AQUATIC BIOMONITORING PROGRAMME FOR TWEEFONTEIN COMPLEX

2015 POST WET SEASON BIOMONITORING SURVEY

Report reference: TFN/A/2015

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EXECUTIVE SUMMARY

This report is based on the results of the biomonitoring and aquatic biodiversity survey conducted during April 2015 on selected sites in the Tweefontein Complex surface rights area. Where applicable, reference is also made previous surveys in order to establish temporal trends. The primary objective of the biomonitoring survey was to monitor the potential impacts of the Tweefontein Complex activities on the receiving water bodies. Sites were selected strategically in the Tweefontein Spruit, Zaaiwater Spruit and its tributaries, pan wetlands and pollution control facilities within the study area. This survey included the application of various protocols, such as aquatic macro-invertebrate sampling (SASS5), habitat assessment and toxicity testing of selected water sources in the study area. Some of the sampling sites were dry at the time of sampling and therefore no biomonitoring protocols could be applied, limiting spatial and temporal trend analyses.

The following conclusions were drawn from the April 2015 biomonitoring survey at Tweefontein complex, with reference to long-term trends where applicable:

Tweefontein Spruit catchment:

- Due to the lack of flow during the April 2015 survey selected biomonitoring protocols could not be applied at the biomonitoring sites.
- As observed during most previous surveys, 2 Seam SPP again indicated high chronic/acute toxicity hazard (Class IV) during April 2015. Based on the definitive testing done for this sample, the safe dilution factor was estimated at very high dilution of 3%.
- It was however promising to note that although some potential sources of toxicity risk (2-seam SPP) was present in the Tweefontein Spruit catchment between sites TFN-US and TFN-DS, the toxicity hazard class again remained the same (Class I - no acute/chronic hazard) at these sites, indicating that these potential sources did not cause an increase in toxicity hazard of the receiving water body.
- The electrical conductivity (EC) measured considerably high at the downstream site TFN-DS (346 mS/m), indicating that potential sources of high salinity reached this area of the Tweefontein Spruit at the time of sampling. Since there was no flow at the upstream site, the highly saline water reaching the downstream site may be originating from the TFN Complex and should be further investigated by the environmental department.
- The composition of mostly tolerant invertebrates as well as a relatively low SASS5 score of 36 indicates that the biotic integrity are poor in the lower Tweefontein Spruit (TFN-DS).
- Long-term SASS5 trends indicate that condition at site TFN-US deteriorated between 2011 and 2013, after which a slight improvement was noted. At site TFN-DS, conditions improved over the period 2011 to 2012, after which a notable deterioration occurred towards 2013. A slight improvement has however been noted during the 2014 and early 2015 survey.
- No indigenous fish have been sampled in this stream since 2009 with the only fish species sampled since 2011 being the alien *Gambussia affinis* (Mosquito fish). The complete absence of any fish (indigenous and alien) during the 2014 survey was indicative of seriously deteriorated biotic integrity prevailing in this reach of the Tweefontein Spruit while no sampling could be performed during 2015. Based on the latest available information the FRAI score for this reach of the Tweefontein Spruit is therefore 0%, falling into a descriptive category F (Critically modified).

Zaaiwater Spruit:

- In the Zaaiwater Spruit catchment, it was important to note that the toxicity assessment classified site ZW1 in a Class III (acute hazard), indicating that water flowing into the Tweefontein complex study area already had some toxicity hazard at the time of sampling. This is an indication that activities upstream of the Tweefontein Complex were responsible for water quality deterioration in the Zaaiwater Spruit at the time of sampling.

- The streams entering the Zaaiwater Spruit from the south-east within this reach (South Witbank Stream, Alpha Stream) also indicated slight toxicity hazard (Class II) and therefore pointed at non-Tweefontein activities also contributing to the toxicity hazard of this reach of the Zaaiwater Spruit.
- It was promising to note that although these streams contributed water with slight toxicity hazard to the Zaaiwater Spruit, the toxicity hazard decreased downstream after flowing through the TNC complex, falling in a Class I (no hazard) at sites ZW3 and ZW4.
- The EC increased notably between sites ZW1 and ZW 3 (sites ZW2 dry) during the April 2015 survey indicating potential sources of pollution entering the Zaaiwater Spruit in this reach. The South Witbank Stream (75.7 mS/m) and especially the Alpha stream (250 mS/m) had relatively high EC levels, and therefore may have contributed to this observed increase in salinity. This is therefore again an indication that non-TFN activities may have been responsible for this observed salinization, although it is recommended that TFN complex further investigate if they possibly contribute to this observed scenario. It was promising to not the EC decreased towards site ZW4 and hence no further sources of salts reached the Zaaiwater Spruit in the lower section.
- The pH level of 5.08 measured on site at site ZW1 exceeded the target water quality ranges again indicated that water of poor quality is flowing into the TFN complex study area due to upstream land-use activities.
- During April 2015, the total SASS5 scores as well as most comparative biotope scores indicated downstream deterioration between site ZW1 (54) and ZW2 (43). The fact that there were no flow at site ZW2 may have contributed to the poor biotic integrity due to evaporation resulting in further concentration of pollutants. It was promising to note that conditions improved towards site ZW4, although there was also no flow at this site during April 2015. Comparison of the SASS5 scores between site ZW1 and ZW4 confirms that conditions in fact improved in the Zaaiwater Spruit after flowing through the TFN complex (also indicated by toxicity testing).
- Long-term trends in SASS5 scores indicated similar temporal trends at sites ZW1 and ZW4, again confirming that the integrity in this reach is mostly driven by conditions upstream of the study area. Deterioration in the biotic integrity of this reach was evident between 2011 and 2013. Some improvement was noted in 2014 and a notable improvement was evident during the early 2015 survey.
- The fact that no indigenous fish and only one alien species was sampled during the April 2015 survey in the Zaaiwater Spruit is indicative of highly deteriorated biotic integrity, based on fish, in this system. There is a strong possibility that no indigenous fish species occur in this reach due to the current level of flow and water quality modification. Due to the absence of indigenous species and the presence of only one alien fish species in April 2015, a very low FRAI score of 2.1% was calculated, placing this reach it in a descriptive category F (critically modified).

Pan wetlands:

- Due to the dry conditions in the study area, most of the pans were still dry or unsuitable for monitoring during April 2015.
- The EC levels in the Boschmans Pan measured very high (515 mS/m) during April 2015 The pH level of this two pans was within general guideline levels for aquatic ecosystems but the very low dissolved oxygen level of 2.31 mg/l exceeded the guideline level and may be limiting to the biotic integrity of this aquatic ecosystem. Due to the fact that the natural cycles of these pans have been altered, it can be expected that the aquatic fauna would have been altered from their natural state.
- As observed during most previous surveys, the EC of Pan 4 (1493mS/m) was also again very high during the April 2015 survey. Some mining activity (non Tweefontein Complex) is evident to the north east of this pan and potentially negative impacts on the water quality due to current mining cannot be excluded. The pH (8.85) and dissolved oxygen levels of Pan 4 were within general guideline levels for aquatic ecosystems during the April 2015 survey.

- A total of 10 aquatic invertebrate taxa were sampled in the Boschmans pan and Makou pan (pan 4) during the April 2015 survey. During April 2015 the SASSpan scores measured 36 at site Boschmans Pan and only 8 at Makou Pan (pan 4). When analyzing the temporal data, it is evident that SASSpan scores have varied greatly between different surveys at all the pans. SASSpan scores are generally higher during the wet season than the dry season (as discussed above). The large variation in SASSpan scores observed at the pans is therefore a reflection of natural phenomenon, although the contribution of human impacts (mining and agriculture) is also expected to contribute to the variation. It is of some concern that most pans indicated a deteriorating long-term trend in SASSpan score (based on regression analyses). The dry conditions prevailing in the study area for the latter part of the study area have contributed to the observed scenario, but water quality deterioration cannot be excluded as a possibility in some of the pans (such as Boschmans and Makou pan).

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1. INTRODUCTION & BACKGROUND

Biological communities reflect overall ecological integrity by integrating different stressors over time and thus providing a broad measure of their aggregate impact. The monitoring of biological communities therefore provides a reliable ecological measure of fluctuating environmental conditions. The biomonitoring protocols applied in this project should give a good reflection of the human impacts on the system under investigation. During this survey, the general habitat condition and availability, as well as biotic integrity based on aquatic macro-invertebrates were assessed to determine the potential impact of mining activities, together with other human impacts on the ecological integrity of the receiving streams and rivers in the vicinity of Tweefontein Complex.

This report is based on the results of the biomonitoring and aquatic biodiversity survey conducted during April 2015 on selected sites in the Tweefontein Complex surface rights area. Where applicable, reference is also made previous surveys in order to establish temporal trends. The primary objective of the biomonitoring survey was to monitor the potential impacts of the Tweefontein Complex activities on the receiving water bodies. Sites were selected strategically in the Tweefontein Spruit, Zaaiwater Spruit and its tributaries, pan wetlands and pollution control facilities within the study area. This survey included the application of various protocols, such as aquatic macro-invertebrate sampling (SASS5), habitat assessment and toxicity testing of selected water sources in the study area. Some of the sampling sites were dry at the time of sampling and therefore no biomonitoring protocols could be applied, limiting spatial and temporal trend analyses.

Refer to Table 1 below for a reference list and status of reports and surveys relevant to the Tweefontein biomonitoring programme.

Table 1: Surveys, activities and reports compiled and planned as part of the Tweefontein biomonitoring programme.

SURVEY	ACTIVITIES	REPORT NO.	STATUS
2011-07	Quarterly toxicity survey.	TFN-A-2011	Completed
2011-08	Dry season biomonitoring survey (toxicity survey, SASS5, Diatom assessment)	TFN-B-2011	Completed
2011-10	Quarterly toxicity survey.	TFN-C-2011	Completed
2011-11/12	Wet season biomonitoring survey (toxicity survey, SASS5, Diatom assessment, fish assessment)	TFN-D-2011	Completed
2012-06	Quarterly toxicity survey.	TFN-A-2012	Completed
2012-08	Dry season biomonitoring survey (including Toxicity survey)	TFN-B-2012	Completed
2012-11	Quarterly toxicity survey.	TFN-C-2012	Completed
2013-02	Wet season biomonitoring survey (including toxicity survey)	TFN-A-2013	Completed
2013-07	Quarterly toxicity survey.	TFN-SS-A-2013	Completed
2013-10	Dry season biomonitoring survey (including Toxicity survey)	TFN-B-2013	Completed
2014-01	Toxicity testing survey	TFN_SS-A-2014	Completed
2014-03	Wet season biomonitoring survey (including toxicity survey)	TFN-A-2014	Completed
2015-04	Wet season biomonitoring survey (including toxicity survey)	TFN-A-2015	This report

2. OBJECTIVES & SCOPE OF WORK

The primary objectives of the biomonitoring programme are as follows:

- Conduct the necessary biomonitoring assessments on potentially receiving water bodies and potential effluents of the Tweefontein Complex.
- Establish and broaden baseline aquatic data against which future changes in the health of these systems can be evaluated.
- Identify river sections that may possibly be affected (in terms of biotic integrity) by mining and/ or other human activities. This information will help to identify potential sources of lowered water quality by pointing water quality assessments in the right direction and hence to recommend remedial measures if required.
- Identify potential risks to receiving water bodies through a process of toxicity testing of potential effluents or pollution sources.
- Develop and maintain a site specific, tailored and integrated biomonitoring program that complies with industry and DWA standards in compliance with the mine's license conditions.

The monitoring programme would therefore form an integrated and useful component of the existing environmental management programme/s at the mine to gauge the success of management procedures and identify aspects/areas of the management programme that may require additional attention.

3. MATERIALS & METHODS

Refer to Appendix 1 for detail regarding the methodology applied.

4. STUDY AREA AND SAMPLING SITES

4.1 Desktop Present Ecological Status (PES), Ecological Importance (EI) and Ecological Sensitivity (ES) of the study area

The Department of Water Affairs developed a classification, mostly as part of the Reserve Determination Process, whereby the ecological status of rivers is categorised between A (Excellent/Pristine) to F (Critically modified). A Desktop assessment was done for the Olifants River water management area during 2010/2011 whereby each sub-quaternary (SQ) reach was assessed and the Present Ecological Integrity (PES), Ecological Importance (EI) and Ecological Sensitivity (ES) were determined by a team of experts. The PES was based on rating of various criteria relating to instream and riparian zone condition (such as continuity, habitat, flow and water quality modification). EI and ES ratings were based on various aspects related to the fish, invertebrates, riparian and instream vegetation and riverine fauna (mammals, birds, amphibians and reptiles).

The two river reaches (sub-quaternary reaches) flowing through the Tweefontein Complex are the B11F-1257 (Tweefontein Spruit) and the B11F-1286 (Zaaiwater Spruit/Klippoortjiespruit). The reach downstream of the confluence of these two streams is B11F-1273 (Tweefontein Spruit, which is bordered by the Tweefontein complex for a short distance in its upper reaches). The next receiving water body downstream of this reach is the Olifants River (B11G-1225) (Table 2).

Based on the draft desktop assessment of the present ecological status (PES) (RFA, 2011), the sub-quaternary reaches of the study area are currently mostly in a largely (Olifants River) to seriously (Tweefontein Spruit and Zaaiwater Spruit) modified ecological status (PES) (Table 2). This is related to current extensive land-use activities in the catchment, especially as a result of agriculture and mining.

Ecological importance (EI) of a river is an expression of its importance to the maintenance of ecological diversity and functioning on local and wider scales (Kleynhans, 1999). Both abiotic and biotic components of the system are taken into consideration in the assessment of ecological importance (Kleynhans, 1999). Based on the desktop assessment (RFA, 2011) of the Ecological Importance (EI) of the sub-quaternary catchments of the study area, it ranges between low to moderate (Tweefontein Spruit) to moderate (Zaaiwater Spruit and Olifants River) (Table 2).

Ecological sensitivity (or fragility) (ES) refers to the system's ability to resist disturbance and its capability to recover from disturbance once it has occurred (resilience) (Resh *et al*. 1988; Milner 1994; Kleynhans 1999). Both abiotic and biotic components of the system are taken into consideration in the assessment of ecological sensitivity (Kleynhans 1999). Based on the desktop assessment of the Ecological Sensitivity (ES) of the sub-quaternary catchments of concern, it ranges in sensitivity between moderate (Tweefontein) to high (Zaaiwater Spruit and Olifants River) (Table 2).

Table 2: Sub-quaternary reaches of concern regarding the Tweefontein Complex study area and their desktop PES, EI and ES ratings.

SQ reach code	B11F1257	B11F1286	B11F1273	B11G1225
Stream name	Tweefontein Spruit	Zaaiwater Spruit /Klippoortjiespruit	Tweefontein Spruit	Olifants River
PES category	E-Seriously modified	E-Seriously modified	E-Seriously modified	D–Largely modified
EI (mean)	Low	Moderate	Low	Moderate
ES (mean)	Moderate	High	Moderate	High

4.2 Tweefontein Complex biomonitoring sites

Biomonitoring protocols were applied at selected sites in the streams and pans within the Tweefontein study area. Sites were selected to best indicate potential impacts from the Tweefontein (TFN) Colliery, but due to the presence of other land users (mining and agriculture), the potential impacts of Tweefontein Colliery could not always be isolated, and are discussed as such in this report. [Double-click on icon below to view sites in Google Earth™ from the MS Word version of report only].

TFN_biomon_2015.kmz

Streams/Rivers

The two primary surface water aquatic ecosystems flowing through the Tweefontein Complex are the Tweefontein Spruit and Zaaiwater Spruit (in some references and maps referred to as Klippoortjie Spruit). Biomonitoring site TFN-US was selected in the upper reaches of the Tweefontein Spruit to determine the biotic integrity of this stream before it enters the Tweefontein Complex (Table 2). Recent disturbance (construction) by Tweefontein Complex in close proximity to this site necessitated that it be relocated further upstream. A new site (TFN-US2) was therefore selected and sampled during the April 2015 survey to replace the previous site TFN-US (Table 2, Figure 1). Another site was selected in the lower reaches of the Tweefontein Spruit (TFN-DS) to determine the biotic integrity of this stream after flowing through and receiving potential impacts from the Tweefontein Complex. The potential impacts between these two sites can primarily be attributed to Tweefontein Complex activities (Table 3).

A similar approach was followed for the Zaaiwater Spruit, where sites were selected both up- and downstream of potential TFN Complex activities. In the case of the Zaaiwater Spruit, it is however more difficult to isolate the potential impact of the Tweefontein Complex, since various other potential impacts also drain into this reach (between ZW1 and ZW4) from the south. In an attempt to determine the potential impact of other users, strategic toxicity testing sites were selected in these tributaries draining from the south (Alpha Stream and South Witbank Stream), and also selected sites on the Zaaiwater Spruit (see Table 5). The location, description and rationale of each of the bioassessment sites are provided in Table 3 and Figure 1 and 2. Photographic views of the sampling sites are provided in Appendix 2.

Table 3: Stream biomonitoring sites for Tweefontein Complex.

Site	Description	Latitude	Longitude
Tweefontein Spruit Ecosystem			
TFN-US (discarded)	*Tweefontein Spruit at R555 road crossing, selected to be upstream of potential Tweefontein Complex impacts.*	*-26.030864°*	*29.112767°*
TFN-US2	New site in Tweefontein Spruit upstream of all potential TFN activities. Will provide an indication of the toxicity of the TFN Spruit before any TFN complex impacts (reference toxicity).	-26.031780°	29.093987°
TFN-DS	Tweefontein Spruit at R547 road crossing and measuring weir, selected to be downstream of potential Tweefontein Complex impacts.	-26.051500°	29.196806°
Zaaiwater Spruit Ecosystem			
ZW1 (ZW-US)	Zaaiwater Spruit at R545 road crossing, selected to be upstream of potential Tweefontein Complex impacts.	-26.090767°	29.095025°
ZW2	Zaaiwater Spruit upstream of all potential Witcons impacts.	-26.085254°	29.126175°
ZW3	Zaaiwater Spruit, upstream of Phoenix dam (includes potential impacts via Alpha and South Witbank streams, excludes Gilfillan)	-26.073167°	29.176967°
ZW4 (ZW-DS)	Zaaiwater Spruit downstream of Phoenix Dam.	-26.063590°	29.197888°

Pan Ecosystems

Various Pan wetland ecosystems are present within the TFN Complex study area. Some of these pans (Boschmans Pan, Farmers Pan and Ephemeral Pan) are in close proximity or within the currently active mining area, while others (Pan1 to 5) are situated in areas where future mining is planned. Sampling sites were selected at all of these pans (Table 4). The location, description and rationale of each of the sites are provided in Table 4 and Figure 1 and 2. Photographic views of the sampling sites are provided in Appendix 2.

Table 4: Pan biomonitoring sites for Tweefontein Complex.

Site	Description	Latitude	Longitude
Farmers Pan	Pan wetland in central area of Tweefontein Complex	-26.061373°	29.136328°
Boschmans Pan	Pan wetland in central area of Tweefontein Complex	-26.048646°	29.140622°
Ephemeral Pan	Ephemeral pan wetland in central area of Tweefontein Complex	-26.056385°	29.143885°
PAN 1	Various Pans in the north-eastern section of the Tweefontein Complex study area (on the farm Makoupan 590 IS).	-26.008041°	29.174430°
PAN 2		-26.003520°	29.177134°
PAN 3		-26.009814°	29.184981°
PAN 4 (Makoupan)		-26.021511°	29.195009°
PAN 5		-26.031611°	29.197444°

Toxicity testing sites

Based on the outcome of the previous biomonitoring and toxicity testing programme, the sampling sites and protocols have been revised. The location, description and rationale of each of the toxicity sites are provided in Table 5 and Figures 1 and 2.

Table 5: Toxicity sampling sites for Tweefontein Complex

Site Name	Sample type	Rationale / Description	Latitude	Longitude
2-seam Sewage Packing Plant (SPP)	Final treated sewage	Sample taken from final treated effluent. This water is currently not released into any aquatic ecosystem but used for irrigation of trees.	-26.00999°	29.13021°
Boschmans lined PCD	PCD	PCD for Boschmans plant. Potential overflows/spill into Tweefontein Spruit.	-26.031744°	29.127273°
TFN-US (discarded)	*Stream*	*Upstream site in Tweefontein Spruit where it flows into TFN complex. Will provide an indication of the toxicity of the TFN Spruit before any TFN complex impacts (reference toxicity).*	*-26.030864°*	*29.112767°*
TFN-US2	Stream	New site in Tweefontein Spruit upstream of all potential TFN activities. Will provide an indication of the toxicity of the TFN Spruit before any TFN complex impacts (reference toxicity).	-26.031780°	29.093987°
TFN-DS	Stream	Site in downstream reaches of Tweefontein Spruit (at gauging weir). Will provide an indication of toxicity of Tweefontein Spruit after flowing through TFN complex (aggregate TFN impacts on Tweefontein Spruit).	-26.051500°	29.196806°
Waterpan PCD	PCD	PCD to contain seepage from Waterpan plant (in process of being demolished). Important to monitor this source as it will reach Tweefontein Spruit through seepage (dam not lined) and spillages.	-26.01633°	29.15403°
ZW1	Stream	Most upstream site in the Zaaiwater Spruit at R545 road crossing, just upstream of Witcons dam.	26° 5'25.50"S	29° 5'42.30"E
Witcons Dam	PCD/Instream dam	Instream dam in Zaaiwater Spruit, upstream of most TFN complex impacts. Would provide indication of toxicity of water close to inflow of Zaaiwater Spruit into TFN complex.	-26.092369°	29.109433°
ZW2	Stream	Zaaiwater Spruit upstream of all potential Witcons impacts.	-26.085254°	29.126175°
ZW3	Stream	Zaaiwater Spruit, upstream of Phoenix dam (includes potential impacts via Alpha and South Witbank streams, excludes Gilfillan)	-26.073167°	29.176967°
ZW4	Stream	Site in downstream reaches of Zaaiwater Spruit (at road crossing downstream of Phoenix Dam). Will provide an indication of toxicity of Zaaiwater Spruit after flowing through TFN complex.	-26.051500°	29.196806°
South Witbank Stream (SWS)	Stream	Site in downstream reaches of South Witbank Stream (at train bridge). Will provide an indication of potential contribution towards toxicity of Zaaiwater Spruit (includes mostly non-TFN Colliery impacts).	-26.089113°	29.152924°
Alpha Stream (AS)	Stream	Site in downstream reaches of Alpha Stream (at train bridge). Will provide an indication of potential contribution towards toxicity of Zaaiwater Spruit (includes non-TFN Colliery impacts).	-26.092273°	29.169714°

PCD: Pollution Control Dam STP: Sewage Treatment Plant RWD: Return Water Dam SPP: Sewage Packing Plant

Figure 1: Aerial view (Google Earth™ image) of northern section of Tweefontein Complex study area (red polygon) indicating biomonitoring and toxicity testing sites.

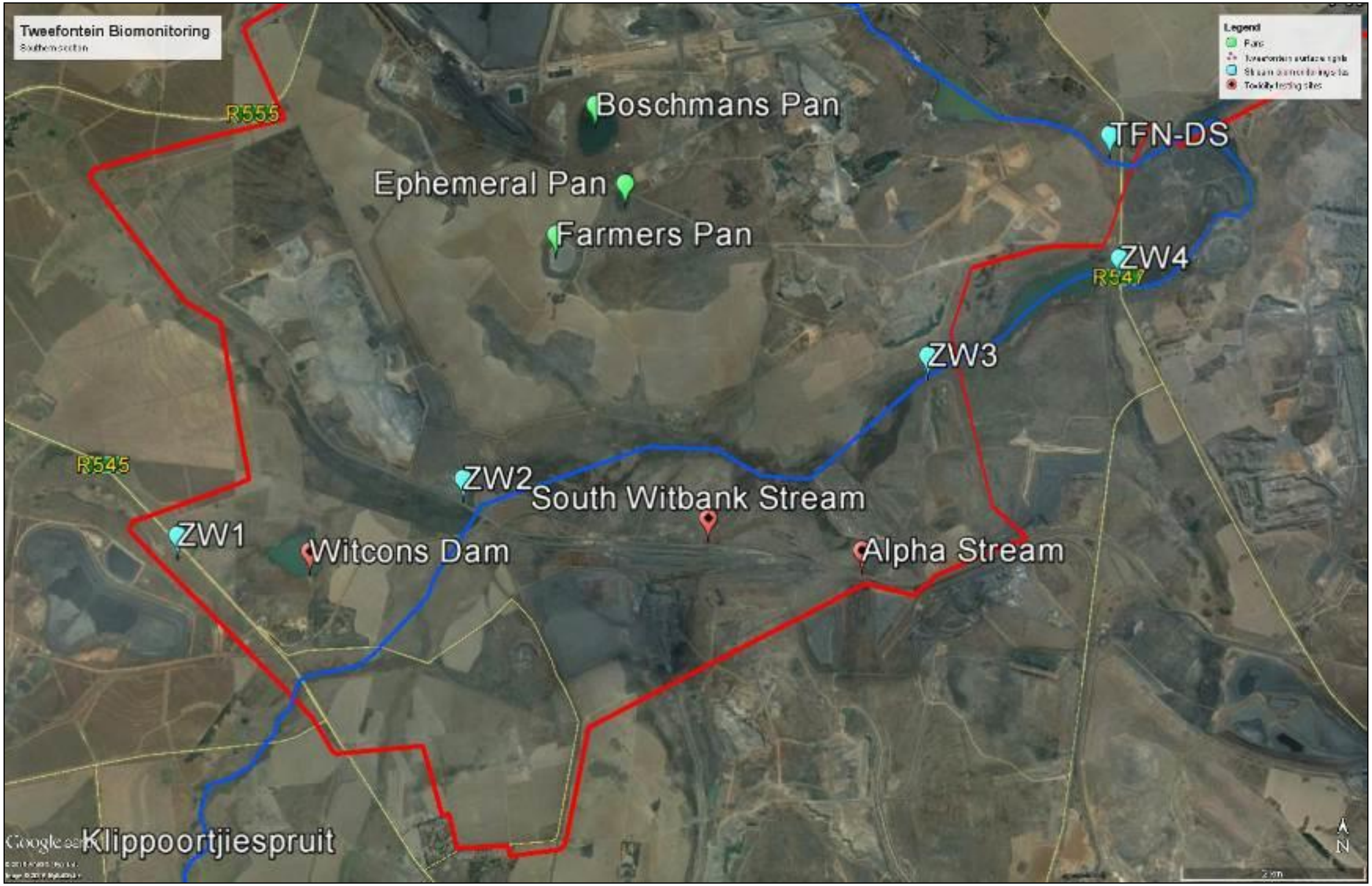

Figure 2: Aerial view (Google Earth™ image) of southern section of Tweefontein Complex study area (red polygon) indicating biomonitoring and toxicity testing sites.

5. RESULTS & DISCUSSION

5.1 Toxicity results

Toxicity testing (as conducted in this biomonitoring programme) is applied by exposing biota under laboratory conditions to water sources (pollution control dams, effluent streams or natural streams) in order to determine the potential risk of such water types to the biota of the receiving water bodies. Toxicity results indicate the potential risk posed within the streams or to the receiving streams in the event of release, seepage or overflow from potential sources of pollution. Consequently, three trophic levels of biota i.e., vertebrates (*Poecilia reticulata*), invertebrates (*Daphnia magna*) and bacteria (*Vibrio fischeri*) are exposed to the source/stream water according to standard procedures under laboratory conditions and thereafter a risk/hazard category is determined by application of the latest **DEEEP**[1] DWA recommended protocols and hazard classification. This risk category equates to the level of acute/chronic risk posed by the selected water source towards the biota of the receiving water bodies. The final risk classification is expressed in terms of **acute**[2]**/chronic**[3] toxicity risk. The *Poecilia reticulata* and *Daphnia magna* individual test results allow for acute interpretation while the *Vibrio fischeri* individual test results allow for chronic toxicity hazard interpretation.

Some of the toxicity samples are tested on a **screening**[4] level while selected pollution control facilities that indicated high toxicity during the previous biomonitoring surveys were upgraded to **definitive**[5] level to allow for the calculation of estimated safe dilution factors should seepage/spills/releases occur. The level and frequency of testing should be guided by the observed hazard. If toxicity levels increase, it may become relevant and useful to increase the frequency of testing. For the same reason, the level of testing for a site/sample should be upgraded to "definitive" in the event that hazards are consistently observed. Definitive testing will allow for the estimation of safe dilution ratios, to negate toxicity hazards, if planned releases or potential spills/overflows are envisaged. The frequency and level of toxicity testing required, will be revised annually based on the outcome of the specific year's assessment.

Hazard classification for *screening* tests (undiluted sample)

After the determination of the percentage effect[6] (EP), obtained with each of the ***battery of toxicity screening*** tests performed, the sample is ranked into one of the following five classes:

Class I	**No acute/chronic hazard -** none of the tests shows a toxic effect.

[1] DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. Consequently a hazard class is determined based on the resulting parameters of the battery of tests.
[2] Acute = Acute refers to an exposure over a relatively short period of the lifespan of biota. The result of an acute test is normally a mortality rate.
[3] Chronic = Chronic refers to prolonged exposures over an extended period of the lifespan of test organisms. The result of a chronic test is normally an inhibition rate, such as growth inhibition.
[4] Screening = A screening toxicity test refers to an undiluted (100% concentration) sample. This is usually performed on a sample from the biomonitoring sites in the receiving water bodies (river/streams) to determine if any toxicity is present. This is performed both up- and downstream of the potential impacts to enable the determination of downstream increases or decreases in toxicity.
[5] 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 (such as effluents and PCD's). The range of dilutions are therefore useful in the event that the 100% sample concentration presents acute toxicity, and allows for the determination of a safe dilution factor, to negate toxicity effects on the receiving water bodies.
[6] EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A 10% effect is regarded as a slight acute toxicity for daphnia and guppies, while a 20% effect is regarded as a slight chronic toxicity for algae and bacteria (vibrio). A 50% effect is regarded as an acute/chronic toxicity for all of the tests (daphnia, guppies, algae and bacteria).

Class II	**Slight acute/chronic hazard** – a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%.
Class III	**Acute/chronic hazard** – the percentage effect level is reached or exceeded in at least one test, but the effect level is below 100%.
Class IV	**High acute/chronic hazard** – the 100% percentage effect is reached in at least one test.
Class V	**Very high acute/chronic hazard** – the 100% percentage effect is reached in all the tests.

Toxicity classification system *definitive* tests (undiluted samples plus range of dilutions)

The samples are classified into one of the following five classes on the basis of the highest toxicity unit (TUa) found in the ***battery of toxicity definitive tests*** performed. The toxicity unit is a function of the L(E)C50, where (TUa) = 100/L(E)C50. The 50% Lethal/Effective concentration (LC50 or LE50) is the linear calculated (derived) concentration at which a 50% mortality or inhibition rate can be expected. Hence, the lower this value is, the higher the acute toxicity level. Conversely, the higher the toxicity unit (TUa) is, the higher the acute toxicity level is. The conversion of L(E)C50 values to TUa values are therefore merely done to achieve a classification scale of increasing values related to increasing toxicity risks:

Class I	**No acute hazard -** none of the tests shows a toxic effect.
Class II	**Slight acute hazard** – the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is blow 50% (TUa is <1).
Class III	**Acute hazard** – the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is below 50% (TUa is between 1 and 10).
Class IV	**High acute hazard** – the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution (TUa is between 10 and 100).
Class V	**Very high acute hazard** – the L(E)C50 is reached in the 100 fold dilution for at least one test (TUa is >100).

Weighing: Each sample is furthermore weighed according to its relative toxicity levels (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

April 2015

The toxicity testing analyses conducted for selected samples at Tweefontein Complex during April 2015 indicated the following:

- Many of the samples tested remained in toxicity hazard Class I, indicating that they have no acute or short chronic toxicity hazard. The samples that fell in this category included TFN-US, Boschmans lined PCD, TFN-DS, Witcons Dam, ZW3, and ZW4 (Table 6).
- As observed during most previous surveys, 2 Seam SPP again indicated high chronic/acute toxicity hazard (Class IV) during April 2015 (Table 6). It is therefore evident that this source is still of concern and could result in acute or chronic impacts on biota and should not be allowed to get into contact with receiving water bodies. Based on the definitive testing done for this sample, the safe dilution factor was estimated at very high dilution of 3%.
- It was promising to note that although some potential sources of toxicity risk (2-seam SPP) was present in the Tweefontein Spruit catchment between sites TFN-US and TFN-DS, the toxicity hazard class again remained the same (Class I - no acute/chronic hazard) between these sites, indicating that these potential sources did not reach or cause an increase in toxicity hazard of the receiving water body.
- In the Zaaiwater Spruit catchment, it was important to note that site ZW1 fell in Class III (acute hazard), indicating that water flowing into the Tweefontein study area already had some toxicity hazard at the time of sampling (the relative safe dilution factor for site ZW1

was 33%). This is an indication that activities upstream of the Tweefontein Complex were responsible for water quality deterioration in the Zaaiwater Spruit at the time of sampling.

- The streams entering the Zaaiwater Spruit in this reach (South Witbank Stream, Alpha Stream) indicated slight toxicity hazard (Class II) and therefore pointed at non-Tweefontein activities also contributing to the toxicity hazard of this reach of the Zaaiwater Spruit (Table 6).
- It was promising to note that although these streams contributed water with slight toxicity hazard to the Zaaiwater Spruit, the toxicity hazard decreased downstream falling in a Class I (no hazard) at sites ZW3 and ZW4.

Temporal (long-term) trends

- The long-term data (Table 7 and Figures 3 to 5) clearly highlights two areas of concern regarding toxicity hazard, namely 2-Seam SPP and Waterpan PCD. These two sources have on a regular basis indicated high (Class IV) toxicity hazard (Table 7, Figure 3). Regression lines indicated that site 2-seam SPP consistently remains high, while site Waterpan PCD indicated a promising improvement (decreased toxicity hazard) over time. This site was also dry during the April 2015 survey and can therefore not contribute to pollution in this catchment at present.
- Another site highlighted by the long-term trends to be regularly of concern is ZW1. Site ZW1 indicates that water of high toxicity flows into the study area and therefore that upstream users are responsible for deterioration (increased toxicity risk) before the potential Tweefontein activities. This trend was again maintained during April 2015 and therefore a consistent trend is evident at this site.
- Most of the other sites in the TFN complex study area has indicated spikes of toxicity at times, but these have generally been short-lived, recovering to no acute hazard during the next survey (Figure 3).

Table 6: Toxicity test results of April 2015 survey (approximate upstream to downstream order)

	Results	TFN-US	Boschmans lined PCD	2-Seam	TFN-DS	ZW-1	Witcons Dam	South Witbank Stream DS	Alpha Stream-DS	ZW-3	ZW-4
[WQ]Water quality	pH	8.3	7.4	7.5	7.8	6.5	7.8	8.1	7.5	7.5	7.5
	EC (Electrical conductivity) (mS/m)	52.9	345	224	301	85.8	119	75.7	250	172.3	130.8
	Dissolved oxygen (mg/l)	8.5	7.5	4.9	7.6	7.9	8	8.2	8.6	8.6	8.6
V. fischeri (bacteria)	Test started on yy/mm/dd	15/05/06	15/05/06	15/05/08	15/05/06	15/05/13	15/05/06	15/05/06	15/04/23	15/04/23	15/04/23
	[%]30min inhibition (-) / stimulation (+) (%)	26	23	-42	12	-91	-3	3	15	20	18
	EC/LC20 (30 mins)	*	*	6	*	33	*	*	*	*	*
	EC/LC50 (30 mins)	*	*	n.r.	*	50	*	*	*	*	*
	Toxicity unit (TU) / Description	no short-chronic hazard	no short-chronic hazard	<1	no short-chronic hazard	2.0	no short-chronic hazard	no short-chronic hazard	no short-chronic hazard	no short-chronic hazard	no short-chronic hazard
D. magna (waterflea)	Test started on yy/mm/dd	15/05/04	15/05/04	15/05/04	15/05/04	15/05/04	15/05/04	15/04/28	15/05/04	15/04/28	15/04/28
	[%]48hour mortality rate (-%)	0	0	-100	0	-100	-5	-10	-10	-5	0
	EC/LC10 (48hours)	*	*	3	*	53	*	*	*	*	*
	EC/LC50 (48hours)	*	*	5	*	65	*	*	*	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard	20.1	no acute hazard	1.5	no acute hazard	S.D.O.T.H.	S.D.O.T.H.	no acute hazard	no acute hazard
P. reticulata (guppy)	Test started on yy/mm/dd	15/05/22	15/05/22	15/05/22	15/05/22	15/05/22	15/05/22	15/04/20	15/05/22	15/05/22	15/05/22
	[%]96hour mortality rate (-%)	0	0	-100	0	-100	0	-10	0	0	0
	EC/LC10 (96hours)	*	*	25	*	55	*	*	*	*	*
	EC/LC50 (96hours)	*	*	58	*	75	*	*	*	*	*
	Toxicity unit (TU) / Description	no acute hazard	no acute hazard	1.7	no acute hazard	1.3	no acute hazard	S.D.O.T.H.	no acute hazard	no acute hazard	no acute hazard
Estimated safe dilution factor (%) [for definitive testing only]				3		33					
Overall classification - Hazard class***		Class I - No acute/chronic hazard	Class I - No acute/chronic hazard	Class IV - High acute/chronic hazard	Class I - No acute/chronic hazard	Class III - Acute/chronic hazard	Class I - No acute/chronic hazard	Class II - Slight acute/chronic hazard	Class II - Slight acute/chronic hazard	Class I - No acute/chronic hazard	Class I - No acute/chronic hazard
Weight (%)		0	0	50	0	75	0	50	25	0	0

WQ = Water quality at the time of starting the *Daphnia magna* testing.
% = for definitive testing, only the 100% concentration (undiluted) sample mortality/inhibition/stimulation is reflected by this summary table. The dilution series results are considered for EC/LC values and
n.r. = not relevant, i.e. the 100% concentration caused less than 10/20/50% (effective concentration) mortalities or inhibition
***** = EC/LC values not determined, definitive testing required if a hazard was observed and persists over subsequent sampling runs
S.D.O.T.H = Some degree of acute/chronic toxic hazard based on this single test organism, refer to overall hazard classification, which takes into account the full battery of test organisms.
******* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as acute/chronic level of toxicity, due to the fact that the *S. capricornutum* (micro-algae) and the *V. fischeri* tests are regarded as short-chronic levels of toxicity tests and the overall classification therefore contains a degree of chronic toxicity assessment.
Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class
site/sample name shaded in purple = screening test
site/sample name shaded in orange = definitive test

Table 7: Temporal variation in toxicity at Tweefontein Complex.

Site Name	Sample type	Aug-11	Oct-11	Nov-11	Jun-12	Aug-12	Nov-12	Feb-13	Jul-13	Oct-13	Jan-14	Mar-14	Apr-15
TFN-US	Stream	Class I – No acute hazard	n/a	n/a	Class I – No acute hazard	DRY	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard
Boschmans lined PCD	PCD	Class II – Slight acute hazard	Class II – Slight acute hazard	Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard
2-seam Sewage Packing Plant (SPP)	Final treated sewage	Class IV-High acute hazard	Class IV-High acute hazard	Class I – No acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	Class III – Acute hazard	Class III – Acute/chronic hazard	Class III – Acute hazard	Class IV-High acute hazard
Waterpan PCD	PCD	Class III – Acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	Class IV-High acute hazard	DRY	Class IV-High acute hazard	Class IV-High acute hazard	Class III – Acute hazard	Class III – Acute hazard	Class III – Acute/chronic hazard	Class III – Acute hazard	DRY
TFN-DS	Stream	Class II – Slight acute hazard	Class II – Slight acute hazard	Class I – No acute hazard	n/a	DRY	Class IV-High acute hazard	Class I – No acute hazard	DRY	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard
ZW1/ZW-US									Class III – Acute hazard	Class III – Acute hazard	Class III – Acute/chronic hazard	Class III – Acute hazard	Class III – Acute hazard
Witcons Dam	PCD/Instream dam	Class II – Slight acute hazard	Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard
ZW2									Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	DRY
South Witbank Stream	Stream	Class I – No acute hazard	n/a	n/a	Class I – No acute hazard	Class I – No acute hazard	Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class II – Slight acute hazard
Alpha Stream	Stream	Class II – Slight acute hazard	n/a	n/a	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class II – Slight acute hazard
ZW3									Class III – Acute hazard	Class III – Acute hazard	Class III – Acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard
ZW4/ZW-DS	Stream	Class II – Slight acute hazard	Class I – No acute hazard	Class I – No acute hazard	n/a	Class IV-High acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute hazard	Class I – No acute/chronic hazard	Class I – No acute hazard	Class I – No acute hazard

PCD: Pollution Control Dam; STP: Sewage Treatment Plant; RWD: Return Water Dam; SPP: Sewage Packing Plant; n/a – not assessed (excluded)

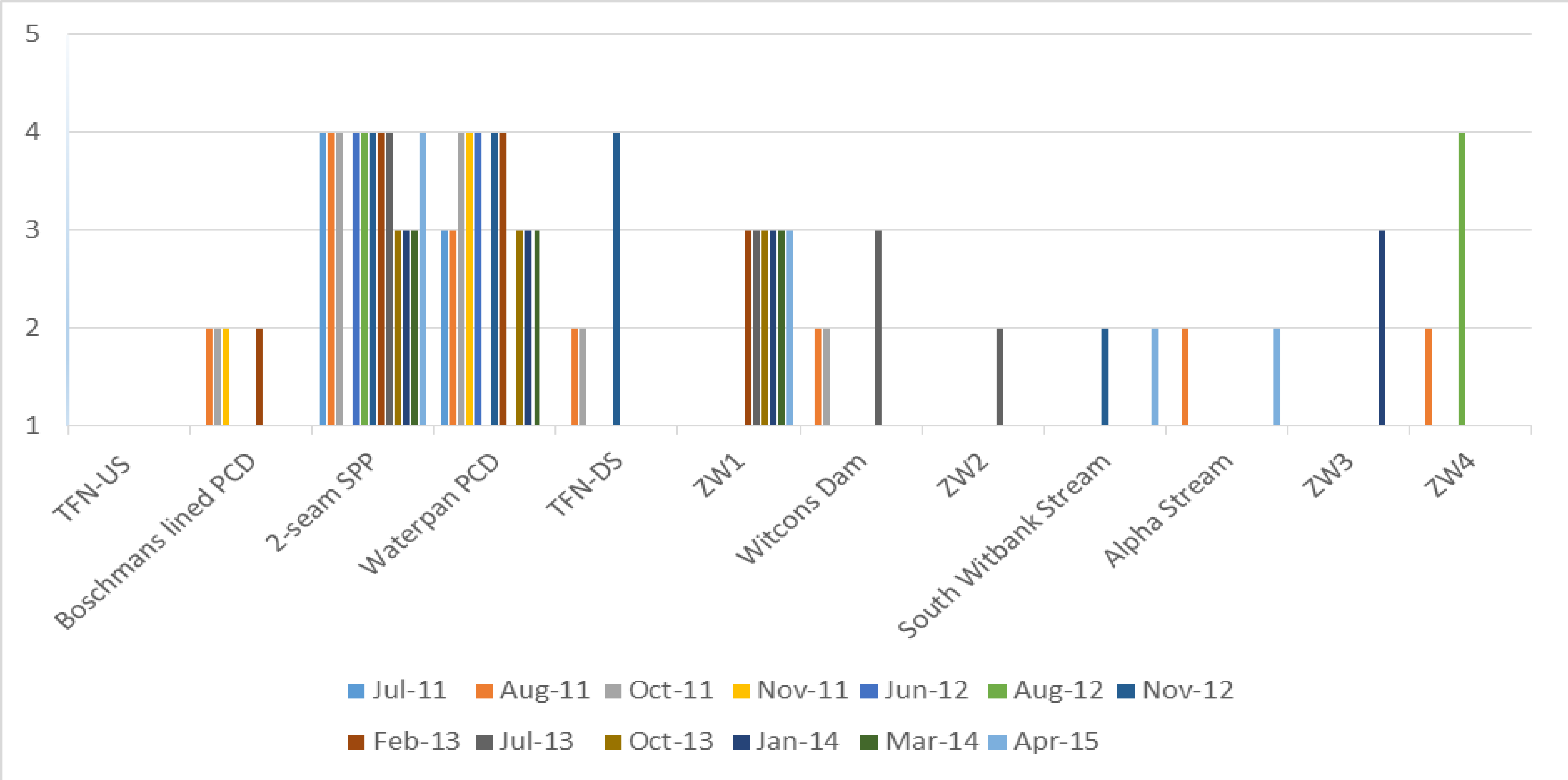

Figure 3: Visual presentation of long-term trends in toxicity hazard of the TFN complex.

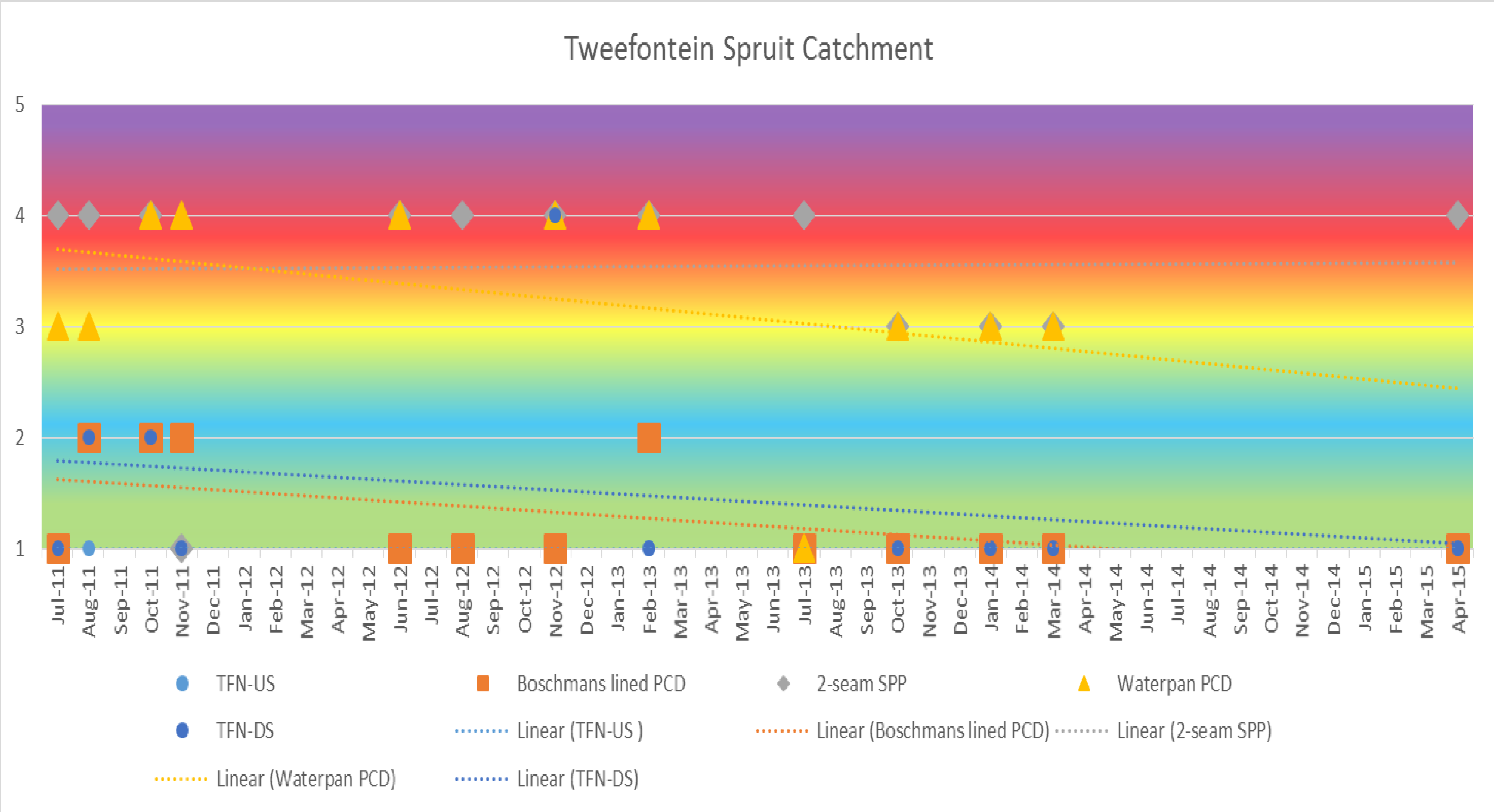

Figure 4: Long-term trends in toxicity hazard categories of the Tweefontein Spruit catchment.

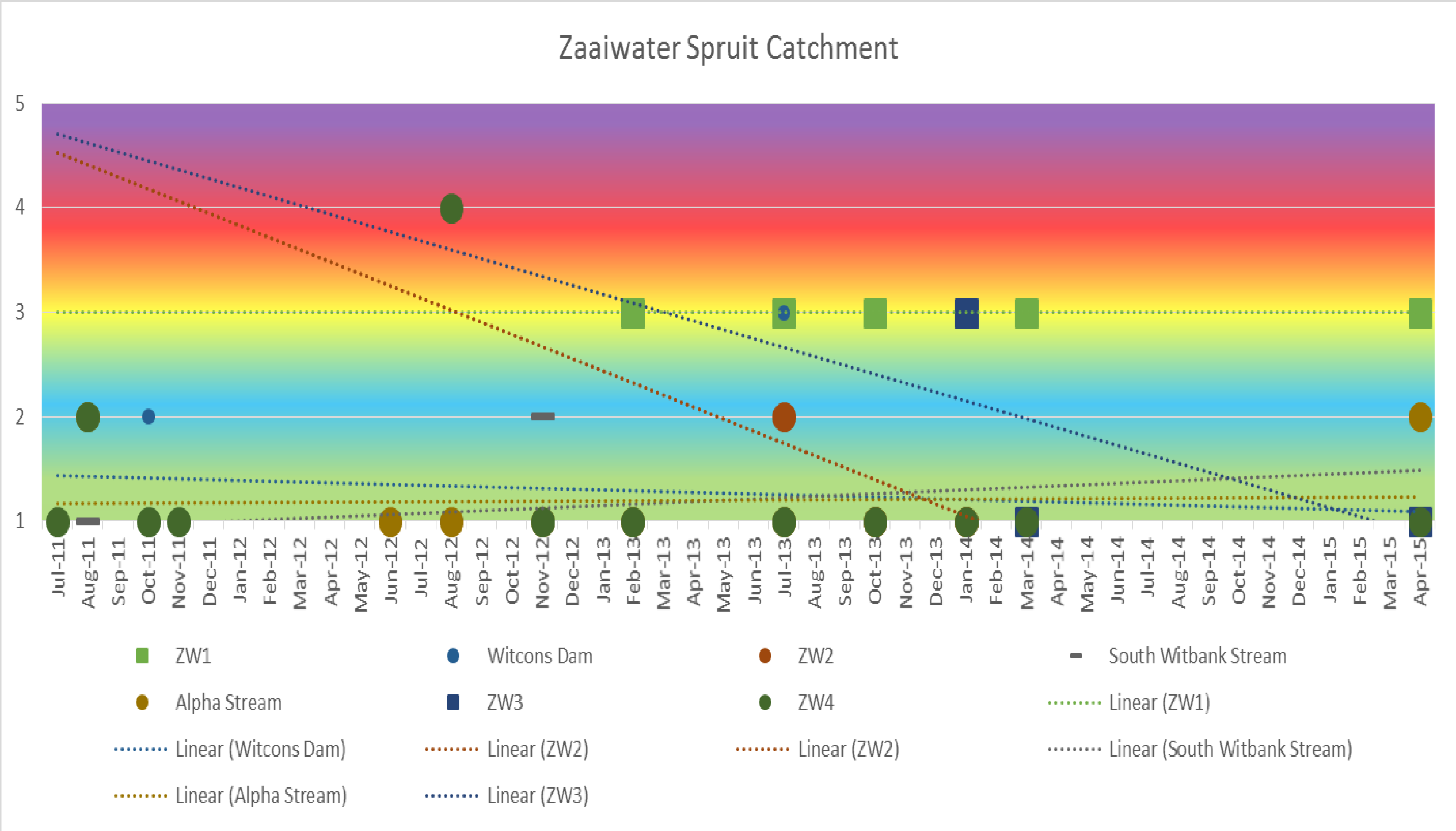

Figure 5: Long-term trends in toxicity hazard categories of the Tweefontein Spruit catchment.

5.2 *In-situ* Water Quality

Selected water quality variables were measured at the biomonitoring sites at the time of biological sampling (Table 8). The purpose of this data is only to assist in the interpretation of biological results at the time of sampling (Refer to Tweefontein Complex surface water monitoring reports for detailed water quality information).

Table 8: *In-situ* water quality assessment results at the time of sampling at the selected biomonitoring sites (April 2015 survey).

Monitoring site	EC (mS/m)	pH	Oxygen saturation (%)	Dissolved oxygen (mg/l)	Water temp (ºC)	Turbidity (visual)
TFN-US	No flow					
TFN-DS	346.0	7.7	94	7.6	17.3	Slight
ZW1	100.4	5.08	73	5.92	15.9	Slight
ZW2	Dry					
ZW3	206.1	7.65	79.5	5.94	18.2	Slight
ZW4	153.9	7.87	103.6	8.05	18.7	Slight
Farmer's Pan						
Boschmans Pan	515.0	7.25	29.8	2.31	18.9	Slight
Ephemeral Pan	Dry					
Pan 1						
Pan 2						
Pan 3						
Pan 4	1493.0	8.85	108.8	8.23	20.1	Slight
Pan 5	Dry					

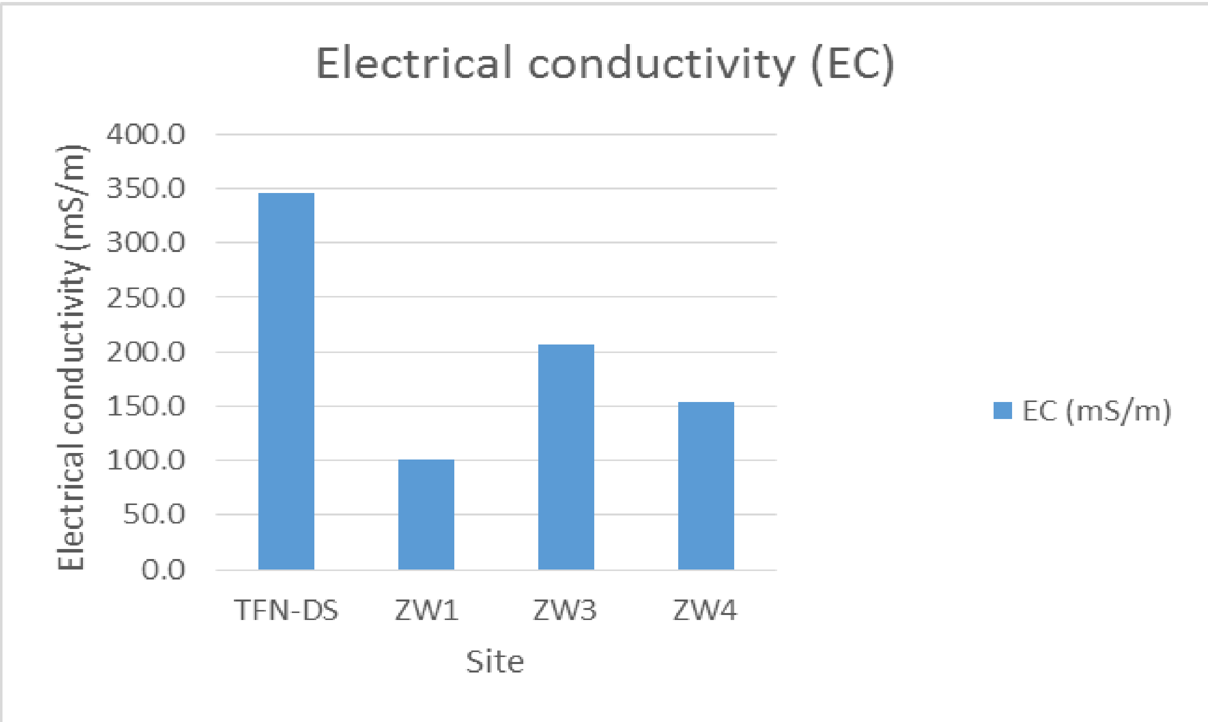

Figure 6: Electrical conductivity levels measured at the stream biomonitoring sites (April 2015)

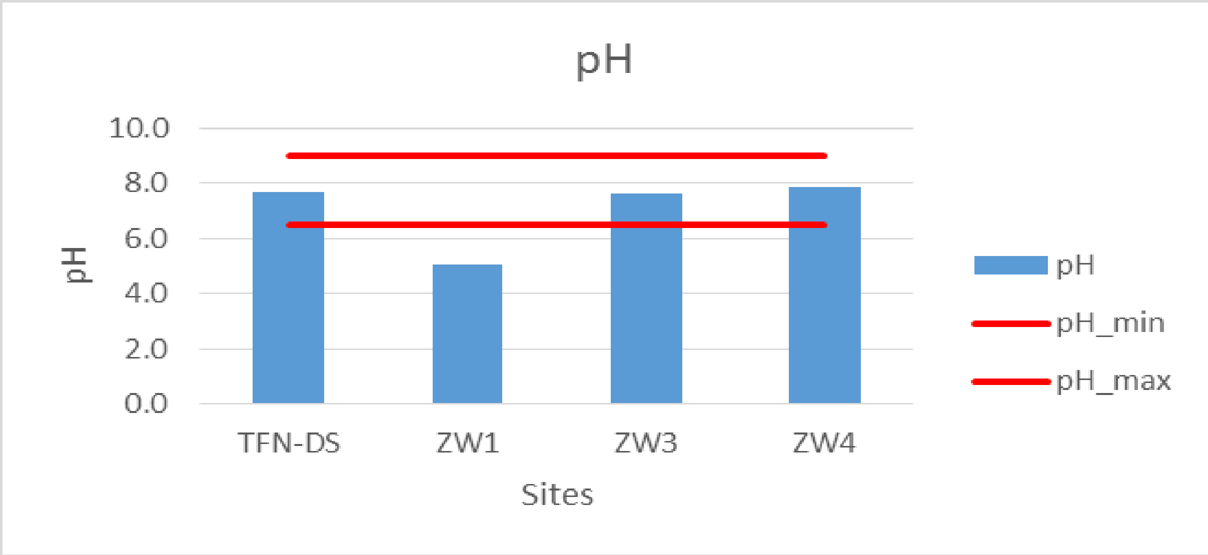

Figure 7: The pH levels measured at the stream biomonitoring sites (April 2015)

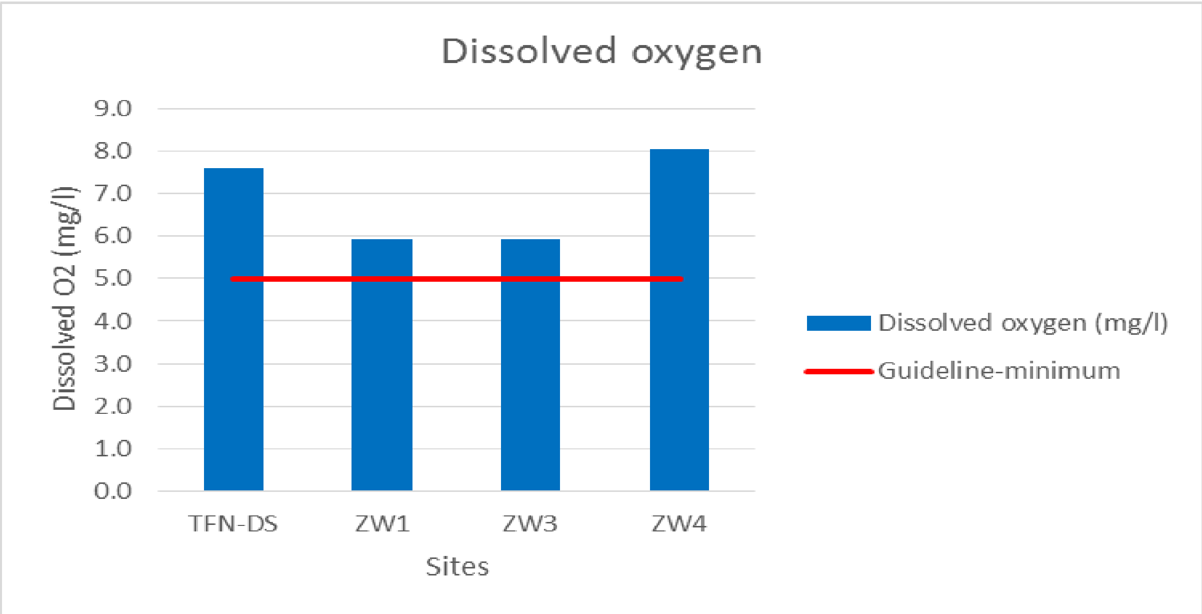

Figure 8: The dissolved oxygen levels measured at stream biomonitoring sites (April 2015)

Tweefontein Spruit

The upstream Tweefontein Spruit had no flow at site TFN-US2 at the time of sampling and limited flow was evident at the downstream site TFN-DS during the April 2015 survey. No spatial comparison of *in-situ* water quality variables was therefore possible for the current biomonitoring survey. The electrical conductivity (EC) measured considerably high at the downstream site TFN-DS (346 mS/m), indicating that potential sources of high salinity reached this area of the Tweefontein Spruit at the time of sampling. Since there was no flow at the upstream site, the highly saline water reaching the downstream site may be originating from the TFN Complex and should be further investigated by the environmental department. Sites Boschmans lined PCD (345 mS/m) and 2-seam SPP (224 mS/m) all had high salinity and may therefore be potential contributors to the observed increased salinity should any seepage or releases occurred in this period.

The pH levels of site TFN-DS (7.7) fell within the target water quality ranges for fish health, irrigation, aesthetics and human health at all of the sites during the April 2015 survey (Table 8, Figure 7). The target for fish health is between 6.5 and 9.0 as it is expected that most aquatic species will tolerate and reproduce successfully within this pH range (DWAF, 1996). During the April 2015 survey, the dissolved oxygen level at site TFN-DS (7.6 mg/l) (Table 8, Figure 8) fell within the guideline value of >5mg/l (Kempster *et. al.,* 1982) for the protection of aquatic ecosystems.

Zaaiwater Spruit

The EC increased notably between sites ZW1 and ZW 3 (sites ZW2 dry) during the April 2015 survey indicating potential sources of pollution entering the Zaaiwater Spruit in this reach. The South Witbank Stream (75.7 mS/m) and especially the Alpha stream (250 mS/m) had relatively high EC levels (Table 6), and therefore may have contributed to this observed increase in salinity. This is therefore an indication that non-TFN activities may have been responsible for this observed salinization, although it is recommended that TFN complex further investigate if they possibly contribute to this observed scenario. It was promising to not the EC decreased towards site ZW4 and hence no further sources of salts reached the Zaaiwater Spruit in the lower section.

The pH level of 5.08 measured on site at site ZW1 (Table 8) exceeded the target water quality ranges for fish health, irrigation, aesthetics and human health during the April 2015 survey. The target for fish health is between 6.5 and 9.0 as it is expected that most aquatic species will tolerate and reproduce successfully within this pH range (DWAF, 1996). The toxicity assessment of this site also indicated that it had a toxicity hazard and the low pH may have been an important variable contributing to the observed scenario. The pH values fortunately improved downstream towards site ZW3 and ZW4 to fall within acceptable limits (this was also confirmed by the toxicity testing that indicated no hazard).

During the April 2015 survey, the dissolved oxygen level at all Zaaiwater Spruit sites fell above the guideline value of >5mg/l (Kempster et. al., 1982) for the protection of aquatic ecosystems and should not be limiting to aquatic biota (Figure 8).

Pan wetlands

Farmers Pan, Ephemeral pan, Pan 1, Pan 2, Pan 3 and 5 was dry at the time of sampling during April 2015.

The EC levels in the Boschmans Pan measured very high (515 mS/m) during April 2015 (Table 8). The Boschmans Pan is currently used as storage and pollution control facility, receiving water from the Tweefontein Dam situated in the lower Tweefontein Spruit. The EC level in the Boschmans Pan is therefore most likely a reflection of the water from the Tweefontein Dam and lower Tweefontein Spruit. EC levels may be high in pans even under natural conditions, and it is therefore uncertain whether the high observed EC levels will be limiting to the natural biota of the pan, which may have a high tolerance to salt level variation. The pH level of this two pans was within general guideline levels for aquatic ecosystems but the very low dissolved oxygenlevel of 2.31 mg/l exceeded the guideline level and may be limiting to the biotic integirty of this aquatic ecosystem. Due to the fact that the natural cycles of these pans have been altered, it can be expected that the aquatic fauna would have been altered from their natural state.

As observed during most previous surveys, the EC of Pan 4 (1493mS/m) was also again very high during the April 2015 survey. This high salt level can be expected to be limiting to some intolerant biota, although the natural indigenous biota of pans will have the ability to adapt to natural seasonal variations of salt that can be expected to occur naturally in pans. The dry conditions that prevailed in the study area during and preceding this survey may have contributed greatly to increased salt levels as a result of evaporation. Anthropogenic activities such as storage of water (polluted or non-polluted), altered hydrological regime through catchment changes (such as ploughing) and water quality deterioration through seepage, spills etc. from surface or ground water origin may however also result in water quality alterations. A few pumps were visible at this site, but is seemed that they were used for abstraction rather than for pumping water of poor quality into the pan. It is therefore uncertain whether this pan is currently being used as a storage facility, which would result in a major negative impact on its water quality, and overall ecological integrity. Some mining activity (non Tweefontein Complex) is evident to the north east of this pan and potentially negative impacts on the water quality due to current mining cannot be excluded. There was also evidence of recreational activities at this pan. The pH (8.85) and dissolved oxygen levels of Pan 4 were within general guideline levels for aquatic ecosystems during the April 2015 survey.

5.3 Aquatic invertebrate assessment

The South African Scoring System (Version 5) is a site-specific index, which, together with associated habitat indices (Habitat suitability scores and IHAS) gives a general perspective of the biotic integrity (based on macro-invertebrates) and the impact of water quality on the biotic integrity of the specific sites (Thirion *et.al*., 1995; Dickens and Graham, 2001). The IHAS scores were used during this biomonitoring survey to provide an indication of the habitat availability and condition for invertebrates, and was therefore applied to determine the comparability of SASS scores between different sites and not to classify the sites into specific habitat categories. Suitability scores, ranging between 0 (unsuitable) to 5 (highly suitable) were also given to the different biotopes (stones-in-current, stones-out-of-current, bedrock, aquatic vegetation, marginal vegetation in-current and out-of-current, gravel, sand and mud) to assist in the habitat evaluation process for each site.

Riverine ecosystems: South African Scoring System (SASS5)

The SASS5 protocol was not completely suitable for application at some of the sites due to the sites being of valley-bottom wetland nature and the fact that the SASS5 protocol was designed for application in permanently **flowing** streams/rivers. The only natural biotope present at all sites was vegetation and this biotope was therefore mainly used for comparative purposes between sites. Due to the relatively low number of taxa surveyed at sites, more emphasis was placed on SASS scores as compared to average scores per taxon (ASPT) values. ASPT values show a large degree of fluctuation under conditions of low diversity and are therefore of lower value as a spatial/temporal indicator of biotic conditions.

Tweefontein Spruit

Conditions were not suitable at site TFN-US2 for the application of the SASS5 protocol during the April 2015 survey (no flow). Site TFN-DS was of limited suitability and hence no spatial comparison in biotic integrity was possible within the Tweefontein Spruit ecosystem. A total of seven aquatic invertebrate taxa were sampled at site TFN-DS in the Tweefontein Spruit during the April 2015 survey (Table 9). No taxa with a high requirement for unmodified water quality was present while only one taxon with a moderate, three with low and five with a very low requirement for unmodified water quality were sampled. A total of eight taxa were sampled at site TFN-DS, No taxa with a high requirements for unmodified water quality were present while only one taxon (Aeshnidae) with a moderate requirement for unmodified water quality was observed. Most of the taxa (five) had a low requirement for unmodified water quality while one had a very low requirement for unmodified water quality were sampled. The composition of mostly tolerant invertebrates therefore indicates that the biotic integrity are generally poor at this site. This was confirmed by the relatively low SASS5 score of 36 calculated for site TFN-DS during April 2015 (Table 10).

Table 9: Aquatic macroinvertebrate taxa sampled at the different stream sites and their relative requirement for unmodified water quality (April 2015).

Taxon	TFN-DS				ZW1				ZW3				ZW4			
	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total
Oligochaeta	-	-	-	-	-	A	-	A	-	-	-	-	-	-	1	1
Atyidae	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	A
HYDRACARINA	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1
Baetidae 1 sp.	-	-	-	-	-	-	-	-	-	-	A	A	-	-	A	A
Baetidae 2 spp.	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	A
Caenidae	-	-	-	-	-	-	-	-	-	-	-	-	A	-	A	A
Coenagrionidae	-	A	-	A	-	B	A	B	-	-	1	1	-	-	-	-
Aeshnidae	-	1	-	1	-	A	-	A	-	-	-	-	-	-	-	-
Gomphidae	-	A	-	A	-	-	-	-	-	-	-	-	-	-	A	A
Libelludae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A
Corixidae*	-	-	-	-	-	A	B	B	-	-	-	-	A	-	-	A
Gerridae*	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Nepidae*	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Notonectidae*	-	-	-	-	-	-	A	A	-	-	-	-	A	-	-	A
Pleidae*	-	-	-	-	-	A	A	B	-	-	A	A	A	-	-	A
Veliidae*	-	1	-	1	-	-	-	-	-	-	A	A	-	-	-	-
Ecnomidae	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-
Dytiscidae (adults*)	-	1	-	1	-	A	1	A	-	A	-	A	1	-	A	A
Hydrophilidae (adults*)	-	-	-	-	-	-	1	1	-	-	1	1	-	-	-	-
Ceratopogonidae	-	-	-	-	-	-	-	-	-	-	A	A	-	-	A	A
Chironomidae	-	-	-	-	-	-	B	B	-	1	A	A	A	-	B	B
Culicidae*	-	-	-	-	-	1	-	1	-	-	A	A	-	-	-	-
Simuliidae	-	A	-	A	-	A	-	A	-	-	-	-	-	-	-	-
Physidae*	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
Sphaeridae	-	-	-	-	-	-	-	-	-	-	-	-	A	-	A	A
Total SASS5 score	0	36	0	36	0	39	31	54	0	7	38	43	43	0	44	67
No. of families	0	7	0	7	0	9	8	13	0	2	9	10	10	0	10	15
ASPT	N/A	5.14	N/A	5.14	N/A	4.33	3.88	4.15	N/A	3.50	4.22	4.30	4.30	N/A	4.40	4.47
Total IHAS				48				46				43				39
IHAS - Habs sampled				18				24				24				14
IHAS - Stream condition				30				22				19				25
Suitability score	0	4	0	4	0	5	1	6	0	4	2	6	4	0	5	9

Table 10: SASS5, ASPT, SASS5 scores per biotope and biotope suitability index scores for different monitoring sites in lotic ecosystems (April 2015) *(auto-colour-coded from red=lowest to green=highest value for each data set)*.

Monitoring site	SASS5 score	ASPT	SASS5-score per biotope			Biotope availability and suitability (Scores)			
			$SASS_{Stones}$	$SASS_{Vegetation}$	$SASS_{GSM}$	Stones	Vegetation	GSM	Combined
TFN-DS	36	5.14	0	36	0	0	4	0	4
ZW1	54	4.15	0	39	31	0	5	1	6
ZW3	43	4.30	0	7	38	0	4	2	6
ZW4	67	4.47	43	0	44	4	0	5	9

Key:
ASPT - Average Score Pre Taxon   S-Stones   Veg-Vegetation   GSM-Gravel, sand & mud

Long-term trends (Figure 9) in SASS5 scores indicate some variation over time, which can be expected naturally in a seasonal wetland type of system. These changes are however further amplified by aspects such as water quality and quantity modification, such as what occurs in the Tweefontein catchment. Conditions at site TFN-US deteriorated between 2011 and 2013, after which a slight improvement was noted. At site TFN-DS, conditions improved over the period 2011 to 2012, after which a notable deterioration occurred towards 2013. A slight improvement has however been noted during the 2014 and early 2015 survey (Figure 9).

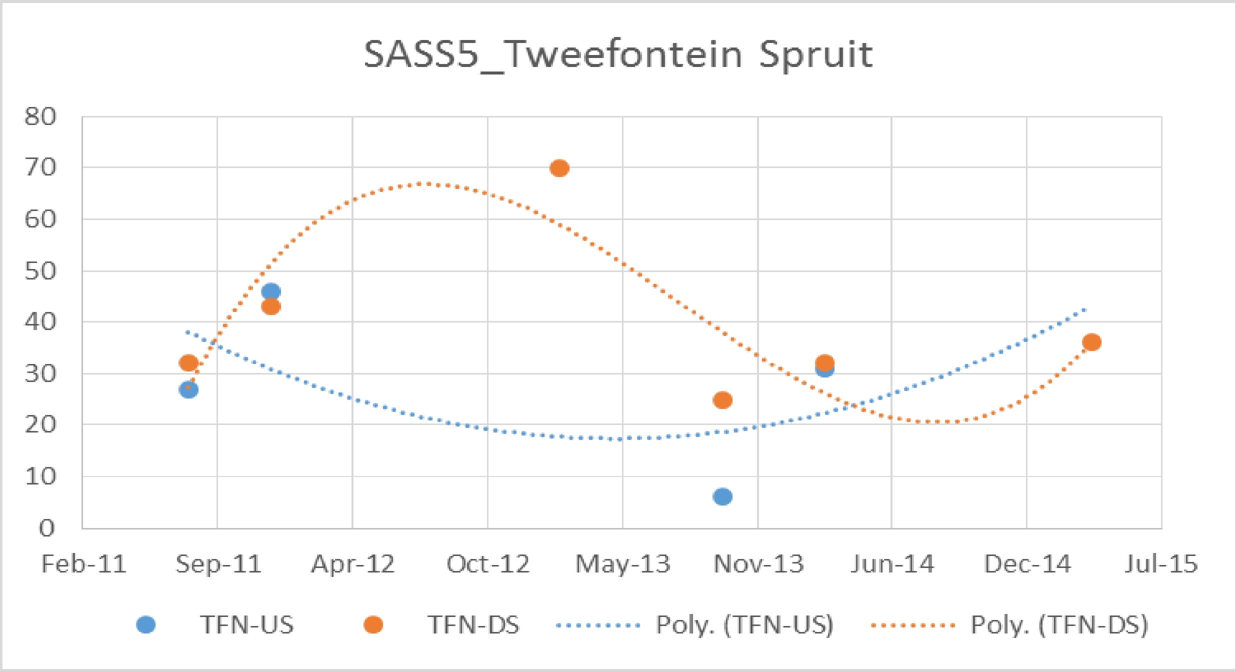

Figure 9: Long-term trends in SASS5 scores in Tweefontein Spruit

Zaaiwater Spruit

A total of thirteen aquatic invertebrate taxa were sampled at site ZW1 in the Zaaiwater Spruit during the April 2015 survey (Table 9). No taxa with a high or moderate requirement for unmodified water quality were present while only two had a moderate requirement for unmodified water quality (Table 9). Most of the taxa observed had a low (6) and very low (5) requirement for unmodified water quality, indicating poor water quality prevailing at this site. A similar scenario was observed at site ZW3 and ZW4 where the majority of the taxa observed had a low to very low requirement for unmodified water quality. The taxon composition at the Zaaiwater Spruit sites therefore indicated poor biotic conditions prevailing at present.

During April 2015, the SASS5 scores indicated downstream deterioration between site ZW1 (54) and ZW2 (43) (ASPT not applicable with low taxa richness) (Table 10). The most comparable biotope, namely vegetation, confirmed that conditions deteriorated between these two sites at the time of sampling. The fact that there were no flow at site ZW2 may have contributed to the poor biotic integrity due to evaporation resulting in further concentration of pollutants. It was promising to note that conditions improved towards site ZW4, although there was also no flow at this site during April 2015. Comparison of the SASS5 scores between site ZW1 and ZW4 confirms that conditions in fact improved in the Zaaiwater Spruit after flowing through the TFN complex (also indicated by toxicity testing).

Long-term trends in SASS5 scores (Figure 10) indicated similar temporal trends at sites ZW1 and ZW4, again confirming that the integrity in this reach is mostly driven by conditions upstream of the study area. Deterioration in the biotic integrity of this reach was evident between 2011 and 2013. Some improvement was noted in 2014 and a notable improvement was evident during the early 2015 survey (Figure 10).

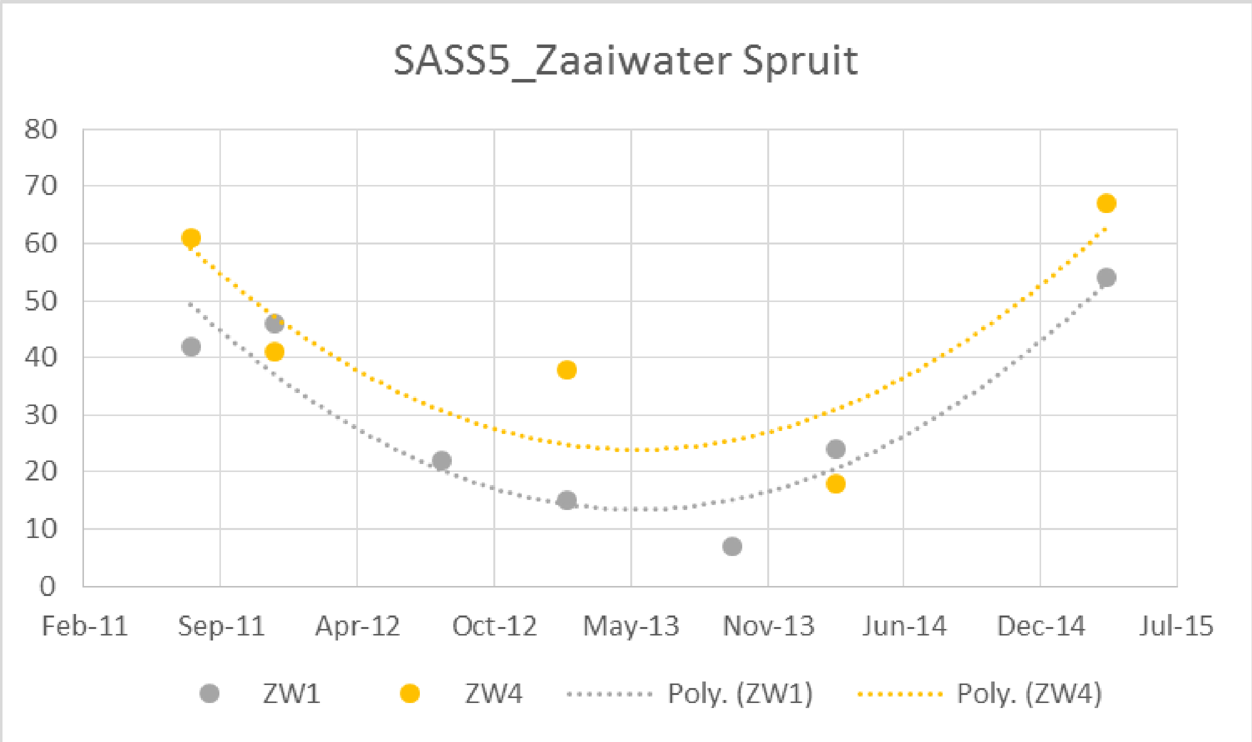

Figure 10: Long-term trends in SASS5 scores in the Zaaiwater Spruit.

Pan wetlands: SASSpan

The SASS5 protocol was designed for application in streams and rivers (lotic/flowing ecosystems) and not for standing/stagnant (lentic) ecosystems. It was however applied and adapted for use in the pan wetlands and referred to as SASSpan. The only purpose of this approach was to determine the aquatic invertebrate composition of the site, and to have a quantitative method for applying biomonitoring to measure changes over time, in terms of the biotic integrity of the site. The results gathered for Pans 1 to 5 is currently baseline information, since Tweefontein colliery has not commenced mining in this area. The information will therefore be valuable to benchmark and to measure future changes should mining take place in the area in future.

Farmers pan, Ephemeral pan, Pan 1, Pan 2, Pan 3 and 5 was dry at the time of sampling during April 2015. A total of 10 aquatic invertebrate taxa were sampled in the Boschmans pan and Makou pan (pan 4) during the April 2015 survey (Table 11). No taxa with a high or moderate requirement for unmodified water quality were sampled in the pans during the April 2015 survey (Table 11). Seven taxa observed in the pans of the study area have a low requirement for unmodified water quality while three have a very low requirement for unmodified water quality. The stones biotope was absent from all pans (as can be expected under natural conditions), with vegetation being the dominant habitat available for aquatic macroinvertebrates.

During April 2015 the SASSpan scores measured 36 at site Boschmans Pan and only 8at Makou Pan (pan 4) (Table 11).

SASSpan scores may be valuable indicators over the long term in determining whether the composition of the pans' macroinvertebrate assemblages change as a result of land use changes. The data gathered therefore contributes to baseline information gathered for the pans of the study area. Very limited information is available for the pans of the study area, and therefore no long-term (temporal) trends can be established at present. When analyzing the temporal data (Figure 4), it is evident that SASSpan scores have varied greatly between different surveys at all the pans. SASSpan scores are generally higher during the wet season than the dry season (as discussed above). The large variation in SASSpan scores observed at the pans is therefore a reflection of natural phenomenon, although the contribution of human impacts (mining and agriculture) is also expected to contribute to the variation. It is of some concern that most pans indicated a deteriorating long-term trend in SASSpan score (based on regression analyses) (Figure 11). The dry conditions prevailing in the study area for the latter part of the study area have contributed to the observed scenario, but water quality deterioration cannot be excluded as a possibility in some of the pans (such as Boschmans and Makou pan).

It must again be stressed that the use of aquatic macroinvertebrates as indicators of pan wetland biotic integrity is still in a testing phase, and the results of this assessment should therefore be viewed with circumspection.

Table 11: Aquatic macroinvertebrate taxa sampled at the different PAN sites and their relative requirement for unmodified water quality (April 2015).

Taxon	Boshman Pan				TFN Pan 4			
	Stones	Veg	GSM	Total	Stones	Veg	GSM	Total
Baetidae 1 sp.	-	B	-	B	-	-	-	-
Coenagrionidae	-	A	-	A	-	-	-	-
Libelludae	-	1	-	1	-	-	-	-
Corixidae*	-	-	-	-	-	-	B	B
Gerridae*	-	B	-	B	-	-	-	-
Notonectidae*	-	1	-	1	-	-	-	-
Pleidae*	-	A	-	A	-	-	-	-
Veliidae*	-	A	-	A	-	-	-	-
Hydrophilidae (adults*)	-	1	-	1	-	-	1	1
Chironomidae	-	1	B	B	-	-	-	-
SASSpan score	0	36	2	36	0	0	8	8
No. of families	0	9	1	9	0	0	2	2
ASPT	N/A	4.00	2.00	4.00	N/A	N/A	4.00	4.00

Key: High requirement for unmodified water quality; Moderate requirement for unmodified water quality; Low requirement for unmodified water quality; Very low requirement for unmodified water quality. Veg=Vegetation

A = 1-10 individuals; B = 11-100 individuals; C = 101-1000 individuals; ASPT = Average score per taxon.

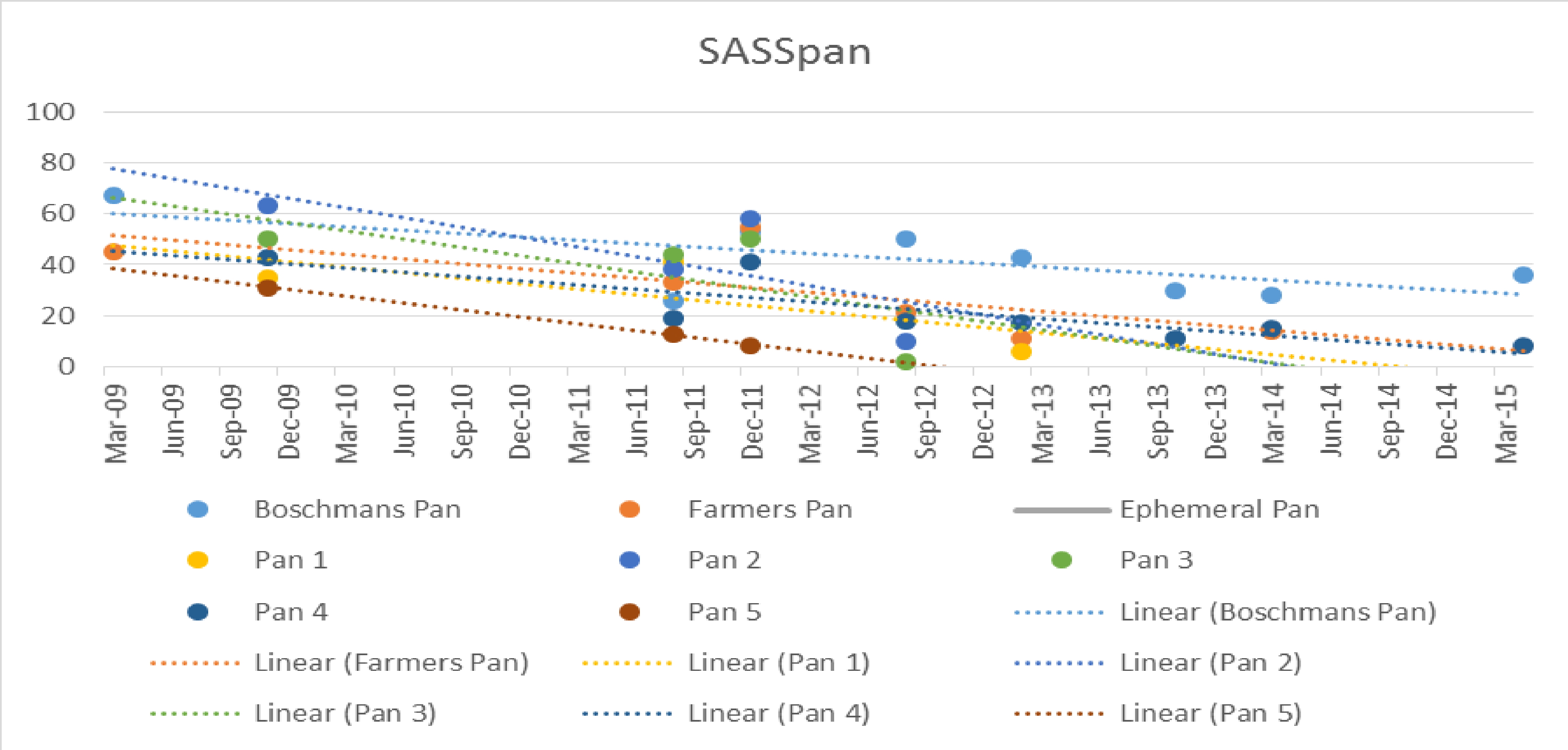

Figure 11: Long-term trends in SASSpan scores in the TFN study area.

5.4 Fish Assessment

Sites TFN-US2, TFN-DS and ZW2 were not suitable for fish assessment during the April 2015 survey.

Fish habitat assessment

The only biotopes available for fish in the Tweefontein Spruit and Zaaiwater Spruit during the April 2015 survey were slow-shallow and slow-deep biotopes (Table 12). The primary cover feature available for fish was provided in the form of macrophytes with limited overhanging vegetation and substrate. In general, habitat availability for fish was low to limiting for fish assemblages.

Table 12: Habitat availability for fish in terms of different velocity-depth classes and cover features (April 2015).

Sites	ZW1	ZW3	ZW4
SLOW-DEEP (>0.5m; <0.3m/s)			
Abundance	4	4	1
Overhanging vegetation	2	2	0
Undercut banks and Root-wads	0	0	0
Substrate	0	0	2
Macrophytes	4	4	0
SLOW-SHALLOW (<0.5m; <0.3m/s)			
Abundance	1	1	2
Overhanging vegetation	2	2	0
Undercut banks and Root-wads	0	0	0
Substrate	0	0	2
Macrophytes	2	3	0
FAST-DEEP (>0.3m; >0.3m/s)			
Abundance	0	0	0
Overhanging vegetation	0	0	0
Undercut banks and Root-wads	0	0	0
Substrate	0	0	0
Macrophytes	0	0	0
FAST-SHALLOW (<0.3m; >0.3m/s)			
Abundance	0	0	0
Overhanging vegetation	0	0	0
Undercut banks and Root-wads	0	0	0
Substrate	0	0	0
Macrophytes	0	0	0

0=Absent; **1**=Rare(<5%); **2**=Sparse(5-25%) **3**=Moderate(25-75%); 4=Extensive(>75)

Site-specific impacts on fish habitats were generally small to none with some moderate to large impacts also identified (Table 13). Channel modification (associated to bridges and weirs) and inundation (bridges and weirs) was the most notable impacts identified on site which could be limiting habitat conditions to the fish assemblage.

Table 13: Site-specific impacts on habitat for fish (April 2015).

	Sampling site		
Activity/Impact on habitat	**ZW1**	**ZW3**	**ZW-DS**
Water abstraction	0	0	0
Flow modification	0	0	0
Bed modification	1	0	0
Channel modification	3	0	0
Inundation	3	0	0
Exotic macrophytes	0	0	0
Solid waste disposal	1	0	0
Indigenous vegetation removal	1	0	0
Exotic vegetation enchroachment	1	0	0
Bank erosion	0	0	0

Key:

0 = no impact on fish habitat
1 = Small impact on fish habitat
2 = Moderate impact on fish habitat
3 = Large impact on fish habitats
4 = Serious impact on fish habitat
5 = Critical impact on fish habitat

Fish species composition

Based on all available information on fish surveys conducted in the study area during the period 2002 to 2015, five indigenous and three alien species were sampled in the Tweefontein Spruit and Zaaiwater Spruit reaches flowing through the Tweefontein Complex (Table 14). Only two indigenous fish species, namely the Southern mouthbrooder and Banded tilapia, were sampled in the Tweefontein Spruit reach (during 2009), while none of these were present during the 2011 to 2015 surveys (Table 14). No indigenous fish have been sampled in this stream since 2009 with the only fish species sampled since 2011 being the alien *Gambussia affinis* (Mosquito fish). The presence of the *G. affinis* is alarming as this species preys on the larvae of indigenous species and competes for food and habitat, having an overall negative impact on biotic integrity. The complete absence of any fish (indigenous and alien) during the 2014 survey was indicative of seriously deteriorated biotic integrity prevailing in this reach of the Tweefontein Spruit while no sampling could be performed during 2015.

Five indigenous fish species have been sampled in the Zaaiwater Spruit ecosystem based on all available information on fish surveys conducted in the study area during the period 2002 to 2015. It is also of concern that three alien fish species, namely the Common carp, Largemouth bass and Mosquito fish have also been sampled in this ecosystem. It was promising to note that after no indigenous fish species were sampled in the Zaaiwater Spruit during surveys conducted in 2009, all five species were again sampled during 2011 (Table 14). Four of these indigenous species were also sampled during the February 2013 survey but is alarming that only one indigenous species (*Barbus anoplus*) was present during the 2014 survey, and none during the 2015 survey. This, together with the presence of only two alien species during 2014 and 2015 is an indication that the Zaaiwater Spruit is in a very poor biotic condition at present.

Table 14: **Fish species sampled (year) at selected sites and overall for the Tweefontein Spruit and Zaaiwater Spruit ecosystem between 2002 and 2015.**

Species	Common Name	TFN-US	TFN-DS	ZW1	ZW-2	ZW 3	ZW4	Overall Tweefontein Complex Streams
Barbus anoplus	Chubbyhead Barb				2013	2002, 2011	2014	2002,2011, 2013, 2014
Barbus neefi	Sidespot Barb					2002, 2011, 2013		2002, 2011, 2013
Clarias gariepinus	Sharptooth catfish						2011	2011
Pseudocrenilabrus philander	Southern Mouthbrooder	2009			2013	2002, 2013	2011	2002, 2009, 2011, 2013
Tilapia sparrmanii	Banded tilapia	2009	2009			2002, 2013	2011	2002, 2009, 2011, 2013
*Cyprinus carpio**	Common carp					2002		2002
*Micropterus salmoides**	Largemouth bass						2014	2014
*Gambussia affinis**	Mosquito fish	2011	2009, 2013	2011	2013	2002, 2009, 2014, 2015	2009, 2014	2002, 2009, 2011, 2014, 2015
Number of indigenous species observed		**2**	**1**	**0**	**3**	**4**	**3**	**5**
Number of alien species		**1**	**1**	**1**	**0**	**2**	**2**	**3**
Number of red data listed species		**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Alien species

Based on the desktop PES-EIS update done for the entire Olifants River water management area (RFA, 2011), nine fish species can be expected to have occurred in the streams and rivers in the vicinity of the Tweefontein Complex (Table 15). It is estimated that at least five indigenous species occurred in the Tweefontein Spruit reach flowing through the Tweefontein complex (B11F-1257). It is of concern that none of these expected species have been sampled since 2009 indicating that they may have been lost from this reach. The Zaaiwater Spruit reach flowing through the Tweefontein complex (B11F-1286) had an estimated six indigenous fish species under natural conditions, of which five were sampled in 2011, four in 2013 and only one in 2014. The river reaches downstream of the Tweefontein Complex (B11F-1273-Tweefontein Spruit and B11G-1225-Olifants River) can be expected to have a higher fish species composition as a result of higher habitat diversity (especially in the Olifants River) (Table 15).

Table 15: Probability of occurrence of fish species in the rivers/streams of the study area

SQ reach code	B11F1257	B11F1286	B11F1273	B11G1225
Species \ Stream name	Tweefontein Spruit	Zaaiwater Spruit /Klippoortjiespruit	Tweefontein Spruit	Olifants River
BARBUS ANOPLUS	0	5	1	3
LABEOBARBUS MAREQUENSIS				3
BARBUS NEEFI	0	5	0	3
BARBUS PALUDINOSUS	0	0	1	3
LABEOBARBUS POLYLEPIS			0	3
CLARIAS GARIEPINUS		5	1	3
CHILOGLANIS PRETORIAE				0
PSEUDOCRENILABRUS PHILANDER	5	5	5	3
TILAPIA SPARRMANII	5	3	5	3

0 - Was RECORDED (or expected to be present under natural/pre-disturbance conditions), but likely absent now
1 = Present, low confidence. The spp has not been recorded in the SQ but based on the local spp "pool", the PES, the spp sensitivity and the SQ similarity to other SQs where the spp occurs (Level 2 ecoregion, Geomorphic zone, altitude and habitats available), is expected to be present.
3= Present, moderate confidence. The spp has not been recorded recently in the SQ, but based on the PES and spp sensitivity it is expected to be present. Where the general PES for the SQ has changed, there are still sections suitable for habitation by the spp.

5 = Present, high confidence. The spp has recently been recorded in the SQ. The PES has not changed to such extent that it would be expected to be absent.

The six indigenous fish species of concern expected under pre-disturbance conditions within the Tweefontein Complex study area is therefore the Chubbyhead barb (*Barbus anoplus*), Sidespot barb (*Barbus neefi*), Straightfin barb (*Barbus paludinosus*), Sharptooth catfish (*Clarias gariepinus*), Southern mouthbrooder (*Pseudocrenilabrus philander*) and Banded tilapia (*Tilapia sparrmanii*). These species are all widespread and common, and none of these species are threatened or near threatened, although *B. anoplus* is considered by provincial conservation authorities and selected national freshwater initiatives to potentially have an elevated conservation status[7] (see Table 16 and footnote). These species all have a preference for slow-shallow and slow-deep habitats, with vegetation (overhanging and aquatic) as the preferred cover feature (Table 17). All the expected species, apart from *Barbus neefi*, can be classified as tolerant to moderately tolerant to changes in the environment (Table 18). *Barbus neefi* with an overall moderately intolerance rating is sensitive to alterations in its trophic structure, habitat, water quality and flow (Table 18). This species was present at site ZW-3 during 2011 and 2013 but absent during the 2014 and 2015 surveys.

Table 16: **Indigenous fish species that can be expected in the Tweefontein and Zaaiwater Spruit ecosystems under pre-disturbed (reference) conditions**

ABBREVIATION	SCIENTIFIC NAME	ENGLISH COMMON NAME	CONSERVATION STATUS
BANO	*BARBUS ANOPLUS* (WEBER, 1897)	CHUBBYHEAD BARB	Widespread and common / Data deficient (taxonomy)
BNEE	*BARBUS NEEFI* (GREENWOOD, 1962)	SIDESPOT BARB	Locally common
BPAU	*BARBUS PALUDINOSUS* (PETERS, 1852)	STRAIGHTFIN BARB	Common
CGAR	*CLARIAS GARIEPINUS (BURCHELL, 1822)*	SHARPTOOTH CATFISH	Common
PPHI	*PSEUDOCRENILABRUS PHILANDER* (WEBER, 1897)	SOUTHERN MOUTHBROODER	Common
TSPA	*TILAPIA SPARRMANII* (SMITH, 1840)	BANDED TILAPIA	Common

[7] Based on International conservation criteria (IUCN red list of threatened species 2014), this species is currently listed as "Least Concern", meaning "evaluated and did not qualify for any other category". The IUCN report includes the following notes on this species:

- "The species complex is widespread with no immediate threats."
- "The *Barbus anoplus* complex is currently under revision and is likely to result in synonymised species being resurrected and others described."
- "If the current taxonomic study confirms that there are separate species, the assessment as LC may need revision in some cases."

This species has however recently been indicated by Mpumalanga Tourism and Parkas Association (MTPA) to be "critically endangered" while the NFEPA project listed this species as "endangered". The author is however of the opinion that this species is widespread and common in the study area (and many areas of Mpumalanga) and that it should rather be classified as Data deficient (taxonomy) until such time as its taxonomy has been reviewed and specific populations, sub-populations, species or sub-species of concern have been identified.

Table 17: **Habitat preferences (flow-depth and cover features) of the expected fish species (Kleynhans, 2003).**

ABBREVIATION	SLOW-DEEP (<0.3 m/s; >0.5 m)	SLOW-SHALLOW (<0.3 m/s; <0.5 m)	FAST-DEEP (>0.3 m/s; >0.3 m)	FAST-SHALLOW (>0.3 m/s; <0.3 m)	OVERHANGING VEGETATION	BANK UNDERCUT	SUBSTRATE	AQUATIC MACROPHYTES	WATER COLUMN
BANO	4.1	4.3	0.9	2.5	4	2.7	2.3	3.2	1.1
BNEE	3.3	4.7	1	1.7	3.9	3.3	4.4	0.5	0.2
BPAU	3.9	3.9	2.2	2.6	4.2	2.4	1.9	3.6	3.5
CGAR	4.3	3.4	1.2	0.8	2.8	2.9	2.8	3	2.6
PPHI	2.6	4.3	0.5	0.9	4.5	3.2	1.9	2.9	0.3
TSPA	3	4.3	0.9	1.5	4.5	1.9	2.5	3.6	1.1

0 = NO PREFERENCE, IRRELEVANT
>0 -0.9 = VERY LOW PREFERENCE -COINCIDENTAL?
>1-1.9 = LOW PREFERENCE
>2-2.9 =MODERATE PREFERENCE
>3-3.9 =HIGH PREFERENCE
>4-5 =VERY HIGH PREFERENCE

Table 18: Relative intolerance ratings of expected fish species (Kleynhans, 2003)

ABBREVIATION	CRITERIA				
	TROPHIC SPECIALIZATION	HABITAT SPECIALIZATION	FLOW REQUIREMENT	REQUIREMENT: UNMODIFIED WATER QUALITY	AVERAGE OVERALL INTOLERANCE RATING
BANO	2.8	2.8	2.3	2.6	2.6
BNEE	3.3	3.4	3.4	3.4	3.4
BPAU	1.6	1.4	2.3	1.8	1.8
CGAR	1	1.2	1.7	1	1.2
PPHI	1.3	1.4	1	1.4	1.3
TSPA	1.6	1.4	0.9	1.4	1.3

1-1.9 = TOLERANT
>2-2.9 = MODERATELY TOLERANT
>3-3.9 = MODERATELY INTOLERANT
>4-5.0 = INTOLERANT

It is expected that most of the pans in the study area may naturally have no fish species present. The absence of fish from pans is often associated with low dissolved oxygen concentrations, as well as the seasonal nature of most pans. Water bodies containing a high plant biomass experience large diurnal fluctuations in dissolved oxygen concentrations and pH due to the alternating processes of photosynthesis and respiration. These large fluctuations will prevent the establishment of a population of all but the most tolerant fish.

Fish Response Assessment Index (FRAI)

The present ecological status (PES) or biotic integrity, based on fish, of the study area was determined through the application of the Fish Response Assessment Index (FRAI) (Kleynhans, 2008). It provides an indication of the present status of the fish assemblage, in relation to what could be expected under natural or unmodified conditions. The present status of the fish assemblage of this river reach was based on all information gathered during this survey, both in terms of fish sampled and derived from available information (such as habitat availability, local input, etc.). The FRAI is designed for application to river reaches, and not per sampling site, and was therefore calculated considering all spatial and temporal available information. The “frequency of occurrence” metrics were therefore based on the frequency of occurrence of a species in the reach (all sites considered) and over time (all surveys considered).

Tweefontein Spruit

The much lower than expected fish species diversity observed at the Tweefontein Spruit biomonitoring sites is a clear indication of poor biotic integrity, based on fish, currently prevailing in this system (Table 22). This was reflected by a very low FRAI score of 0%, falling into descriptive category F (Critically modified) (Table 20). It is evident from the FRAI calculation that all metrics considered (velocity-depth classes, cover, flow dependence, water quality, migration and alien species) have been altered to some or other extent. It is therefore evident that the current poor condition of the fish assemblage cannot be attributed to a single impact, but is related to long-term exposure to various impacts. It is therefore evident that both Tweefontein Complex and non-Tweefontein-Complex activities have contributed to the present status of the fish assemblage in the Tweefontein Spruit. Aspects of concern specifically associated with Tweefontein complex are as follows:

- Flow modification by dams (Tweefontein Dam) and weirs (measuring weir) and abstraction reduce flow in the river, and hence habitat suitability and availability for fish. These and other structures (road crossings) create migration barriers for fish, limiting their natural distribution ranges and migration activity.
- Water quality deterioration especially through agricultural runoff and mining activities will negatively impact on various species and different life-stages of a species. Poor water quality may also act as chemical migration barriers preventing the natural movement of fish.
- Presence of alien fish species: alien fish species compete with indigenous species for food and habitat, and predacious species can eradicate indigenous fish species from an area while some species transform and disturb habitats (especially breeding habitats). The presence of alien species in the study area is not thought to be related to Tweefontein Complex mining area, and the mine should not allow any stocking of alien species on its property.

Table 19: Estimated frequency of occurrence of indigenous fish species under reference and present conditions in the Tweefontein Spruit

ABBREVIATIONS: REFERENCE SPECIES	SCIENTIFIC NAMES: REFERENCE SPECIES	REFERENCE CONDITION (EXPECTED) FREQUENCY OF OCCURRENCE	PRESENT FREQUENCY OF OCCURRENCE: PES
BANO	BARBUS ANOPLUS WEBER, 1897	4	0
BNEE	BARBUS NEEFI GREENWOOD, 1962	2	0
BPAU	BARBUS PALUDINOSUS PETERS, 1852	3	0
PPHI	PSEUDOCRENILABRUS PHILANDER (WEBER, 1897)	5	0
TSPA	TILAPIA SPARRMANII SMITH, 1840	5	0

0=ABSENT
1=PRESENT AT VERY FEW SITES AND/OR DURING VERY FEW SURVEYS (<10%)
2=PRESENT AT FEW SITES AND/OR DURING FEW SURVEYS (>10-25%)
3=PRESENT AT ABOUT >25 TO 50% OF THE SITES AND/OR DURING >25 TO 50% OF THE SURVEYS
4=PRESENT AT MOST SITES AND/OR DURING MOST SURVEYS (>50- 75%)
5=PRESENT AT ALMOST ALL SITES AND/OR DURING ALL SURVEYS (>75%)

Table 20: Fish Response Assessment Index (FRAI) results for the Tweefontein Spruit reach.

METRIC GROUP	METRIC	*RATING (CHANGE)	METRIC GROUP WEIGHT (%)
VELOCITY-DEPTH CLASSES METRICS	Response of species with high to very high preference for FAST-DEEP conditions	0	97
	Response of species with high to very high preference for FAST-SHALLOW conditions	0	
	Response of species with high to very high preference for SLOW-DEEP conditions	-5	
	Response of species with high to very high preference for SLOW-SHALLOW conditions	-5	
COVER METRICS	Response of species with a very high to high preference for overhanging vegetation	-5	100
	Response of species with a very high to high preference for undercut banks and root wads	-5	
	Response of species with a high to very high preference for a particular substrate type	-5	
	Response of species with a high to very high preference for instream vegetation	-5	
	Response of species with a very high to high preference for the water column	-5	
FLOW DEPENDANCE METRICS	Response of species intolerant of no-flow conditions	0	76
	Response of species moderately intolerant of no-flow conditions	-5	
	Response of species moderately tolerant of no-flow conditions	-5	
	Response of species tolerant of no-flow conditions	-5	
PHYSICO-CHEMICAL METRICS	Response of species intolerant of modified physico-chemical conditions	0	69
	Response of species moderately intolerant of modified physico-chemical conditions	-5	
	Response of species moderately tolerant of modified physico-chemical conditions	-5	
	Response of species tolerant of modified physico-chemical conditions	-5	
MIGRATION METRICS	Response in terms of distribution/abundance of spp with catchment scale movements	n/a	47
	Response in terms of distribution/abundance of spp with requirement for movement between reaches or fish habitat segments	5.0	
	Response in terms of distribution/abundance of spp with requirement for movement within reach or fish habitat segment	5.0	
INTRODUCED SPECIES METRICS	The impact/potential impact of introduced competing/predaceous spp?	0	63
	How widespread (frequency of occurrence) are introduced competing/predaceous spp?	0	
	The impact/potential impact of introduced habitat modifying spp?	n/a	
	How widespread (frequency of occurrence) are habitat modifying spp?	n/a	
FRAI SCORE (%)		**0**	
FRAI CATEGORY		**F**	
FRAI CATEGORY DESCRIPTION		**Critically modified**	

*GUIDELINES FOR RATING/CHANGE (0-->5)
-5=Extreme loss from reference (absent); -4=Serious loss from reference; -3=Large loss from reference; -2=Moderate loss from reference, -1= Small loss from reference; 0=No change from reference; 1= Small increase from reference; 2=Moderate increase from reference; 3=Large increase from reference; 4=Serious increase from reference; 5=Extreme increase from reference (completely dominant).

Zaaiwater Spruit

The fact that no indigenous fish and only one alien species was sampled during the April 2015 survey in the Zaaiwater Spruit is indicative of highly deteriorated biotic integrity, based on fish, in this system. There is a strong possibility that no indigenous fish species occur in this reach due to the current level of flow and water quality modification (Table 21). Due to the absence of indigenous species and the presence of only one alien fish species in April 2015, a very low FRAI score of 2.1% was calculated, placing this reach it in a descriptive category F (critically modified) (Table 22). Similar to what was observed in the Tweefontein Spruit, the current status of the fish in the Zaaiwater Spruit is also a reflection of long-term exposure to various impacts, potentially by both Tweefontein Complex and non- Tweefontein-Complex activities. Aspects of concern are similar to those described above for the Tweefontein Spruit.

Table 21: Estimated frequency of occurrence of indigenous fish species under reference and present conditions in the Zaaiwater Spruit

ABBREVIATIONS: REFERENCE SPECIES	SCIENTIFIC NAMES: REFERENCE SPECIES	REFERENCE CONDITION (EXPECTED) FREQUENCY OF OCCURRENCE	PRESENT FREQUENCY OF OCCURRENCE: PES
BANO	BARBUS ANOPLUS WEBER, 1897	4	0
BNEE	BARBUS NEEFI GREENWOOD, 1962	2	0
BPAU	BARBUS PALUDINOSUS PETERS, 1852	3	0
CGAR	CLARIAS GARIEPINUS (BURCHELL, 1822)	1	0
PPHI	PSEUDOCRENILABRUS PHILANDER (WEBER, 1897)	5	0
TSPA	TILAPIA SPARRMANII SMITH, 1840	4	0

Table 22: Fish Response Assessment Index (FRAI) results for the Zaaiwater Spruit Reach.

METRIC GROUP	METRIC	*RATING (CHANGE)	METRIC GROUP WEIGHT (%)
VELOCITY-DEPTH CLASSES METRICS	Response of species with high to very high preference for FAST-DEEP conditions	0.0	97
	Response of species with high to very high preference for FAST-SHALLOW conditions	0.0	
	Response of species with high to very high preference for SLOW-DEEP conditions	-5.0	
	Response of species with high to very high preference for SLOW-SHALLOW conditions	-5.0	
COVER METRICS	Response of species with a very high to high preference for overhanging vegetation	-5.0	100
	Response of species with a very high to high preference for undercut banks and root wads	-5.0	
	Response of species with a high to very high preference for a particular substrate type	-5.0	
	Response of species with a high to very high preference for instream vegetation	-5.0	
	Response of species with a very high to high preference for the water column	-5.0	
FLOW DEPE NDAN CE METRI CS	Response of species intolerant of no-flow conditions	0.0	76
	Response of species moderately intolerant of no-flow conditions	-5.0	
	Response of species moderately tolerant of no-flow conditions	-5.0	
	Response of species tolerant of no-flow conditions	-5.0	
PHYSICO-CHEMICAL METRICS	Response of species intolerant of modified physico-chemical conditions	0.0	69
	Response of species moderately intolerant of modified physico-chemical conditions	-5.0	
	Response of species moderately tolerant of modified physico-chemical conditions	-5.0	
	Response of species tolerant of modified physico-chemical conditions	-5.0	
MIGRATI ON METRIC S	Response in terms of distribution/abundance of spp with catchment scale movements	n/a	47
	Response in terms of distribution/abundance of spp with requirement for movement between reaches or fish habitat segments	2.0	
	Response in terms of distribution/abundance of spp with requirement	1.0	

	for movement within reach or fish habitat segment		
INTRODUCED SPECIES METRICS	The impact/potential impact of introduced competing/predaceous spp?	4.0	63
	How widespread (frequency of occurrence) are introduced competing/predaceous spp?	4.0	
	The impact/potential impact of introduced habitat modifying spp?	0	
	How widespread (frequency of occurrence) are habitat modifying spp?	0	
FRAI SCORE (%)		**2.1**	
FRAI CATEGORY		**F**	
FRAI CATEGORY DESCRIPTION		**Critically modified**	

*GUIDELINES FOR RATING/CHANGE (0-->5)
-5=Extreme loss from reference (absent); -4=Serious loss from reference; -3=Large loss from reference; -2=Moderate loss from reference, -1= Small loss from reference; 0=No change from reference; 1= Small increase from reference; 2=Moderate increase from reference; 3=Large increase from reference; 4=Serious increase from reference; 5=Extreme increase from reference (completely dominant).

6. CONCLUSIONS & RECOMMENDATIONS

The following conclusions were drawn from the April 2015 biomonitoring survey at Tweefontein complex, with reference to long-term trends where applicable:

Tweefontein Spruit catchment:

- Due to the lack of flow during the April 2015 survey selected biomonitoring protocols could not be applied at the biomonitoring sites.
- As observed during most previous surveys, 2 Seam SPP again indicated high chronic/acute toxicity hazard (Class IV) during April 2015. Based on the definitive testing done for this sample, the safe dilution factor was estimated at very high dilution of 3%.
- It was however promising to note that although some potential sources of toxicity risk (2-seam SPP) was present in the Tweefontein Spruit catchment between sites TFN-US and TFN-DS, the toxicity hazard class again remained the same (Class I - no acute/chronic hazard) at these sites, indicating that these potential sources did not cause an increase in toxicity hazard of the receiving water body.
- The electrical conductivity (EC) measured considerably high at the downstream site TFN-DS (346 mS/m), indicating that potential sources of high salinity reached this area of the Tweefontein Spruit at the time of sampling. Since there was no flow at the upstream site, the highly saline water reaching the downstream site may be originating from the TFN Complex and should be further investigated by the environmental department.
- The composition of mostly tolerant invertebrates as well as a relatively low SASS5 score of 36 indicates that the biotic integrity are poor in the lower Tweefontein Spruit (TFN-DS).
- Long-term SASS5 trends indicate that condition at site TFN-US deteriorated between 2011 and 2013, after which a slight improvement was noted. At site TFN-DS, conditions improved over the period 2011 to 2012, after which a notable deterioration occurred towards 2013. A slight improvement has however been noted during the 2014 and early 2015 survey.
- No indigenous fish have been sampled in this stream since 2009 with the only fish species sampled since 2011 being the alien *Gambussia affinis* (Mosquito fish). The complete absence of any fish (indigenous and alien) during the 2014 survey was indicative of seriously deteriorated biotic integrity prevailing in this reach of the Tweefontein Spruit while no sampling could be performed during 2015. Based on the latest available information the FRAI score for this reach of the Tweefontein Spruit is therefore 0%, falling into a descriptive category F (Critically modified).

Zaaiwater Spruit:

- In the Zaaiwater Spruit catchment, it was important to note that the toxicity assessment classified site ZW1 in a Class III (acute hazard), indicating that water flowing into the Tweefontein complex study area already had some toxicity hazard at the time of sampling. This is an indication that activities upstream of the Tweefontein Complex were responsible for water quality deterioration in the Zaaiwater Spruit at the time of sampling.

- The streams entering the Zaaiwater Spruit from the south-east within this reach (South Witbank Stream, Alpha Stream) also indicated slight toxicity hazard (Class II) and therefore pointed at non-Tweefontein activities also contributing to the toxicity hazard of this reach of the Zaaiwater Spruit.
- It was promising to note that although these streams contributed water with slight toxicity hazard to the Zaaiwater Spruit, the toxicity hazard decreased downstream after flowing through the TNC complex, falling in a Class I (no hazard) at sites ZW3 and ZW4.
- The EC increased notably between sites ZW1 and ZW 3 (sites ZW2 dry) during the April 2015 survey indicating potential sources of pollution entering the Zaaiwater Spruit in this reach. The South Witbank Stream (75.7 mS/m) and especially the Alpha stream (250 mS/m) had relatively high EC levels, and therefore may have contributed to this observed increase in salinity. This is therefore again an indication that non-TFN activities may have been responsible for this observed salinization, although it is recommended that TFN complex further investigate if they possibly contribute to this observed scenario. It was promising to not the EC decreased towards site ZW4 and hence no further sources of salts reached the Zaaiwater Spruit in the lower section.
- The pH level of 5.08 measured on site at site ZW1 exceeded the target water quality ranges again indicated that water of poor quality is flowing into the TFN complex study area due to upstream land-use activities.
- During April 2015, the total SASS5 scores as well as most comparative biotope scores indicated downstream deterioration between site ZW1 (54) and ZW2 (43). The fact that there were no flow at site ZW2 may have contributed to the poor biotic integrity due to evaporation resulting in further concentration of pollutants. It was promising to note that conditions improved towards site ZW4, although there was also no flow at this site during April 2015. Comparison of the SASS5 scores between site ZW1 and ZW4 confirms that conditions in fact improved in the Zaaiwater Spruit after flowing through the TFN complex (also indicated by toxicity testing).
- Long-term trends in SASS5 scores indicated similar temporal trends at sites ZW1 and ZW4, again confirming that the integrity in this reach is mostly driven by conditions upstream of the study area. Deterioration in the biotic integrity of this reach was evident between 2011 and 2013. Some improvement was noted in 2014 and a notable improvement was evident during the early 2015 survey.
- The fact that no indigenous fish and only one alien species was sampled during the April 2015 survey in the Zaaiwater Spruit is indicative of highly deteriorated biotic integrity, based on fish, in this system. There is a strong possibility that no indigenous fish species occur in this reach due to the current level of flow and water quality modification. Due to the absence of indigenous species and the presence of only one alien fish species in April 2015, a very low FRAI score of 2.1% was calculated, placing this reach it in a descriptive category F (critically modified).

Pan wetlands:

- Due to the dry conditions in the study area, most of the pans were still dry or unsuitable for monitoring during April 2015.
- The EC levels in the Boschmans Pan measured very high (515 mS/m) during April 2015 The pH level of this two pans was within general guideline levels for aquatic ecosystems but the very low dissolved oxygen level of 2.31 mg/l exceeded the guideline level and may be limiting to the biotic integrity of this aquatic ecosystem. Due to the fact that the natural cycles of these pans have been altered, it can be expected that the aquatic fauna would have been altered from their natural state.
- As observed during most previous surveys, the EC of Pan 4 (1493mS/m) was also again very high during the April 2015 survey. Some mining activity (non Tweefontein Complex) is evident to the north east of this pan and potentially negative impacts on the water quality due to current mining cannot be excluded. The pH (8.85) and dissolved oxygen

levels of Pan 4 were within general guideline levels for aquatic ecosystems during the April 2015 survey.

- A total of 10 aquatic invertebrate taxa were sampled in the Boschmans pan and Makou pan (pan 4) during the April 2015 survey. During April 2015 the SASSpan scores measured 36 at site Boschmans Pan and only 8 at Makou Pan (pan 4). When analyzing the temporal data, it is evident that SASSpan scores have varied greatly between different surveys at all the pans. SASSpan scores are generally higher during the wet season than the dry season (as discussed above). The large variation in SASSpan scores observed at the pans is therefore a reflection of natural phenomenon, although the contribution of human impacts (mining and agriculture) is also expected to contribute to the variation. It is of some concern that most pans indicated a deteriorating long-term trend in SASSpan score (based on regression analyses). The dry conditions prevailing in the study area for the latter part of the study area have contributed to the observed scenario, but water quality deterioration cannot be excluded as a possibility in some of the pans (such as Boschmans and Makou pan).

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APPENDIX 1: Materials and Methods

1. *In-situ* Water quality

A Hach HQ40d Multimeter was used to measure the following parameters:

- Dissolved oxygen levels (mg/l) and saturation (%)
- Electrical conductivity (EC) in µS/m
- pH
- Water temperature (°C)

2. Toxicity testing

Toxicity testing (as applied in this biomonitoring programme) is applied by exposing biota to water sources in order to determine the potential risk of such waters to the biota/biological integrity. Consequently a range of biota, namely bacteria (*Vibrio fischeri*), micro-algae (*Selenastrum capricornutum*), fish (guppies, *Poecilia reticulata*) and invertebrates (Water flea, *Daphnia magna*) were exposed to selected samples, according to standard procedures under laboratory conditions. This information was then applied to determine a risk category based on the percentage of mortalities/growth inhibition of the exposed biota. This risk category (hazard classification) equates to the potential risk posed by the water of a specific source should it be released or come into contact with the biota of the receiving water bodies.

Test conditions

All tests were conducted in environmental controlled rooms using the following internationally standardized methods:

<u>Vibrio fischeri bioluminescent test</u>

Standard method:	EN ISO 11348-3, 1998
Deviation from standard method:	None
Test species:	*Vibrio fischeri* (NRRL B-11177)
Exposure period:	15 and 30 minutes
Test sample volume:	500 ul
Measurement equipment:	Luminoscan TL, Hygiene Monitoring System
Test endpoint:	Screening test - % growth inhibition or stimulation relative to control
Statistical method used:	EXCEL spreadsheet

<u>Selenastrum capricornutum growth inhibition test</u>

Standard method:	OECD Guideline 201, 1984
Deviation from standard method:	None
Test species:	*Selenastrum capricornutum*, Printz (CCAP 278/4 Cambridge, UK)
Exposure period:	72h
Test sample volume:	25 ml
Test chamber type:	10 cm long cell
Test temperature:	21-25°C
Measurement equipment:	Jenway 6300 spectrophotomenter
Test endpoint:	Screening test - % growth inhibition or stimulation relative to control
Statistical method used:	EXCEL spreadsheet

<u>*Daphnia magna* acute toxicity test</u>

Standard method:	US EPA, 1993
Deviation from standard method:	None
Test species:	*Daphnia magna*

Test species age:	Less than 24h old
Exposure period:	24 and 48h
Test sample volume:	25 ml
Number of test organisms per beaker:	12
Replicate number beakers per sample:	4
Test temperature:	21±2°C
Test endpoint: Screening test -	%mortality
Statistical method used:	EXCEL spreadsheet

Poecilia reticulata **acute toxicity test**

Standard method:	US EPA, 1996
Deviation from standard method:	None
Test species:	*Poecilia reticulata*
Test species age:	Less than 21 days
Exposure period:	96h
Test sample volume:	200 ml
Number of test organisms per beaker:	12
Replicate number beakers per sample:	2
Test temperature:	21±2°C
Test endpoint: Screening test -	%mortality
Statistical method used:	EXCEL spreadsheet

Toxicity test results classification systems

Criteria, as suggested by the Direct Estimation of the Ecological Effect Potential (DEEEP[⇓]) approach (DWAF, 2003), for the ecological hazard assessment for discharges, has been based on criteria provided for the TEM method by RIZA in the Netherlands. After the determination of the percentage effect (EP), obtained with each of the ***battery of toxicity screening*** tests performed, the sample is ranked into one of the following five classes:

After the determination of the percentage effect[8] (EP), obtained with each of the ***battery of toxicity screening*** tests performed, the sample is ranked into one of the following five classes:

Class I	**No acute hazard** - none of the tests shows a toxic effect.
Class II	**Slight acute hazard –** a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%.
Class III	**Acute hazard –** the percentage effect level is reached or exceeded in at least one test, but the effect level is below 100%.
Class IV	**High acute hazard** – the 100% percentage effect is reached in at least one test.
Class V	**Very high acute hazard –** the 100% percentage effect is reached in all the tests.

[⇓] DEEEP = Direct Estimation of Ecological Effect Potential. This is a battery of tests that can measure toxicity of complex mixtures based on a set of parameters stemming from the results of effects, even if all constituents are not known. Consequently a hazard class is determined based on the resulting parameters of the battery of tests.

[8] EP (Percentage effect) = an effect measured either as a mortality rate or inhibition rate (depending on the type of test). A 10% effect is regarded as a slight acute toxicity for daphnia and guppies, while a 20% effect is regarded as a slight acute toxicity for algae and bacteria (vibrio). A 50% effect is regarded as an acute toxicity for all of the tests (daphnia, guppies, algae and bacteria)

Toxicity classification system definitive tests (undiluted samples plus range of dilutions)

The samples are classified into one of the following five classes on the basis of the highest toxicity unit (TUa) found in the ***battery of toxicity definitive tests*** performed. The toxicity unit is a function of the L(E)C50, where (TUa) = 100/L(E)C50. The 50% Lethal/Effective concentration (LC50 or LE50) is the linear calculated (derived) concentration at which a 50% mortality or inhibition rate can be expected. Hence, the lower this value is, the higher the acute toxicity level. Conversely, the higher the toxicity unit (TUa) is, the higher the acute toxicity level is. The conversion of L(E)C50 values to TUa values are therefore merely done to achieve a classification scale of increasing values related to increasing toxicity risks:

Class I	**No acute hazard** - none of the tests shows a toxic effect.
Class II	**Slight acute hazard –** the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is blow 50% (TUa is <1).
Class III	**Acute hazard** – the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is below 50% (TUa is between 1 and 10).
Class IV	**High acute hazard –** the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution (TUa is between 10 and 100).
Class V	**Very high acute hazard –** the L(E)C50 is reached in the 100 fold dilution for at least one test (TUa is >100).

Weighting: Each sample was furthermore weighted according to its level of toxicity (no acute hazard to very high acute hazard) observed for each different test performed. If none of the tests detected any toxicity, the sample would have a weight of 0%, while very high acute hazards detected by all tests will result in a weight of 100%, with a sliding scale for any variation of these extremes. The weighting system therefore provides a measure to compare relative toxicity on a scale between 0 and 100, and toxicity hazards can therefore be compared between samples that fall within the same class.

3. Aquatic invertebrate assessment: South African Scoring System, Version 5.

Benthic macro-invertebrate communities of the selected sites were investigated according to the South African Scoring System, version 5 (SASS5) approach (Dickens & Graham, 2001). This method is based on the British Biological Monitoring Working Party (BMWP) method and has been adapted for South African conditions by Dr. F. M. Chutter (Thirion *et al.*, 1995). The SASS method is a rapid, simple and cost effective method, which has progressed through four different upgrades/versions. The current upgrade is Version 5, which is specifically designed to comply with international accreditation protocols.

<u>Sample Collection</u>

An invertebrate net (30 x 30cm square with 1mm mesh netting) was used for the collection of the organisms. The available biotopes at each site were identified on arrival. Each of the biotopes was sampled by different methods explained later (samples should not be collected when the river is in flood).

The biotopes were combined into three different groups, which were sampled and assessed separately:

A) Stone (S) Biotopes:

Stones in current (SIC) or any solid object: *Movable stones of at least cobble size (3 cm diameter) to approximately 20 cm in diameter, within the fast and slow flowing sections of the river.* Kicksampling is used to collect organisms in this biotope. This is done by putting the net on the bottom of the river, just downstream of the stones to be kicked, in a position where the current will carry the dislodged organisms into the net. The stones are then kicked over and against each other to dislodge the invertebrates (kicksampling) for ± 2 minutes.

Stones out of current (SOOC): *Where the river is still, such as behind a sandbank or ridge of stones or in backwaters.* Collection is again done by the method of kicksampling, but in this case the net is swept across the area sampled to catch the dislodged biota. Approximately 1 m^2 is sampled in this way.

Bedrock or other solid substrate: Bedrock includes stones greater than 30cm, which are generally immovable, including large sheets of rock, waterfalls and chutes. The surfaces are scraped with a boot or hand and the dislodged organisms collected. Sampling effort is included under SIC and SOOC above.

B) Vegetation (VG) Biotopes:

Marginal vegetation (MV): *This is the overhanging grasses, bushes, twigs and reeds growing on the edge of the stream, often emergent, both in current (MvegIC) and out of current (MvegOOC).* Sampling is done by holding the net perpendicular to the vegetation (half in and half out of the water) and sweeping back and forth in the vegetation (± 2m of vegetation).

Submerged vegetation (AQV): *This vegetation is totally submerged and includes Filamentous algae and the roots of floating aquatics such as water hyacinth.* Sampled by pushing the net (under the water) against and amongst the vegetation in an area of approximately one square meter.

C) Gravel, Sand and Mud (GSM) biotopes:

Sand: *This includes sandbanks within the river, small patches of sand in hollows at the side of the river or sand between the stones at the side of the river.* This biotope is sampled by stirring the substrate by shuffling or scraping of the feet, which is done for half a minute, whilst the net is continuously swept over the disturbed area.

Gravel: *Gravel typically consists of smaller stones (2-3 mm up to 3 cm).* Sampling similar to that of sand.

Mud: *It consists of very fine particles, usually as dark-collared sediment.* Mud usually settles to the bottom in still or slow flowing areas of the river. Sampling similar to that of sand.

D) Hand picking and visual observation:

Before and after disturbing the site, approximately 1 minute of “hand-picking” for specimens that may have been missed by the sampling procedures was carried out.

Sample preparation

The organisms sampled in each biotope group were identified and their relative abundance also noted on the SASS5 datasheet.

SASS-Habitat Assessment

A SASS-habitat assessment index, according to the habitats sampled, was performed due to the fact that changes in habitat can be responsible for changes in SASS5 scores. This was done by the application of Integrated Habitat Assessment System (IHAS version 2) (McMillan, 1998). Suitability scores, ranging between 0 (unsuitable) to 5 (highly suitable) were also given to the different biotopes (Stones-in-current, stones-out-of-current, bedrock, aquatic vegetation, marginal vegetation in-current and out-of-current, gravel, sand and mud) to assist in the habitat evaluation process for each site.

Fish assessment

Fish Response Assessment Index (FRAI)

Fish sampling was performed at each site using a SAMUS electrofisher. All representative habitat types (biotopes) were sampled to gain a representative fish sample of the site. All fish were identified up to species level and returned to the river.

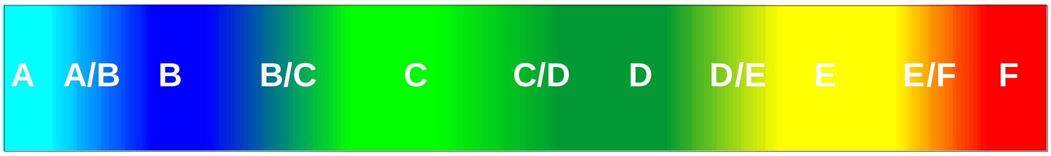
The determination and description of the present ecological status (PES) of the aquatic ecosystems in the study area, in terms of fish, was done according to the methodology described for River EcoClassification during Reserve Determinations (Kleynhans & Louw, 2008) using the Fish Response Assessment Index (FRAI) (Kleynhans, 2008). The results were then used to classify the present state of the fish assemblage into a specific descriptive category (A to F) (Table A1).

The FRAI is not in its conventional form designed for the application per site, but rather to a reach with a few sites. Metrics are therefore based on spatial frequency of occurrence of a species within the reach.

Table A1: Descriptive categories used to describe the present ecological status (PES) of biotic components (adapted from Kleynhans, 1999).

CATEGORY	BIOTIC INTEGRITY	DESCRIPTION OF GENERALLY EXPECTED CONDITIONS
A	Excellent	Unmodified, or approximates natural conditions closely. The biotic assemblages compares to that expected under natural, unperturbed conditions.
B	Good	Largely natural with few modifications. A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modifications. Most aspects of the biotic assemblage as expected under natural unperturbed conditions.
C	Fair	Moderately modified. A lower than expected species richness and presence of most intolerant species. Most of the characteristics of the biotic assemblages have been moderately modified from its naturally expected condition. Some impairment of health may be evident at the lower end of this class.
D	Poor	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant and moderately intolerant species. Most characteristics of the biotic assemblages have been largely modified from its naturally expected condition. Impairment of health may become evident at the lower end of this class.
E	Very Poor	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant and moderately tolerant species. Most of the characteristics of the biotic assemblages have been seriously modified from its naturally expected condition. Impairment of health may become very evident.
F	Critical	Critically modified. Extremely lowered species richness and an absence of intolerant and moderately tolerant species. Only intolerant species may be present with complete loss of species at the lower end of the class. Most of the characteristics of the biotic assemblages have been critically modified from its naturally expected conditions. Impairment of health generally very evident.

It must be emphasized that the A→F scale represents a continuum, and that the boundaries between categories are notional, artificially-defined points along the continuum (as presented below). This situation falls within the concept of a fuzzy boundary, where a particular entity may potentially have membership of both classes (Robertson *et al*. 2004). For practical purposes, these situations are referred to as boundary categories and are denoted as B/C, C/D, and so on.



APPENDIX 2: Photographic views of sampling sites.

Site TFN-US/ TFN-US2


August 2012


February 2013


October 2013


March 2014


April 2015

Site TFN-DS


August 2012


February 2013


October 2013


March 2014


April 2015

Site ZW-US


August 2012


February 2013


October 2013


March 2014


April 2015

Site ZW-DS


August 2012


February 2013


October 2013


March 2014

Site ZW2


February 2013


October 2013


March 2014


April 2015

Site ZW3


February 2013


October 2013


March 2014


April 2015

Boschmans Pan


August 2012


February 2013


October 2013


April 2015

Farmers Pan


August 2012


February 2013


October 2013


April 2015

Ephemeral Pan


August 2012


February 2013


October 2013


April 2015

Pan 1


August 2012


February 2013


March 2014


April 2015

Pan 2


August 2012


February 2013


October 2013


April 2015

Pan 3


August 2012


February 2013


October 2013


April 2015

Pan 4 (Makoupan)

November 2011

February 2013

October 2013

April 2015

Pan 5

November 2011

February 2013

March 2014

April 2015

Boschmans Lined PCD

October 2013

2-seam SPP

October 2013

Waterpan PCD

April 2015

Witcons Dam

April 2015

South Witbank Stream

October 2013

Alpha Stream

October 2013