils)	X	
	In-system sampling (D-frame net)		X
	Invertebrate identification		X
	Water chemistry (in-situ)		X
	Surface water/ecotoxicology		X
	Statistical analysis		X
	Landscape assessment	X	
	Functional and integrity assessments		X*
On-going	Soil samples		
	Invertebrate incubation		
	Invertebrate identification		
	Water chemistry (laboratory inundated soils)		
	In-system sampling (D-frame net)		X
	Invertebrate identification		X
	Water chemistry (in-situ)		X
	Surface water/ecotoxicology		X
	Statistical analysis		X
	Landscape assessment	X	
	Functional and integrity assessments		X (9 pans/annum)

*To be derived from the GroundTruth (2018) wetland study report. However, should significant changes have occurred the pans would have to be reassessed.

4. OFFSET RECEIVING AREA: MONITORING

The monitoring of the offset receiving areas (**Figure 4-1**) is considered to be crucial in terms of ensuring that the mitigation targets have been attained and are maintained into the future. Furthermore, Condition 1.7 of the WUL stipulates that the "ecological class of rivers and wetlands (seasonal pans) that will remain within the catchment and the offset (PES of B) must not be lowered. Recommended Ecological Class (REC) for wetland must be set as a class B".

The following monitoring requirements described below have been developed in accordance with the principles outlined in WET-RehabEvaluate V2 (Walters et al. 2019), with specific monitoring being recommended for the anticipated outputs and outcomes of the project. The monitoring includes the collection of baseline and routine monitoring information to enable the evaluation of the rehabilitation effectiveness at least five years after completion of the rehabilitation activities, and to ensure that the systems are being maintained at the desired level. It is anticipated that the impacts associated with the expanding mining activities are limited to the systems described in Section 3 of the report, and therefore, water quality and ecotoxicology sampling is not required for the offset receiving areas (refer to Condition 1.1 and Condition 5.1 of the WUL).

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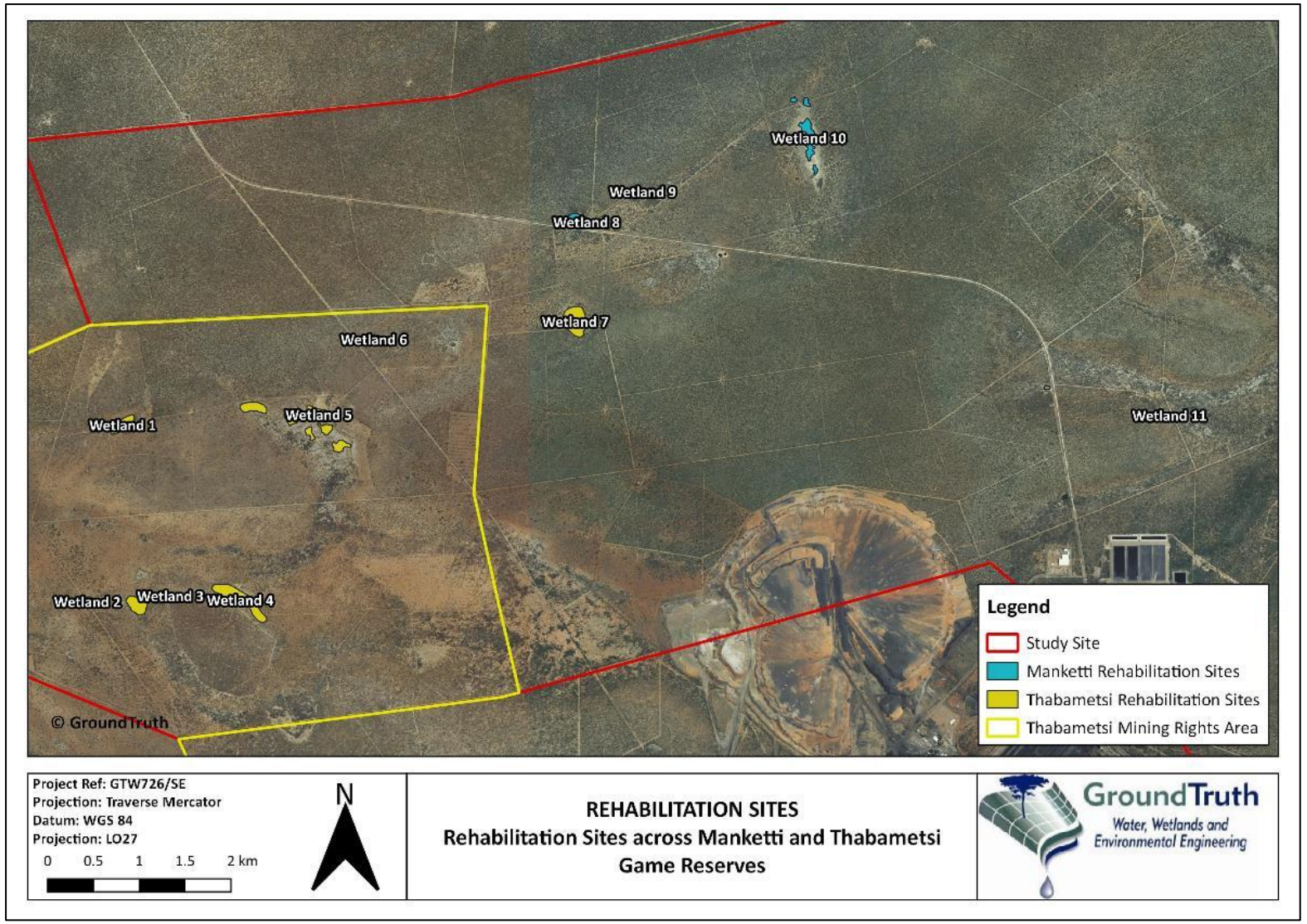

Figure 4-1 Overview of the non-perennial pans identified as suitable offset receiving sites across the Manketti Game Reserve and the Thabametsi site (GroundTruth 2020, p46).

4.1 Monitoring of interventions

The assessment of the structural integrity would be undertaken based on the specific criteria outlined in **Table 4-1** and focus on the long-term stability of the interventions and the likelihood of achieving the stated objectives. This assessment would serve to identify weaknesses or strengths of the selected interventions within the wetlands.

Table 4-1 Criteria used for monitoring earthen structural integrity of wetland rehabilitation interventions (Modified from Walters et al. 2019).

Earthen structures/works:	Revegetation
• Dimensions according to specifications • Authorised deviations from plan • Excessive settling of the soil (>10% of overall height) • Erosion on the bank • Establishment of vegetative cover • Scouring downstream • Evidence of outflanking • Adequate compaction of soil • Backfill material has sagged below the level of the adjacent ground • Backfill is raised above the adjacent ground level • Materials used during implementation not removed e.g., sandbags	• Poor vegetation cover (i.e., extensive bare ground based according to regional vegetation type) • High density of invasive alien plant cover in the revegetated area • Poor representation of the native species **Alien Plant Control** • Evidence of alien vegetation sprouting/coppicing • Felled trees resulting in other in-system damage e.g., causing an obstruction within a stream

The rehabilitation interventions are considered to be 'soft interventions' as none of them involve major earthworks or construction of any concrete or stone structures. Brush-packing and potholing are temporary veld management measures designed to assist in veld regeneration processes. Therefore, the majority of the monitoring required will need to be observation-based. Specific veld assessments can be carried out on a seasonal basis to assess the change in veld conditions in the post-rehabilitation landscape.

4.2 Fixed point photography/site photographs

Pre- and post-implementation photographs must be recorded for each rehabilitation site. These should be collected in the form of Fixed-Point Photographs, as outlined in WET-RehabEvaluate V2, to allow repeated monitoring to be undertaken.

4.3 Wetland assessments

The ecological integrity and functioning of the wetlands should be monitored with:

- WET-Health (Macfarlane et al. 2020) and WET-EcoServices (Kotze et al. 2020), collected during the planning process being used as the baseline; and
- Subsequent monitoring being undertaken two (2) and five (5) years after completion of the rehabilitation strategy, to provide the final assessment of the benefits and effectiveness of the rehabilitation activities.

In addition, it should be noted that one of the systems in the Thabametsi mining rights area falls relatively close to infrastructure planned for the Thabametsi Project, which is proposed to be implemented in 30+ years. The system is Rehabilitation Site 4, which falls approximately 320m away from the planned South West Pit (IPP 145 Mt Pit as per the TIPP 1 layout provided in drawing **ECG-T01-IGN-SP-SP-00002 –** refer to **Figure 4-2**). The potential impacts on the system are likely to be negligible, as the system, for the most part, is hydrologically isolated from the IPP 145 Mt Pit and would not be negatively affected upon by hydrologically derived impacts. However, it would be important to monitor both Rehabilitation Site 4 and Rehabilitation Site 3 (which lies approximately 530m away from the pit) as there may be negative impacts derived from aeolian transport of sediment and coal dust associated with the IPP 145 Mt Pit. These systems should be assessed in terms of their integrity and functionality before the implementation of the Thabametsi Project and for several years thereafter, depending on the progress of the remainder of the Thabametsi Project. The approach described in Section 3 should then be adopted for these two systems.

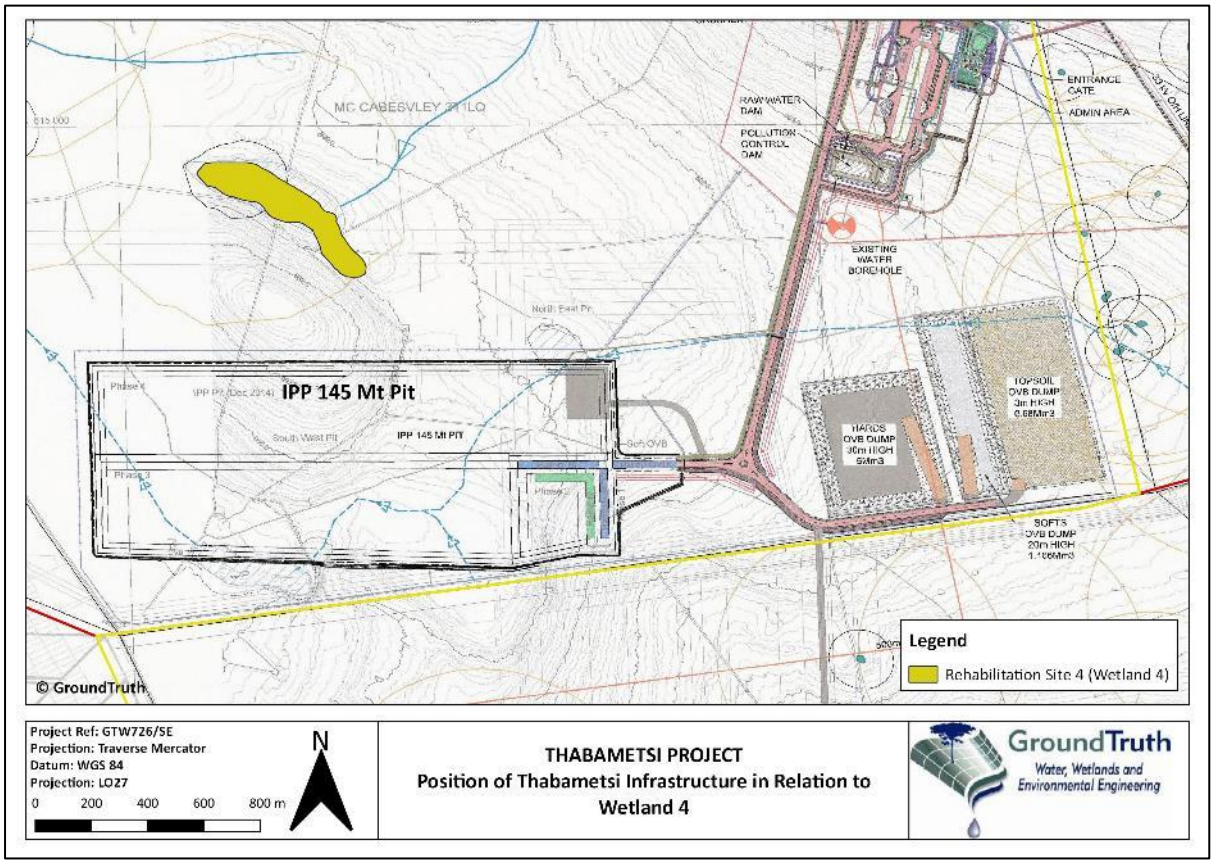

Figure 4-2 Location of Rehabilitation Site 4 (Wetland 4) in relation to the Thabametsi Project infrastructure – specifically the IPP 145 Mt Pit.

4.4 Wetland rehabilitation effectiveness

All of the above-mentioned monitoring should be used to inform the evaluation of the effectiveness of the rehabilitation and enhancement. This would be undertaken once the required monitoring information has been collected, five years following the completion of the wetland rehabilitation activities.

5. CONCLUSION

As part of the Water Use Licence requirements stipulated by DWS, a monitoring protocol for the identified non-perennial pans to be retained in the landscape was to be compiled. Due to the nature of the pans within the landscape, the generally prescribed methods, such as SASS5; are deemed unsuitable. As such, this monitoring protocol has presented an approach that should be adopted for the baseline monitoring of the non-perennial pans and for the annual ongoing monitoring, providing the data required by DWS.

During the wetland study undertaken by GroundTruth (2018) sixty-three (63) systems were identified of which three (3) were classified as artificial systems, whilst sixty (60) were natural systems. Of the sixty systems, it is anticipated that thirty-three (33) systems will be lost due to the expanding mining activities and its associated infrastructure, leaving twenty-seven (27) systems requiring ongoing monitoring to ensure the integrity of the systems is not reduced below the B category REC, as stipulated in the conditions of the WUL. Due to the sheer number of systems, the proposed ongoing monitoring activities have been separated into routine monitoring, which includes the biomonitoring, water chemistry and ecotoxicology sampling; whilst rotational functioning and integrity assessments should be undertaken. Nine (9) pans will be assessed on an annual basis, and thus within a three-year period all of the pans would have been assessed once.

The proposed monitoring activities for the non-perennial pans within the offset receiving area has also been included in this report. Based on the proximity of the pans to the mine, water quality and ecotoxicology sampling is not required, but rather observation-based monitoring and the assessment of rehabilitation interventions, functionality and integrity, to ensure that the mitigation targets are attained. Two (2) systems located within the Thabametsi Project area, should be monitored in more detail (refer to Section 3) as soon as the Project is initiated, to ensure the proposed activities are not negatively impacting the systems.

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7. APPENDICES

7.1 Appendix 1: Life history/ecology notes

(Excerpt from Day et al. 2010, Appendix 3, p. 109)

APPENDIX 3

BACKGROUND INFORMATION ON THE LIFE HISTORIES AND OTHER CHARACTERISTICS OF MAJOR GROUPS OF INVERTEBRATES THAT HATCHED OUT DURING LABORATORY TRIALS

A3.1 Ostracods (Crustacea, Ostracoda: seed shrimps)

Ostracods form an important part of the zoobenthos in African inland waters. Many species can survive harsh environmental conditions. Most species are relatively unselective scavengers. Ostracods are small- (<1 mm) to medium-sized (8 mm) bivalved crustaceans. Their bodies are completely enclosed by the carapace, which consists of two lateral valves. There are eight larval instars, the ninth instar being the adult. The animal no longer moults after it reaches the adult stage and matures: in other words, growth is determinate. All Cypridoidea, the superfamily to which the ostracods in this study belong, can produce drought-resistant eggs able to survive in desiccated form, sometimes for many decades (see Martens, 2001 for further details).

Megalocypris princeps (Sars 1898) is a remarkable ostracod. The subfamily to which it belongs is almost entirely endemic to Africa and the individuals of most species of the subfamily are very large. *Megalocypris princeps* is the largest non-marine ostracod in the world, reaching a length of 8 mm. It is typical of temporary vleis in the Western Cape province of South Africa.

A3.2 Phyllopods (Crustacea: Class Branchiopoda: clam and fairy shrimps)

A3.2.1 Conchostracans (clam shrimps)

The Conchostraca, commonly known as clam shrimps, are small (<20 mm), primitive freshwater crustaceans. They occur on all the continents except for Antarctica. In general conchostracans are found in temporary rainwater or snow-melt pools that regularly dry up completely or partially. Most conchostracans are laterally compressed, with a bivalved carapace that completely encloses the body and limbs. They reach between 3 mm and 18 mm in length, depending on species. The antennae are large and are used for swimming and burrowing. The trunk is generally composed of many segments, each with a pair of flattened phyllopodous limbs used for locomotion, feeding

and respiration. Conchostracans are mainly benthic animals. Most burrow into the surface layers of the substratum, where they lie with their ventral surfaces pointed upwards, and feed non-selectively on detritus and algae in suspension. They produce feeding currents by beating their thoracic limbs. Some species, including some of the leptestheriids (the type found in our samples), bumble about on the bottom, often permanently *in copulo,* the male holding the female in front of and at right angles to himself.

Conchostracans breed continuously throughout the adult stage. Resting eggs within resistant cysts are usually dropped when the female moults. These cysts can survive extremely unfavourable conditions. The time of hatching is often capricious and triggered by specific environmental conditions. The hatchlings are free-swimming metanauplius larvae with three pairs of appendages and a median eye. There are about five naupliar stages. From the moment of hatching it is a race against time for the organism to reach the adult stage and produce the maximum number of cysts before the pools dry up through evaporation (see Brendonck, 2000 for more details).

The conchostracan in our samples was *Leptestheria rubidgei* (Baird 1862), which is widespread in South Africa.

A3.2.2 Anostracans (fairy and brine shrimps)

Anostracans are small (in southern Africa, <30 mm in length), primitive crustaceans, most of which live in fresh water, although the common brine shrimp, *Artemia*, lives in extremely saline inland salt pans. Fairy shrimps occur on all continents bar Antarctica and are found only in temporary pools that regularly dry up. Anostracans are mid-water animals, normally swimming on their backs and filtering particulate matter, often algae, from the water or scooping organic material from the surface. Their flattened limbs provide propulsion for swimming, currents for feeding, and a large surface area for respiration. Reproduction is usually sexual, the eggs developing to metanauplii (second-stage larvae) within the egg sac of the female. The metanauplii are retained within the 'egg' shell, more properly called a cyst, which is covered by a hard, resistant coat before being laid. The larvae within their cysts are able to survive desiccation for many months or years. The cysts of all southern African species appear to require desiccation before they will hatch (see Brendonck, 2000 for more details).

The fairy shrimps in our samples belonged to the genus *Streptocephalus*. Only adult male anostracans can be identified to species level, and none of those from the incubation experiments grew to adulthood, so we cannot be sure of the species. Both *S. dendyi* and *S. purcelli* are known from the wetlands we sampled.

A3.3 Cladocerans (Crustacea: Class Branchiopoda: Order Cladocera)

The Cladocera are commonly known as 'water fleas'. Most species are found in fresh water at more or less neutral pH values. They vary in length from <1 mm to nearly 5 mm. The head is not covered by a carapace. The antennae are large and biramous and are the primary organs of locomotion. The body is covered by a folded, unhinged carapace consisting of two valves. The five or six pairs of leaf-like thoracic limbs are greatly modified for food gathering. Most species are filter-feeders, feeding being aided by the beating of the thoracic legs. Other species are benthic scavengers. Dorsally the carapace forms a brood chamber into which eggs are laid. Reproduction is often parthenogenetic. Unfertilized eggs are laid in the brood chamber and after a few days the juveniles, which look like small adults, are released into the surrounding water. After a further two or three days, the young females produce their own eggs. Sexual reproduction generally occurs during times of stress. Males are produced and mating results in the production of fertilized ephippial eggs, which are surrounded by a hard, resistant shell and constitute the life stage that is able to resist desiccation (see Seaman *et al.*, 2000 for details).

7.2 Appendix 2: Overview of eDNA

7.2.1 *eDNA – An Introduction*

Each species carries a unique genetic signature in all cells. When an organism's cells are shed into the environment (e.g., through excretion, shedding, mucous secretions, saliva, eggs, or tissue breakdown) its genetic signature is also released. eDNA is short for 'environmental DNA'. eDNA contains the unique animal cells left in the environment (soils, water, marine sediments, snow) through excretion and secretion. eDNA is easily collected in water or sediment samples taken at a site, filtered, and analysed using metabarcoding, which rapidly detects and then identifies species present at the site (see **Figure 7-1**). In this process, signatures from different organisms can be isolated from water or sediment samples - or from complex mixed-species samples of invertebrates (e.g., kick sample, or sweep net methods) and used to determine which species are present in an environment. eDNA thus has the potential to revolutionise the way in which biodiversity is monitored/assessed (Harper et al. 2018, Ruppert et al. 2019). eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from the organism, particularly in running water. In contrast eDNA in soil can bind to organic particles and persist for a very long time (sometimes hundreds or thousands of years). eDNA is sampled in low concentrations and can be degraded (i.e., broken into short fragments), which limits the analysis options. These DNA-based monitoring approaches make it possible to directly monitor large numbers of species at large spatial and temporal scales.

1) ***Visit habitat where animals have been present and shedding their cells.***
2) ***Collect and filter the water using NatureMetrics sampling kit and filter.***
3) ***Inject supplied buffer solution & preservative into the provided filter and seal. Send filter to the NatureMetrics laboratory for analysis.***
4) ***NatureMetrics extract the DNA and individual sequences from the filter.***
5) ***NatureMetrics use well developed and optimised bioinformatics pipelines to match sequence data obtained from filters to individual named species.***
6) ***NatureMetrics provide a summary report of taxa detected which can be used to monitor trends over time and generate species diversity indices.***

Figure 7-1 NatureMetrics' six step process in detecting and identifying species present at a site.

7.2.1.1 *Why use DNA for monitoring*

With nature in a state of decline, responsive environmental management has never been more important; but this requires a constant stream of information about the living world. It is increasingly clear that the conventional set of tools and techniques for deriving species information are too low throughput, expensive, and slow to meet the data requirements for effective environmental management.

Ecologists are limited by a lack of biological data, usually as a result of a sampling bottleneck, a taxonomic bottleneck, or both. Combined these issues mean that (a) the true distribution and status of most species is unknown, and (b) total community level biodiversity is rarely assessed.

Specific issues limiting environmental management include low detection rates, low throughput, inconsistency and inaccuracy among taxonomists (**Figure 2-8**):

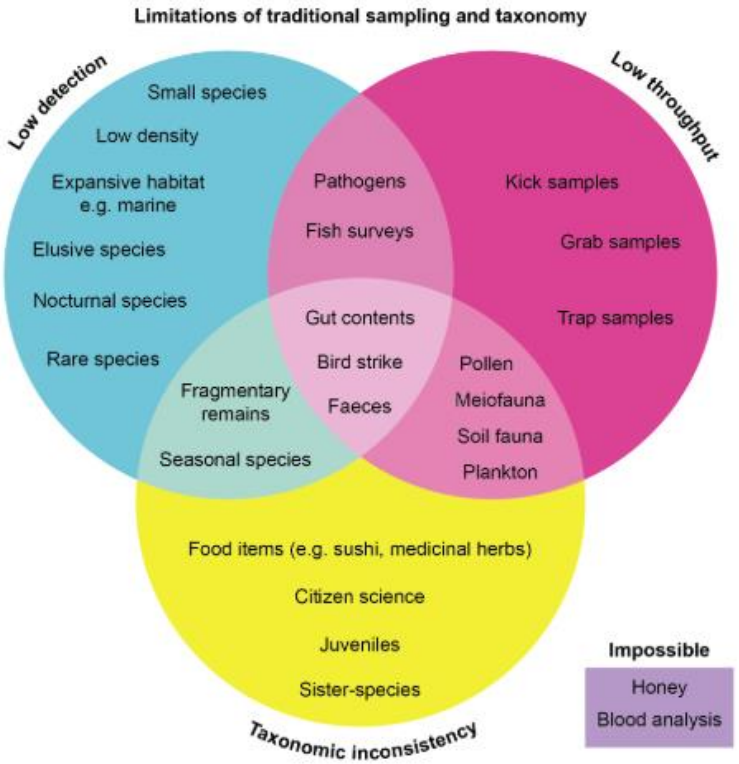

Figure 7-2 Limitations with traditional sampling methods and taxonomy

Low Detection Rates: Finding rare, elusive, or cryptic taxa is difficult by definition, and these taxa may be particularly important for management decisions (e.g., finding invasive species before they establish, or conserving rare populations before they become extinct). Environmental DNA can be used to detect organisms present in the wider area without requiring their presence in the physical sample. Moreover, eDNA detection is independent of life stage, which may yield greater opportunities for detection (e.g., from the presence of eggs and larvae).

eDNA detection has been shown to be more sensitive than traditional survey methods across a wide variety of rare, invasive, and cryptic organisms.

Low Throughput: Traditional taxonomy is not conducive to high throughput, which is vital for long-term and routine monitoring. The scalability of biotic surveys has traditionally been limited in scale or

scope due to the demands of specimen sorting and species identification – particularly in the case of invertebrates.

Passive trapping and active sampling techniques can capture a vast number and diversity of organisms, but their extensive deployment is limited by the taxonomic bottleneck. Using DNA Metabarcoding, analysis of these diverse samples can be scaled up to allow hundreds of samples to be processed in parallel.

Inconsistency and inaccuracy among taxonomists: Traditional taxonomy is difficult to standardise. Taxonomists vary in their experience and skill levels, while dichotomous keys also vary and can be based on subjective characters that may be interpreted differently. Consequently, the same query materials can be identified differently by different taxonomists. These inconsistencies may be absent for highly trained taxonomists identifying large, easily identifiable species, but trained taxonomists are an endangered species in themselves. Inconsistencies and inaccuracies are a more significant problem for cryptic and neglected taxa such as the meiofauna. For example, documenting the diversity of marine life is challenging because many species are cryptic, small, and rare, and belong to poorly-known groups.

DNA sequences are digital and can be easily curated and databased. This information is easily auditable and third-party verifiable.

The reduced reliance on expert taxonomists means that DNA taxonomy can be combined with citizen science programs to generate enormous datasets at large spatial and temporal scales.

7.2.1.2 *Advantages of eDNA for monitoring*

As a result of the limitations mentioned above and others, there are several clear advantages to using eDNA for monitoring biodiversity. Seven main advantages are highlighted below:

- Many times faster
    - eDNA metabarcoding can generate data across thousands of species in just a few weeks as it can identify species at any life stage and at low detection limits.
- Morphology and life-stage independent
    - Species level data can be generated for morphologically intractable groups - e.g., cryptic species.
- Reduced taxonomic bias
    - There is no need to select for body size for ease of recognition.
- Statistically powerful datasets
    - The large datasets generated are statistically powerful and can detect even subtle changes.
- Third-party verifiable
    - DNA can be stored for re-analysis thus DNA samples collected become even more powerful and useful over time. The DNA can be revisited if you decide you want to focus on different species groups or can be reanalysed to include more up-to-date sampling. As new data are added to the database, more can be learnt about the samples over time.
- Reduced observer bias
    - Samples can be collected by anyone, anywhere and identification does not require an expert to be present when the samples are collected, instead identifications are

inferred from the DNA sequences during metabarcoding analysis - so there is no reliance on field ID skills.

- Non-invasive low effort field sampling method
    - Samples can be collected, and species detected without harming, handling, or interfering with wild animals. Sample collection is faster, cheaper, and more comprehensive.

7.2.1.3 *Key Considerations and limitations*

It is important to be aware of the inherent uncertainties in the use of eDNA for monitoring, particularly in terms of the distance that eDNA can travel from the source organism while still remaining detectable. This means that in flowing water the eDNA signal should be regarded as characteristic of the diversity in the wider upstream catchment rather than only reflecting the species present at the sampling site itself. In still water, eDNA moves less far from source and can be patchily distributed. Our sampling protocols take account of the waterbody type in order to account for the different levels of expected mixing.

Detection probability is strongly influenced by species ecology. For instance, species can be more easily detected when they are highly active in the water and when they are breeding. It is therefore, important to take the species lifecycle into account when planning a sampling campaign.

Case Study: a comprehensive and revolutionary species identification in the Peruvian Amazon

In collaboration with WWF Peru, NatureMetrics analysed 44 e-DNA samples of 500ml of river water from the Maranon River Basin in Northern Peru, to identify river dolphins, Amazon manatee and migratory catfish.

The analysis revealed that migratory catfish were present throughout the river, Manatee at 12 sites, and river dolphins in all lowland sites. NatureMetrics also detected over 375 species of vertebrates as well as 155 mammals, including the rarely observed night monkeys and kinkajou, 74 amphibians, 65 birds and six reptiles.

In addition to the afore-mentioned considerations and/or limitations, the following three aspects are considered to be limitations within the South African context and include:

- eDNA is a new technology within South Africa, even though it has been implemented internationally.
- Due to it being new technology within South Africa, there are limited service provides available to assist with eDNA/molecular studies.
- Soil and/or water samples are currently analysed in the United Kingdom, and thus is dependent on international courier services, the exchange rate (Rand/Pound), and obtaining the relevant export/import permits for the samples.