

AQUATIC MACROINVERTEBRATE BIOMONITORING RESULTS FOR A SITE ON THE CROCODILE RIVER IN THE KRUGER NATIONAL PARK, INCOMATI CATCHMENT, MPUMALANGA PROVINCE, SOUTH AFRICA.



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¹ Upstream view of the Crocodile River at the X2CROC-MAROE site (15 September 2022).

Table of Contents

1	INTRODUCTION	5
2	METHODS.....	5
2.1.	DATA INTERPRETATION	5
2.1.1	STREAM FLOW	5
2.1.2	IN SITU WATER QUALITY	6
2.1.3.	SASS5.....	6
2.2.	SITE SAMPLED.....	8
2.2.1.	SITE DETAILS.....	9
2.2.2.	SITE SAMPLING HISTORY.....	12
3	RESULTS	13
3.1.	FLOW	13
3.2.	IN SITU AND CHEMICAL WATER QUALITY	15
3.3.	BIOMONITORING RESULTS	18
3.3.1.	AQUATIC MACROINVERTEBRATES (SASS)	18
3.3.3	ODONATA.....	22
4	DISCUSSION	25
4.1	FLOW	25
4.2	WATER CHEMISTRY	25
4.3	INSTREAM HABITAT	26
5	CONCLUSION & RECCOMENDATIONS	27
6	REFERENCES	29
7	APPENDIX A – SASS5 DATA SHEETS	31
8	APPENDIX B – Site photographs.....	34

List of Tables

Table 2-1.	Description of ecological stream conditions as guidelines for allocation of ecological categories (based on Kleynhans 1996, 1999 & Government Gazette, 30 December 2016, No. 1616, Department of Water and Sanitation).	7
Table 2-2.	List and details of site sampled in the Crocodile River in the Kruger National Park during September 2021 and 2022.....	9
Table 2-3.	List indicating data available for previous sampling events at the Maroela site in the Crocodile River (X2CROC-MAROE).	12
Table 3-1.	A summary of average daily flows for the period 1959 to 1999 compared to the 2000 to 2021 period.	15
Table 3-2.	Results of in situ measurements collected during the September 2020 and 2021 surveys.	16

Table 3-3. In situ results of sites in the Crocodile River, relatively close to the Maroela site, sampled in the 1960s.	16
Table 3-4. A summary of stream conditions per site based on the November 2021 SASS5 monitoring results. Arrows indicate an improvement (upward), deterioration (downward) or no change (horizontal).	19
Table 3-5. A summary of average daily flow during sampling, and stream community family level-based responses.	19
Table 3-6. A summary of responses of the stream community to water quality.	20
Table 3-7. A summary of responses of the stream community to instream habitat quality. .	21
Table 3-8. A summary of the presence of invasive taxa.	21
Table 3-9. Ecospecs based on specific indicators	22
Table 3-10. Odonata encountered during different site visits. Abundances were rated as A (1-2), B (3-5), C (6-10), D (11-20), E >20), and F >1000. Life stages are indicated as imago (im), emergent (em), nymph (ny), and oviposition (ov) and copulation (cp).	23

List of Figures

Figure 2-1. Sketch map of the Crocodile sub-catchment, indicating the main tributaries. ...	10
Figure 2-2. Map indicating the site location of the Maroela site (X2CROC-MAROE) as well as the location of two flow gauging weirs, X2H017 and X2H016.	11
Figure 3-1. Average daily flow for the period 1959 to 1999 compared to the 2000 to 2021 period.	14
Figure 3-2. Daily flow conditions from May to end September expressed as percentage for the period 1 Sep 1960 to 30 November 2021. Daily flow for the 2020 and 2021	15
Figure 3-3. Water temperature in situ measurements plotted for the 1960s sampling event (Matthews 1969) at Malelane and Crocodile Bridge compared to the 2020 and 2021 sampling event at Maroela.	17
Figure 3-4. Selected chemical results summarised from the Van Graan Gauging Weir (X2H017) compared, indicating % change when comparing monthly data from 1977 – 1999 to 2000 – 2009.	17
Figure 3-5. A histogram representing average daily flows 6 weeks prior to each sampling event.	18
Figure 7-1. A view of some of the habitats available and sampled at the X2CROC-MAROE site on 15 September 2020.	35
Figure 7-2. A view of some of the habitats available and sampled at the X2CROC-MAROE site on 14 September 2021.	36

Abbreviations

ASPT	=	Average Score Per Taxon
CPOM	=	Coarse Particulate Organic Matter
DWAF	=	Department of Water Affairs and Forestry
DWS	=	Department of Water Affairs and Sanitation
EPT %	=	Ephemeroptera, Plecoptera, Trichoptera %
FAM	=	Family - number of SASS5 taxa

FBI	=	Family Biotic Index
FPOM	=	Fine Particulate Organic Matter
LIFE	=	Lotic-invertebrate Index for Flow Evaluation
MIRAI	=	Macro-Invertebrate Response Assessment Index
PES	=	Present Ecological State
PES-EIS	=	Present Ecological State, Ecological Importance & Sensitivity
PSI	=	Pollution Sensitive Index
RHP	=	River Health Programme
SASS5	=	South African Scoring System, Version 5
SS	=	SASS Score
SSS	=	Salinity Sensitivity Index

1 INTRODUCTION

Biomonitoring was carried out at the Maroela site (X2CROC-MAROE) in the Crocodile River on the southern boundary of the Kruger National Park. Previous SASS biomonitoring at the selected sites was carried out September 1996, 2011, August 2012, September 2013, August 2014, September 2015, July 2016, August 2017, September 2020. This field work was carried out in September 2021, using aquatic macro-invertebrates as indicators. This report presents the historical and the 2021 results, with notes on deterioration or improvements, as well as recommendations towards improvements.

2 METHODS

The biomonitoring of streams, using aquatic macro-invertebrates as indicators, was carried out at two sampling sites. In situ physical and chemical measurements were taken, and in addition to the aquatic biomonitoring, invasive weed species recognised were recorded and the degree of weed infestation estimated. Visible site-specific infield anthropogenic impacts potentially affecting stream conditions negatively, were also recorded. Only impacts well documented and supported in available scientific literature are recorded.

Aquatic macro-invertebrates were collected using the SASS5 method (Dickens & Graham 2002). Taxa are collected in three different biotopes (e.g., stones, vegetation, and gravel/sand/mud) represented at each site. Taxa are allocated sensitivity ratings (1 – 15), with 1 representing tolerant taxa and 15 sensitive.

Physio-chemical measurements were collected infield at each sampling site. The saturated dissolved oxygen and water temperature was measured with an Economical Rugged Series Dissolved Oxygen Meter. The total dissolved solids (TDS), pH, conductivity, and salinity were measured with a Multi-Parameter Testr™ 35 Series. Water clarity was measured with a 120 cm turbidity tube.

2.1. DATA INTERPRETATION

Different indexes (LIFE, PSI, FBI, %ST, MIRAI, etc.) are used per site on each data set to guide the interpretation of results. As with any community, individuals respond differently to different impacts, and **no index will provide definitive answers**. The lack of what we know about an individual species response to various environmental variables and their interactions serve as limitations to our understanding for the reasons or causes of presence and absence. A higher the degree of confidence in data interpretation is directly linked to the amount of data available.

2.1.1 STREAM FLOW

Where flow gauging stations managed by the Department of Water Affairs and Sanitation (DWS) are present and relevant, monthly flow data were summarised. Where there are no flow gauging stations, flow was visually rated as zero, trickle, low, medium, high, or flood.

There are two flow station up- and downstream from the X2CROC-MAROE site. Data for the upstream gauging weir (X2H017 at Van Graan Dam) is available from 2 August 1959 to 1 September 1998, and the downstream gauging weir (X2H016) at the Tenbosch weir from 1 September 1960 to 20 November 2020.

2.1.2 IN SITU WATER QUALITY

The *in-situ* water quality parameters are measured to assist with the interpretation of SASS data. The Department of Water Affairs and Forestry's (DWAF) Guidelines for Aquatic Ecosystems are used where relevant.

2.1.3. SASS5

All site data are summarised per sampling site in terms of years sampled and sampling season. A combination of the summarised data linked to community composition are used, combined with instream habitat ratings and results from aquatic ecoregion biological bands. Dallas (2007) collated data across the country from different ecoregions, and, on a broader scale, geomorphological zones. In the Dallas (2007) approach, biological bands were calculated as percentiles of the SASS scores. The author uses the same approach with all available data, to guide interpretation. The Macro-Invertebrate Response Assessment Index (MIRAI) is based on individual taxa (i.e., family level or even broader), rated based on their overall perceived preference for different flow regimes, habitat biotopes, and water quality (Thirion 2008). These ratings are combined with importance ratings (subjective) of the different flow velocities, habitat type, and water quality.

The percentage of sensitive taxa and adjusted ASPT incorporates taxa abundances, based on an approach used in Australia (Chessman 2003). The PSI refers to the pollution sensitive index developed for sedimentation (Extence et al. 2013). Taxa sensitivity to sedimentation is rated and community composition express an overall rating from 0 to 1.0, with values <0.3 heavily sedimented, 0.3 – 0.4 moderately sedimented, and >0.5 slightly to naturally or moderately sedimented.

The Family Biotic Index (FBI) is a guideline to the sensitivity of the stream community organic pollution. The overall community is rated out of ten, with low values (0 – 5) potentially representing no to limited organic pollution, >5 to 6 moderate organic pollution, and >6 – 10 very high organic pollution (Hillsenhof 1988).

The Lotic-invertebrate Index for Flow Evaluation (LIFE) is a flow index, with high values (8 – 12) suggesting taxa dominant with a preference for moderately strong to strong flow, and lower values (1 – 7) associated with predominantly slower flows (Extence et al. 1999).

A salinity index was developed by Horrigan et al. (2005) for river systems in Australia. The authors conducted sensitivity analysis with predictive artificial neural network models. Parallel with taxon-specific mean conductivity values a salinity sensitivity score (SSS) were assigned to each taxon (1—very tolerant, 5—tolerant, 10—sensitive). The cumulative SSS are then used as a measurement of change in macroinvertebrate communities because of salinity increase, referred to as the Salinity index (SI). Family level sensitivity indexes from the Australian work was used for the South African taxa to provide a broad indication of any changes in the stream community linked to increased salinity.

Index values are compared against the different survey events per site to determine change if any, and likely causes. Classes assigned are ultimately based on the Dallas (2007) percentile approach to biological bands per aquatic ecoregion, but MIRAI results are

considered. As more data per site becomes available, the confidence of assigned class improves. Stream condition classes are broadly divided into A to F, with A being unmodified or natural, and F critically to extremely modified. A description of each class is included in Table 2-1 that follows.

Table 2-1. Description of ecological stream conditions as guidelines for allocation of ecological categories (based on Kleynhans 1996, 1999 & Government Gazette, 30 December 2016, No. 1616, Department of Water and Sanitation).

ECOLOGICAL CATEGORY	GENERIC DESCRIPTION OF ECOLOGICAL CONDITIONS
A	Unmodified/natural, close to natural or close too predevelopment conditions within the natural variability of the system drivers, hydrology, physico-chemical and geomorphology. The habitat template and biological components can be considered close to natural or to pre-development conditions. The resilience of the system has not been compromised.
A/B	The system and its components are in a close to natural condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a B category.
B	Largely natural with few modifications. A small change in the attributes of natural habitats and biota may have taken place in terms of frequencies of occurrence and abundance. Ecosystem functions are essentially unchanged.
B/C	Close to largely natural most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a C category.
C	Moderately modified. Loss and change of natural habitat and biota have occurred in terms of frequencies of occurrence and abundance. Basic ecosystem functions are still predominantly unchanged. The resilience of the system to recover from human impacts has not been lost and it is ability to recover to a moderately modified condition following disturbance has been maintained.
C/D	The system is in a close to moderately modified condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a D category.
D	Largely modified. A large change or loss of natural habitat, biota and basic ecosystem functions have occurred. The resilience of the system to maintain the category has not been compromised and the ability to deliver ecological goods and services have been maintained.
D/E	The system is in a close to largely modified condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of an E category. The resilience of the system is often under severe stress and may be lost permanently if adverse impacts continue.
E	Seriously modified. The change in the natural habitat template, biota and basic ecosystem functions are extensive. Only resilient biota may survive, and it is highly likely that invasive and problem (pest) species may dominate. The resilience of the system is severely compromised as is the capacity to provide ecological goods and services. However, geomorphological conditions are largely intact but extensive restoration may be required to improve the system's hydrology and physico-chemical conditions.
F	Critically / Extremely modified. Modifications have reached a critical level and the system has been modified completely with an almost complete change of the natural habitat template, biota, and basic ecosystem functions. Ecological goods and services have largely been lost. This is likely to include severe catchment changes as well as hydrological, physico-chemical, and geomorphological changes. In the worst instances, the basic ecosystem functions have been destroyed and the changes are irreversible. Restoration of the system to a synthetic but sustainable condition acceptable for human purposes and to limit downstream impacts is the only option.

2.2. SITE SAMPLED

The Maroela site sampled on the Crocodile River is located roughly 269.8 km downstream from its source, draining a catchment surface area (topographic divide) of approximately 9,057 km² (Table 2-2). The Crocodile catchment upstream from the Maroela site drains four different aquatic ecoregions, and nine different level II aquatic ecoregions. The Maroela site and PESEIS reach node falls within the Lowveld aquatic ecoregion (level II = 3.07). The site is located downstream from the seasonal Lwakahale tributary, overlooking the Mjejane upmarket estate on the southern banks of the Crocodile River outside the park, but within the 5 km buffer. Further up- and downstream, agricultural activities dominated by sugarcane falls mostly within the 5 km buffer. The site location details are included in the Table 2-2, the location within the Crocodile sub-catchment roughly illustrated on a sketch map (Figure 2-1) and for more accurate visual location in Figure 2-2.

2.2.1. SITE DETAILS

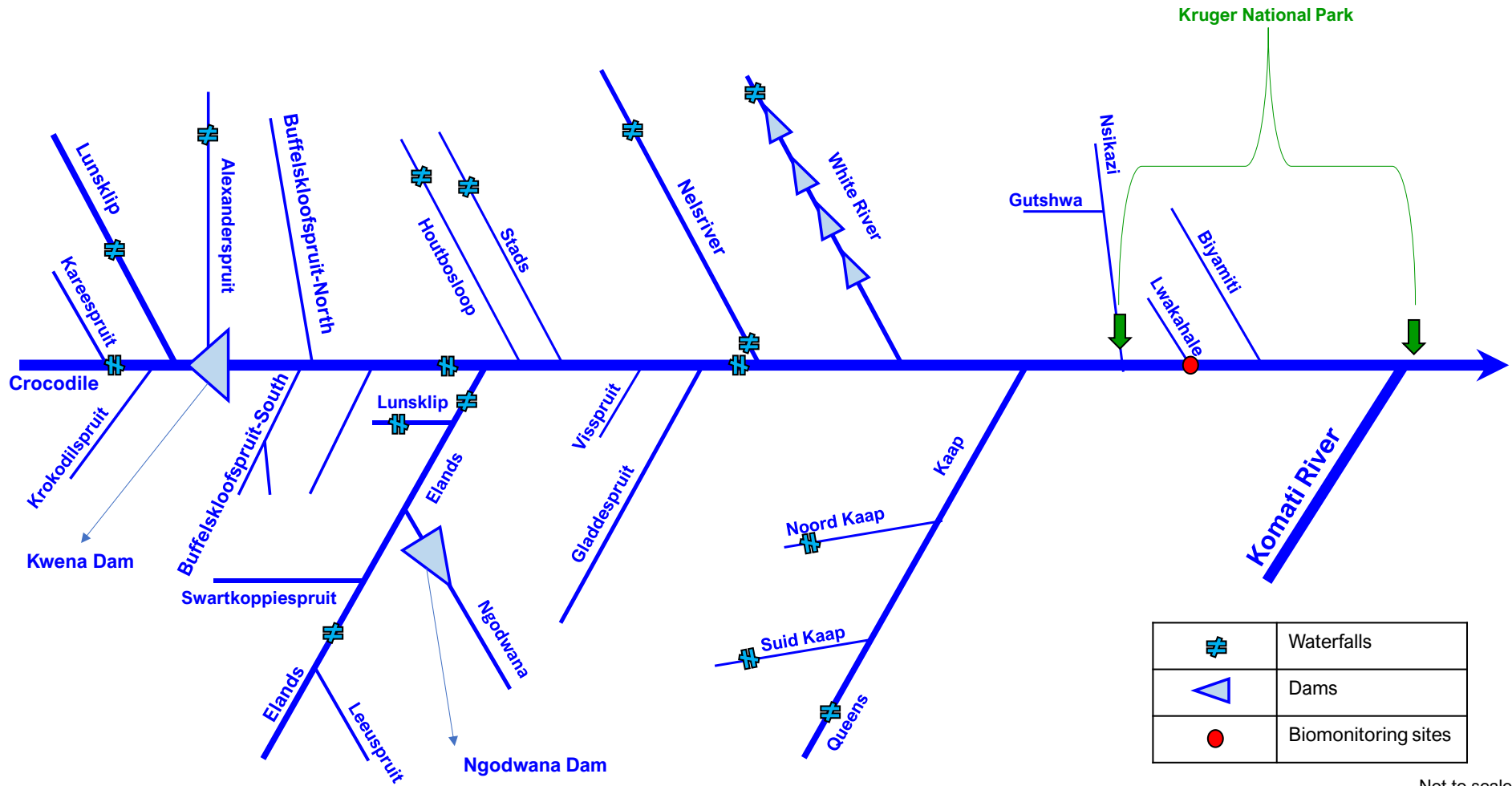
South Africa has been divided into 31 aquatic level I ecoregions based on physiography, climate, rainfall, geology, and potential natural vegetation. These 31 ecoregions have further been subdivided into level II aquatic ecoregions, based on more detailed similarity (Kleynhans et al. 2005). The site listed below (Table 2-2) was sampled in September 2021 and 2022.

Table 2-2. List and details of site sampled in the Crocodile River in the Kruger National Park during September 2021 and 2022.

SITE CODE	SITE NAME	RIVER	ECOREGION Lev II	LATITUDE² (S)	LONGITUDE (E)	ELEVATION (M a.s.l.)	Topographic Divide (km²)	PESEIS Reach Node	Distance from Source (km)
X2CROC-MAROE	Maroela	Crocodile	3.07	-25.37922	31.70796	213	9 057	X24F-00953	269.8

² Map Datum WGS84, with co-ordinate format decimal degrees, and elevations were retrieved from a Garmin GPSMAP 64s with software version 5.00.

CROCODILE-INCOIMATI CATCHMENT



Not to scale

Figure 2-1. Sketch map of the Crocodile sub-catchment, indicating the main tributaries.

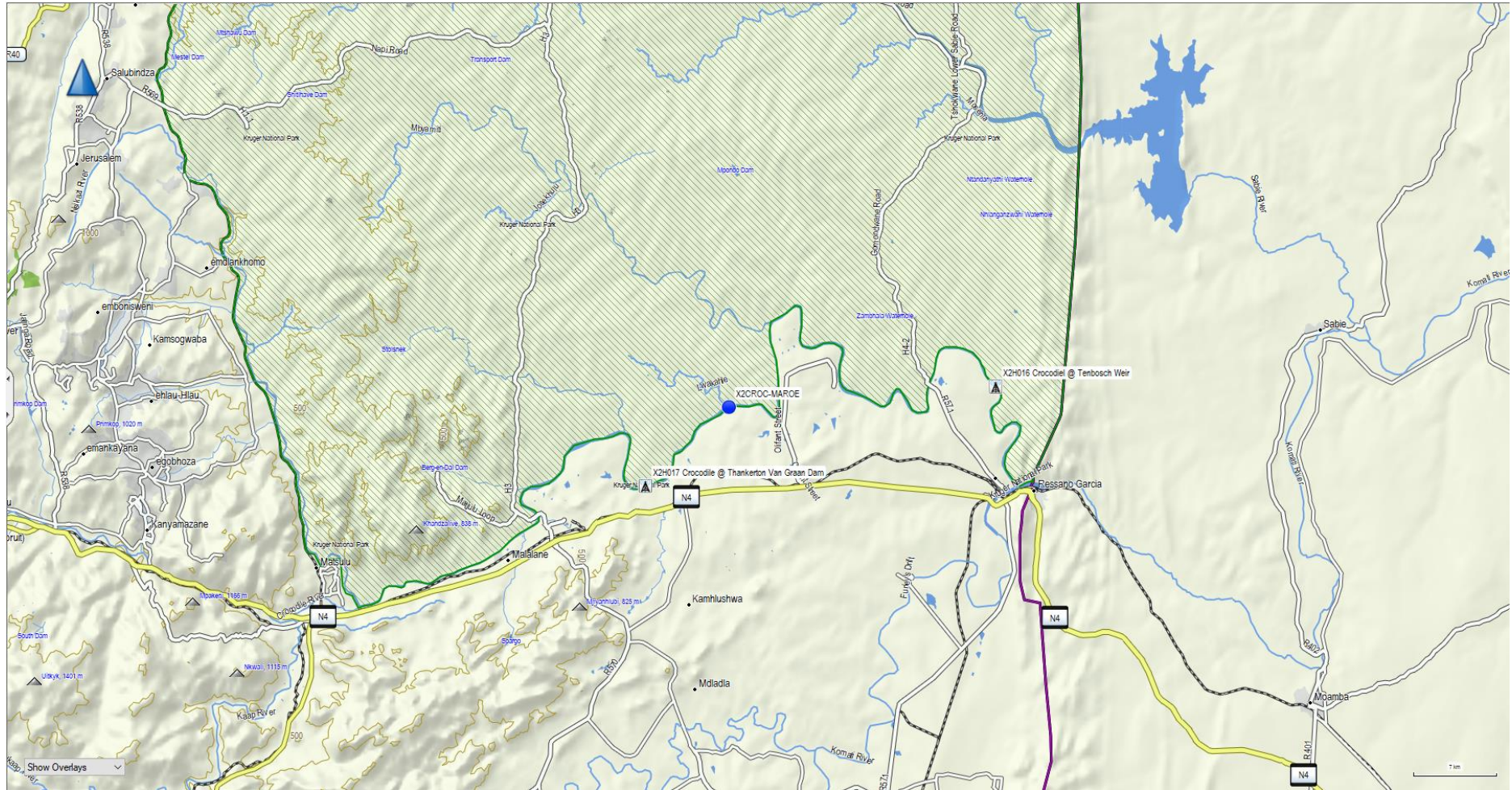


Figure 2-2. Map indicating the site location of the Maroela site (X2CROC-MAROE) as well as the location of two flow gauging weirs, X2H017 and X2H016.

2.2.2. SITE SAMPLING HISTORY

A survey of Ephemeroptera (mayflies) was carried out across the Incomati Catchment (South Africa, at 31 sites during 1966/67 (Matthew 1968). During the 1966/67 survey site were at Malelane and Crocodile Bridge, up- and downstream from the Maroela site respectively.

Biomonitoring data using SASS is available for September 1998, and then August – September from 2011 onwards. Data for 2018 and 2019 was not available for this report. The 1998 to 2021 period therefore amounts to 10 sampling events (Table 2-3), representing 0.0005 to 0.0008 % of the total time³ lapse between the first and last sampling dates. The ebbs and flows of the Crocodile River between 11 September 1996 and 14 September 2021 site visits represent 219,216 hours flow. Biomonitoring represents the brief (0.0005 – 0.0008%) opening of a window into the temporal scale of the river at the Maroela sampling point, used to determine present conditions.

As an example, during surveys in 1966 and 1967, seven Ephemeroptera families were encountered in the Crocodile River between Malelane and Crocodile Bridge, whereas only five were encountered between September 1996 and September 2021. Families absent were Prosopistomatidae and Oligoneuridae. The family Tricorythidae was present and relatively abundant at the Malelane site but absent at the Crocodile Bridge site during the 1966/67 survey. Within the 1998-to-2021-year sampling period at the Maroela site (X2CROC-MAROE) Tricorythidae was only encountered 13 August 2014 (FROC = 10%).

Table 2-3. List indicating data available for previous sampling events at the Maroela site in the Crocodile River (X2CROC-MAROE).

Site Code	Old Codes	River	Site Name	Sampling Date										Sampling Events
				Sep-96	Sep-11	Aug-12	Sep-13	Aug-14	Sep-15	Jul-16	Aug-17	Sep-20	Sep-21	
X2CROC-MAROE	X2CROC-LUKWA CR26	Crocodile	Maroela	1	1	1	1	1	1	1	1	1	1	10

Sampling was mostly carried out during late winter to spring. Winter and especially spring are generally considered to represent the low flow season.

³ The actual sampling (time physically sampling) takes anything from 7 to 10 minutes.

3 RESULTS

Results are focusing on the drivers of change in the river, and then on how the aquatic macroinvertebrates are potentially responding to any potential changes. Historical flow conditions, flow conditions prior to the sampling event (1 May – 31 Sep), and average flow on the day compared to average daily flow for the sampling month is compared.

In situ water quality was measured on the day of sampling, and these results are compared (broadly) to historical results if available. Historical water chemistry results were plotted monthly for the available 1977 to 2009 period, to determine how much the water chemistry habitat changed over time. No data for the period post 2009 was available.

Aquatic macroinvertebrate results are compared to historical distribution data available, and against expected taxa on species, genus, and family level. A sampling event is summarised in terms of the stream community's preference or associations with different flow velocities, instream substrate and habitat, and perceived water quality. The percentage change is calculated based on available site data. In Appendix A, copies of the completed SASS5 data sheets for the September 2020 and 2021 sampling events included.

3.1. FLOW

Flow conditions (Figure 3-1) at the site was rated based on readings obtained from the Tenbosh Weir flow gauging station (Figure 2-2) at the Department of Water Affairs and Sanitation's website. High flows are generally encountered between the months of December to April, with the highest in February. Low flows are from May to November, with the lowest flows during the months of September and October. Flow conditions affect the instream habitat template, and in turn the functioning of the ecosystem and responses of aquatic taxa dependant on these flowing systems.

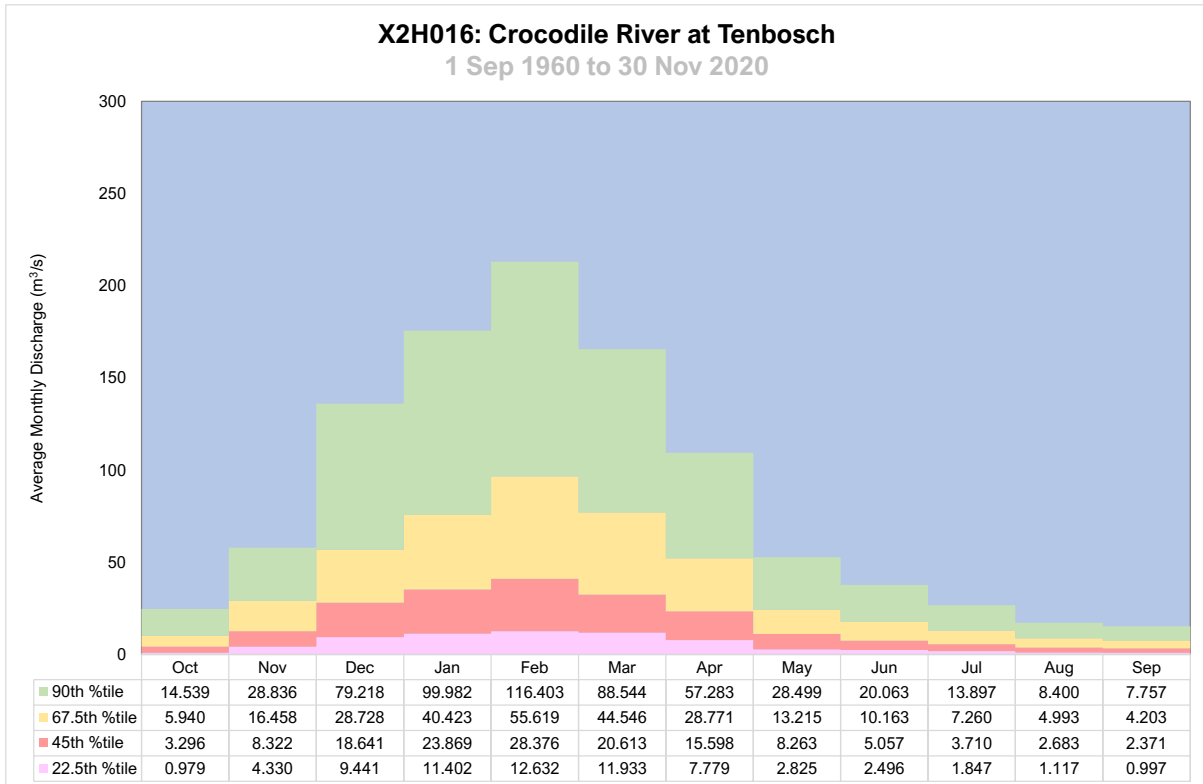


Figure 3-1. Average daily flow for the period 1959 to 1999 compared to the 2000 to 2021 period.

The average daily flows from 1960 to 2021 were plotted from 1 May to 30 September, and percentage increase and decrease plotted as dotted lines. The 01 May to 30 September 2020 and 2021 period were also plotted, to roughly ascertain flow conditions prior to and during sampling (Figure 3-2).

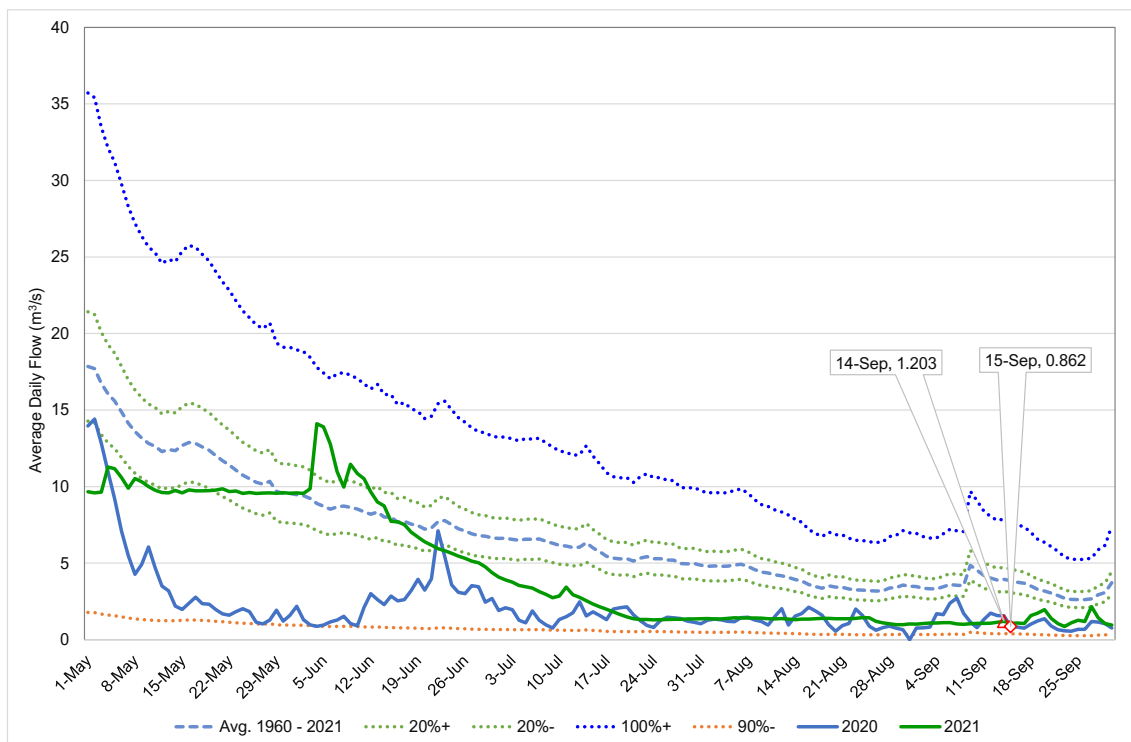


Figure 3-2. Daily flow conditions from May to end September expressed as percentage for the period 1 Sep 1960 to 30 November 2021. Daily flow for the 2020 and 2021

Flow conditions was lower during the 2020 and 2021 sampling events when compared to historical data. The 2021 sampling event experienced consistent high flow during May to June, decreasing in July towards September, compared to the 2020 flows which were already low in May with a slight peak flow event in June (Figure 3-2).

Table 3-1. A summary of average daily flows for the period 1959 to 1999 compared to the 2000 to 2021 period.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
1959 - 1999	7.202	13.799	31.622	36.380	64.126	43.552	24.704	13.434	8.555	6.448	4.475	3.883
2000 - 2021	3.981	9.653	23.014	44.316	35.387	40.622	22.812	11.215	6.898	4.590	2.908	2.647
% Decrease	45%	30%	27%	-22%	45%	7%	8%	17%	19%	29%	35%	32%

The results in Table 3-1 suggest an increase of 22% in average daily flows during the month of January when comparing 1959 to 1999 to the 2000 to 2021 period. The biggest decrease in average monthly flows is for the July to December and February period.

3.2. IN SITU AND CHEMICAL WATER QUALITY

In situ results for sites sampled in 2020 and 2021 are listed (Table 3-2). Temperature (Temp.) represents water temperature measured in degrees Celsius (°C), EC represents electrical conductivity, and DO the dissolved oxygen expressed as a percentage.

Table 3-2. Results of *in situ* measurements collected during the September 2020 and 2021 surveys.

Site	Date	Time	In Situ Measurements			
			Temp. °C	pH	EC µS/cm	TDS ppm
X2CROC-MAROE	15/xi/2020	14:10	26.1	8.1	481.3	
	14/xi/2021			Not available		

From historical and current *in situ* results, it is evident that the electrical conductivity values in the Crocodile River in the Kruger National Park have increased considerably since the 1960s (Table 3-2 and Table 3-3). Looking at the increase in ions which would drive an increase in electrical conductivity, sulphate (SO₄), chloride (Cl), and calcium (Ca) showed the highest increases between 2000 and 2009 when compared to the 1977 to 1999 period (Figure 3-4). Unfortunately, additional data for the 2010 to 2021 period could not be traced, but based on the *in situ* EC values, there are still ions entering the system in the form of pollutants.

Table 3-3. *In situ* results of sites in the Crocodile River, relatively close to the Maroela site, sampled in the 1960s.

Site	Habitat Type	Date	Stream Discharge (m/s)	Water Temperature (°C)	pH	EC (µS/cm)
Crocodile Bridge	Sand-edge	Oct-66		26.3	7.7	295
Crocodile Bridge	Sand-edge	Jan-67		28.9	7.8	112
Crocodile Bridge	Pool bottom	Oct-66		25.9	7.7	280
Crocodile Bridge	Pool bottom	Jan-67		28.9	7.8	112
Crocodile Bridge	Marginal veg	Apr-66	0.137	22.5	8.5	170
Crocodile Bridge	Marginal veg	Jul-66	0.145	19.4	8.4	212
Crocodile Bridge	Marginal veg	Oct-66	0.222	26.3	7.7	300
Crocodile Bridge	Marginal veg	Jan-67		28.9	7.8	112
Crocodile Bridge	Riffle	Apr-66	0.350	22.5	8.5	190
Crocodile Bridge	Riffle	Jul-66	0.469	19.4	8.5	210
Crocodile Bridge	Cobble-riffle	Apr-66	0.853	22.5	8.5	170
Crocodile Bridge	Cobble-riffle	Jul-66	0.828	19.4	8.5	210
Crocodile Bridge	Sandy substrate	Apr-66	0.213	22.5	8.5	170
Crocodile Bridge	Sandy substrate	Jul-66	0.231	19.4	8.5	215
Malelane	Sand-edge	Apr-66		22.7	7.7	180
Malelane	Sand-edge	Jul-66		17.9	8.3	174
Malelane	Rocky-edge	Apr-66		22.8	8.0	174
Malelane	Rocky-edge	Jul-66		19.0	8.4	177
Malelane	Pool bottom	Apr-66		22.5	7.8	172
Malelane	Marginal veg	Apr-66		22.7	7.8	182
Malelane	Marginal veg	Jul-66		17.7	8.4	183
Malelane	Marginal veg	Oct-66	0.145	24.8	7.9	180
Malelane	Marginal veg	Jan-67	0.137	27.2	8.2	100
Malelane	Shallow stream	Apr-66	0.231	22.7	8.3	170
Malelane	Riffle	Apr-66	0.367	22.6	8.3	170
Malelane	Riffle	Jul-66	0.521	17.4	8.4	172
Malelane	Cobble-riffle	Apr-66	0.862	22.6	8.3	170
Malelane	Cobble-riffle	Jul-66	0.879	17.4	8.4	167
Malelane	Sandy substrate	Jul-66	0.376	17.8	8.4	175

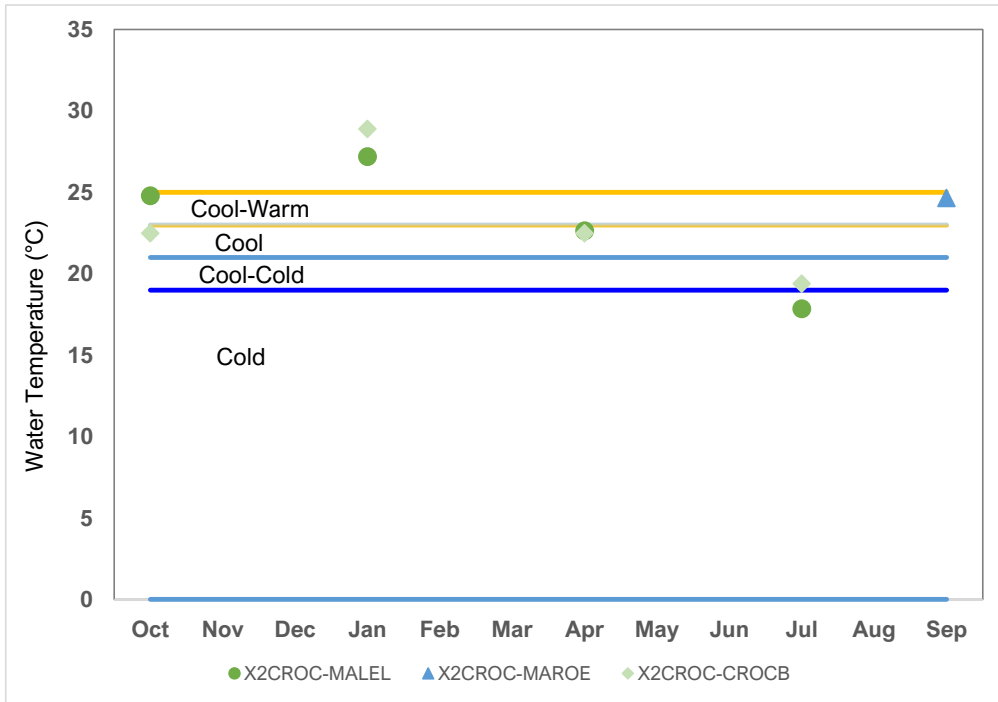


Figure 3-3. Water temperature in situ measurements plotted for the 1960s sampling event (Matthews 1969) at Malelane and Crocodile Bridge compared to the 2020 and 2021 sampling event at Maroela.

Daylight stream temperatures range between cool-warm to warm in summer months, with cold to cool water dominant in the 1960s during July.

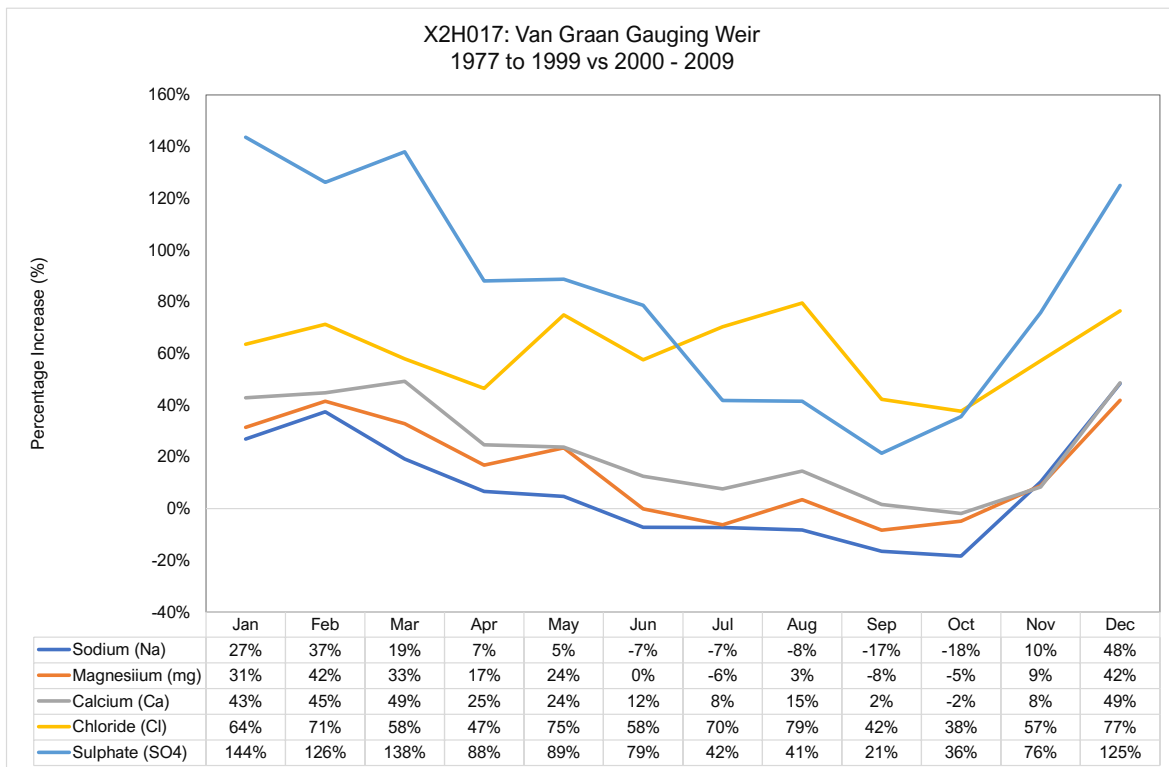


Figure 3-4. Selected chemical results summarised from the Van Graan Gauging Weir (X2H017) compared, indicating % change when comparing monthly data from 1977 – 1999 to 2000 – 2009.

3.3. BIOMONITORING RESULTS

Where a species sampled are known their observed and documented ecological requirements are considered to improve an understanding of responses. The lowest taxonomic resolution of taxa encountered is recorded to provide more insight into responses to change. Following is a summary of available results for the different sampling events, with the focus on change in 2021 compared previous surveys.

3.3.1. AQUATIC MACROINVERTEBRATES (SASS)

Conditions were categorised using MIRAI, based on expected taxa (reference) compared to previous and current SASS5 results. Conditions were mostly rated as moderately modified (C-category) but ranged from largely modified (D) to largely natural (B).

Table 3-4 presents MIRAI and SASS5 scores for the different sampling events. The percentage change refers to the change in 2021 results when compared to previous results. A change of $\leq 10\%$ is considered small, $>10\%$ to $\leq 49\%$ moderate, and $\geq 50\%$ large. Colour codes green, yellow, and red are used to highlight the highest negative change, with red a negative change.

Based on the results in Table 3-4, the MIRAI model suggests improved conditions (B-category) in 2021, and an improvement of 2% when compared to 90th percentile of all previous results. There was a considerable increase in the total SASS5 score (29%) and the number of taxa (21%), but the change in average score per taxon (ASPT) was very limited (3%).

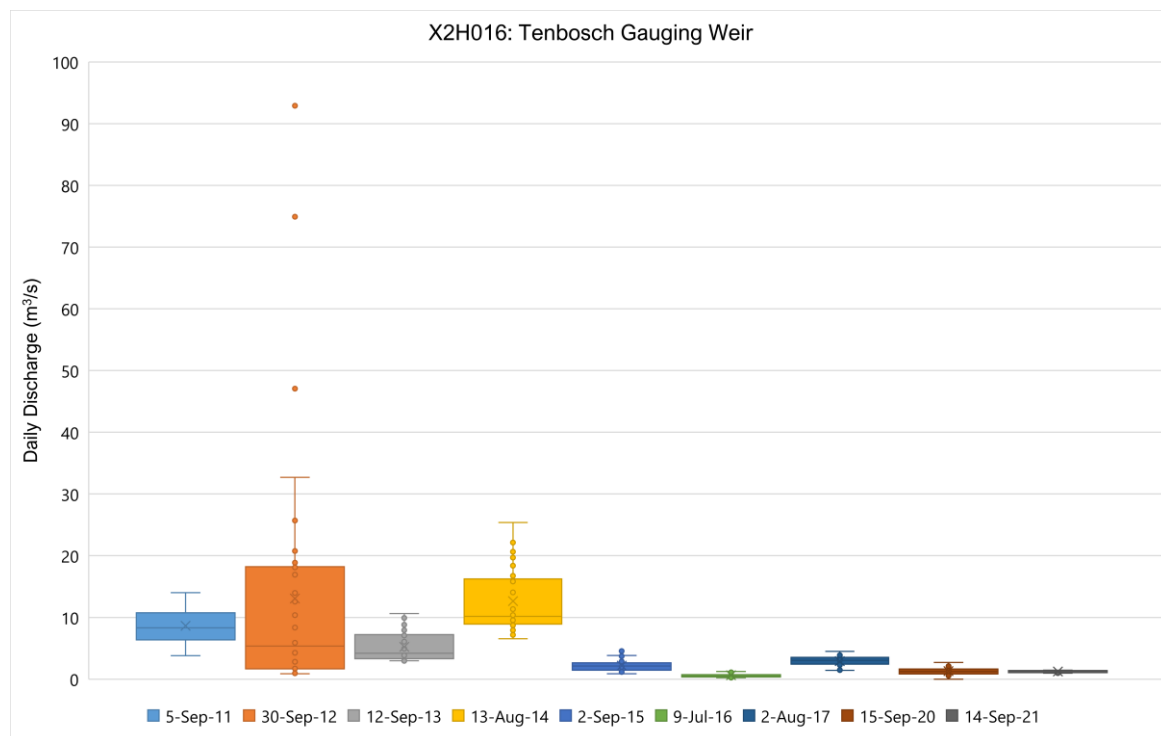


Figure 3-5. A histogram representing average daily flows 6 weeks prior to each sampling event

Table 3-4. A summary of stream conditions per site based on the November 2021 SASS5 monitoring results. Arrows indicate an improvement (upward), deterioration (downward) or no change (horizontal).

X2CROC-MAROE	Sampling Date										% Change
	Sep 1996	Sep 2011	Aug 2012	Sep 2013	Aug 2014	Sep 2015	Jul 2016	Aug 2017	Sep 2020	Sep 2021	
MIRAI	62	62	71	78	80	68	69	66	71	83	2% ↗
SASS5 Total Score	139	126	159	159	177	127	144	132	127	182	29% ↑
No. of SASS Taxa	21	23	27	29	31	2	26	28	24	32	21% ↑
ASPT	6.6	5.5	5.9	5.5	5.7	5.3	5.5	4.7	5.3	5.7	3% ↗

Table 3-5. A summary of average daily flow during sampling, and stream community family level-based responses.

X2CROC-MAROE	Flow & Flow Responses											% Change
	Sep 1996	Sep 2011	Aug 2012	Sep 2013	Aug 2014	Sep 2015	Jul 2016	Aug 2017	Sep 2020	Sep 2021		
Avg. Daily Discharge (m ³ /s)	49.398 ⁴	3.762	1.726	3.007	6.551	2.160	0.484	3.225	0.862	1.203		
Flow Conditions	Wet-LF	Wet-LF	Dry-LF	Mod-LF	Wet-LF	Mod-LF	Dry-LF	Mod-LF	Dry-LF	Dry-LF		
Fast – Mod Flow (%)	38%	26%	33%	8%	26%	29%	27%	14%	33%	28%	1% ↗	
Slow flow - Stagnant (%)	62%	74%	67%	72%	74%	71%	73%	86%	67%	72%	0% →	
LIFE (Family)	8.0	7.3	7.3	7.3	7.2	7.3	6.7	6.6	7.3	7.5	3% ↗	

In Figure 3-5 the average daily flow prior to the sampling date is plotted to visually compare flow conditions experience by taxa prior to sampling. The same approach is illustrated in Figure 3-2, plotting the May to September average flow and 2020 and 2021 flows, highlighting the different flow scenarios experience by instream biota. Flow conditions are rated as a dry, moderate, or wet low flow event based on the average daily flow (Table 3-5). On a community level, the changes from September 2021 when compared to previous results in low. Wadeable habitat accessible and sampled differs during the different flow events, which could skew flow related results when the instream habitat remains stable.

⁴ No flow data available for the Tenbosch Weir (X2H016) over the sampling period due to very high flows, but flow measured at the Van Graan Weir (X2H017) in was 10.474 m³/s on 9 Jul 1996 and 49.398 m²/s on 16 Nov 1996. There are no flow records for the July and November 196 dates.

Table 3-6. A summary of responses of the stream community to water quality.

X2CROC-MAROE	Water Quality Response										% Change
	Sep 1996	Sep 2011	Aug 2012	Sep 2013	Aug 2014	Sep 2015	Jul 2016	Aug 2017	Sep 2020	Sep 2021	
Organic Pollution Tolerance	3.6	5.0	4.3	4.1	4.8	4.1	5.1	5.4	5.2	4.7	1% ↓
Salt Tolerance Index	6.0	5.8	4.4	4.2	3.1	4.4	3.2	4.1	5.0	3.6	17% ↑
% Ephemeroptera	31%	7%	9%	29%	14%	9%	4%	25%	11%	24%	18% ↓
% Trichoptera	19%	2%	2%	3%	5%	1%	5%	5%	3%	1%	84% ↓*
% Odonata	24%	55%	29%	5%	18%	7%	8%	10%	3%	5%	84% ↓*
% Diptera	13%	11%	17%	31%	6%	33%	21%	24%	46%	22%	37% ↑*
% Sensitive Taxa	44%	12%	34%	42%	34%	20%	10%	29%	22%	23%	45% ↓
% Tolerant Taxa	56%	88%	66%	58%	66%	80%	90%	71%	78%	77%	32% ↑

When considering percentage change, responses of the stream community sensitive to water quality is more related to the flow conditions than that of the stream community response to flows (Table 3-5 versus Table 3-6). The FBI (organic pollution tolerance index) indicates a decrease in organic pollution in 2021 when compared to previous results. A low number (e.g., 3.6) indicates low potential for organic pollution based on the stream community encountered, while a high number (e.g., 5.4) indicates a high potential of organic pollution. Generally, the highest potential for organic pollution was reflected during extreme low flow events.

Taxa tolerant to salts appeared to have increased, with 6.0 representing taxa with low salt tolerance dominating, while 3.1 suggest taxa tolerant to salts dominant. The 2021 data therefore suggests a 17% increase in salt tolerant taxa despite higher flows. When looking at the response of sensitive and tolerant orders, Ephemeroptera (mayflies), Trichoptera (caddisflies), and Odonata (dragon- and damselflies) suggested a decrease in numbers and diversity in 2021, while Diptera (flies) which are generally more tolerant to water quality changes increased. The percentage sensitive taxa decreased (45%) while tolerant taxa increased (32%) in 2021 when compared to previous surveys (Table 3-6).

Table 3-7. A summary of responses of the stream community to instream habitat quality.

X2CROC-MAROE	Instream Habitat Response										% Change
	Sep 1996	Sep 2011	Aug 2012	Sep 2013	Aug 2014	Sep 2015	Jul 2016	Aug 2017	Sep 2020	Sep 2021	
Cobble	53%	39%	27%	42%	23%	42%	31%	33%	57%	33%	9% ↘
Vegetation	12%	11%	23%	14%	32%	10%	31%	30%	20%	20%	1% ↗
Gravel-Sand-Mud	24%	38%	23%	21%	15%	38%	32%	25%	18%	43%	73% ↕
Generalists	10%	12%	28%	23%	29%	10%	5%	12%	6%	4%	62% ↘

Based on the responses of the stream community to instream habitat, there was a considerable increase (73%) in taxa associated with the gravel-sand-mud biotope in 2021, likely associated with increased availability of this biotope. There was also a decrease in generalist taxa, while the cobble and vegetation biotopes appear to be more stable.

Table 3-8. A summary of the presence of invasive taxa.

X2CROC-MAROE	Invasive Taxa										% Change
	Sep 1996	Sep 2011	Aug 2012	Sep 2013	Aug 2014	Sep 2015	Jul 2016	Aug 2017	Sep 2020	Sep 2021	
% Invasives	0%	1%	2%	2%	8%	4%	19%	19%	8%	20%	↑

The increase in the percentage of invasive taxa is considerable (20% in 2021). This is concerning especially since a negative impact on the ecosystem is expected now and over time based on other available invasive response publications, but in South Africa is still poorly known. Bunn & Arthington (2002) indicated that exotic taxa frequently proliferate in systems with modified flow regimes, and decreased flow and increased water temperature driven by climate change will further benefit the spread of invasive taxa (Dodds & Whiles 2010).

Table 3-9. Ecospecs based on specific indicators

ECOSPECS		TPCs	Sep-96	Sep-11	Aug-12	Sep-13	Aug-14	Sep-15	Jul-16	Aug-17	Sep-20	Sep-21	
			Wet-LF	Wet-LF	Dry-LF	Mod-LF	Wet-LF	Mod-LF	Dry-LF	Mod-LF	Dry-LF	Dry-LF	
MIRAI	Range: B - 80 to 89%	MIRAI >62%	62	71	71	78	80	68	69	66	71	83	
Abundance	Abundance: No Ds	No Taxa D-abundance	None	None	Simuliidae	None	None	None	None	None	Simuliidae	Baetidae Simuliidae Physidae Thiaridae	
Water Quality	% Sensitive Taxa	%ST >40	44%	12%	34%	42%	34%	20%	10%	29%	22%	23%	
	Family Biotic Index	Organic Tolerant Taxa	3.6	5.0	4.3	4.1	4.8	4.1	5.1	5.4	5.2	4.7	
	Ratio: Sensitive-Tolerant	High; Mod; Low	>Mod	<Mod	<Mod	>Mod	<Mod	<Mod	<Mod	>Mod	<Mod	<Mod	
Flow	Taxa Flow Preference	>0.3 - G 0.3 - 0.1 Y <0.1 - R	0.3 - 0.1	<0.1	<0.1	<0.1	<0.1	<0.1	>0.3	<0.1	<0.1	<0.1	
	Ratio: V Fast-Fast vs Mod-Slow	>1; =1; or <1	0.7	0.1	0.2	0.1	0.4	0.1	0.1	0.1	0.1	0.1	
Habitat	SIC Biotope:		11	10	10	12	12	8	9	8	10	12	
	Perleidæ - A-abundance		A	1		A	A						
	Tricorythidae: <i>Tricorythus</i> sp. - B-abundance						A						
	Hydropsychidae >2 sp. - B-abundance		2 sp. - B	1 sp. - A	1 sp. - B	2 sp. - A	1 sp. - A	1 sp. - A	2 sp. - A	1 sp. - A	1 sp. - B	2 sp. - B	
	Philopotamidae: <i>Chimarra</i> sp. - A-abundance		A		A				A		A	A	
	Elmidae: A-abundance		A	1	B	A	A	A	A	1	B	B	
	SOOC-biotope:												
	Heptageniidae (<i>Afronunus</i> sp.) - A-abundance		A	A	A	A	B	A	B	B	B	B	
	Leptophlebiidae - A-abundance		B	B	A	A	B	B	B	B	B	B	
	Vegetation-biotope:												
	Heptageniidae (<i>Componeuria njalensis</i>) - B-abundance					A					B		A
	Coenagrionidae (<i>Pseudagrion</i> sp.) - B-abundance			A	A	A		A	A				A
	Leptoceridae - B-abundance		A				A	A	B	A			A
GSM - Biotope:													
Polymitarcidae		A											
Gomphidae		B	B	A	B	B	A	A	A	A	A		
Key Indicators	Thirteen Key Taxa:												
	% of Community		38%	39%	26%	31%	29%	38%	27%	25%	33%	25%	
	Perleidæ												
	Heptageniidae												
	Leptophlebiidae												
	Tricorythidae												
	Polymitarcidae												
	Coenagrionidae												
	Aeshnidae												
	Libellulidae												
	Gomphidae												
	Hydropsychidae												
	Leptoceridae												
Elmidae													
Psephenidae													
Water Quality:													
Prosoptomatidae (<i>Prosoptoma</i> sp.)		Present/Absent											
Tricorythidae (<i>Tricorythus</i> sp. 'Lowveld')		Present/Absent				A							
Unionidae: (<i>Unio caffer</i>)		Present/Absent											
Migration:													
Palaemonidae (<i>Macrobrachium lepidactylus</i>)		Present/Absent						1					
Exotics	Exotic Taxa:												
	% of Community		>0% = R; 0% = G	0%	2%	4%	3%	10%	29%	39%	22%	17%	39%
	Parastacidae: <i>Cherax quadricarinatus</i>		Present/Absent	?	?	?	?	?	?	Present	?	Present	Present
	Physidae: <i>Physa acuta</i> - Absent		Present/Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Thiaridae: <i>Tarebia granifera</i> - Absent		Present/Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

The Ecospecs table (Table 3-9) is an attempt to provide a visual overview of conditions, highlighting specific problems highlighted linked to indicator taxa. For example, the MIRAI model provides a B-category in 2021, which is considered largely natural. In 2021 the abundance was high which “promotes” a high Ecstatus in the MIRAI model. The key indicator taxa however provide a different picture.

3.3.3 ODONATA

Species of Odonata (dragonflies and damselflies) expected are listed and those encountered during different site visits identified as larvae, exuviae, or imago (adults) are listed. Odonata were not the targeted indicator, but those species encountered are listed and their ecological requirements considered. Long-term species level monitoring is very valuable to track changes over time.

Table 3-10. Odonata encountered during different site visits. Abundances were rated as A (1-2), B (3-5), C (6-10), D (11-20), E (>20), and F >1000. Life stages are indicated as imago (im), emergent (em), nymph (ny), and oviposition (ov) and copulation (cp).

ODONATA	Sites			
	13 Jan 2013	10 Apr 2017	15 Sep 2020	14 Sep 2021
Discharge ⁵ (m ³ /s)	33.145	9.193	0.862	1.203
Flow Condition Rating	Mod-HF	Dry-HF	Dry-LF	Dry-LF
Calopterygidae				
<i>Phaon iridipennis</i>	A-im			
Coenagrionidae				
<i>Aciagrion gracile</i>				
<i>Africallagma glaucum</i>				
<i>Ceriagrion glabrum</i>				
<i>Ischnura senegalensis</i>	B-m			B-im
<i>Pseudagrion acaciae</i>	C-im			B-im
<i>Pseudagrion coeleste</i>				
<i>Pseudagrion commoniae</i>				
<i>Pseudagrion gamblesi</i>				
<i>Pseudagrion hageni</i>				
<i>Pseudagrion hamoni</i>		C-im		
<i>Pseudagrion kersteni</i>				
<i>Pseudagrion massaicum</i>	A-im			
<i>Pseudagrion sjoestedti</i>				
<i>Pseudagrion sublacteum</i>	C-im	D-im	C-im	C-cp
<i>Pseudagrion sudanicum</i>	A-im			
Lestidae				
<i>Lestes pallidus</i>				
<i>Lestes plagiatus</i>				
Platycnemididae				
<i>Elatoneura glauca</i>	C-im			
<i>Mesocnemis singularis</i>				
Aeshnidae				
<i>Anax ephippiger</i>				
<i>Anax imperator</i>	A-im			
<i>Anax speratus</i>			1-im	
<i>Anax tristis</i>				
Gomphidae				
<i>Ceratogomphus pictus</i>				
<i>Crenigomphus hartmanni</i>				A-ny
<i>Gomphidia quarrei</i>				
<i>Ictinogomphus ferox</i>	B-im			
<i>Lestinogomphus</i> sp. 1				
<i>Mastigogomphus</i> sp.				
<i>Neurogomphus zambeziensis</i>				
<i>Paragomphus elpidius</i>				
<i>Paragomphus genei</i>			A-im	A-em
<i>Paragomphus magnus</i>				
<i>Paragomphus sabicus</i>	A-im			
<i>Phyllogomphus selysi</i>				
Libellulidae				
<i>Acisoma variegatum</i>				

⁵ Data from Tenbosch Gauging Weir (X2H016)

ODONATA	Sites			
	13 Jan 2013	10 Apr 2017	15 Sep 2020	14 Sep 2021
<i>Brachythemis lacustris</i>	C-im	C-im		B-im
<i>Brachythemis leucosticta</i>	C-im	B-im		
<i>Crocothemis erythraea</i>	B-im		A-im	B-im
<i>Crocothemis sanguinolenta</i>				
<i>Dipacodes levebfrii</i>				
<i>Diplacodes luminans</i>				
<i>Hemistigma albipunctum</i>				
<i>Nesciothemis farinosa</i>	C-im	B-im		
<i>Olpogastra lugubris</i>				
<i>Orthetrum abbotti</i>				
<i>Orthetrum brachiale</i>				
<i>Orthetrum chrysostigma</i>	B-im	B-im	B-im	B-im
<i>Orthetrum stemmale</i>				
<i>Orthetrum trinacria</i>				
<i>Palpopleura lucia</i>				
<i>Pantala flavescens</i>	B-im	B-im		B-im
<i>Tramea basilaris</i>				
<i>Trithemis annulata</i>	C-im	C-im	C-im	
<i>Trithemis arteriosa</i>		B-im		A-em
<i>Trithemis donaldsoni</i>	A-im	A-im		
<i>Trithemis kirbyi</i>	A-im	B-im		
<i>Trithemis weneri</i>				
<i>Urothemis assignata</i>				
<i>Urothemis edwardsii</i>				
<i>Zygonoides fueleborni</i>		A-im	B-ny	B-ny
<i>Zygonyx natalensis</i>		A-im		B-ny
<i>Zygonyx torridus</i>		B-im	C-ny-im	C-ny
Macromiidae				
<i>Phyllomacromia contumax</i>				
<i>Phyllomacromia picta</i>				
Number of species	19	14	8	13

A total of 27 of the expected 66 species were recorded at the Maroela sampling site. Adult species present during all four sampling events are predominantly associated with flowing waters in open exposed habitats, while the dominant larval habitats are lotic-slow and lentic-permanent waters, with claspers and sprawlers dominating. The absence of specific species is of concern, but more field time or eDNA samples are required to confirm presence-absence.

4 DISCUSSION

A brief discussion of results follows, focusing firstly on the three main drivers of change, and then on how the aquatic macroinvertebrates responded to each driver.

4.1 FLOW

Flow conditions changed when compared to historical results, with the biggest change occurring during low flows (Table 3-1). Flow conditions during the September 2020 and 2021 was considerably lower and were categorised as dry low flow conditions both those periods. That suggests that average daily flow on 15 September 2020 and 14 September 2021 was very low when compared to historical average daily flow for the month of September. The September 2020 flow was slightly lower than the September 2021 flows, but the most obvious difference is the high flows preceding the September 2021 sampling event (Figure 3-2). A prolonged period of high flow during the right time of year makes increase instream habitat available and allows for oviposition and larvae or nymphs to mature towards emergence. Based on aquatic macroinvertebrate diversity and abundances, the September 2021 flows provided better a long-term instream habitat template than in September 2020.

4.2 WATER CHEMISTRY

In situ results suggest a change in water electrical conductivity when comparing the 2020 results to the available 1966 to 1967 results for the Crocodile River a few kilometres up- and downstream from the Maroela sampling site (Table 3-2, Table 3-3). *In situ* results of sites in the Crocodile River, relatively close to the Maroela site, sampled in the 1960s (Table 3-3). When considering water chemistry data available for the 1977 to 2009 period measured at the van Graan Gauging Weir (X2H017), the ions responsible for increased conductivity in September over the 1977 to 2009 period are mainly sulphate (SO₄), chloride (Cl), and calcium (Ca). The major sources of increases in SO₄, Ca, and Cl are considered the weathering of evaporites and or pollution (Eby 2004). Potential activities contributing excessive ions as pollutants can originate from from municipall water treatment (e.g., Ca, Mg, Na, Cl), waste water treatment (SO₄ & Cl), untreated waste water (e.g., Ca, Mg, Na, Cl, SO₄), mining activities (SO₄), and chemical manufacturing (Cl, SO₄, & Na).

The water temperature regime⁶ is one of the major factors affecting the length of egg incubation, hatching, growth, maturation of specific aquatic macroinvertebrate species (Ward 1992), but also the rate of chemical interactions, dissolved oxygen, and pH. On a family level, the stream community seems to indicate very little change in terms of taxa preference within specific temperature ranges, but on a species level it is expected to be different.

Community responses in terms of water quality, however, indicates a decrease in the percentage sensitive taxa represented in the stream community, despite the availability of relatively good instream habitat. Sensitive groups decreasing includes Ephemeroptera, Trichoptera, and Odonata, while Diptera are increasing (Table 3-6). When applying the Australian Salinity Sensitivity Score (SSS) to South African taxa on a family level, it indicates an increase in salt tolerant taxa since biomonitoring was initiated in 1996 (Table 3-6). The

⁶ Magnitude, timing, frequency, duration

increase in salt tolerant taxa correlates well with the water quality results, which indicates an increase in electrical conductivity (Table 3-2 and Table 3-3), driven by increases in sulphates, sodium, and chlorides (Figure 3-4). Of specific concern in terms of water quality is the absence of Ephemeroptera: Prosopistomatidae and Tricorythidae, with Prosopistomatidae last recorded in the 1960s, and Tricorythidae only recorded once at a low abundance over 10 sampling events. Tricorythidae is sensitive to increased electrical conductivity, especially manganese sulphates and calcium sulphates (Palmer & Scherman 2000).

The last record of the bivalve Unionidae: *Unio caffer* in the Crocodile River was reported by Haas (1936) in Oberholzer & van Eeden (1967). In the Americas Unionidae has been identified as one of the most imperiled group of aquatic animals with the decline in populations attributed to environmental contamination (Williams et al. 1993; Strayler et al. 2004; Haag 2012). The Natural Heritage Network in the United States and Canada listed, 202 of the nearly 300 known unionoid species as presumed extinct, possibly extinct, critically imperilled, imperilled, or vulnerable (Master et al. 2000 in Lydeard, et al. 2004). The longevity in freshwater mussels ranges for species globally between 4 and 190 years, with slow-growing species living the longest (Haag & Rypel 2011). Wang et al. (2017) indicated that freshwater mussels were among the more sensitive species to alachlor, ammonia, chloride, potassium, sulfate, copper, nickel, and zinc.

The absence of species previously present and determining the cause for absence is clearly of more value in the understanding and protection of valuable freshwater ecosystems than providing subjective ecological categories.

4.3 INSTREAM HABITAT

Biota responded strongly to the gravel-sand-mud substrate, increasing by 73% when compared to previous results (Table 3-7). In terms of those taxa associated with cobble and marginal vegetation, response was less obvious.

The results suggest an increase in sediment-gravel-mud inputs and deposition, while the other biotopes are mostly intact.

4.4 ODONATA

The larvae, exuviae, and adults of 27 (41%) of an expected 66 species have been encountered thus far. The adult species present prefer open vegetative river-stream habitat, with some species with a preference for semi-shaded habitats present but limited. Some species often also associated with lakes and large dams are also present, but they are not dominant. Permanent water species dominated temporal water species.

In terms of larvae, lotic - slow flowing water species were dominant, with lotic moderate flowing water species well represented and lotic fast flowing species present. Species for which the larval mode are categorised as claspers and sprawlers dominate, with shallow burrowers and hiders present. Deep burrowers were absent from samples.

Emergents encountered during surveys included Gomphidae: *Paragomphus genei* and Libellulidae: *Trithemis arteriosa*.

5 CONCLUSION & RECCOMENDATIONS

Ecstatus

The present ecological status (PES) of the Crocodile River at the Maroela sampling site for September 2021 is a B (largely natural) when applying the MIRA model. It is clear from the current and historical results that the water quality of the Crocodile River has altered the instream community, with several taxa previously recorded now absent. The MIRA model in this instance indicates a B-class because the number of taxa encountered in 2021 was high. The PES provided in the MIRA model provides a “false sense of security” when looking at the condition of the Crocodile River. It blurs out issues in our aquatic ecosystems which requires to be addressed in terms of research and management. The presence and absence of taxa historically encountered and now absent is, based on current local and global studies, most likely linked to poor water quality. A PES of B-class suggests that everything is in order.

Monitoring

In the 1980s Sappi Ngodwana Pulp and Paper Mill addressed the quality of their effluent to limit chlorides because the Tabaco Industry could indicate that chloride concentrations of greater than 50 mg/L burns the leaf surfaces when irrigated. Freshwater studies in our aquatic ecosystems in the last 20 years have mostly been focused on monitoring to providing broad indications of “ecosystem health”. Studies focusing on what was and is in our river, why and when they are in an aquatic system and why not, are extremely limited. That means that when it comes to water quality, broad guidelines are provided by licensing authorities, despite the continuous decrease and possible disappearance of certain species. Water quality criteria and other guidance to protect our aquatic ecosystems needs to be updated on at least reach level, taking the natural water quality (pre humanoid) to which species within the system have evolved into consideration. Understanding the percentage change from natural and which chemicals, ions drive the change, help with identifying and addressing sources. Companies discharging effluent needs to have a clear indication of what the water quality discharged allowed needs to be, so that they can apply the knowledge of the chemical engineers to improve the factory/mill processes (e.g., Sappi Pulp Mill Tabaco Industry example). If species are considered indicators, that is exactly what the focus of studies should be. Broad generic guidelines are clearly not adequate for the protection of our aquatic ecosystems.

At an educational level, academic institutions should focus on the ecology of individual species, and rather testing of the ecstatus models than teaching application. More focus should be placed on identifying indicator species for specific drivers in specific parts (e.g., ecoregion, ecological reach, etc.) of every catchment.

Climate Change

One of the biggest global threats to the future of all current species on the planet is human induced climate change (Gummer 2001, World Economic Forum 2016). The recent IPCC (2022) report section on Terrestrial and Freshwater Ecosystems and their Services indicated

with very high confidence⁷ **“Multiple lines of evidence, combined with strong and consistent trends observed on every continent, make it very likely that many observed changes in ranges, phenology, physiology and morphology of terrestrial and freshwater species can be attributed to regional and global climate changes, particularly increases in frequency and severity of extreme events”**. Temperature changes are associated with losses of cold-water species (Root et al. 2003). The challenge is therefore to identify and prioritise “climate refugia”, and the removal of migration or movement barriers. To understand climate induced water temperature changes, water temperature data loggers should be installed permanently at all regular long term biomonitoring sites. That way, species presence absence data can be correlated with water temperature regimes, building knowledge on potential changes in water temperature, stream communities and individuals.

⁷ In the report a level of confidence is expressed using five qualifiers: very low, low, medium, high, and very high, and typeset in italics, e.g., *medium confidence*. For a given evidence and agreement statement, different confidence levels can be assigned, but increasing levels of evidence and degrees of agreement are correlated with increasing confidence.

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7 APPENDIX A – SASS5 DATA SHEETS

SASS5 Data Sheets for the September 2020 and 2021 surveys.

8 APPENDIX B – Site photographs



Figure 8-1. A view of some of the habitats available and sampled at the X2CROC-MAROE site on 15 September 2020.



Figure 8-2. A view of some of the habitats available and sampled at the X2CROC-MAROE site on 14 September 2021.