A STUDY OF THE IMPACT OF ANTHROPOGENIC ACTIVITIES IN THE CROCODILE RIVER, MPUMALANGA.

by

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I declare that **THE STUDY OF IMPACT OF ANTHROPOGENIC ACTIVITIES IN THE CROCODILE RIVER, MPUMALANGA** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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SIGNATURE DATE

(Mr M I Soko**)**

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ABSTRACT

In South Africa water is recognized as a crucial element in the battle against poverty, the cornerstone of prosperity, and a limiting factor to growth. The National Water Act 36 of 1998 recognizes that basic human and environmental needs should be met and that the use of water in all aspects must be sustainable. The Crocodile River (East) is situated in the north east of the Republic of South Africa and it is recognized as a stressed catchment in South Africa. The main impacts are domestic, industrial, agricultural, mining and afforestation activities. These activities pollute the river by discharging effluent as well as seepage from areas that support mining and intensive agriculture in to the river. The river catchment has been a center of research studies for many scientists either focusing on water quality or biological indicators separately. The aim of study was to determine the present ecological condition and the health of the Crocodile River. The objectives were to determine water quality status, identify possible sources of pollution and assess the spatial and temporal trends in ecological state. Fourteen monitoring sites were selected from the Crocodile River and its tributaries. The macro-invertebrates data were collected using the SASS 5 protocol and fish were collected using an electroshocker- catch and release method during high and low flow conditions of the year 2013. Water quality data was obtained by sampling using a polyethylene bottle from different sites within the Crocodile River and its tributaries from September 2012 until August 2013. The samples were analyzed by Mpumamanzi laboratory in Nelspruit and Waterlab in Pretoria. Additional water quality data was obtained from the Department of Water Affairs. Multivariate statistical methods were used to analyze all the data obtained. The multivariate statistical methods indicated that fish and macro-invertebrates species abundance, richness and evenness increase with the river flow distance downstream. Water temperature was one of the leading environmental variables for the structuring of fish and macro-invertebrates assemblage in the Crocodile River and its tributaries. A group formation of site during high and low flow condition by the Bray Curtis similarity and NMDS ordination indicated that many sites share similar macro-invertebrates or fish species. The one way ANOVA analysis indicated that there was no significance difference between macro-invertebrates richness and abundance during both flow conditions but there was a significance difference in fish

richness and abundance between the two flow conditions. The PCA indicated that sodium has the highest physico-chemical impact amongst the physico-chemical parameters in the bi-plot followed by ammonium and nitrate. A correlation of physico-chemical parameters such as chlorine, total dissolved solid, electrical conductivity, sulphate and pH was observed. The levels of total dissolved solids and electrical conductivity were found to increase longitudinally as the river flows downstream. The dominance of single species within the macro-invertebrates and fish communities was an indication of imbalance of the communities within the sites. The presence of the macro-invertebrate family Beatidae and the fish species *Chiloglanis pretoriae* at sites CR3 and CR4 indicates a good water quality in the upper reaches during low flow condition as these species are sensitive to changes in water quality, while the presence of the macro-invertebrate family Pleidea and fish species *Barbus viviparus* at sites CR10 and KR1 during low flow condition indicated possible water pollution as these species tolerate changes in water quality and this was linked to the discharge of effluent from industrial, abandoned mines and run-offs from agricultural activities in the downstream reaches of the river. The low flow condition was dominated by sensitive species especially in the upper reaches than during high flow condition. The macro-invertebrates assessment index indicated that during low flow condition the Crocodile River was mostly at ecological class B (largely natural with few modification) above the Kwena dam, but from downstream of the dam the ecological category was in C class (moderately modified), while its tributaries are in ecological category B (largely natural with few modification). The fish response assessment index indicated that the ecological category for fish was mostly at C class (moderately modified) in the Crocodile River. The changes in macro-invertebrates communities and fish in the Crocodile River were believed to be associated with change of water quality and habitat modification due to flow modification. Agricultural activities in the upper reaches and a combination of industrial and mining activities in the middle and lower reaches of the Crocodile River were believed to be the sources of pollution that results in the change of water quality, fish and macro-invertebrates assemblage in the Crocodile River. Investing in the health of the Crocodile River is important for many Mbombela citizens and river dwellers as they rely on the functioning of the river for survival.

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LIST OF ABBREVIATION

ABBREVIATION	MEANING
ASPT	AVARAGE SCORE PER TAXA
DWA	DEPARTMENT OF WATER AFFAIRS
DWAF	DEPARTMENT OF WATER AFFAIRS AND FORESTRY
EC	ECOLOGICAL CATEGORY CLASS
FISHER'S LSD	FISHER'S LEAST SIGNIFICANT DIFFERENCE
FRAI	FISH RESPONSE ASSESSMENT INDEX
GSM	GRAVEL, SAND AND MUD
H	HIGH FLOW
IVI	IMPRTANT VALUE INDEX
L	LOW FLOW
MIRAI	MACROINVERTEBRATES RESPONSE ASSESSMENT INDEX
NMDS	NON-METRIC MULTIDIMENSIONAL SCALING
NWA 36 OF 1998	NATIONAL WATER ACT, 36 OF 1998
PCA	PRINCIPAL COMPONENT ANALYSIS
POL	POLYNOMIAL
RDA	REDUNDANCE ANALYSIS
RHP	RIVER HEALTH PROGRAM
SASS	SOUTH AFRICAN SCORING SYSTEM
SPSS	STATISTICAL PRODUCT AND SERVICE SOLUTIONS
TDS	TOTAL DISSOLVED SOLIDS
VEG	VEGETATION

CHAPTER 1

1. GENERAL INTRODUCTION

Water is life for all living things. It plays a crucial role in human for drinking, health, sanitation and agriculture. Moreover, water is important for industry, power generation, mining operations and tourism. According to Basson *et al*., (1997), water is recognized as a crucial element in the battle against poverty, the cornerstone of prosperity, and a limiting factor to growth. The South African new water law recognizes that basic human and environmental needs should be provided (DWAF, 1998) and that the exploitation of water in all aspects must be sustainable (Davies and Day, 1998). Protecting the needs of the environment requires tools that can be used to monitor environmental conditions as well as for setting ecological objectives to ensure the proper and sustainable management of the resource (Roux *et al*., 1999). The River Health Program (RHP) was developed to serve as a source of information regarding the overall ecological status of river ecosystems in South Africa. The RHP primarily makes use of in-stream and riparian biological communities to characterize the response of the aquatic environment to multiple disturbances. The rationale is that the integrity or health of the biota inhabiting the river ecosystems provides a direct and integrated measure of the health of the river as a whole (Karr and Chu, 1997).

1.1. Global overview of water resources and human population

Water covers 71% of the Earth's surface, mostly in oceans and other large water bodies, with 1.6% of water below ground in aquifers and 0.001% in the air as vapour, clouds and precipitation (U.S. Geological Survey, 2000). As the demand for water increases in line with human population pressure and economic development activities, river ecosystems will continue to deteriorate unless they are managed in a sustainable way. The exponential increase in the world population, the growing sophistication of its needs and activities for the maintenance of present day life style and the process of industrialization have not only resulted in vastly increased pressure and depletion of water resource but they have also caused generation of enormous quantities of waste (Fuggle and Rabie, 1992). According to Ashton *et al*., (2008), the deterioration of the water resource is due to increased pollution caused by anthropogenic activities such as industry, urbanization, afforestation, mining, agriculture, power generation and accidental water pollution.

Anthropogenic activities result in a significant decrease of surface water quality of aquatic ecosystem in catchments (May *et al*., 2006). Water resources in a catchment play a major role in assimilating or carrying municipal and industrial waste water and run off from agricultural land. River inflows contributes main pollutants to the water resource in a catchment, thereby tending to induce serious ecological and sanitary problems (Gilbert and Wendy, 2003; Kunwar *et al.,* 2005). In South Africa environmental pollution problems started during the first half of the 19th century, with the development of towns and industries and associated accumulation of wastes in built-up areas (DWAF, 1998). The pollution of rivers by human induced activity is now becoming a threat to water resource and its biodiversity.

Pollution means the direct or indirect alteration of the physical, chemical or biological properties of a water resource so as to make it less useful to the intended use (NWA, 36 of 1998). In reference to Weale (1992), water is said to be polluted when it is impaired by contaminants and either does not support a human use, such as drinking water or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish and macro-invertebrates. The National Water Act 36 of 1998 states that activities that pollute or degrade water resource require authorization by the Department of Water Affairs and Forestry (DWAF), to ensure proper management of the river. Even though water quality monitoring is part of the conditions of a water use license, rivers such as the Crocodile River continues to deteriorate. These activities may pollute the river by discharging effluent as well as seepage.

1.2. Overview of the Crocodile River (East) Catchment

The Crocodile River (East) is located in the north east of the Republic of South Africa, Mpumalanga Province and is a relatively large river basin and one of the most economically productive in the country and a source of life for all living organism in this area (DWAF, 1995). It has a total main stem river length of approximately 320 km draining a catchment area of about 10450 km^2. The River is characterized by a broad range of riverine habitats ranging from cold mountains streams of Drakensberg to the slow flow warm water where the river meanders the Lowveld. As a result of the diverse habitats the river is also one of the most biologically diverse systems in South Africa with at least 49 fish species occurring (SCR, 1998).

The lower reaches of the Crocodile River is considered to have poor water quality due to agricultural runoff and return flows, as well as additional mining activities (Kleynhans, 1999). In turn, these water quality changes have important consequences for all segments of society as well as the natural ecosystems that depend on the water resources (Oberholster *et al.*, 2008). The quantities of water abstracted for irrigation, as well as the decreased inflows caused by increased afforestation, have resulted in a marked decline in winter flows from many tributaries and the main stem of the Crocodile River. According to Rainhaverst (2012), the Crocodile River is fast becoming dangerous to be used for watering of crops and swimming and the degradation of water quality in the Crocodile River may also cause a change in the plants, invertebrate and fish communities in the river.

The impact of the state of the local river has forced the Department of Water Affairs, the Inkomati Catchment Management Agency (ICMA) as well as the Ehlanzeni and the Mbombela Municipalities to intervene by issuing directives to defaulting water users. Water quality remains an issue in the Crocodile River and such problems can be aggravated when a low flow is combined with a high load of point or non-point source pollution that can exceed the so-called dilution capacity of the river. According to Ballance *et al.,* 2001, the modifications imposed by the Kwena Dam have already been reported to decrease fish biodiversity in the reaches of the Dam.

The issue surrounding the Crocodile River and its tributaries can be solved by continuous monitoring of water quality and management of the river.

1.3. Study Rationales

The Crocodile River is utilized by many water users (Agricultural, Mining, Domestic and Industrial) until it confluence with Komati River in Komatipoort. These water users pose a threat to the health of the river due to abstraction, discharging and seepage of chemicals to the catchment. In recent years the Crocodile River and associate systems have been the center of research programs from many researchers including the implementation of the River Health Program (DWAF, 1995). Most researchers are focusing on the water quality; physico-chemical and the biological indicators separately. This study intends to identify the possible sources of pollution in the Crocodile River using both biological indicators (fish and Macro-invertebrates) and physico-chemical parameter to give an insight or overview of the ecological state of the river. It is believed that the information gathered during the research will be used in the Crocodile Catchment Water Quality Management Plan.

1.4. Aims and Objectives

The main aim of the study was to determine the current state (health) of the Crocodile River (East) in terms of anthropogenic impacts and identify possible sources of pollution along the river using biological indictors and water quality parameters. The objectives were to;

- Determine water quality status
- Identify sources of pollution
- Assess the ecological state of aquatic ecosystems
- Assess the spatial and temporal trends in ecological state
- Contribute to water quality objectives of the river

1.5. Research Questions

The following questions were to be answered at the end of the research:

- What is the quality status of the water in the Crocodile River?
- What are the possible sources of pollution?
- What is the impact of pollution on aquatic biodiversity – fish and macroinvertebrates?
- What is the river health in terms of ecological classes?
- How does the community make use of the river?

CHAPTER 2

2. LITERATURE REVIEW

2.1. Introduction

Rivers have long been used and abused for the disposal of waste. In South Africa environmental pollution problems started during the first half of the 19th century, with the development of towns and industries and associated accumulation of wastes in built-up areas (DWAF, 1998). The pollution of rivers by human induced activity is now becoming a threat to water resource and its biodiversity. According to Oberholster and Ashton (2008), the deteriorating of water quality can adversely affect human health and has economic implications for various sectors of the economy including agriculture and industry. Polluted water contains viruses, bacteria, intestinal parasites, and other harmful microorganisms, which can cause waterborne diseases such as diarrhea, dysentery, and typhoid. According to Smith (2003), although the rivers have the capacity of self-purification, this capacity is altered due to anthropogenic activities in the river catchment, leading to the destruction of this important ecosystem. Surface water is most exposable to pollution because of their easily accessibility for the disposal of wastewaters (Samarghadi *et al*., 2007). The anthropogenic influences such as urban, industrial and agricultural activities increase exploitation of the water resources.

The introduction of industry, urbanization, afforestation, and mining, agriculture and power generation has long been studied to cause modification in water resource. The change of water quality cause by the effluent, discharge and seepage from these activities also change the ecological processes that naturally purify water quality. The qualities of rivers are good indication of the way of life within a community through which it is flowing. It is an indicator of the socio-economic conditions and environmental awareness and attitude of its users and everything that happens in a catchment area is reflected in the quality of the water that flows through it because the results of human activity and lifestyle ultimately end up in rivers, through runoff.

As the demand for water increases in line with human population pressure and economic development activities, river ecosystems will continue to deteriorate unless they are managed in a sustainable way. The exponential increase in the world population, the growing sophistication of its needs and activities for the maintenance of present day life style and the process of industrialization have not only resulted in vastly increased pressure and depletion of the earth's essential natural resources but they have also caused generation of enormous quantities of waste (Fuggle and Rabie, 1994). According to Parsons and Jolly (1994), the production of unwanted by-product or waste of all human activities is characteristic of mankind and inevitable in modern society. The more advanced the level of civilization, the greater the production of waste, in liquid as well as in solid form. Thus, utilizations and protection of this resource in a sustainable manner is essential for the future of the country. Protecting the needs of the environment requires tools that can be used to monitor environmental conditions as well as for setting ecological objectives to ensure the proper and sustainable management of the resource (Roux *et al*., 1999).

In Mpumalanga the provincial implementation initiative was being driven by the Mpumalanga Parks Board and the Kruger National Park as part of the application of the RHP on the main rivers of Mpumalanga and the first complete monitoring exercise of this nature took place on the Crocodile and Elands River during late 1996 and early 1997.

2.2. River Health Programme (RHP)

The national monitoring programme that focuses on measuring and assessing the ecological state of riverine ecosystem was designed for South Africa and it was implemented in the Mpumalanga Province especially in the Crocodile and Elands Rivers in 1996 and early 1997 (Roux *et al.,* 1999). The river health program was developed with the overall goal of expanding the ecological basis of information on aquatic resources in order to support the rational management of these systems (Roux, 1997). The formal design was initiated in 1994 by the Department of Water

Affairs and Forestry and the main purpose was for the programme to serve as a source of information regarding the overall status of riverine ecosystem in South Africa. The River health programme makes use of instream biological response monitoring in order to characterize the response of the aquatic environment to multiple disturbances and the rational is that the integrity of the biota inhabiting the river provides a direct, holistic and integrated measure of the integrity of the river as a whole (Karr and Chu, 1997). Indices such as Fish Response Assessment Index and Macro-Invertebrates Assessment Index have been developed for instream assessment and are forming part of the river health programme. Monitoring as used in the river health programme makes use of Eco-classification procedure to assess the severity of change from reference conditions. The development of the eco-classification procedure which uses all the indices has assisted South Africa to assess the present ecological state of some of the rivers in the country including Crocodile and Elands Rivers. The last decade has seen the development and increasing application of biological indices with the purpose of expressing and interpreting how similar an assemblage at a site is to its potential if it were undisturbed (Karr, 1991). These indices are typically additive i.e., the sum of several measurement or calculated variables known as metrics which are obtained from sampling the assemblage. In reference to (Karr *et al.,* 1986; Barbour *et al.,* 1995), a metrics is defined in this usage as an ecological attribute of the assemblage estimated from a collection of organisms and responsive perturbation or disturbance.

2.3. Biomonitoring

Monitoring programmes of rivers have long been established for chemical and physical characteristics of water and were performed regularly in many parts of the country. The utilization of biological monitoring has only recently become a point of focus for organizations interested in determining the biological characteristics and status of rivers in South Africa. In Mpumalanga the Crocodile River and some of its tributaries are monitored quarterly by the Department of Water Affairs while the Mpumalanga Tourism and Parks Agency (MTPA) monitor the river bi-annually using macro-invertebrates and fish as their biological indicators.

2.3.1. Macro Invertebrates

Aquatic macro-invertebrates have been commonly used than any other biological group to assess the biological integrity of stream ecosystems with relatively good success throughout the world (Resh *et al.*, 1995; Barbour and Gerritsen, 1996; O'Keeffe and Dickens, 2000) and because they exhibit a wide variation of response to pollutants they have been extensively used in lotic water bodies to evaluate water quality and complement physico-chemical surveys (Hawkes, 1979; Shutes, 1985). Macro-invertebrates are organisms that are large enough to be seen with the naked eye and lack a backbone They inhabit all types of running waters, from fast flowing mountain streams to slow moving muddy rivers and these include insects in their larval or nymph form, crayfish, clams, snails, and worms. Most live part or most of their life cycle attached to submerged rocks, logs, and vegetation.

Indices based on macro-invertebrate assemblages have proven to be useful measures of river health and are widely applied today in South Africa (Rosenberg and Resh, 1993) and this include the South African Scoring System which was developed by Chutter (1997). The SASS index is based on the presence of families of aquatic macro-invertebrates and their sensitivity to water quality changes and is currently in its fifth stage of development. The calculated results are expressed as score as (SASS score) and average score per taxa (ASPT value).

2.3.2. Ichthyofauna

As a result of the diverse habitants of the Crocodile River, it is also one of the most biologically diverse systems in South Africa with at least 49 fish species occurring (SCR, 1998). Fishes are cold-blooded vertebrate animals, living in water and breathing by means of gills and having fins for stability and movement. According to Sprague (1973), fishes are common used as bioassay organisms, but they have rarely been used in comprehensive monitoring (Hocutt and Stauffer, 1980) and effort on using fish in field monitoring have been directed towards bioassay of contaminants, often using representative important species (USEPA, 2005).

Fish communities have a high degree of natural variability; they can be useful indicators of ecosystem health (Moyle, 1994). The presence and abundance of fish species can be related to water chemistry, physical habitat, and land-use activities to provide a more complete picture of water quality across a river basin. Fish have been given consideration in biological water-quality monitoring of streams because they are generally perceived by the public to be ecologically relevant, and they are directly related to legislative mandates because of human health and endangered species concerns (Berkman *et al*., 1986). In South Africa Fish received general attention with reference to the intolerance of certain species to particular environment (Kleynhans *et al*., 1992).

2.3.3. Water Quality

Typically, water pollution problems are identified through sampling and analyzing parameters such as ammonia, biochemical oxygen demand (BOD), dissolved oxygen (DO), total dissolved solids (TDS), heavy metals, nitrate, pesticides, pH, phosphorus, total suspended solids (TSS) and turbidity. Kleynhans (1999) monitored the lower reaches of the Crocodile River and concluded that industrialization, urbanization and agricultural practice have direct impact on the deterioration of water quality. A study conducted by Ramshoo and Muslim (2011) concluded that land use activities (such as agricultural activities) influence nutrients loading and discharge in a river. The finding of their studies were similar to the study conducted by Kleynhans (1999), revealing that the upper reaches of the Crocodile River has good water quality, but more susceptible to eutrophication due to agricultural activities. A study conducted by Heath and Claassen (1999), concluded that the section from Nelspruit to the confluence with the Kaap River is associated with domestic runoff, littering and an increase in nutrients. Industrial effluents from Nelspruit cause an increase in manganese and boron concentrations, while major sewage treatment works at the towns of Nelspruit, Matsulu and Kanyamazane are sources of high nutrient loads in the river (Heath and Claassen, 1999).

2.4. Study Area and Site selection

2.4.1 Study Area

The Crocodile River (East) catchment is located within the Inkomati Water Management area, the first Water Management Area (WMA) in South Africa to have a Catchment Management Agency (CMA). The Crocodile River originates north of Dullstroom in western part of the catchment area and it rises at an altitude of approximately 2000 m.a.s.l near Dullstroom in Steenberg Mountains. It is a relatively large river basin with a total main-stem of approximately 320 km and covers a catchment of about 10450 km^2.

According to DWAF (1995), the main form of land use occurring in the Crocodile River catchment are as follows:

Forestry - the western half of the catchment, with annual rainfall >800 mm, has the largest number of exotic plantations. Some 1 722 km^2, or 16.5% of the catchment is covered by exotic plantations.

Dryland agriculture - are located primarily in the central parts of the catchment and take the form of maize, subtropical fruits, nuts, citrus, coffee and vegetable cultivation.

Irrigated agriculture - the largest area of irrigation are located in the central and eastern region of the catchment and about 91 000 ha of crops are irrigated with sugar-cane (21 000 ha) and citrus (20 000 ha) being the most important.

Nature conservation - the major area of nature conservation activity in the catchment occurs within the southern portion of the Kruger National Park.

Mining and quarrying - the majority of mining activity has occurred along the Kaap River and to some degree in the lower Crocodile River.

Domestic and industrial land use - the towns of Nelspruit and White River are the focus of domestic and industrial land use in the catchment, with smaller centers at strategic points across the catchment. A large paper mill is situated at Ngodwana next to the Elands River.

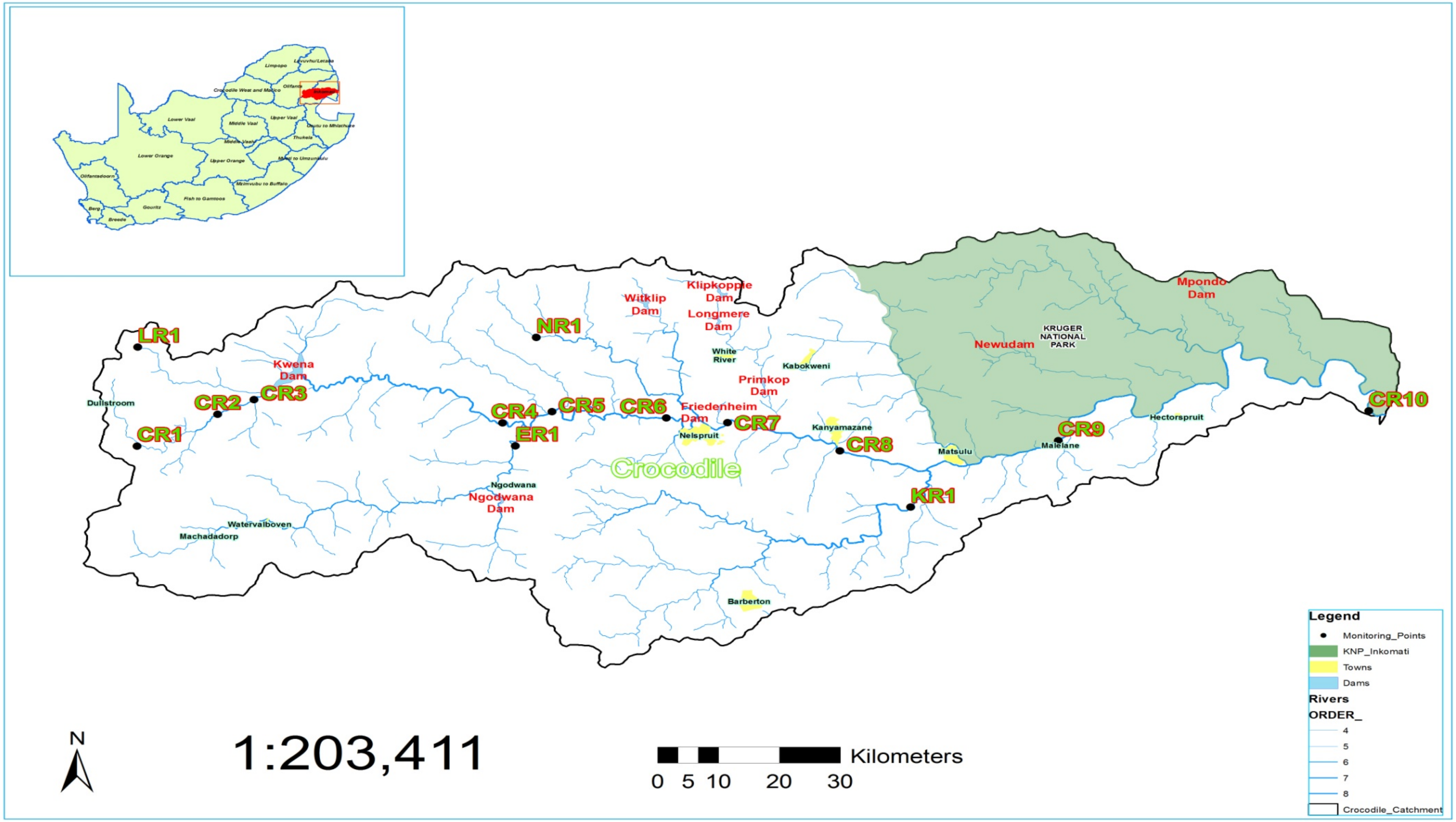


Figure 2.1: Map showing the monitoring sites in the Crocodile River Catchment.

2.4.2. Site Selections

Site selection for this study was based on the activities in the catchment and the main aim of the study. Fourteen sites were selected from the main stem of the Crocodile River and its tributaries (Elands, Nels and Kaap River) to represent different sections of the river and activities in the catchment. Moreover, accessibility and safety of the sites were taken into consideration. The river was categorized in to three sections whereby site CR1-CR4 were the upper reaches including site LR1 as a tributary, CR5-CR8 were the middle reaches including site ER1 and NR1 as tributaries and CR9-CR10 lower reaches including site KR1 as a tributary.

Table 2.1: The methods used, river sampled and co-ordinates of the selected sites.

Site Names	River	Assessment Used	Co-ordinates
CR1	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4937 30.1447
CR2	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4349 30.2637
CR3	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4078 30.3176
CR4	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4521 31.6810
CR5	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4329 30.7549
CR6	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4633 30.9629
CR7	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4504 31.0172
CR8	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.5027 31.1845
CR9	Crocodile River	SASS 5, FRAI and Water Quality Sample	-25.4838 31.5059
CR10	Crocodile river	SASS 5, FRAI and Water Quality Sample	-25.3937 31.9768
ER1	Elands River	SASS 5, FRAI and Water Quality Sample	-25.4933 30.7036
NR1	Nels River	SASS 5 and FRAI	-25.6068 31.288
KR1	Kaap River	SASS 5, FRAI and Water Quality Sample	-25.6068 31.288
LR1	Lunsklip River	FRAI	-25.3105 30.1456

CHAPTER 3

3. ICHTHYOFAUNA

3.1. Introduction

Maintaining species diversity of stream dwelling fishes is one of the ecological functions of streams. The identification of patterns of variations in stream dwelling fish assemblages and their potential causal mechanisms is a central theme in stream ecology (Mathews, 1998). Fish have received general attention with reference to the intolerance of certain species to particular environmental conditions in South Africa (Kleynhans *et al*., 1992). Since fish are relatively long lived and mobile they are considered good indicators of long term influences on the general habitat conditions within a reach. The numbers of species of fish that occur in a specific reach, as well as factors such as different size classes and the health of fish can be used as indicators of river health. In South Africa fishes are considered to be one of the important indicators of river health and their responses to modified environmental conditions are measured in terms of the Fish Response Assessment Index (Kleynhans, 2008).

Factors influencing fish assemblages involved the physico-chemical environment which is spatial heterogeneous and temporary variable and biotic interaction such as competitions and predation (Harvey and Stewart, 1991; Gorman, 1998; Grossman *et al.,* 1998; Dauwalter *et al.,* 2008). According to Ashton (2007), several factors such as development; agricultural pollution, domestics and industrial effluent, and water withdrawal also threatened the freshwater Ichthyofauna in South Africa. The survival of the fauna depends largely on the success of conservation efforts outside protected areas (Skelton *et al*., 1995).

The Crocodile River has diverse habitats and is considered to be the most biological diverse system in South Africa with forty nine (49) species occurring (Ballance *et al.,* 2001). Some of the fish species that are likely to occur in the Crocodile River include:

Tilapia sparmanii, Chiloglanis swierstrai, Amphilius uranoscopus, Barbus anoplus, Amphilius natalensis, Labeobarbus marequensis, Barbus argenteus, Chiloglanis pretoriae, Chiloglanis parutus, Macasenius macrolepidotus, Barbus neefi, Oreochromis mossambicus, Barbus treurensis, Barbus unitaeniatus, Barbus eutaenia, Labeo rosae, Labeo cylindricus, Anguilla mossambica, Barbus birficus ,Pseudocrenilabrus philander, Opsaridium peringueyi, Barbus annectes, Barbus paludinosus, Glossogobius callidus, Hydrocynus vittatus, Hypophthalmichthys molitrix, Mesobola brevianalis, Labeo molybdinus, Glossogobius guiris, Petrocephelus wesselsi, and Synodontis zambezensis (Kleynhans, 1999; KNP, 2014).

Although species such as exotic largemouth bass, *Micropterus salmoides*, exotic carp, *Cyprinus carpio* and sharp tooth catfish, *Clarias gariepinus*, are indigenous species in South Africa, they have been introduced in this part of the system and were released in the Kwena Dam due to their habitat preference (Kleynhans, 1988). Alien species such as *Oncorhynchus mykiss* (rainbow trout) also occurs in the upstream of the catchment. Fish diversity in rivers is related to habitat complexity, which is influenced by depth, type of substratum, and water current velocity. The more pristine the river, the greater and more stable the species diversity throughout the seasons. The relationship between habitat traits and presence or absence of fish species suggests that the majority of fishes in small streams are habitat specialists (Gorman and Karr, 1978).

Although the Crocodile River support a vast variety of fish diversity, studies conducted by Kleynhans (1999), indicated that the presence of the Kwena dam has negative impact on biodiversity in the downstream reaches. According to Kleynhans (1999), the relative fish assessment index integrity score per fish habitat segment decreases longitudinally in the Crocodile River and this was linked to the activities that pose a threat to the river. Thus, ecological studies focusing on biodiversity patterns are crucial for the management and conservation of natural resources in the tropics (Galacatos *et al*., 1996).

3. 2. Materials and Methods

3.2.1. Field Survey

Fish surveys were conducted in the above mentioned bio-monitoring sites during high and low flow conditions in the year 2013. During the survey three sections of the river were sampled per site using an electric shocker (SAMUS-725MP) as the one that was effective for this study. The data was collected in different velocity depth classes and for each flow depth class, the presence of features that provide cover for fish was taken into consideration. Information on the general habitat and cover preferences of fish species was obtained from the available literature and personal experience. Fish data collected in different velocity depth was kept separate for analysis and the results were recorded as a number of fish caught per time unit (Kleynhans, 1999).

3.2.2. Data analysis

Analysis of data for this study focused on quantifying the spatial and temporal variation in species richness and abundance, and identification of environmental variables explaining variation across the study sites. Species richness and abundance were used directly in the analysis because the sampling effort was similar across sites and seasons. The species dominance was assessed by the importance value index (IVI) using the frequency of occurrence and relative abundance of species (Krebs, 1989). A one-way ANOVA was used to test the differences in species richness and abundance across sites during high and low flow conditions. The significant main effects were analyzed using the Fishers' LSD when appropriate. The SPSS version 18 was used to perform statistical analyses and alpha was set at $P<0.05$.

Discrete temporal and spatial patterns in fish assemblages were identified using the PRIMER version 5 (Clarke and Gorley, 2001). The richness and abundance data collected were log (x+1) transformed to meet the assumption of multivariate normality and to moderate the influence of extremes in richness and abundance.

Transformed sample data were then used to create a Bray-Curtis similarity matrix calculated for all pair-wise sample comparisons (Bray and Curtis, 1957). Analysis of similarities (ANOSIM) was used to compare fish assemblage from the sites during low and high flow conditions. A two way ANOSIM without replication was used to test for site and seasons effects since fish at each site were sampled once in every season. A two-way nested ANOSIM was used to test for the Kwena Dam effect on fish biodiversity in the Crocodile River. The relationship amongst assemblages from each site was graphically represented using a cluster analysis and a Non-Metric Multi-Dimensional Scaling analysis (NMDS). The contribution of each species to the differences among assemblage groups was identified using SIMPER (Clarke and Warwick, 2001).

A Detrended Correspondence Analysis (DCA) was performed to identify the strongest gradient of assemblage composition independent of the environmental variables and standard deviation redundancy analysis (RDA) was selected for the evaluation of the variability in the assemblage structure in relation to the measured environmental factors. The multivariate statistical analysis was performed using CANOCO version 4.5 (ter Braak and Smilaeur, 2002).

A Fish Response Assessment Index (FRAI) was performed to determine the ecological condition of the river. The FRAI is a rule based model recently developed by Kleynhans (2008), and is based on the environment intolerances and preference of the reference fish assemblage and the response of a constituent species of the assemblage to a particular groups of environmental determinates or a drivers. These intolerance and preference attributes are categorised into metric groups with constituents metric that relates to the environmental requirements and preferences of individual species.

3.3. Results and Discussion

3.3.1 Spatial and temporal trend analysis

A total of 1504 fish were caught in the Crocodile River and its tributaries during both high and low flow surveys, representing 30 species. The highest number of species as indicated in Table 3.1 and 3.2, were sampled during low flow at site CR9 and certain species such as *Amphilius uranoscopus, Barbus unitaeniatus and Labeo cylindricus* were sampled only during low flow survey. Sites CR7, CR8 and CR10 followed a similar trend. The unavailability of these species at the specified sites during the high flow might be due to difficulties of sampling during high flow conditions and the change in habitat within the sites.

Tables 3.1 and 3.2 indicate the expected fish species (as documented in previous studies) and species obtained during this study (and their numbers) for high and low flow conditions respectively.

Table 3.1: Expected fish species in the Crocodile River and its tributaries and the number of fish caught per site during high flow conditions (Kleynhans, 1999; KNP, 2014).

Expected fish species in the Crocodile River and its tributaries	**Number and types of fish caught per site during study**												
	CR1H	**CR2H**	**CR3H**	**CR4H**	**CR5H**	**CR6H**	**CR7H**	**CR8H**	**CR9H**	**CR10H**	**ER1H**	**NR1H**	**KR1H**
FAMILY: AMPHILIIDEA													
Amphilius natalensis	-	-	-	-	-	-	-	-	-	-	-	-	-
Amphilius uranoscopus	-	-	1	2	2	-	-	-	-	-	-	-	-
FAMILY: ANGUILLIDEA													
Anguilla marmorata	-	-	-	-	-	-	-	-	-	-	-	-	-
Anguilla mossambica	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: CYPRINIDAE													
Barbus anoplus	13	3	-	-	-	-	-	-	-	-	-	-	-
Barbus argenteus	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus brevipinnis	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus eutaenia	-	-	-	-	-	-	20	14	14	-	-	-	-
Barbus hamilton	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus neefi	-	-	3	-	-	-	-	-	-	-	-	-	-
Barbus paludinosus	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus radiatus	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus trimaculatus	-	-	-	-	-	-	-	-	-	4	-	-	-
Barbus unitarians	-	-	-	-	-	-	10	5	-	-	-	-	-
Barbus viviparus	-	-	-	-	-	-	-	-	-	40	-	-	-
Barbus annectens	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo congoro	-	-	-	-	-	-	-	-	-	1	-	-	-
Labeo cylindricus	-	-	-	-	-		2			5			5
Labeo molybdinus	-	-	-	-	-	-	-	-	4	-	-	-	-

Table 3.1: Continued

Expected fish species in the Crocodile River and its tributaries	Number and types of fish caught per site during study												
	CR1H	**CR2H**	**CR3H**	**CR4H**	**CR5H**	**CR6H**	**CR7H**	**CR8H**	**CR9H**	**CR10H**	**ER1H**	**NR1H**	**KR1H**
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	-	3
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeobarbus marequensis	-	-	-	-	-	15	29	20	4	4	10	2	-
Labeobarbus polylepis	-	-	-	-	-	-	-	-	-	-	-	-	-
Opsaridium peringueyi	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: MOCHOKIDEA													
Chiloglanis bifurcus	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	-	-	-	-	-	-	-	-	4	10	-	-	-
Chiloglanis pretoriae	-	-	45	4	14	10	11	14	7	-	25	-	-
Chiloglanis swierstrai	-	-	-	-	-	-	-	-	2	4	-	2	3
FAMILY: CLARIIDAE													
Clarias gariepinus	-	-	-	-	-	-	-	-	-	1	-	-	-
FAMILY: GOBIIDEA													
Glossogobius callidus	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossogobius guiris	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: CHARACIDAE													
Hydrocynus vittatus	-	-	-	-	-	-	-	-	-	-	-	-	-
Micralestes acutidens													
FAMILY: MORMYRIDAE													
Marcusenius macrolepidotus	-	-	-	-	-	-	-	-	2	-	-	-	-
Petrocephelus wesselsi	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.1: Continued

Expected fish species in the Crocodile River and its tributaries	Number and types of fish caught per site during study												
	CR1H	**CR2H**	**CR3H**	**CR4H**	**CR5H**	**CR6H**	**CR7H**	**CR8H**	**CR9H**	**CR10H**	**ER1H**	**NR1H**	**KR1H**
FAMILY: CICHLIDAE													
Pseudocrenilabrus philander	-	-	-	-	-	-	10	-	4	-	-	-	-
Tilapia rendalli	-	-	-	-	-	-	-	-	5	-	-	-	-
Tilapia sparmanii	-	-	-	-	-	-	-	-	-	-	-	-	-
Oreochromis mossambicus	-	-	-	-	-	-	1	-	-	-	-	-	-

Table 3.2: Expected fish species in the Crocodile River and its tributaries and the number of fish caught per site during low flow conditions (Kleynhans, 1999; KNP, 2014).

Expected fish species in the Crocodile River and its tributaries	**Number and types of fish caught per site during study**													
	CR1L	**CR2L**	**CR3L**	**CR4L**	**CR5L**	**CR6L**	**CR7L**	**CR8L**	**CR9L**	**CR10L**	**LR1L**	**ER1L**	**NR1L**	**KR1L**
FAMILY: AMPHILIIDEA														
Amphilius natalensis	-		2	-	-	-	-	-	-	-	-	-	-	-
Amphilius uranoscopus	-	1		9	6		1	2	4		1	-	-	-
FAMILY: ANGUILLIDEA														
Anguilla marmorata	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anguilla mossambica	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: CYPRINIDAE														
Barbus anoplus	8	-	-	-	-	-	-	-	-	-	2	-	-	
Barbus argenteus	-	-	-	21	-	-	-	-	-	-	-	-	-	
Barbus brevipinnis	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Barbus eutaenia	-	-	-	-	-	-	56	19	8	-	-	-	-	4
Barbus hamiltons	-	-	-	-	-	-	-	-	-	8	-	-	-	-
Barbus neefi	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus paludinosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbus radiatus	-	-	-	-	-	-				3	-	-	-	-
Barbus trimaculatus	-	-	-	-	-	-		8	11		-	-	-	-
Barbus unitaeniatus	-	-	-	-	-	-	17	5	2		-	-	-	-
Barbus viviparus	-	-	-	-	-	-	-	-	1	142	-	-	-	5
Barbus annectens	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeo congoro	-	-	-	-	-	-	-	-	-	4	-	-	-	-
Labeo cylindricus	-	-	-	-	-	-	2	14	6	8	-	-	-	-

Table 3.2: Continued

Expected fish species in the Crocodile River and its tributaries	**Number and types of fish caught per site during study**													
	CR1L	**CR2L**	**CR3L**	**CR4L**	**CR5L**	**CR6L**	**CR7L**	**CR8L**	**CR9L**	**CR10L**	**LR1L**	**ER1L**	**NR1L**	**KR1L**
Labeo molybdinus	-	-	-	-	-	-	-	6	4	-	-	-	-	-
Labeo rosae	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Labeo ruddi	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyprinus carpio	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Varicorhinus nelspruitensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Labeobarbus marequensis	-	-	-	-	81	11	22	35	5	12	-	-	-	9
Opsaridium peringueyi	-	-	-	-	-	-	-	14	-	-	-	-	-	-
Labeobarbus polylepis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: MOCHOKIDAE														
Chiloglanis bifurcus	-	-	-	-	19	-	-	-	-	-	-	-	-	-
Chiloglanis paratus	-	-	-	-	-	-	9	-	9	15	-	-	-	-
Chiloglanis pretoriae	-	20	76	23	26	17	19	51	25	-	26	-	-	-
FAMILY: CLARIIDAE														
Clarias gariepinus	-	-	-	-	-	1	1		4	1	-	-	-	-
FAMILY: GOBIIDAE														
Glossogobius callidus	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Glossogobius guiris	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: CHARACIDAE														
Hydrocynus vittatus	-	-	-	-	-	-	-	-	-	5	-	-	-	-
Micralestes acutidens	-	-	-	-	-	-			17	3	-	-	-	-

Table 3.2: Continued

Expected fish species in the Crocodile River and its tributaries	**Number and types of fish caught per site during study**													
	CR1L	**CR2L**	**CR3L**	**CR4L**	**CR5L**	**CR6L**	**CR7L**	**CR8L**	**CR9L**	**CR10L**	**LR1L**	**ER1L**	**NR1L**	**KR1L**
FAMILY: MORMYRIDAE														
Marcusenius macrolepidotus	-	-	-	-	-	-	4	4	-	-	-	-	-	-
Petrocephelus wesselsi	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FAMILY: CICHILIDAE														
Oreochromis mossambicus	-	-	-	-	-	-	3	2	8	21	-	-	-	-
Pseudocrenilabrus philander	-	5	6	-	11	2	34	8	4	-	-	-	-	-
Tilapia rendalli	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Tilapia sparmanii	-	-	-	-	-	-	-	-	-	-	-	-	-	2

A polynomial trend line (Figure 3.1), indicated an increase of total number of fish species caught in the middle (sites CR7 and CR8) and lower reaches (sites CR9 and CR10). The increase of the total number of fish species was believed to be associated with the presence of habitat diversity especially for the species which prefer slow flowing water as this part of the river is characterized by such habitat. Figure 3.1 further indicated that during low flow conditions higher number of species occurs in the system compared with high flow conditions. Species such as *Tilapia sparmanii, Labeo rosae and Labeo molybdinus* dominated the downstream part of the Crocodile River.

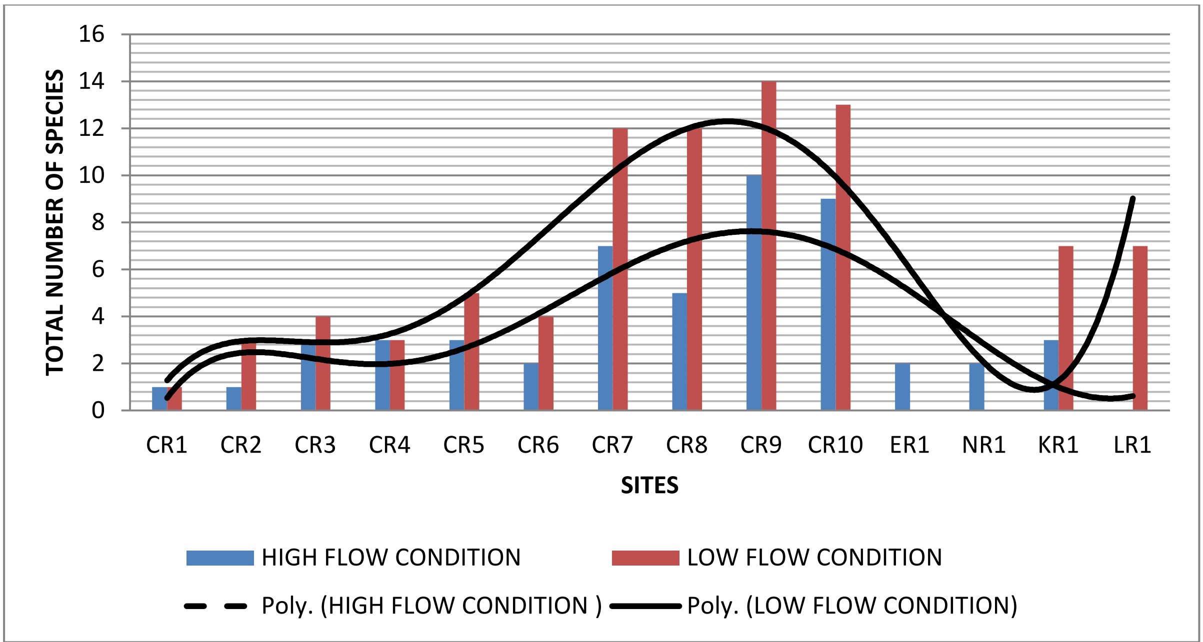


Figure 3.1: Total number of fish caught during the low flow and high flow survey in the Crocodile River and its tributaries with a polynomial trendline.

Sites CR1, CR2 and CR4 in the upper reaches of the Crocodile River had low diversity of species or only a small number of species were caught, especially at site CR1 where only one species (*Barbus anoplus*) occurs. The low diversity of species at site CR2 was believed to be caused by the presence of predator species (*Oncorhychus mykiss,* rainbow trout) which is known to occur in this part of the river. Furthermore, the release of water from the Kwena Dam has resulted in natural changes at site CR4 which was below the dam and this was observed by the limited diversity of species sampled at this site due to change in habitat as compared to other sites below the dam.

A Primer version 5 was used to complete a range of univariate diversity tests, which included Margalef richness index, Pielous evenness index and Shannon-Wiener index. Species richness is a measure of the total number of species present for a given number of individuals. Evenness is a measure of how evenly the individuals are distributed among different species. The diversity index incorporates both of these parameters. Richness ranges from 0 (low richness) to 12 (high richness), evenness ranges from 0 (low evenness) to 1 (high evenness), diversity ranges from 0 (low diversity) to 5 (high diversity). Margalef richness index is an indication of species richness and abundance. The polynomial trend line that has been overlaid on the Margalef species richness graph (Figure 3.2), shows a similar pattern with the total number of species graph (Figure 3.1), with an increase in species richness and abundance at site CR9 during low flow condition.

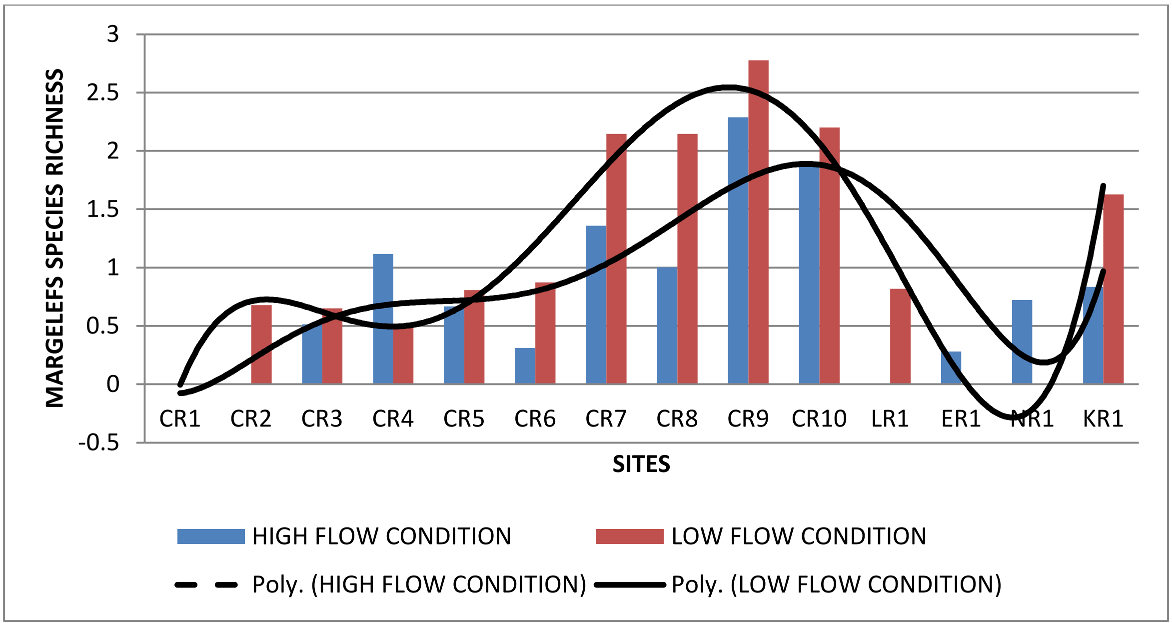


Figure 3.2: Margalef species richness of the Crocodile River and some of its tributaries during low flow and high flow surveys.

The increase in abundance and species richness at some sites in a river system may be due to the fact that some of the species that might be present in the river were tolerant to water pollution. When comparing sites CR9 and CR10 during both flow conditions, a decrease of species richness and abundance were observed after the Malelane town at site CR10 and this might be due to change in water quality caused by the discharge of sewage in to the system downstream. Site CR10 recorded species that prefer slow flowing water like *Tilapia rendalli* and *Labeo congoro* (Kleynhans, 1999*),* hence the lower reaches contains high species richness and abundance when compared to the middle and upper reaches of the river during both flow conditions.

The upper reaches sites CR1, CR2, CR3 which were above the Kwena Dam and CR4 which was below the Kwena Dam had low species richness and abundance compared to the middle and lower reaches of the river during low flow condition. Site CR4 had high species richness when compared to other sites (CR5 and CR6) of the middle reaches during high flow condition. Although the sites upstream of the river had lower species richness site CR4 indicated relatively high species richness especial during high flow condition compared to it associate sites. The low species richness at sites CR1, CR2, CR3 and CR4 can be attributed to the Kwena Dam which reduces fish richness and abundance upstream by limiting fish movement. A study conducted by Kleynhans (1999), indicated that the presence of the dam has impact on habitat on the downstream reaches of the dam especially during low flow condition. Other studies conducted in subtropical small stream of the Haungshan Mountain in China indicated that dam construction can reduce upstream fish richness by eliminating or reducing fish movement. Dams can also replace native warm water assemblages with non-native coldwater assemblages by decreasing downstream water temperature (Holmquist *et al*., 1998; March *et al*., 2003; Bonner and Wilde, 2000; Minckley *et al*., 2003; Quinn and Kwak, 2003). Thus, the reason why the upstream reaches have low species richness and abundance which might be due to such obstructions.

After the confluence of the Elands River at site CR5 the fish species richness started to increase gradually passing through the Nelspruit town (CR7) and Kanyamazane town (CR8). The increase in species richness and abundance at these sites were related to the presence of habitat preference for the fish species caught at those sites. A higher fish species richness and abundance was observed at site KR1 when compared to the other tributaries sampled during the survey. Species richness in the Crocodile River was observed to increase longitudinally in the downstream reaches.

The evenness index (Pielous evenness index) was also an important component of the diversity indices. It expresses how equally individuals were distributed among the different species (Figure 3.3). The polynomial trend line that has been overlaid to the Pielous evenness index indicated that site CR3 lack evenness during both flows, which was an indication that the site might be dominated by one species (*Chiloglanis pretoriae)* and which was a indication of good water quality as this species is susceptible to change in water quality and are dependent on flowing-water habitats and have a preference for substrate cover (Kleynhans, 1999).

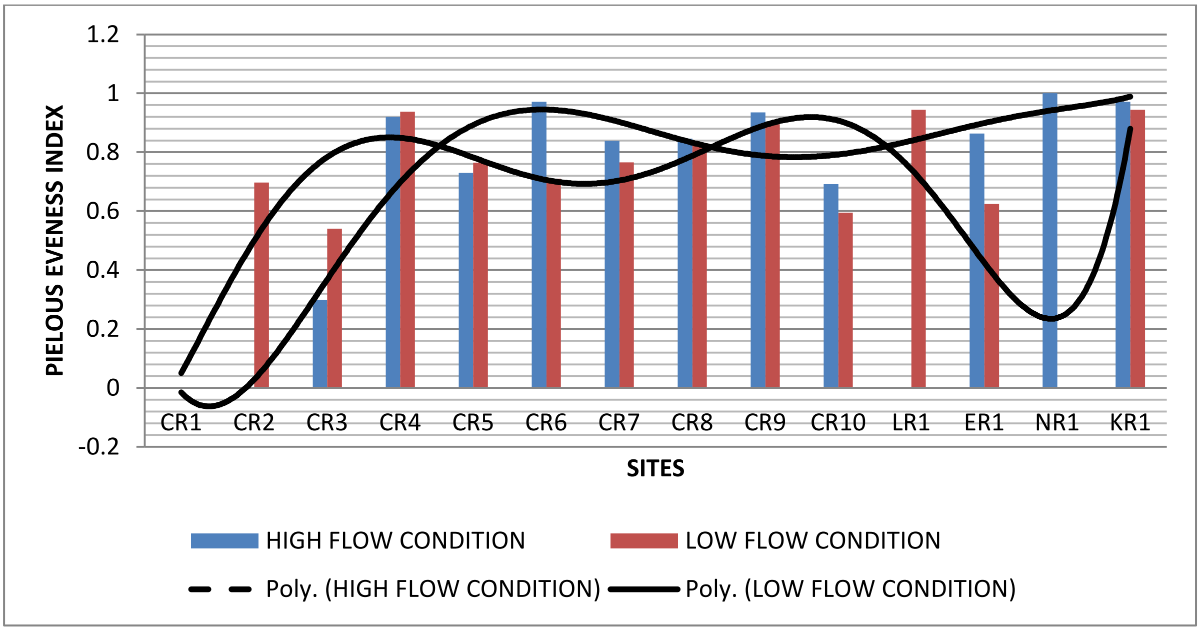


Figure 3.3: Pielous richness index of the Crocodile River and some of its tributaries during low flow and high flow surveys.

Site CR10 was also dominated by one species (*Barbus viviparus*) during both flow conditions and this species is moderately tolerant to pollution. The presence of this fish species indicated that the lower reaches of the Crocodile River had poorer water quality. At site LR1 fish species were not evenly distributed during low condition. The lack of evenness at other sites especially CR1 was due to the fact that the site was dominated by one species, *Barbus anoplus*. The presence of this species in CR1 indicated that the upper reaches of the Crocodile River was susceptible to change in water quality as this species is moderately tolerant to poor water quality (Kleynhans, 1999) .

The Shannon Wiener diversity index also followed a similar pattern as Pielous richness index. The polynomial trendline in figure 3.4 also indicate the lack of diversity in the upper reaches of the Crocodile River.

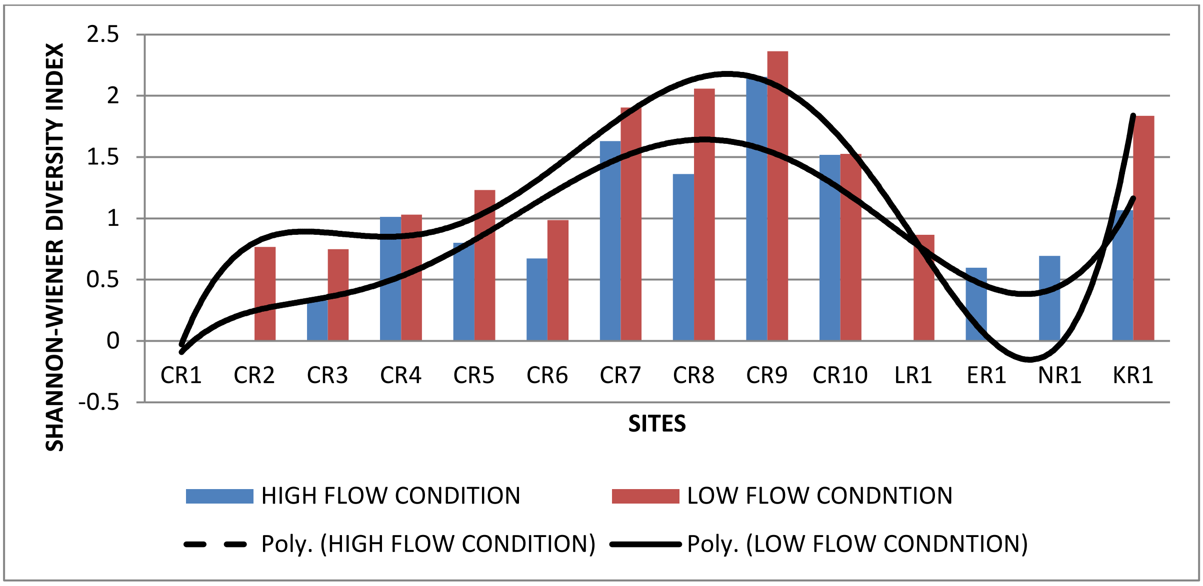


Figure 3.4: Shannon Wiener diversity index of the Crocodile River and some of its tributaries during low flow and high flow surveys.

The low diversity in the upper reaches (Figure 3.4), was attributed to habitat diversity, water quality characteristics and the presence of predator species. This implies that the upper reaches of the Crocodile River had low fish diversity which was due to poor habitat diversity and substrate complexity.

A Bray-Curtis cluster analysis and NMDS ordination was performed in order to obtain an indication of the temporal and spatial trends of fish communities in the Crocodile River (Figure 3.5). The data used for this analysis was transformed in log (x+1). The Bray Curtis cluster analysis (Figure 3.5A), indicated that there were seven distinctive groupings of sites formation in the system which was a good indication of similarities although other grouping had only one site. The formation of similarity grouping was observed at similarity value of 40 and 50% (Figure 3.5B). Site ER1 was the only tributary site that was grouped together with other sites in the Crocodile River during high flow. The formation of grouping within the sites was attributed to the fact that they had similarities in species occurrence within specific sites (Figure 3.5).

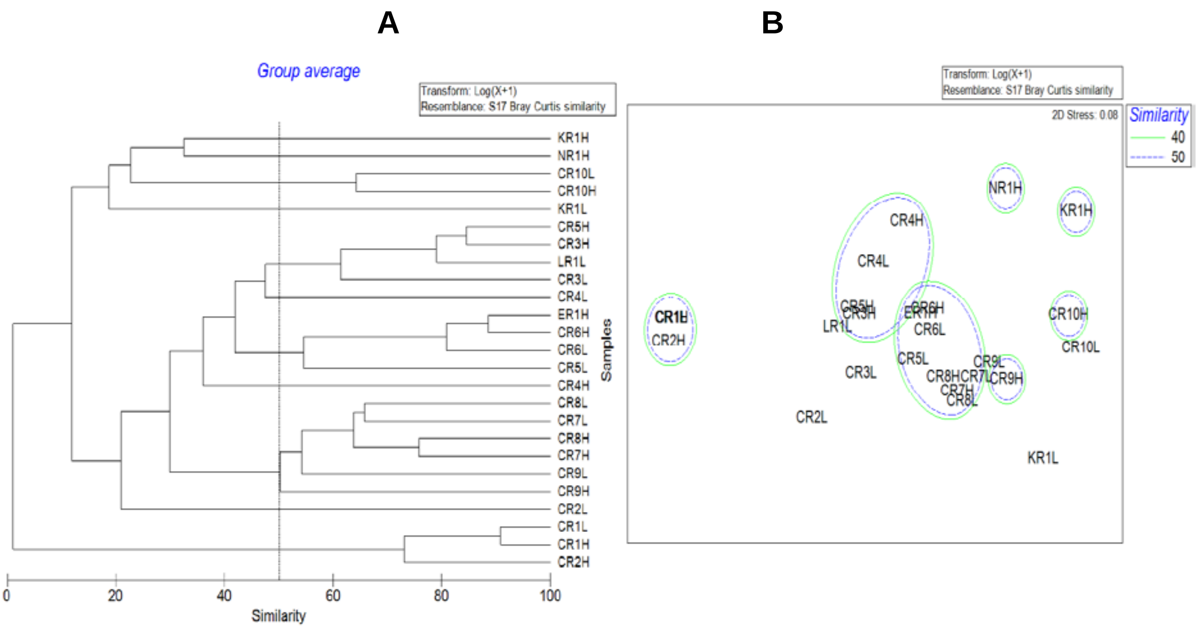


Figure 3.5: Bray-Curtis similarity matrix-based cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the Ichthyofauna collected in the Crocodile River and its tributaries during low flow and high flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.08.

The separation of sites was linked to different species composition occurring at those sites. The separation of sites CR2, KR1 and CR3 during low flow condition from the rest of the grouping was attributed to change in water quality within the vicinity as many agricultural activities occur at their vicinity. A group formation of sites CR1 and CR2 was observed during high flow condition and the group formation was as a results of species composition within the sites. Another group formation of sites CR3 and CR5 (during high flow condition) and site CR4 (during both flow condition) was observed. The similarity was linked to the change of flow conditions and habitat disturbance at these sites, as this sites were susceptible to change of flow from small tributaries coming directly to the sites while CR4 was susceptible to change of flow due to the release of water from the dam. The NMDS ordination (Figure 3.5B), for all the sites sampled in the Crocodile River and its tributaries also indicated that the same grouping as the Bray-Curtis cluster analysis (Figure 3.5A), and the generated stress values was 0.08, which according to a rough rule of thumb for two dimensional ordinations a stress with a value of <0.05 gives an excellent representation with no prospect of misinterpretation (Clarke and Warwick, 1994).

A plot of Cluster analysis and NMDS ordination for high flow condition (Figure 3.6) indicated that fish assemblage was separated in two groups at a Bray-Curtis similarity value of 40%. The first branch representing the assemblage of sites KR1, NR1, CR10 and CR9 and the second branch representing fish assemblage of sites CR5, CR3, CR4 CR8, CR7, CR6 and ER1. A further similarity was observed at Bray Curtis similarity of 60% (Figure 3.6). The further similarity of fish assemblage during high flow conditions was a results of habitat diversity, water quality and species occurrence within the sites.

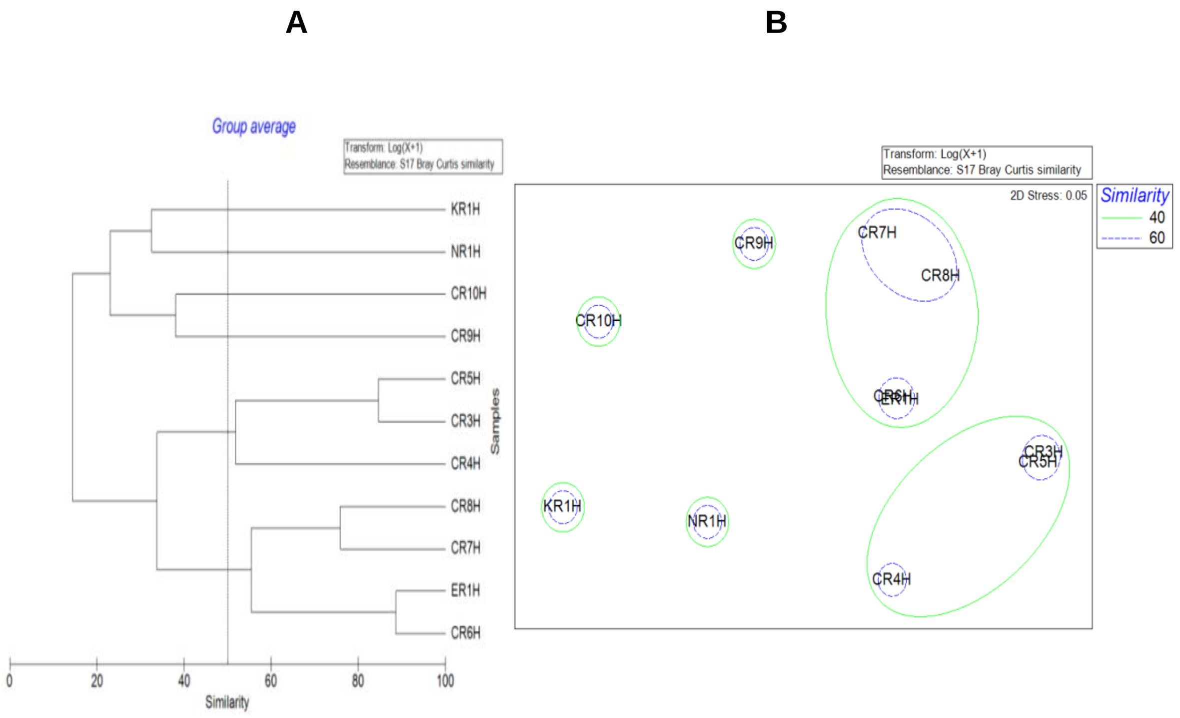


Figure 3.6: Bray-Curtis similarity matrix-based cluster analysis (A) and the NMDS ordination (B) of the Ichthyofauna collected at the sites on the Crocodile Rivers during high flow conditions.

The NMDS for high flow conditions indicated that site CR9, CR10, NR1 and KR1 were separated from the rest of the group (Figure 3.6B). The separation was associated with the fish response to pollutants and habitat during high flow condition. A difference in fish species occurrence at sites CR 9 and CR10 (lower reaches) and KR1 and NR1 (tributaries) were also a factor for the separation. Figure 3.7B, also correspond to the groups identified in the cluster analysis and two distinct group formations were observed and the stress value of 0.05.

A group formation of ten sites within the Crocodile River and two sites in its tributaries was observed at similarity 60 and 40% (Figure 3.7B). At similarity 40% sites such as CR5, CR6, CR7, CR8 and CR9 were grouped together but separated at similarity 60% and this was linked to the change in species composition within the sites.

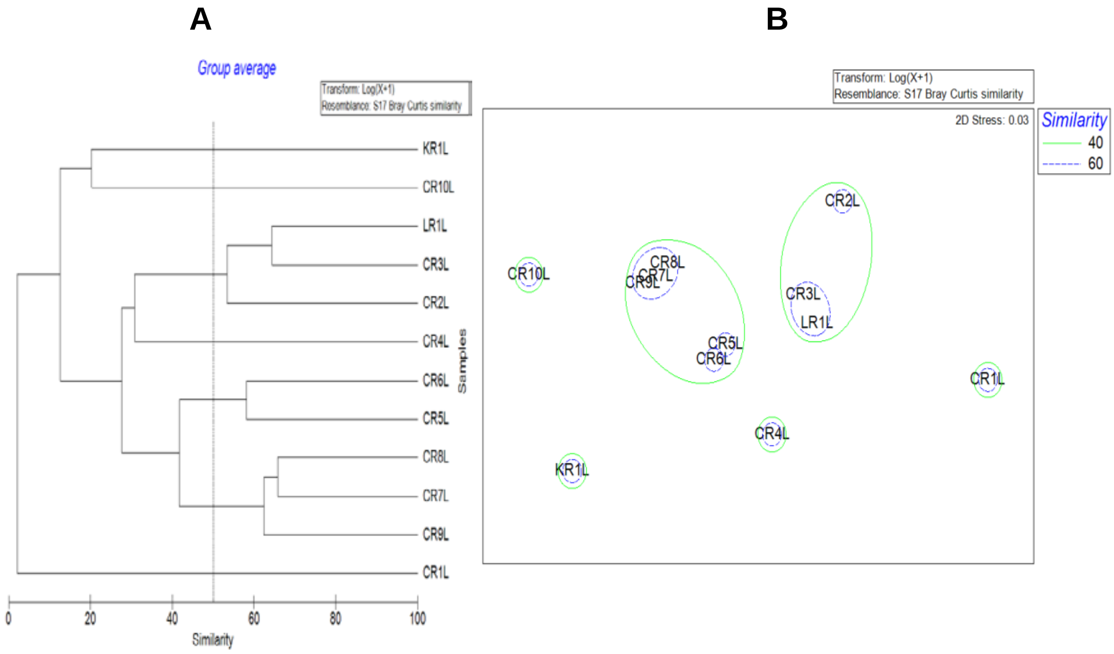


Figure 3.7: Bray-Curtis similarity matrix-based cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the Ichthyofauna collected at the sites on the Crocodile Rivers and its tributaries during low flow condition. The NMDS ordination was completed with 30 iterations and showed a stress of 0.03.

Another group formation of sites was also observed at the same similarity 40% between sites LR1, CR3 and CR2. The group formation of this sites were believed to be linked to the fact that these sites were upstream of the Kwena Dam and they might share similar fish species. Site CR3 and LR1 were grouped together at similarity 60% while site CR2 separated from the grouping. The group formation of sites CR3 and LR1 was believed to be associated to the fact that this sites were closer to the dam. According to species similarities matrix the formation of two distinct groups by hierarchy clustering seems reasonable and satisfactory. The generated stress value for the NMDS was 0.03 and according to a rough rule of thumb for two dimensional ordinations, stress of <0.05 gives an excellence representation with no prospect of misinterpretation. It was clear that only eight sites can form a two distinct groups out of the twelve. Sites CR10, KR1, CR4 and CR1 had one site grouping and they were separated from the two distinct group formation and their separation was linked to different species diversity, water quality characteristics, habitat diversity and flow modification.

A K-dominance curve (Figure 3.8), for the Ichthyofauna community was plotted which shows the cumulative dominance percentage against the species rank. The K-dominance curve indicated that during high flow condition 100% of single species dominated the fish communities at sites CR1 and CR2 and above 80% at site CR3 (Figure 3.8). This species include *Barbus anoplus* (moderately tolerant to pollutants) at both site CR1 and CR2, while at site CR3 was *Chiloglanis pretoriae* (species intolerant to pollutants) (Kleynhans, 1999). The k dominance curve further indicated that during high flow condition above 60% of single species dominated the fish communities at site ER1 and CR6 while during low flow condition site CR3, CR2, CR10 and CR 4 were dominated by single species at the same percentage.

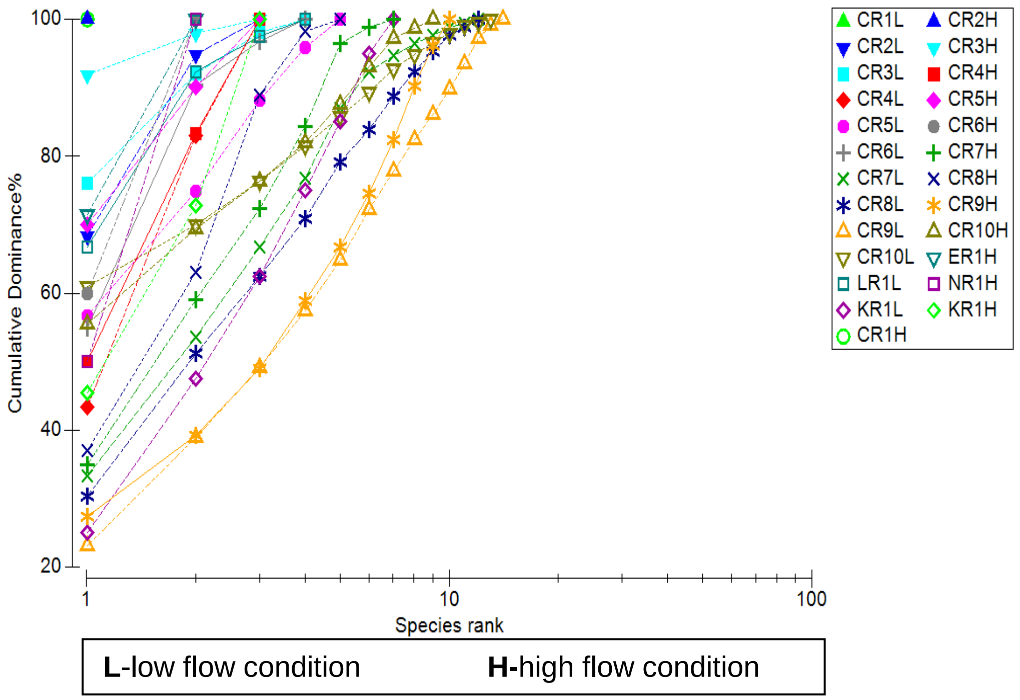


Figure 3.8: Ranked species K-dominance curves for the Ichthyofauna communities collected at the sites on the Elands and Crocodile Rivers during high and low flow conditions.

These sites were dominated by same species *Chiloglanis pretoriae* except site CR10 which was dominated by species such as *Barbus viviparus* (moderately tolerant) and CR6 which was dominated by *Labeobarbus marequensis*. The single species dominance at these sites were linked to the change in habitat preference for other species occurring on the sites as fish communities in a river are structured typically by substrate complexity, stream flow and water quality (Gorman and Karr, 1978). The presence of the *Chiloglanis pretoriae* in the upper reaches of the Crocodile River during low flow condition indicated that the river is not highly polluted as these species dependent on flowing-water habitats and have a preference for substrate cover. Moreover they are associated with good water quality while the presence of *Barbus viviparus* in the downstream reaches indicated that the lower reaches might be susceptible to pollutants (Kleynhans, 1999). The pollutants in the downstream reaches were associated with industrial and agricultural activities as this segment is characterized by these activities..

The SIMPER analysis for both flow conditions indicates the intergroup relationship between fish species (Table 3.3). The ten groups identified by Bray Curtis and NMDS ordination are as follows:

Group 1: CR1H, CR2H & CR1L
Group 2: NR1H (less than 2 sites in a group)
Group 3: CR6H, ER1H,ER1L, CR5L, CR6L, CR7H, CR7L, CR8H & CR8L
Group 4: CR9L (Less than 2 samples in a group)
Group 5: CR3H, CR5H, CR3L, CR4L, LR1L & CR4H
Group 6: CR5H, CR3H,CR6H,ER1H,ER1L, CR4L & CR4H
Group 7 : CR9 (Both flow conditions)
Group 8: KR1H (Less than 2 samples in a group)
Group 9: CR10L (less than 2 sites in a group)
Group 10: CR10H (less than 2 sites in a group)

Table 3.3: The contribution of various species with the group determined using SIMPER for both flow conditions.

	Species	Average abundance (per site)	Average similarity	Contribution %	Cumulative %
Group1	*Barbus anoplus*	2.07	79.04	100.00	100.00
Group 2	Less than 2 sites in a group				
Group 3	*Barbus eutaenia*	2.95	13.19	22.89	22.89
	Labeobarbus marequensis	2.76	11.50	19.96	42.85
	Chiloglanis pretoriae	2.78	11.19	19.43	62.28
	Pseudocrenilabrus philander	1.89	5.42	9.42	71.70
	Barbus unitaeniatus	1.66	5.22	9.07	80.77
	Oreochromis mossambicus	1.19	3.13	5.43	86.19
	Labeo cylindricus	1.14	2.06	3.58	89.77
	Chiloglanis paratus	1.04	1.57	2.72	92.50
Group 4	Less than 2 sites in a group				
Group 5	*Chiloglanis pretoriae*	2.96	34.86	50.56	50.56
	Labeobarbus marequensis	3.02	32.44	47.06	97.62
Group 6	*Chiloglanis pretoriae*	3.47	41.44	67.20	67.20
	Barbus neefi	1.65	13.50	21.89	89.09
	Amphilius uranoscopus	0.96	6.73	10.91	100.00
Group 7	*Barbus viviparus*	4.34	17.17	26.73	26.73
	Chiloglanis paratus	2.59	11.09	17.26	43.98
	Labeo cylindricus	1.99	8.29	12.90	56.88
	Chiloglanis swierstrai	2.00	7.44	11.58	68.46
	Labeobarbus marequensis	2.09	7.44	11.58	80.05
	Oreochromis mossambicus	2.24	6.41	9.98	90.02
Group 8	Less than 2 sites in a group				
Group 9	Less than 2 sites in a group				
Group 10	Less than 2 sites in a group				

The average similarity within group 1 was 79.04% with only *Barbus anoplus* contributing 100% of the abundance within this group. Species that have 50% or higher contribution to the formulation of the group may be good indicators of what is taking place at the site. The presence of *Barbus anoplus* species within this group was an indication that water quality within this group was poor as this species is tolerant to pollutants. There was a higher dissimilarity of 100% when comparing group 1 with the other groups and this was an indication that group 1 doesn't share any species with the other grouping. In group 3 the average similarity was 57.6% with *Barbus eutaenia, Labeobarbus marequensis, Chiloglanis pretoriae,* *Pseudocrenilabrus philander, Barbus unitaeniatus, Oreochromis mossambicus, Labeo cylindricus* and *Chiloglanis paratus* contributing 92.50% of the abundance within this group. Species such as *Barbus eutaenia, Labeobarbus marequensis* and *Chiloglanis pretoriae* were the most dominant species within this group indicating that water quality within this group was not degraded to such an extent. There was a higher dissimilarity between this group (group 3 and 2 (87.11%), group 3 and 4 (82.23%), group 3 and 5 (59.93%), group 3 and 6 (76.54%), group 3 and 9 (86.25%), group 3 and 9 (86.25%) and group 3 and 10 (89.89%)). The sites within group 5 showed an average similarity of 68.94%, with *Chiloglanis pretoriae* and *Labeobarbus marequensis* making large contribution of 97.62% within this group. When group 5 was compared with the other groups a dissimilarity was high (group5 and 2 (77.42%), group 5 and 3 (58.9, group 5 and 4 (70.86%), group 5 and 6 (58.14%), group 5 and 7 (84.79%,) group 5 and 8 (77.42%), group 5 and 9 (76.51%), group 5 and 10 (100%)). In group 6, *Chiloglanis pretoriae, Barbus neefi, and Amphilius uranoscopus* had an average similarity of 61.66% with a large contribution of 100% within this group indicating that water quality within the group were not impacted. Group 7 had an average similarity of 64.26% with *Barbus viviparus, Chiloglanis paratus, Labeo cylindricus, Chiloglanis swierstrai, Labeobarbus marequensis* and *Oreochromis mossambicus* contributing 90.02% within the group and when compared to the other grouping the dissimilarity was above 70%. Groups 2, 4, 8, 9, 10 had less than two samples in a group and their similarities were not determined hence their dissimilarity were also high.

The one way ANOVA analysis indicated that fish abundance and richness had a significant difference between low flow and high flow ($P < 0.05$). The LSD was analyzed to determine which mean is significant from each other during both flow regimes and the LSD Fishers' result were found to be LSD=43.5 and the mean was less than the LSD Fishers' value. It was concluded that fish richness and abundance are higher during low flow than high flow condition.

The RDA triplot for both flow regimes (Figure 3.9), indicated as with Bray-Curtis similarity matrices and NMDS plot (Figure 3.5), that there was similarity of sites in the Crocodile River and its tributaries.

In RDA plot the length of the arrow was related to the strength of the correlation. In general, the longer the arrow, the more highly related that variable to species composition and the approximation correlation is positive when the angle was acute and negative when the angle was larger that 90 degrees.

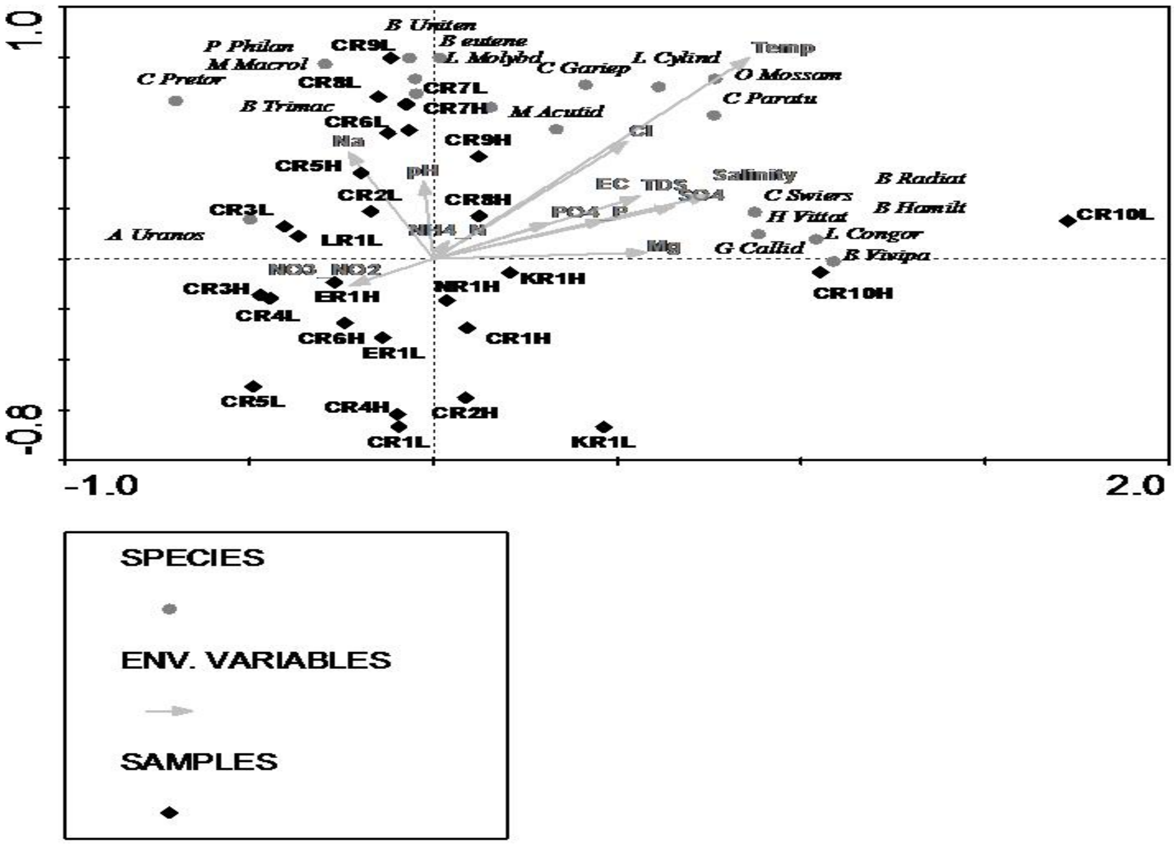


Figure 3.9: RDA tri-plot illustrating the similarities in the fish communities between the various sites and surveys with the physico-chemical variables superimposed. The tri-plot describes 61.8% of the variation with 34.5% being described on the first axis and 38.9% on the second axis. Only the taxa of with more than 20% is explained by the model is visualized.

The distance between the sampling sites in the diagram indicated the similarity of their fish community as they were measured by their Euclidean distance. The environmental variables super imposed (Figure 3.9), indicated that fish species such as *Labeo cylindricus, Clarias gariepinus, Oreochromis mossambicus, Chiloglanis paratus, Micralestes acutidens, Labeo molybdinus, Chiloglanis swierstrai, Labeo congoro, Glossogobius callidus, Barbus viviparus, Hydrocynus vittatus, Barbus hamiltons, Barbus radiatus and Barbus eutaenia* at site CR7, CR9 and CR10L which was separated from sites CR7 and CR9 correlated positively with water temperature in the river. The positive correlation between water temperature and the species was an indication that the fish assemblage within the Crocodile River and its tributaries were also structured based on the favorable temperature condition. According to River-Moore *et al.,* (2004), water temperature is one of the primary environmental drivers structuring fish communities. The collected fish data for high and low flow condition was further used to assess the ecological category class of the river compared with other studies conducted in this system using the Fish Response Assessment Index model (Kleynhans, 2008).

Table 3.4: Fish Response Assessment Index Class ratings (Kleynhans, 2008).

Class rating	**Description of generally expected conditions for integrity classes**	**Relative FRAI rating score (% of expected)**
A	Unmodified, or approximate natural conditions closely	90 to 100
B	Largely natural with few modifications..	80 to 90
C	Moderately modified.	60 to 79
D	Largely modified.	40 to 59
E	Seriously modified.	20 to 39
F	Critically modified.	0 to 19

It was clear that the ecological class for the Crocodile River system was low during high flow condition when compared to the low flow condition (Figure 3.10), and this was linked to the fact that during high flow condition, it was not possible to sample all the fish habitat in some segments of the river. Thus, fish species such as *Labeo polylepis, Tilapia sparmanii, Oreochromis mossambicus, Micralestes acutidens* and *Chetia brevis* were present in low abundance during the survey and they were not ruled out if they were not caught on that specific site.

The Fish Response Assessment Index Scores for site CR1 was 80.7% and 79% during low and high flow condition respectively (Figure 3.10). These scores percentage were an indication that site CR1 ecological category was in class B/C (largely natural with few modifications) and class C (moderately modified) class. Kleynhans (1999), however concluded that this site was in ecological category A class which was an indication that the site was in a natural state or condition.

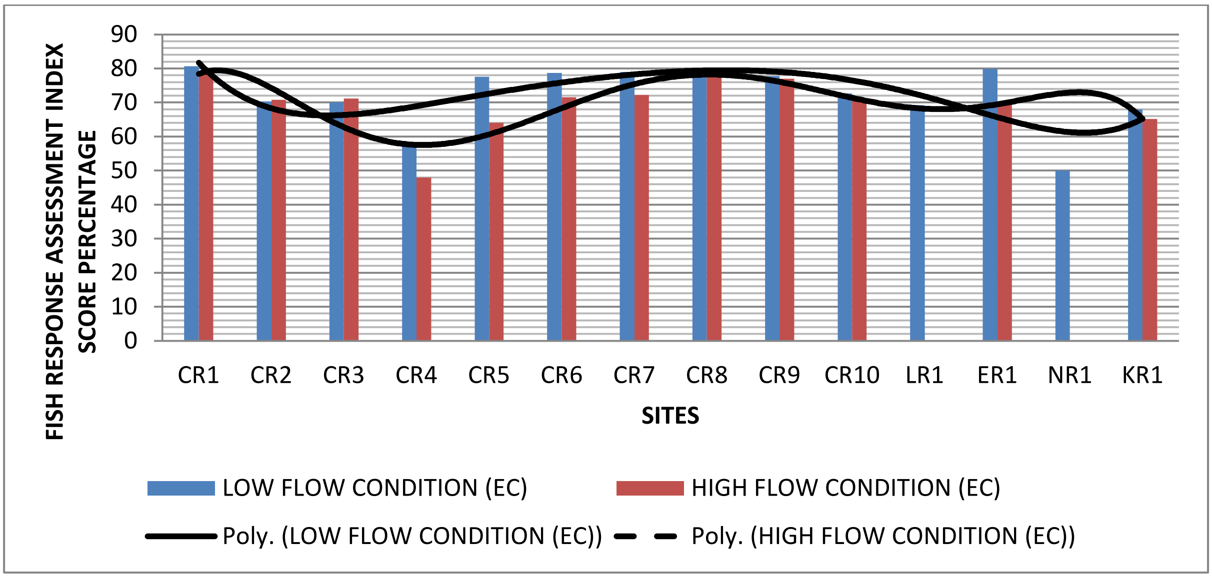


Figure 3.10: The ecological category (EC) percentage of the fish sampled in the Crocodile River and its tributaries during low and high flow condition done using the fish response assessment index.

The deterioration of the ecological class of site CR1 was associated with the presence of predator species such as *Oncorhychus mykiss* and water quality changes that affected the presence of the species. At site CR2 which was a site upstream of the Kwena dam the ecological category class was lower compared with the other upstream site CR1. The Fish Response Assessment Index score for site CR2 was rated 70.77% and 70.3% during high and low flow condition and the ecological class was C which was an indication that the site was moderately modified. The modification of this site was linked to the change in water quality, the presence of predator species and flow modification. Species such as *Chiloglanis bifurcus* was not sampled during the current survey in this segment but in reference to Kleynhans (1999), this species was sampled in 1978, but not in 1996. However, a single specimen was again caught in 1998 (Kleynhans, 1999), but it was not available in the survey conducted in 1996. The presence of the Kwena Dam also has an impact on the migratory species that are within the segment as it inhibits them for free movement. A study conducted by Kleynhans (1998) concluded that site CR2 had an ecological category C class (79.9%), which was close to a B class. This was an indication that the sites were slightly deteriorating in species availability. Downstream of the Kwena dam the ecological category was low compared to site CR4. The ecological category class for this site was C/D (57.9%) and D (48%) during low and high flow condition respectively. A study conducted by Ferreira (2005), concluded that the site was in ecological category C class (moderately modified) while a study conducted by Kleynhans (1999), concluded that the site was in ecological category class B (largely natural with few modification). This is an indication that the ecological status of this site has deteriorated. The deterioration of the ecological status is due to the change of habitat and flow modification as a result of the release of water from the Kwena dam.

At site CR5, from (Figure 3.10), the ecological category improves from class C/D to an ecological class C (77.57%) which indicated that the segment was moderately modified and this can be due to change in flow condition also caused by the Kwena Dam, weirs and influx of water coming from the Elands River. Exotic fish species such as *Xiphophorus helleri* which have a small impact (such as feeding habit due to its high number) on the indigenous species also occurs in this segment. The Elands River, site ER1 of the current study had an ecological class category C (69.5%) moderately modified. This site has exotic species such as *Oncorhychus mykis*s (rainbow trout) and *Micropterus salmoides* (largemouth bass) which are known to have an impact (such as predating other fish species) on the indigenous species. A study conducted by Ferreira (2005), indicated that this site sampled has ecological category class B during low flow which indicated that the site was largely natural with few modifications. The low ecological category class of this site for this current study was due to the fact that the species were responding to a change in water quality or the presence of predator species and other factors such as the influx of effluent coming from the processing of paper mill which caused an increase in water volume downstream of the mill. Downstream of the Nelspruit and Kanyamazane town the river is mostly dominated by pools and limited riffles.

During the current study these sites (CR7 and CR8) of the Crocodile River was in ecological category C (71.5%) which was the same ecological class obtained by Kleynhans (1999). This sites of the river have high diversity of fish species and approximately eighteen species occur here but during the survey only thirteen of the expected species were caught. The change in water quality and the over abstraction of water which result in change in flow pattern of the river might be the causes for the absence of other species such as *Opsaridium peringueyi* which was a species that prefer good water quality. Its absence also indicated that the sites might be slightly deteriorated in water quality. Sites CR9 and CR10 which forms part of the lower reach of the Crocodile River in the current study was found to be moderately modified with an ecological category C class with ecological category percentage of 77.7% and 72.7% respectively during the low flow conditions. Sites CR9 and CR10 were mostly dominated by species that prefer slow flowing water such as *Tilapia sparmanii, Tilapia rendalli, and Labeo congoro* and only twenty fish species out of the thirty six species expected were caught in this reach. This was believed to be associated with change of habitat in the downstream reaches. Intolerant fish species such as *Amphilius uranoscopus, Chiloglanis pretoriae and Chiloglanis paratus* were available in this segment although they were in low abundance as this segment was dominated by pools and rapids. This site was also known to contain species such as *Hypophthalmichthys molitrix* which has a huge impact in habitat modification due to its activities, e.g. feeding habits. The absence of other species at these sites was linked to lack of habitat preference as indicated above and poor water quality caused by agricultural activities and industries.

The Kaap River which was site KR1 of the current study had an Ecological Category of C (68.8%) indicating that the river was moderately modified and species such as *Chiloglanis swierstrai and Barbus brevipinnis* were only sampled during low flow condition in low abundance. This was linked to the change in water quality through seepage from mining industries and run off from agricultural activities taking place in this river. Site LR1 had an ecological category C (moderately modified) class with 69.2% during low flow condition and it was sampled once during the survey. Site NR1 had an ecological category D (largely modified) class with 50% during low flow condition. Agricultural activities in this vicinity were believed to have an influence on the water quality on the sites.

3.4. Conclusion

The multivariable statistical methods used to identify the diversity and richness of the Ichthyofauna in the Crocodile River and its tributaries indicated that the diversity, richness and abundance of the Ichthyofauna in the Crocodile River increase longitudinally in the downstream reaches of the river and occurs during low flow condition and there was a significant difference between richness and abundance during both flow conditions. The high diversity, abundance and richness of fish species in the downstream reaches of the Crocodile River were associated with an increase in habitat complexity, while the low diversity and richness of Ichthyofauna in the upper reaches was associated with the presence of weirs an impoundment which limit fish species migration in the upper reaches and poorer habitant diversity. The ecological category class of the Crocodile River was observed to decrease longitudinally in the downstream reaches of the river during both flow condition. Although the ecological category class were similar during both flow condition, the low flow condition was better than the high flow condition as the ecological class percentage increased slightly to be close to Class B in other sites except at sites CR4 and NR1 which were largely modified. The modification of the ecological category were believed to be associated with the presence of predator species, change in habitat, water quality and flow modification during both flow conditions. A number of tolerant fish species (e.g. *Barbus anoplus* and *Barbus viviparus*) also confirmed that during high flow condition the Crocodile River has poor water quality as a result of agricultural run-off or return flow in the upper reaches, industrial and sewage effluent and mining seepage in the middle and lower reaches of the Crocodile River. From the results it was evident that fish assemblage in the Crocodile River is affected by different factors such as water quality, change in habitat and flow modification resulting from anthropogenic activities such as dams, weirs, agricultural and industrial run-offs and effluents especially during high flow conditions.

CHAPTER 4

4. MACRO-INVERTEBRATES

4.1. Introduction

South Africa is a dry country and as the demand for water increases in line with human population pressure and economic development activities, river ecosystems will continue to deteriorate unless they are managed in a sustainable way. According to Roux *et al*. (1999), the protection of the environment requires tools that can be used to monitor environmental conditions as well as for setting ecological objectives to ensure proper and sustainable management of the resource. Thus, a biomonitoring program was designed to monitor the health of the river systems in South Africa.

Macro-invertebrates have been commonly used than any other biological group to assess the biological integrity of stream ecosystems with relatively good success throughout the world because they exhibit a wide variation of response to pollutants and have been extensively monitored in lotic water bodies to evaluate water quality and complement physico-chemical surveys (Hawkes, 1979; Shutes, 1985). Macro-invertebrates are measured using a recognized biomonitoring method such as SASS5 (Dickens and Graham, 2002). Biomonitoring measures such as SASS and ASPT (average score per taxon) score are related to each ecological class.

The SASS protocol was designed by Chutter (1998) and currently is in its fifth version of development. SASS makes use of the natural sensitivity or tolerance to adverse water quality of the wide variety of benthic invertebrates in a river, aggregating the effects of water quality over time. It provides an ideal system to measure the response of aquatic fauna to general water quality conditions in a river. The SASS method produces three different and complimentary scores SASS Score, Number of Taxa and ASPT and it was designed for running water. Thus, it is not used to set reserve of ephemeral rivers and standing waters (Dallas, 2000; Dickens and Graham, 2002).

The Crocodile River is a perennial river which has rifles, runs and pool biotopes which support different families of macro-invertebrates. Macro-invertebrates live in different places in the water body e.g. some live in water surface, some in the water itself, others in sediment or on bottom or on submerged rocks, logs, and leaf litter, and identification of these macro-invertebrates can indicate whether the river is in the poor or good condition.

4.2. Materials and Methods

4.2.1. Field survey

Macro-invertebrates were collected during the low and high flow of the year, 2013 at the sites selected for this study. The collection of the macro-invertebrates was done using the SASS 5 protocol (Dickens and Graham, 2002). Three groups of habitats included: 1. Stone (S)-which referred to stone in and out of current, 2. Vegetation (V)-which refer to marginal vegetation, instream vegetation and 3. Gravel, Sand and Mud (GSM) which refers to fine stone, silt and mud which are found in the river and these biotopes were sampled as follows:

4.2.1.1. Stone biotopes

4.2.1.1.1. Stone in current (SIC)

Stone in-current are free/loose stones (pebbles and cobles) situated where movement of water prevents the settling out of fine silts. For macro-invertebrates collection in this habitat, a net was placed close but downstream of the stones to be kicked and this was in a position where the current carried the dislodged biota in to the net and bedrocks were rubbed with hands or boots. The kicking of stone continued for approximately two minutes. In stone that were embedded or difficult to move the sampling continued up to a maximum of five minutes.

4.2.1.1.2. Stone out of current (SOC)

Stone out of current are moveable stones out of any perceptible current such that fine sediments settle on their upper surface. The kicking of stone out of current was one minute which included scraping them with hands or feet while continuously sweeping the net through the disturbed area. All the samples collected in and out of current were combined into single stone biotopes samples.

4.2.1.2. Vegetation biotopes

4.2.1.2.1. Marginal vegetation (Mveg/C)

Marginal vegetation is vegetation that is hanging at the edge of the stream often emergent both in current and out of current. Sampling of the marginal vegetation was conducted in a total length of approximately two meters and it was spread over one or more locations especially where different kinds of marginal vegetation were present in different flow velocities. The net was kept below the water surface to avoid the tendency of collecting organisms from above the water surface and it was pushed vigorously in to the vegetation moving back and forwards in the same area to dislodge any invertebrates.

4.2.1.2.2. Aquatic vegetation

The net was pushed repeatedly against and through the vegetation under the water to dislodge and collect invertebrates over an area of approximately one meter square. Samples collected in and out of current were combined into a single vegetation biotopes sample.

4.2.1.3. Gravel Sand and Mud biotopes (GSM)

These biotopes include small stones, sand grains, mud, silts and clay particles. The GSM was stirred by shuffling or scraping with the feet whilst continuously sweeping the net over the disturbed area to catch dislodged biota for one minute. Samples collected in and out of current were combined in to a single GSM biotopes sample.

4.2.1.4. Hand picking and visual observation

Approximately one minute of hand picking for specimens that may have been missing by the sampling procedure was taken in to consideration.

4.2.1.5. Sample preparation and analytical procedure

Before the collected samples above were tipped in to separate trays, they were washed down to the bottom of the net until the water passing through the net runs clear. Sufficient water was then added to the trays to immerse the samples and before the identification of organisms began, the larger obstructing leaves, twigs and other loose debris and stones were removed from the tray. The organisms listed on the SASS5 scoring sheet were identified to family level. Viewing and identification was done for a maximum of 15 minutes per biotope if new species were observed but if new taxon were not observed for approximately 5 minutes the operation stopped. The Abundances of the identified families was rated as 1=1, A=2-10, B=10-100, C=100-1000 and D=>1000, as outlined on the SASS score sheets. To determine the River Health Class the ASPT was used with the following benchmark ranges (Table 4.1).

Table 4.1: River health classes and descriptions (SORR, 2006).

ASPT	Class	Description
7	*Natural*	There is negligible modification of the instream and riparian habitats. The biota is also unmodified.
6	*Good*	The ecosystem is largely unmodified, and is essentially in a good state with largely intact biodiversity.
5	*Fair*	The ecosystem is heavily modified with dominating species being tolerant or opportunistic. There is a very low abundance (if present) of sensitive species.
<5	*Poor*	The ecosystem is unacceptably modified. Mainly tolerant species are present; there is invasion by alien species and disrupted population dynamics. The species are often diseased.

After the completion of sample identification and scoring the samples were returned to the river. Three principal indices were then calculated for SASS and this included SASS Score, Number of Taxa and ASPT. The calculation of results was done by noting or ticking any families observed in any biotope in the total columns of the scoring sheet. Quality scores for each taxon noted in the total column were assigned on the scoring sheet and were summed to provide the SASS score. The ASPT was calculated by dividing the SASS score with the Number of taxa found.

4.2.2. Data analysis

Analysis of data for this study focused on quantifying the spatial and temporal variation in species richness and abundance, and identification of environmental variables explaining variation across the study sites. Species richness and abundance were used directly in the analysis because the sampling effort was similar across sites and seasons. The species dominance was assessed by the importance value index (IVI) as proposed by Krebs (1989), using the frequency of occurrence and relative abundance of species. A one way ANOVA was used to test the differences in species richness and abundance across sites during low flow and high flow sampling. The SPSS version 18, was used to perform statistical analyses and alpha was set at $P<0.05$.

Discrete temporal and spatial patterns in macro-invertebrates assemblages were identified using the PRIMER version 5 (Clarke and Gorley, 2001). The richness and abundance data collected were log(x+1) transformed to meet the assumption of multivariate normality and to moderate the influence of extremes in richness and abundance. Transformed sample data were then used to create a Bray-Curtis similarity matrix calculated for all pair-wise sample comparisons (Bray and Curtis, 1957). Analysis of similarities (ANOSIM) was used to compare macro-invertebrates assemblage from the sites across two seasons. A two way ANOSIM without replication was used to test for sites and seasons effects since macro-invertebrates at each site were sampled once in every season. A two way nested ANOSIM was used to test for the Kwena Dam effect on macro-invertebrates biodiversity in the Crocodile River. The relationship between assemblages from each site was graphically represented using a cluster analysis and a non-metric Multi-Dimensional Scaling analysis (NMS). The contribution of each species to the differences among assemblage groups was identified using SIMPER (Clarke and Warwick, 2001).

A Detrended Correspondence Analysis (DCA) was performed to identify the strongest gradient of assemblage composition independent of the environmental variables and standard deviation redundancy analysis (RDA) was selected for the evaluation of the variability in the assemblage structure in relation to the measured environmental factors. The multivariate statistical analysis was performed using CANOCO version 4.5 (ter Braak and Smilauer, 2002).

The Macro-Invertebrates Assessment Index (MIRAI) was also used to interpret the Ecological condition of the macro-invertebrates of the sites. The MIRAI is a rule based model recently developed by (Thirion, 2008). It integrates the ecological requirements of the invertebrates taxa in a community of assemblage to their response to modified habitat condition.

4.3. Results and Discussion

A total of 6396 individuals belonging to 54 macro-invertebrates taxa were collected in the Crocodile River and its tributaries during high flow and low conditions. The highest number of families was sampled in sites KR1 and CR5 during high flow and this was linked to change in habitat modification and change in water quality. Deteriorating water quality was among the major factors contributing to the disappearances of some families in many rivers and streams. Karr *et al*., (1985), Ganasan and Hughes (1998); Soto-Galera *et al*., (1998); Ellen *et al., (*1999); Waite and Carpenter, (2000), found that pollution effect reflected decreasing richness at communities. Polluted habitats are poor in species richness and at the community level pollution reduces both species diversity and abundance and only certain species and only few individuals can survive pollution. Tables 4.2 and 4.3 indicate taxon sampled during high and low flow conditions in the Crocodile River and its tributaries.

Table 4.2: Expected macro-invertebrates sampled during high flow condition in the Crocodile River and its tributaries (Thirion, 2012).

Taxon	CR1H	CR2H	CR3H	CR4H	CR5H	CR6H	CR7H	CR8H	CR9H	CR10H	ER1H	NR1H	KR1H
Class: Turbellaria	4	1	1	6	3	0	0	0	6	27	1	0	0
PHYLUM: ANNELIDA													
Class: Oligochaeta	1	2	1	3	20	0	0	0	0	3	1	4	1
Class: Hirudinae	0	0	0	1	0	0	0	0	10	2	0	0	0
ORDER: DECAPODA													
Family Potamonautidae	1	6	0	1	4	0	0	0	0	0	1	0	0
Family Atyidae	0	0	0	0	0	0	0	0	1	6	0	0	7
Family Palaemonidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxon: Hydracarina	0	0	1	0	2	22	0	0	16	32	1	0	8
ORDER: PLECOPTERA													
Family Notonemouridae	0	0	0	0	0	0	0	0	0	0	0	0	0
Family Perlidae	4	1	2	0	9	0	1	0	25	0	10	0	1
ORDER: EPHEMEROPTERA													
Family Baetidae	43	74	74	92	6	8	2	19	18	21	15	9	21
Family Caenidae	28	21	10	9	4	2	9	0	2	0	3	12	0
Family Heptageniidae	35	80	25	21	6	0	0	12	0	0	4	35	7
Family Leptophlebiidae	0	0	18	15	0	0	0	0	0	0	4	10	8
Family Oligoneuridae	6	0	0	0	0	0	0	0	0	0	0	0	0
Family Prosopistomatidae	4	1	1	0	0	0	0	0	0	0	0	0	0
Family Telagonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Family Trichorythidae	11	37	15	3	0	0	0	0	0	0	0	112	0

Table 4.2: Continued

Taxon	CR1H	CR2H	CR3H	CR4H	CR5H	CR6H	CR7H	CR8H	CR9H	CR10H	ER1H	NR1H	KR1H
ORDER: ADONATA													
Family Chlorocyphidae	0	0	0	0	0	0	2	0	0	0	1	0	12
Family Coenagrionidae	0	0	3	3	0	0	0	0	0	2	0	0	1
Family Aeshnidae	4	16	30	3	7	0	0	0	0	0	1	5	0
Family Corduliidae	0	0	0	0	0	0	0	0	0	0	0	0	1
Family Gomphidae	2	11	5	2	12	0	9	0	19	3	15	5	4
Family Libellulidae	0	0	2	0	2	3	3	6	2	2	9	0	0
ORDER: HEMIPTERA													
Family Corixidae	1		4	8	8	0	0	0	0	15	0	0	0
Family Gerridae	0	0	0	0	0	0	0	0	1	0	0	0	0
Family Naucoridae	0	1	0	0	0	4	4	7	5	0	0	0	0
Family Notonectidae	1	0	0	0	0	0	0	0	0	1	0	0	0
Family Pleidea	0	0	0	0	3	5	0	0	0	0	0	0	0
Family Veliidae	0	0	1	0	0	21	0	0	0	19	0	0	0
ORDER: TRICHOPTERA													
Family Ecnomidae	0	0	0	0	0	0	0	0	0	0	0	0	1
Family Hydropsychidae	16	7	5	11	2	0	5	50	0	0	1	18	9
Family Philopotamidae	0	4	5	10	2	0	0	0	0	0	7	10	0
Family Hydroptilidae	0	0	0	0	12	0	0	0	5	0	0	0	0
Family Leptoceridae	0	1	2	0	0	0	6	0	0	0	0	1	13
Family Petrothrincidae	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.2: Continued

Taxon	CR1H	CR2H	CR3H	CR4H	CR5H	CR6H	CR7H	CR8H	CR9H	CR10H	ER1H	NR1H	KR1H
ORDER: COLEOPTERA													
Family Dytiscidae	0	0	0	0	1	0	0	0	0	0	0	0	1
Family Elmidae	5	1	2	2	3	0	4	0	5	0	4	5	6
Family Gyrinidae	5	1	2	3	5	3	1	0	0	0	5	2	5
Family Helodidae	0	0	0	0	0	0	1	0	0	0	0	0	0
Family Hydrophilidae	0	1	1	0	0	0	0	0	0	0	0	0	0
Family Psephenidae	7	5	6	0	0	5	0	0	0	0	1	0	0
ORDER: DIPTERA													
Family Athericidae	0	0	0	0	2	0	3	0	0	0	0	0	5
Family Ceratopogonidae	0	2	14	0	10	1	0		42	22	6	8	5
Family Chironomidae	9	75	15	7	7	2	7	47	60	2	7	7	9
Family Culicidae	1	1	0	1	0	0	1	0	0	0	0	0	0
Family Muscidae	0	0	0	0	0	0	4	0	3	2	0	1	0
Family Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Family Simuliidae	10	8	13	0	5	6	2	78	35	2	65	6	1
Family Syrphidae	0	0	0	7	0	0	0	0	0	0	0	0	0
Family Tabanidae	0	1	5	2	0	0	2	3	5	0	10	0	2
Family Tipulidae	2	8	0	0	0	0	0	0	0	0	2	2	1
Family Ancylidae	0	0	0	8	0	0	1	0	0	0	3	0	1

Table 4.2: Continued

Taxon	CR1H	CR2H	CR3H	CR4H	CR5H	CR6H	CR7H	CR8H	CR9H	CR10H	ER1H	NR1H	KR1H
PHYLUM: MOLLUSCA													
CLASS: GASTROPODA													
Family Physidae	0	0	0	0	0	0	0	0	4	26	0	0	0
Family Planorbinae	0	0	5	0	0	0	0	0	0	0	0	0	0
Family Thiaridae	0	0	0	0	0	0	0	0	20	194	1	0	5
Family Viviparidae	0	0	0	0	0	0	0	0	0	0	0	0	0
CLASS: BIVALVIA (PELECYPODA)													
Family Corbiculidae	0	0	0	1	0	0	0	0	19	15	0	0	1

Table 4.3: Expected taxa sampled during low flow condition in the Crocodile River and its tributaries (Thirion, 2012).

Taxon	CR1L	CR2L	CR3L	CR4L	CR5L	CR6L	CR7L	CR8L	CR9L	CR10L	ER1L	NR1L	KR1L
Class: Turbellaria	1	1	10	0	9	0	0	0	0	88	0	0	0
PHYLUM: ANNELIDA													
Class: Oligochaeta	0	0	15	9	7	0	6	4	1	0	0	2	8
Class: Hirudinea	0	0	0	0	0	0	0	0	0	0	0	0	1
ORDER: CRUSTECEA													
Family: Potamonautidae	12	9	1	10	113	0	0	0	1	0	1	11	9
Family: Atyidae	0	0	0	0	0	0	0	0	5	2	0	0	1
ORDER: PLECOPTERA													
Family: Perlidae	0	1	1	9	72	0	4	0	0	0	3	6	8
Family: Baetidae	114	9	116	107	50	0	4	8	16	3	10	13	12
ORDER: EPHEMEROPTERA													
Family: Caenidae	15	8	65	0	2	0	14	15	12	50	0	0	3
Family: Heptageniidae	9	9	78	8	5	0	0	4	2	5	6	5	8
Family: Leptophlebiidae	5	14	7	3	0	0	0	0	0	0	4	4	8
Family: Oligoneuridae	0	0	9	4	0	0	0	0	0	0	0	1	0
Family: Polymitarcyidea	0	0	0	0	5	0	0	0	0	0	0	0	0
Family: Prosopistomatidae	99	4	0	0	0	0	0	0	0	0	0	0	0
Family: Teloganodidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Trichorythidae	2	8	27	1	0	0	0	0	0	0	0	9	1

Table 4.3: Continued

Taxon	CR1L	CR2L	CR3L	CR4L	CR5L	CR6L	CR7L	CR8L	CR9L	CR10L	ER1L	NR1L	KR1L
ORDER: ODONATA													
Family: Chlorocyphidae	0	1	1	0	0	0	0	0	0	0	0	0	0
Family: Chlorolestidae	0	0	0	0	5	0	0	0	0	0	0	0	0
Family: Coenagrionidae	0	6	3	1	0	0	0	39	4	2	0	0	0
Family: Aeshnidae	3	6	1	0	0	0	0	17	0	0	0	10	4
Family: Corduliidae	0	0	0	0	7	0	0	0	1	0	0	2	0
Family: Gomphidae	7	16	6	0	0	0	11	0	100	17	1	0	2
Family: Libellulidae	0	0	1	0	0	0	18	10	0	2	56	1	1
ORDER: HEMIPTERA													
Family: Belostomatidae	0	0	0	0	0	0	0	12	0	0	0	0	0
Family: Corixidae	1	0	0	0	0	0	0	0	0	16	3	0	0
Family: Gerridae	0	0	0	0	0	0	0	8	0	0	0	0	0
Family: Naucoridae	0	3	0	0	16	0	0	0	0	40	2	0	0
Family: Nepidae	0	1	0	0	0	0	0	0	1	0	0	0	0
Family: Notonectidae	0	0	0	0	0	0	0	0	10	0	0	0	0
Family: Pleidea	0	0	0	0	8	0	0	0	7	129	0	0	0
Family: Veliidae	0	1	2	0	17	0	4	8	0	9	0	0	0

Table 4.3: Continued

Taxon	CR1L	CR2L	CR3L	CR4L	CR5L	CR6L	CR7L	CR8L	CR9L	CR10L	ER1L	NR1L	KR1L
ORDER: TRICHOPTERA													
Family: Hydropsychidae	5	2	8	10	10	0	8	70	1	9	4	2	10
Family: Philopotamidae	4	1	18	1		0	0	0	4	0	0	1	1
Family: Hydroptilidae	0	0	0	0	10	0	0	0	0	0	0	0	0
Family: Leptoceridae	0	0	0	0	0	0	0	0	4	5	1	1	1
ORDER: COLEOPTERA													
Family: Dytiscidae	2	0	0	0	5	0	0	0	0	0	0	0	0
Family: Elmidae	1	0	1	1	2	0	0	0	3	0	5	1	10
Family: Gyrinidae	1	0	1	8	8	0	8	65	2	3	0	0	0
Family: Hydraenidae	0	0	0	0	0	0	0	0	0	6	0	0	0
Family: Hydrophilidae	3	0	0	0	0	0	0	0	0	0	0	2	0
Family: Psephenidae	2	4	8	0	2	0	0	0	0	0	2	3	8
ORDER: DIPTERA													
Family: Athericidae	0	0	0	1	0	0	4	0	0	0	0	0	0
Family: Ceratopogonidae	0	3	0	0	0	0	0	0	2	0	4	0	1
Family: Chironomidae	2	19	29	1	19	0	148	88	22	15	12	1	0
Family: Culicidae	1	0	0	0	0	0	0	0	0	0	0	1	0
Family: Ephydridae	0	0	0	0	0	0	0	0	1	0	0	0	0

Table 4.3: Continued

Taxon	CR1L	CR2L	CR3L	CR4L	CR5L	CR6L	CR7L	CR8L	CR9L	CR10L	ER1L	NR1L	KR1L
Family: Muscidae	0	0	0	0	0	0	14	0	0	0	0	0	0
Family: Simuliidae	0	1	1	0	20	0	157	0	2	7	98	0	1
Family: Syrphidae	16	0	0	0	0	0	0	0	0	0	0	1	0
Tabanidae	5	0	1	0	0	0	4	4	1	0	2	0	8
Family: Tipulidae	0	5	1	10	0	0	13	0	0	0	14	15	1
PHYLUM: MOLLUSCA													
ORDER: GASTROPODA													
Family: Ancylidae	11	0	1	1	34	0	0	0	0	0	1	0	0
Family: Bulininae	0	0	0	1	0	0	0	0	0	0	0	0	0
Family: Lymnaeidae	0	0	0	0	0	0	0	18	0	0	0	0	0
Family: Physidae	0	0	0	0	0	0	0	0	2	0	0	0	0
Family: Planorbinae	0	0	0	0	0	0	0	6	0	0	0	0	0
Family: Thiaridae	0	0	0	0	0	0	0	0	4	12	0	0	237
Family: Viviparidae	0	0	0	0	0	0	0	0	0	0	0	0	0
CLASS: BIVALVIA (PELECYPODA)													
Family: Corbiculidae	0	0	0	3	0	0	0	0	1	4	0	0	1

The overlaid polynomial line in the total number of macro-invertebrates taxa graph indicated that generally higher abundance of taxa were sampled during low flow condition (Figure 4.1).

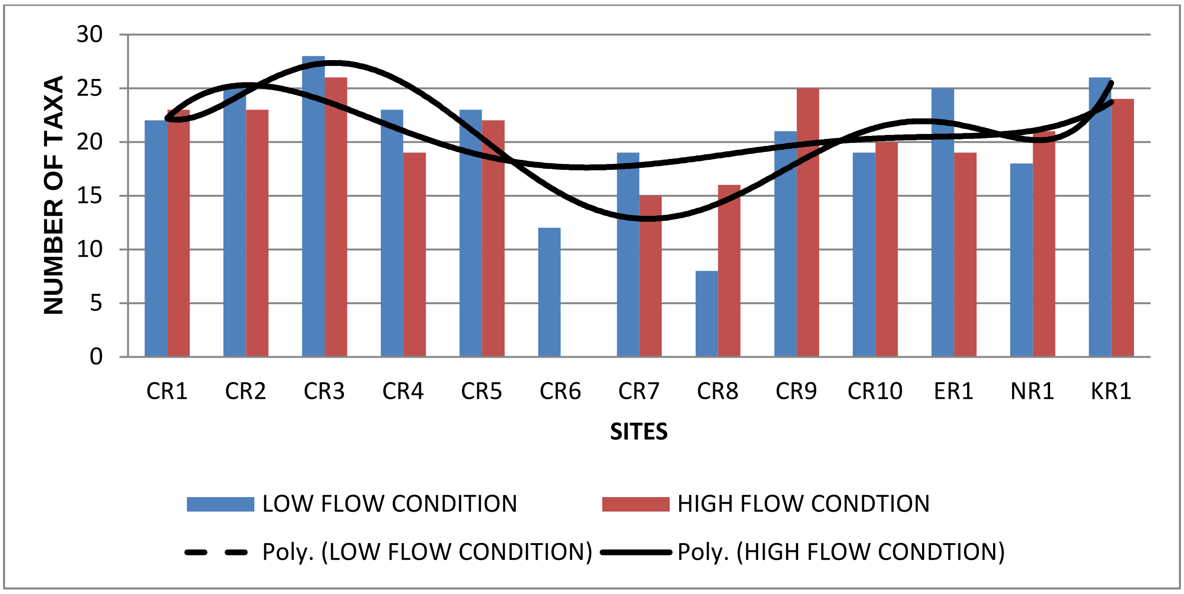


Figure 4.1: Total number of macro-invertebrates taxa sampled in the Crocodile River and its tributaries during both flow conditions.

The higher number of taxa sampled during low flow condition was linked to the presence of complex substrates such as gravel, vegetation and stone. The Margalef species richness index graph also indicated that the highest level of abundance of macro-invertebrates was found during low flow condition while only sites such as site CR2, CR8, CR10 and NR1 recorded high abundance during high flow condition (Figure 4.2).

The decrease in taxon richness was observed between site CR3 and CR4 during both flow conditions and this was an indication of the change in habitat downstream of the Kwena Dam and low water bridge which are situated upstream. The macro-invertebrates community richness rises at site CR5 during low flow condition which was due to good habitat availability at the site (Figure 4.2).

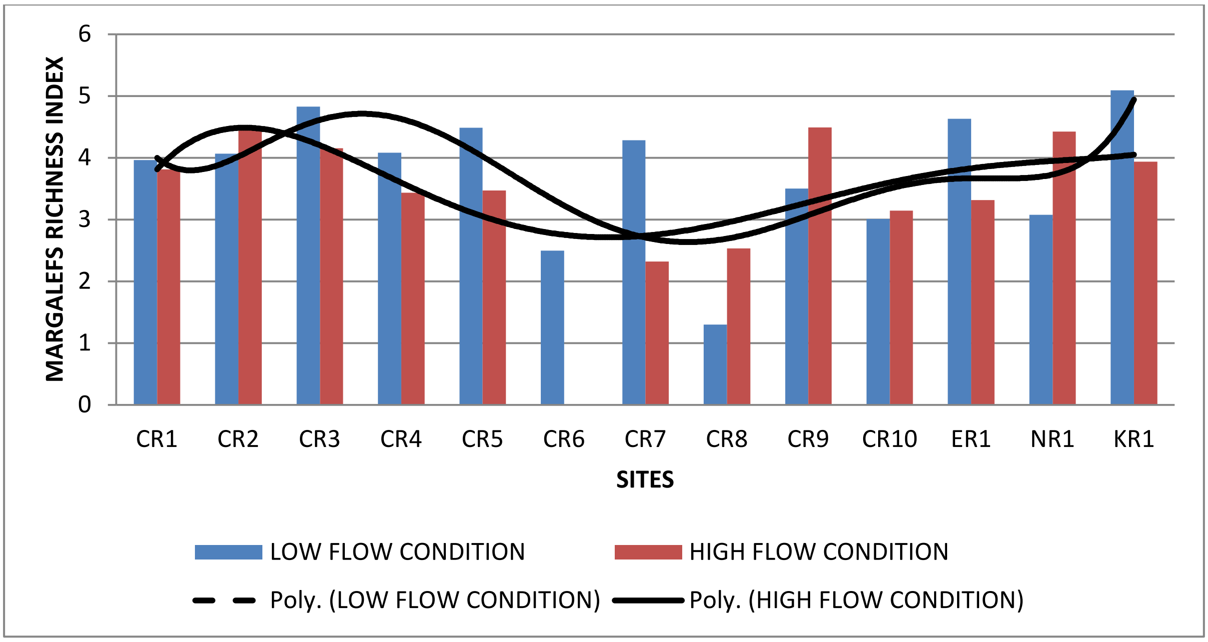


Figure 4.2: Margalef species richness of the Crocodile River and its tributaries during both flow conditions

When site CR7 was compared with site CR8 decrease of macro-invertebrates richness at site CR8 was observed. The decrease was linked to alteration of water quality upstream of site CR8 due to sewage works effluent discharged in the river. Although there was a variation of macro-invertebrates richness, both sites, were dominated by Chironomidae and Simullidae families and these families are associated with low water quality sensitivity score (Dickens and Graham, 2001). Thus, their presence at the sites represented poor water quality which was associated with the presence or discharge of sewage upstream. A similar condition was observed at sites CR9 and CR10 as deterioration of community richness was also observed. The tributaries of the Crocodile River system that were sampled indicated that they have limited alteration of macro-invertebrates community richness for the system as most of them had high richness during low flow condition.

The Pielous Evenness Index (Figure 4.3), indicated that at site CR5 the macroinvertebrate communities was evenly distributed as compared to all the other sites sampled and this was related to different habitat and substrate availability.

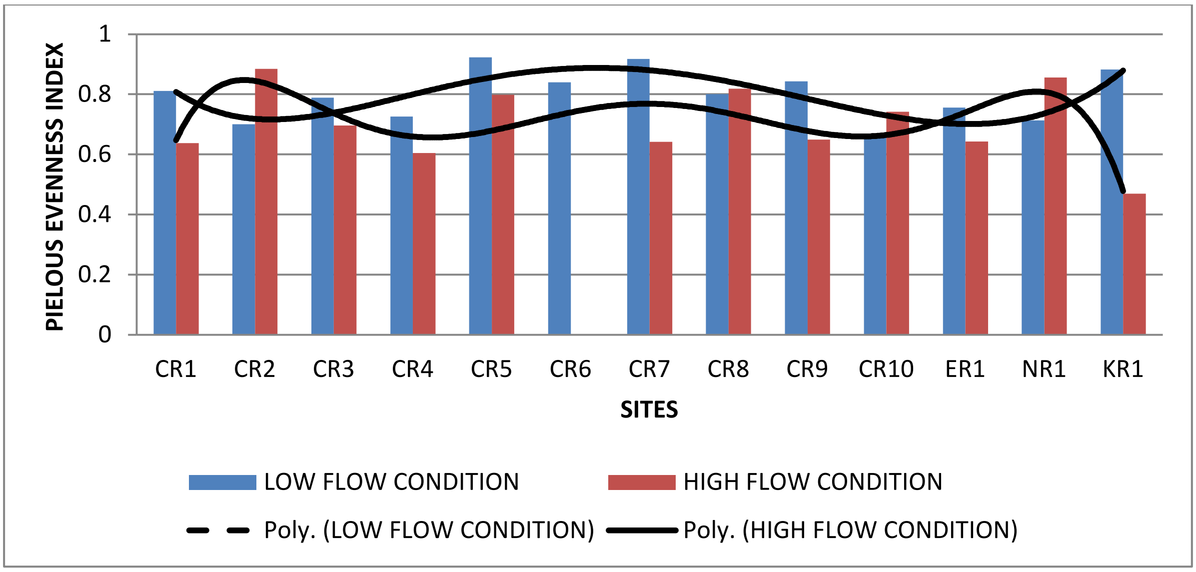


Figure 4.3: Pielous richness index of the Crocodile River and its tributaries during both flow conditions**.**

At site CR4 the evenness was less when compared to site CR3. Site CR7 was evenly distributed compared to site CR8 which was downstream of the Kanyamazane township. The univariate analysis used to identify the evenness, diversity and richness of macro-invertebrate communities in the Crocodile River, indicated variation in seasonality during both flows and with a single domination of taxon. The overlaid polynomial line (Figure 4.4) indicated a similar pattern as the Pielous evenness index graph.

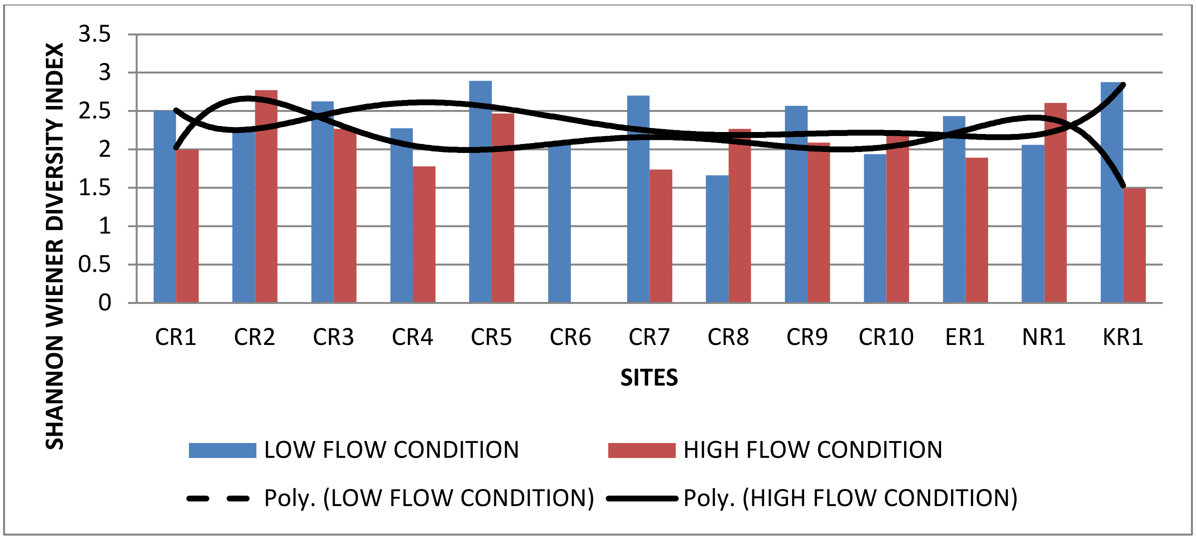


Figure 4.4: Shannon-Wiener diversity index of the Crocodile River and its tributaries during both flow conditions.

The variation in macro-invertebrates diversity during both flow conditions was related to change in habitat complexity or substrate complexity and water quality in the Crocodile River and its tributaries as less diversity was observed during high flow conditions.

A Bray-Curtis Cluster similarity analysis and a NMDS ordination of invertebrates sampled in the Crocodile River and its tributaries during both flow conditions is displayed as Figure 4.5. The figure indicates formation of sites grouping at similarity approximately 40% and a further formation of sites grouping was observed at similarity approximately 50% and 65%. The similarity of sites was linked to the presence of similar macro-invertebrates assemblages occurring at those sites. The cluster analysis also indicated the separation of sites CR1, NR1, CR4, CR5 and CR8 from the group formations during low flow condition. This formation is due to the change in macro-invertebrates assemblage at these sites due to habitat availability, flow condition and water quality.

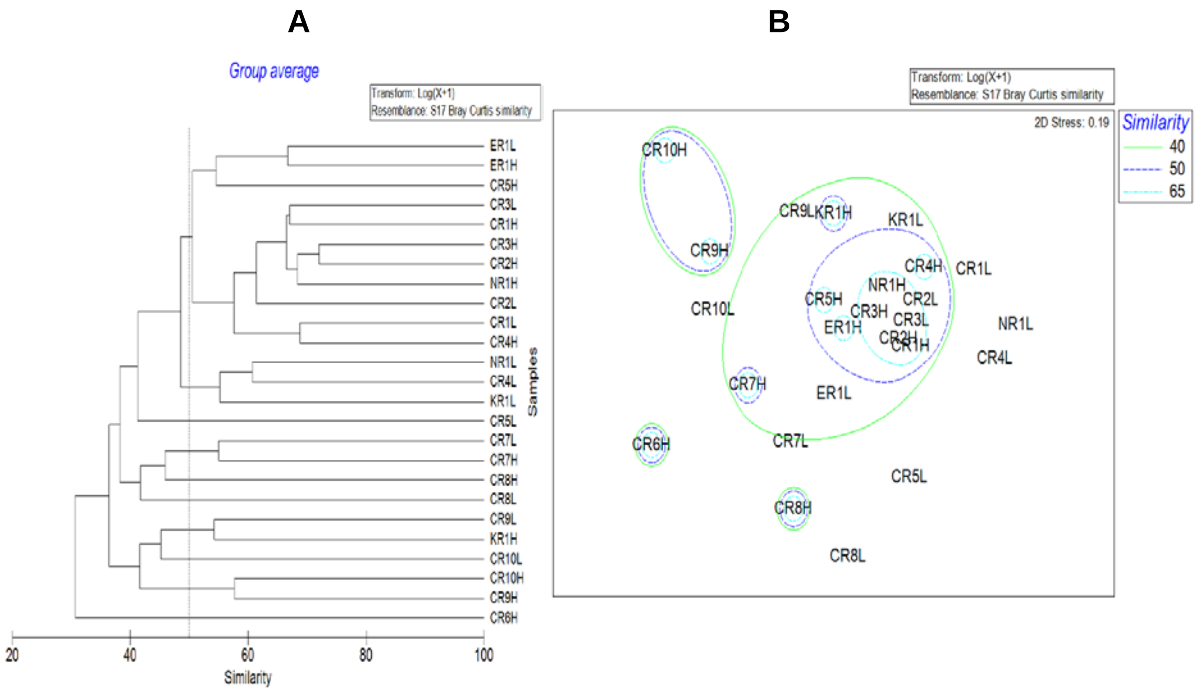


Figure 4.5: Bray-Curtis similarity matrix-based cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the Macro-invertebrates collected at the sites Crocodile Rivers and tributaries during high and low flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.19.

The NMDS ordination with a stress value of 0.19 indicated the same trend for the community structure during low and high flow conditions (Figure 4.5B). The formation of grouping at the sites at different percentages was due to the fact that macro-invertebrates at other sites were affected by various factors (water quality constituents). The presence of agricultural activities, settlements and industries can also be linked to the change in macro-invertebrates assemblages in the river.

The Bray-Curtis Cluster analysis and NMDS ordination similarity of invertebrates sampled in the Crocodile River and its tributaries during high flow condition (Figure 4.6), indicated group formation of sites at similarity approximately 50% and 60%. The group formation of sites was as a result of the presence of similar families at those sites. The analysis further indicated that sites CR7, CR8, CR6 and KR1 had less than two sites group formation and this was an indication that these sites had different macro-invertebrates assemblages. This was an indication of the impact of changes of water quality and habitat characteristics as these sites are in the vicinity of industrial and agricultural activities which pose a threat to the river.

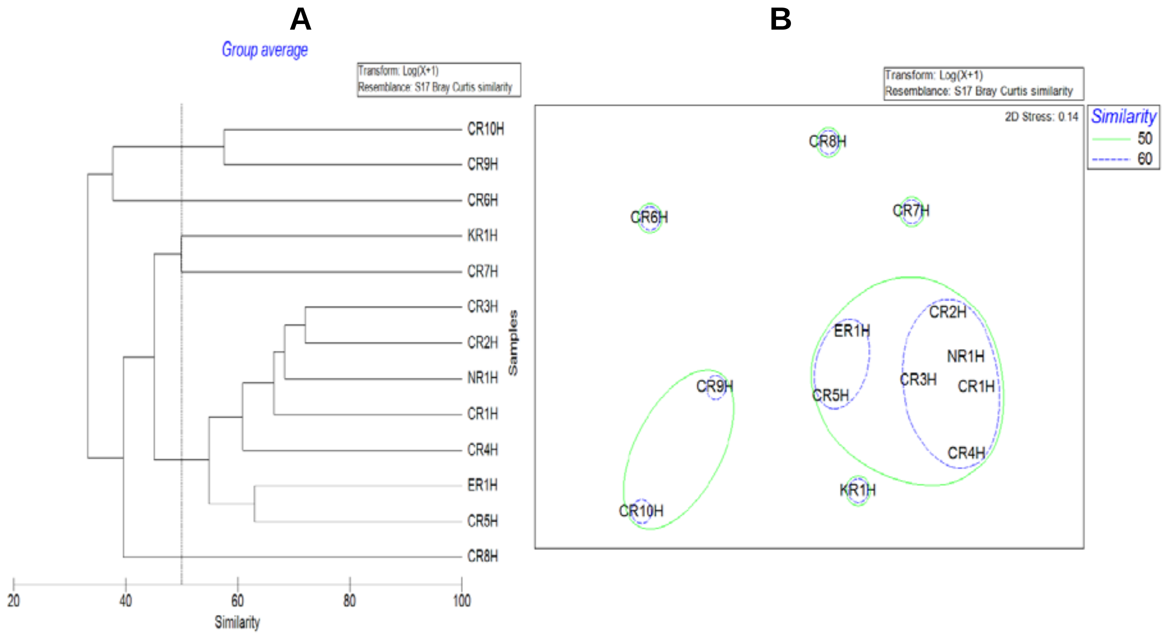


Figure 4.6: Bray-Curtis similarity matrix-based cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the macro-invertebrates collected at the sites on the Crocodile Rivers and tributaries during high flow condition. The NMDS ordination was completed with 30 iterations and showed a stress of 0.14.

The NMDS ordination with a stress value of 0.14 indicated the same trend when looking at the community structure during high flow conditions (Figure 4.6B). It was clear that the group formation of sites in the Crocodile River and its tributaries occur at difference percentage level. At approximately 60% they were eight group formation of sites and at similarity approximately 50% they were only six group formation of sites the formation of grouping as indicated in Figure 4.6. It can be linked to the fact that during the high flow condition the site might share similar macro-invertebrates assemblages due to change in habitat caused by high flow, change in water quality and habitat amongst the sites.

The Bray-Curtis Cluster analysis similarity of invertebrates and the NMDS ordination (Figure 4.7), macro-invertebrates sampled in the Crocodile River and its tributaries during low flow condition indicated that there was a group formation of sites at similarity approximately 50% and 60% similar to that of Figure 4.6. The group formation of sites was linked to the sharing of families within the sites.

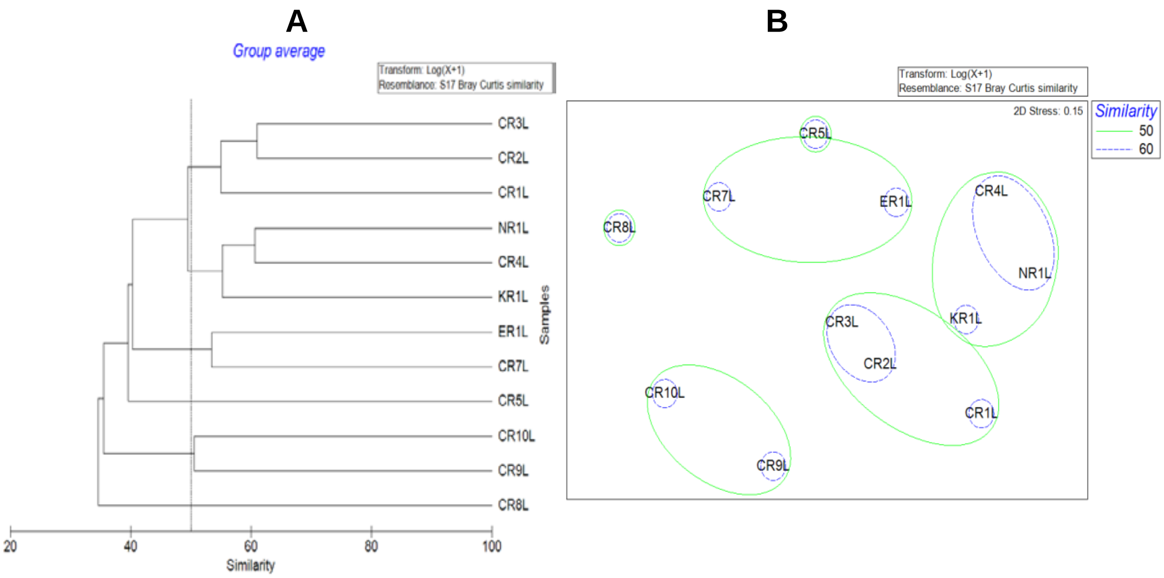
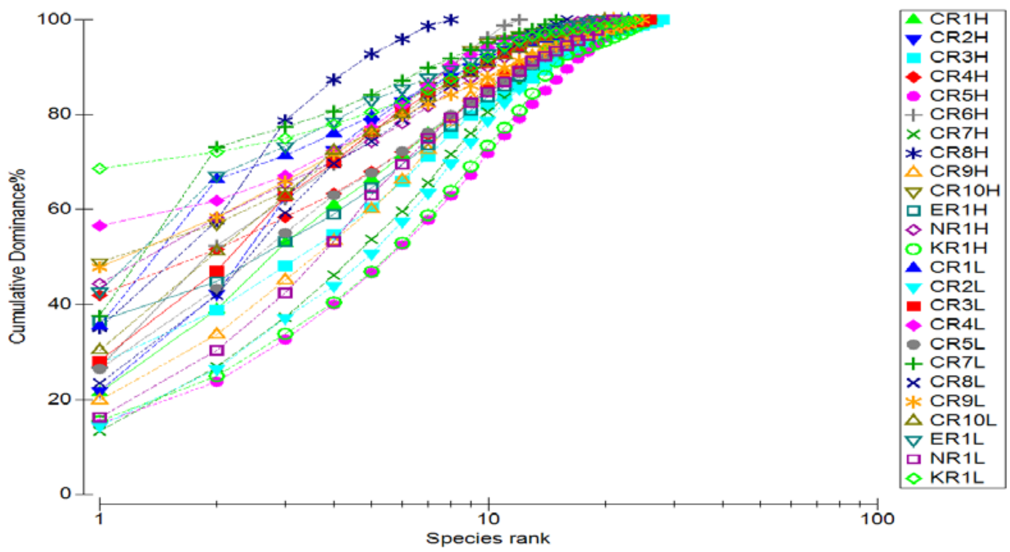


Figure 4.7: Bray-Curtis similarity matrix-based cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the macro-invertebrate collected at the sites on the Crocodile Rivers and tributaries during low flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.15.

The cluster analysis (Figure 4.7A), further indicated that site CR8 had less than two site group formation at both percentages which was linked to the fact that this site might receive pollutants for the discharge of sewage (Kanyamazane Township) during low flow condition and it does not share similar macro-invertebrates assemblages with other sites. The NMDS ordination with a stress value of 0.15 indicated the same trend when looking at the community structure during low flow conditions (Figure 4.7B). The NMDS ordination indicated clearly that group formation of sites occurs at different percentages in the Crocodile River and some of its tributaries. There were five group formation at similarity 50% and ten group formation of site at approximately 60%. The formation of grouping at different percentages within the river indicated that although there might be differences in macro-invertebrates assemblages due to different factors other sites were identical in having similar macro-invertebrates assemblage.

A K-dominance plot was constructed to determine any increased dominance of species at some sites and during specific seasons. The K-dominance curves presented in Figure 4.8**,** were in the form of ranked species abundance curves. From the K-dominance curve it can be seen that other site were dominated by a single taxon of macro-invertebrates during both flow conditions. Above 60% of a single family namely Thiaridae dominated the macro-invertebrates communities at site KR1 during low flow condition.



L-low flow condition	**H**-High flow condition

Figure 4.8: Ranked species K-dominance curves for the macro-invertebrate communities collected from the Crocodile River and its tributaries during high and low flow conditions.

The dominance curves further indicated that at site CR3, CR4, CR9 and ER1 during low flow and CR10 and NR1 during high flow condition were dominated by single taxon which contributed above 40%. These dominant families include the Simuliidae at ER1, Beatidae at CR3 and CR4, Gomphidae at CR9, and Pleidae at CR10. The presence of Beatidae at site CR3 and CR4 indicates a good water quality as this family is sensitive to change in water quality, while the presence of family Pleidea at site CR10 indicated possibility of water pollution as the taxon can tolerates change in water quality and this was linked to the discharge of effluent from industrial activities and run-offs from agricultural activities.

The dominance of single species at other sites indicated an imbalance of community of aquatic macro-invertebrates during low flow and high flow conditions. The dominance of single species meant that when the abundance of macro-invertebrates decreased, the diversity also decreased.

The results of Simper analysis indicating the group relationship between sites or Taxa and the groups identified by the Bray-Curtis analysis and NMDS ordination (Figure 4.5) are as follows:

Group1: CR6H (Less than 2 group in a site)
Group 2: CR9H and CR10H
Group 3: CR10L (less than 2 sites in a group)
Group 4: KR1H, KR2H and CR9L
Group 5: CR8L (less than 2 sites in a group)
Group 6: CR8H (less than 2 sites in a group)
Group 7: CR7H, CR7L
Group 8:CR5L (less than 2 sites in a group)
Group 9: CR4L, NR1L
Group 10: CR1H,CR2H,CR3H,CR4H,CR5H,ER1H,NR1H,CR1L,CR2L,CR3L,ER1L

The identification of the groups above using the Bray-Curtis analysis and NMDS ordination further elaborated the similarities of taxa between sites in percentage.

The average similarity in group 2 was 56.6%, group 2 was all the sites (CR9 and CR10) of the lower reaches of the river during high flow condition (Table 4.5). Five families; Ceratopogonidae, Thiaridae, Beatidae, Hydracarina and Corbiculidae contributed more than 50% of the abundance within the group (Table 4.5). The taxa that may have 50% or higher contribution to the formulation of group and may be good indictors of pollution occurring on the site. Within the group twelve families represented more than 50% of the total composition in the group. The most abundant families were Ceratopogonidae followed by Thiaridae, Beatidae, Hydracarina, Corbiculidae, Turbellaria, Muscidae, Gomphidae, Chironomidae, Hirudinea, Libellulidae and Physidae. Although the presence of these families in group 2 might be an indication of good water quality within the sites, there was a concern about the presence of Thiaridae family which contributed about 11.77% of the abundance in group 2, as this family is tolerant to change in water quality. There was a higher percentage dissimilarity between group 2 and the other groups. The order of dissimilarity were (group 2 and 1 (62.3%), group2 and 3 (56.01%), group 2 and 4 (60.38%), group 2 and 5 (77.56%), group 2 and 6 (69.63%), group 2 and 7 (67.28%), group 2 and 8 (68.13%), group 2 and 9 (82.0%), group 2 and 10 (68.11%)).

Table 4.4: The contribution of taxon within a group formation in the Crocodile River and its tributaries determined using SIMPER for both flow conditions.

	Taxon	Av.Abund	Av.Sim	Contrib%	Cum.%
Group 1	Less than 2 site within the group				
Group 2	Ceratopogonidae	3.45	6.98	12.13	12.13
	Thiaridae	4.16	6.78	11.77	23.90
	Baetidae	3.02	6.56	11.39	35.29
	Hydracarina	3.16	6.31	10.96	46.24
	Corbiculidae	2.88	6.18	10.72	56.97
	Turbellaria	2.64	4.33	7.53	64.49
	Physidae	2.45	3.59	6.22	70.72
	Gomphidae	2.19	3.09	5.36	76.08
	Chironomidae	2.60	2.45	4.25	80.32
	Hirudinea	1.75	2.45	4.25	84.57
	Libellulidae	1.10	2.45	4.25	88.82
	Muscidae	1.24	2.45	4.25	93.07
Group 3	Less than 2 sites within the group				
Group 4	Baetidae	2.83	6.76	13.74	13.74
	Thiaridae	2.96	4.25	8.64	22.39
	Elmidae	1.91	4.00	8.13	30.52
	Heptageniidae	1.79	3.62	7.36	37.88
	Gomphidae	2.44	3.23	6.57	44.45
	Hydropsychidae	1.80	3.11	6.33	50.78
	Atyidae	1.52	2.70	5.49	56.27
	Leptoceridae	1.65	2.54	5.17	61.44
	Ceratopogonidae	1.19	2.11	4.29	65.73
	Tabanidae	1.33	2.10	4.28	70.01
	Chironomidae	1.81	1.96	3.98	73.99
	Leptophlebiidae	1.46	1.84	3.74	77.74
	Corbiculidae	0.69	1.76	3.59	81.32
	Oligochaeta	1.19	1.76	3.59	84.91
	Simuliidae	0.83	1.76	3.59	88.50
	Caenidae	1.32	1.19	2.42	90.92
Group 5	Less than 2 sites within the group				
Group 6	Less than 2 sites within the group				
Group 7	Caenidae	2.51	7.22	13.13	13.13
	Gomphidae	2.39	7.22	13.13	26.25
	Chironomidae	3.54	6.52	11.86	38.11
	Hydropsychidae	1.99	5.61	10.21	48.32
	Muscidae	2.16	5.04	9.18	57.50
	Athericidae	1.50	4.34	7.90	65.40
	Libellulidae	2.17	4.34	7.90	73.31
	Baetidae	1.35	3.44	6.26	79.57
	Simuliidae	3.08	3.44	6.26	85.83
	Tabanidae	1.35	3.44	6.26	92.10
Group 8	Less than 2 sites within the group				

Table 4.4: continued.

	Taxon	Av.Abund	Av.Sim	Contrib%	Cum.%
Group 9	Baetidae	3.66	8.79	14.48	14.48
	Potamonautidae	2.44	7.98	13.16	27.64
	Tipulidae	2.59	7.98	13.16	40.80
	Perlidae	2.12	6.48	10.68	51.48
	Heptageniidae	1.99	5.97	9.83	61.31
	Leptophlebiidae	1.50	4.62	7.61	68.92
	Hydropsychidae	1.75	3.66	6.03	74.95
	Oligochaeta	1.70	3.66	6.03	80.98
	Chironomidae	0.69	2.31	3.80	84.78
	Elmidae	0.69	2.31	3.80	88.59
	Oligoneuridae	1.15	2.31	3.80	92.39
Group 10	Baetidae	3.47	6.71	11.78	11.78
	Heptageniidae	2.94	5.64	9.91	21.69
	Chironomidae	2.53	4.94	8.68	30.37
	Caenidae	2.35	4.05	7.11	37.49
	Gomphidae	1.92	3.57	6.28	43.77
	Hydropsychidae	1.87	3.45	6.07	49.84
	Simuliidae	1.92	2.49	4.38	54.22
	Aeshnidae	1.62	2.49	4.38	58.60
	Leptophlebiidae	1.63	2.36	4.14	62.74
	Trichorythidae	1.97	2.34	4.11	66.85
	Philopotamidae	1.51	2.19	3.84	70.69
	Elmidae	1.15	1.97	3.46	74.15
	Psephenidae	1.14	1.48	2.60	76.74
	Ceratopogonidae	1.21	1.46	2.57	79.31
	Gyrinidae	1.00	1.46	2.56	81.87
	Potamonautidae	1.08	1.41	2.48	84.35
	Turbellaria	0.98	1.28	2.25	86.60
	Perlidae	0.99	1.17	2.05	88.65
	Oligochaeta	1.09	1.15	2.01	90.66

As reflected in Table 4.5, group 4 had an average similarity of 49.17% and the families that contributed to more than 50% within the group were: Beatidae, Thiaridae, Elmidae, Heptageniidea, Gomphidae and Hydropsychidae . Beatidae had higher abundance compared to all the families within the group, followed by family Thiaridae. The second high abundance of family Thiaridae in the group indicated that the site might have moderate water pollution. There was a higher percentage dissimilarity between group 4 and others; (group 4 and 1 (72.97%), group 4 and 2 (60.38%), group 4 and 3 (59.20%), group 4 and 5 (68.13%), group 4 and 6 (66.96%), group 4 and 7 (60.5%), group 4 and 8 (64.39%), group 4 and 9 (58.24%), group 4 and 10 (52.51%)). The high dissimilarities within the groups was an indication that there were variables that played a role in these dissimilarities.

Group 7 had an average similarity of 54.97% during both flow conditions. The high similarities within this group was an indication that the sites within this group share same families or taxa. This group was characterized by tolerant and sensitive families which had a high abundance contribution such as families Caenidae, Gomphidae, Chironomidae, Hydropsychidae and Muscidae which contributed 57.50% of the abundance within the group. Although the presence of a tolerant taxon was an indication of pollution taking place, taxa such as families Caenidae and Gomphidae were the dominant taxa within this group. This was an indication that even though there is pollution taking place the sites might be in good condition. The was also a high percentage dissimilarity (group 7 and 1 (65.71%), group 7 and 2 (67.28%), group 7 and 3 (57.88%), group 7 and 4 (60.50%), group 7 and 5 (59.89%), group 7 and 6 (54.09%), group 7 and 8 (65.28%), group 7 and 9 (71.29%), group 7 and 10 (57.36%)) within this group when compared with other group in this study. In reference to Ferreira (2005), the dissimilarities within the grouping might be an indication that they were different variables acting on the group.

In group 9 families such as: Beatidae, Potamonautidae, Tipulidae, Perlidae, Heptageniidae and Leptophlebiidae contributed 68.92% of the abundance within the group. The high abundance was an indication of good water quality as it is known that these taxa are sensitive to pollutants (Table 4.5). This group also had high dissimilarity (group 9 and 1 (81.94%), group 9 and 2 (82.0%), group 9 and 3 (79.53%), group 9 and 4 (58.24%), group 9 and 5 (71.24%), group 9 and 6 (71.45%), group 9 and 7 (71.29%), group 9 and 8 (59.99%), group 9 and 10 (52.75%)) when compared to other groupings.

Group 10 had an average similarity of 56.92%. The family Beatidae had a high abundance contribution of 11.78% within this group followed by family Heptagenidae with an abundance contribution of 9.91%, which were both sensitive to water quality. The high abundance of Chironomidae (8.89%) which was the third abundance contribution in this group was an indication that although the group might have sensitive water quality families, there might be slight water quality problem at the site. Group 10 was formed by nineteen families which had a total contribution of 90.66% within the group. When group 10 was compared with other groupings a high percentage dissimilarity was observed : group 10 and 1 (70.05%), group 10 and

2 (68.11%), group 10 and 3 (61.10%), group 10 and 4 (52.51%), group 10 and 5 (62.29%), group 10 and 6 (60.03%), group 10 and 7 (57.36%), group 10 and 8 (58.13%), group 10 and 9 (52.75%) amongst the grouping. Groups 1, 3, 5, 6 and 8 had less than two sites in each group and their similarities were not determined. Their dissimilarities were higher as compared the other groups were high.

A one way ANOVA used indicated that there was no significant difference ($P>0.05$) between the macro-invertebrates abundance and richness during both flow regimes.

The RDA triplot for both flow regimes (Figure 4.9) indicates, as with Bray-Curtis similarity matrices and NMDS plot (Figures 4.5 and 4.6) that there was similarity between sites in the Crocodile River and its tributaries in terms of macro-invertebrates taxa. In RDA plot the length of the arrow was related to the strength of the correlation. In general, the longer the arrow, the more highly related that variable was to species composition and the approximation correlation is positive when the angle is acute and negative when the angle is larger than 90 degrees. The distance between the sampling sites in the diagram indicated the similarity of their macro-invertebrates community as they were measured by their Euclidean distance. When the environmental variables were superimposed on the RDA plot, a positive correlation between macro-invertebrates families such as Chironomidae, Gomphidae and Libellulidae at sites CR7L, CR8L, CR8H, CR9L, CR10L, ER1H and ER1L with water temperature, Chloride and sodium was observed and water temperature was the leading environmental variable for the correlation. The taxa Physidae, Hydracarina, Ceratopogonidae, Thiaridae, Hirudinae and Corbicullidae at site CR9H, KR1H, CR5H correlated positively with environmental variables such as salinity, total dissolved solid, electrical conductivity, magnesium and sulphate.

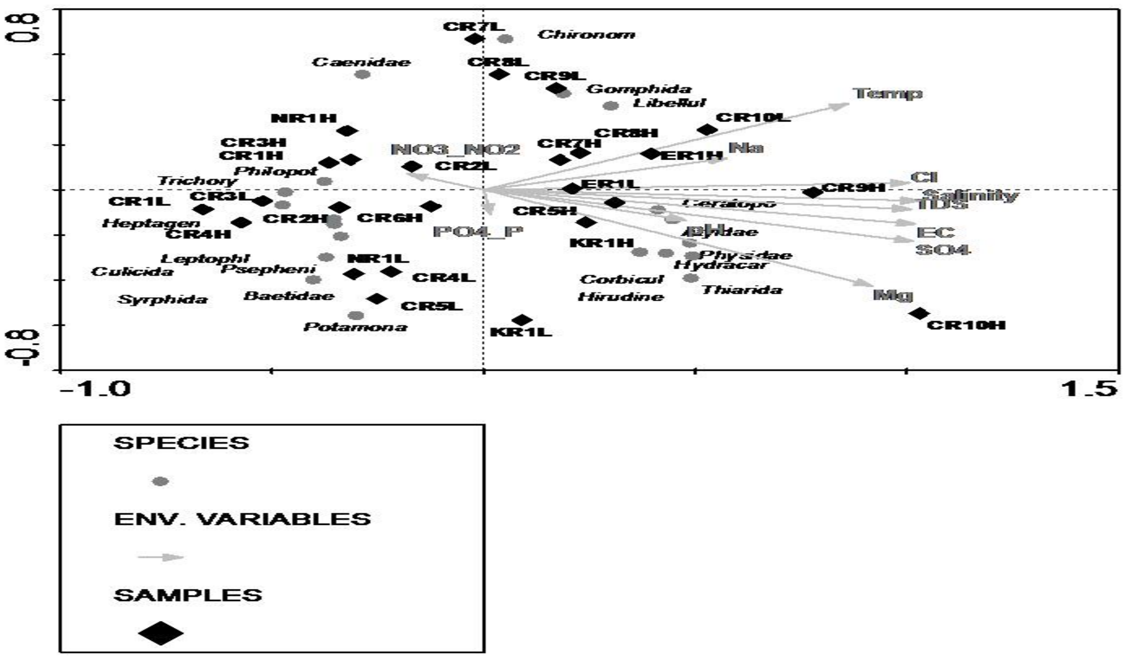


Figure 4.9: RDA tri-plot illustrating the similarities in the invertebrate communities between the various sites (and surveys) with the water quality variables superimposed. The tri-plot describes 42.9% of the variation with 27.4% being described on the first axis and 26.2 % on the second axis. Only the taxon of with more than 20% is explained by the model is visualized and these taxon includes : Chironomidae, Gomphidae, Libellulidae, Ceratopogonidae, Atyidae, Physidae, Hydracarina, Thiaridae, Corbicullidae, Hirudinae, Beatidae, Potanomitidae, Syrphidae, Culicidae, Leptophlebiidae, Heptagenidae, Trychoridae, Philopotamidae, Caenidae.

The correlation of macro-invertebrates taxa with the environmental variables at different sites was an indication that macro-invertebrates at different sites within the river might be evenly distributed based on their water quality tolerance and habitat preference.

The average score per taxa (ASPT) and the South African Scoring System (SASS) for both high and low flow conditions were used to determine the health of the Crocodile River and its tributaries (Table 4.6 and 4.7). The ASPT results for the upper Crocodile River was in good to natural condition compared to the middle and lower reaches which range from fair to good condition. The ASPT for site CR1 was

6.5 during low flow condition indicated that the site was in good condition and (7.1) during high flow condition which indicated that the site was in natural state (Table 4.6 and 4.7). Site CR2 remain in a good condition during both flow while site CR3 (ASPT of 7.5) and CR4 (ASPT of 7.3) were in a natural state during low flow condition. Site CR3 was in a good condition with an ASPT of 6.5 while site CR4 was in a fair state of 5.7 during high flow condition. The change in ASPT in the upper reaches of the Crocodile River was linked to the change in water quality, and habitat during high flow condition caused by run-offs from agricultural activities. The ASPT of site CR5 which was downstream of the confluence of the Crocodile River and the Elands River was 6.1 which indicated that the site was in a good condition during low flow and fair with an ASPT of 5.9 high during high flow. The change in ASPT during high flow conditions at this site was linked to the influx of water coming from the Elands River. Sites CR7 and CR8 which are in the middle reaches of the river had fair condition with 5 and 4.7 respectively during low flow condition and they were in good condition during high flow condition.

The lower reaches of the Crocodile River remained in a fair state during both flow conditions with an ASPT ranging from 4-5. The tributaries of the Crocodile River were all in a good condition with ASPT of >6 during both flow conditions. Therefore, the ASPT of the Crocodile River decrease longitudinal with the increase in the river flow downstream as a result of the anthropogenic activities along the river. Tables 4.6 and 4.7 have all the scores of the SASS related assessments for the low and high flow conditions respectively.

Table 4.5: SASS Score, Number of Taxa and ASPT within different biotopes sampled and the Macro-Invertebrates Response Assessment Index Ecological Class Category during low flow condition in the Crocodile River and its tributaries.

	BIOTOPES	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**NR1**	**KR1**
SASS SCORE	STONE	126	137	152	100	130	-	64	75	52	39	68	103	127
	VEG	51	54	52	34	80	-	52	72	54	45	30	66	37
	GSM	47	49	39	44	62	-	49	67	47	59	90	21	22
	TOTAL	**149**	**174**	**166**	**131**	**135**	**-**	**75**	**75**	**121**	**104**	**127**	**146**	**156**
NO OF TAXA	STONE	17	18	20	13	19	-	12	16	10	9	12	15	18
	VEG	8	8	8	6	14	-	10	15	11	9	7	10	8
	GSM	9	9	7	7	13	-	11	14	10	11	12	4	6
	TOTAL	**23**	**25**	**22**	**18**	**22**	**-**	**15**	**16**	**25**	**20**	**19**	**21**	**24**
ASPT	STONE	7.4	7.6	7.6	7.7	6.8	-	5.3	4.7	5.2	4.3	5.7	6.9	7.1
	VEG	6.4	6.8	6.5	5.7	5.7	-	5.2	4.8	4.9	5.0	4.3	6.6	4.6
	GSM	5.2	5.4	5.6	6.3	4.8	-	4.5	4.9	4.7	5.4	7.5	5.3	3.7
	TOTAL	**6.5**	**6.9**	**7.5**	**7.3**	**6.1**	**-**	**5**	**4.7**	**4.8**	**5.2**	**6.7**	**6.9**	**6.5**
MIRAI	**EC%**	**78**	**80**	**80**	**62**	**78**	**-**	**62**	**71**	**73**	**73**	**83**	**82**	**62**
CLASS	**A-F**	**C**	**B**	**B**	**C**	**C**	**-**	**C**	**C**	**C**	**C**	**B**	**B**	**C**

ASPT= Average Score Per Taxon, EC= ecological category, MIRAI= Macro-Invertebrates Assessment Index, GSM=Gravel Sand and Mud, VEG= Vegetation

Table 4.6: SASS score, Number of taxa and ASPT within different biotopes sampled and the Macro-Invertebrates Response Assessment Index Ecological Class Category during high flow condition in the Crocodile River and its tributaries.

	BIOTOPES	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**NR1**	**KR1**
SASS SCORE	STONE	164	142	144	116	105	54	139	45	87	63	145	101	139
	VEG	48	107	61	33	89	38	19	47	0	0	72	83	88
	GSM	67	63	71	129	60	49	22	31	42	49	62	73	63
	TOTAL	**170**	**177**	**180**	**137**	**136**	**81**	**140**	**49**	**111**	**78**	**160**	**119**	**174**
NO OF TAXA	STONE	20	18	18	19	17	9	20	7	17	15	22	14	19
	VEG	6	16	11	7	18	7	5	8	0	0	10	13	13
	GSM	12	12	16	21	11	10	6	7	9	12	12	11	12
	TOTAL	**24**	**26**	**28**	**24**	**23**	**13**	**21**	**9**	**21**	**19**	**26**	**18**	**27**
ASPT	STONE	8.2	7.9	8	6.1	6.2	6	6.9	6.4	5.1	4.2	6.6	7.2	7.3
	VEG	8	6.7	5.5	4.7	4.9	5.4	3.8	5.9	0	0	7.2	6.4	6.8
	GSM	5.6	5.3	4.4	6.1	5.5	4.9	3.7	4.4	4.7	4.1	5.2	6.6	5.3
	TOTAL	**7.1**	**6.8**	**6.4**	**5.7**	**5.9**	**6.2**	**6.7**	**5.4**	**5.3**	**4.1**	**6.2**	**6.6**	**6.4**
MIRAI	**EC%**	**75**	**74**	**74**	**75**	**77**	**77**	**64**	**72**	**71**	**71**	**82**	**80**	**64**
CLASS	**A-F**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**B**	**B**	**C**

ASPT= Average Score Per Taxa, EC= ecological category, MIRAI= Macro-Invertebrates Assessment Index, GSM=Gravel Sand and Mud, VEG= Vegetation.

The change in ASPT score in the lower reaches of the river can be due to the absence of certain habitats such as aquatic vegetation. The water flow in the lower reached was different from the flow at the upper reaches. Riffles, rapids were lacking at the lower reaches so sensitive families such as Beatidae and Heptagenidae were also limited. A comparison of the number of taxa sampled indicated that the middle reaches of the river has low number of taxa sampled which ranged from 15-22 while the upper reach ranged from 18-25 and the lower reach which include CR9 and CR10 ranged from 20-25 during low flow condition. There was a difference in the total number of taxa during low and high flow conditions especially at site CR4 (Tables 4.6 and 4.7). The change in number of taxa at this site was linked to the change in habitat caused by the Kwena Dam. Sites CR7 and CR8 had less number of taxa and they were dominated by the families Chironomidae and Simullidae. The presence of these families at the site indicated poor water quality which was associated with sewage.

The results obtained using the SASS 5 protocol were further used to obtain the Macro-invertebrates Ecological Category Class using the Macro-invertebrates Response Assessment Index (MIRAI) and were compared with the Class rating for Macro-invertebrates and other studies conducted in the Crocodile River Catchment.

Table 4.7: The Macro-invertebrates Response Assessment Index (MIRAI) Ecological Class Ratings.

Class rating	**Description of generally expected conditions for integrity classes**	**Relative MIRAI Rating score (% of expected)**
A	Unmodified, or approximate natural conditions closely	90 to 100
B	Largely natural with few modifications	80 to 90
C	Moderately modified	60 to 79
D	Largely modified	40 to 59
E	Seriously modified	20 to 39
F	Critically modified	0 to 19

The Macro-Invertebrates Response Assessment Index (Figure 4.10), indicated that the upstream of the Kwena dam was in ecological category B class for both sites CR2 and CR3 during low flow condition. This category indicated that the sites were largely natural with few modifications. Site CR1 which is upstream of these sites was in ecological category C class, indicating that the site was moderately modified (Figure 4.13). A study conducted by Roux *et al*., (1999) concluded that site CR1 had an ecological category B class (largely natural with little modification), CR2 and CR3 had an ecological category A class (natural without any modification).When comparing this finding with the current study, a deterioration of the ecological category was observed during high flow and low flow conditions at these sites. The deterioration of ecological category was believed to be associated with the change in habitat, flow modification and water quality during rainy season as more run-offs from agricultural activities might have an impact on the river. The impact of Kwena Dam on habitat and flow downstream was observed at site CR4 (Figure 4.10) as the ecological category deteriorated when compared with the upstream sites of the dam especially during low flow condition.

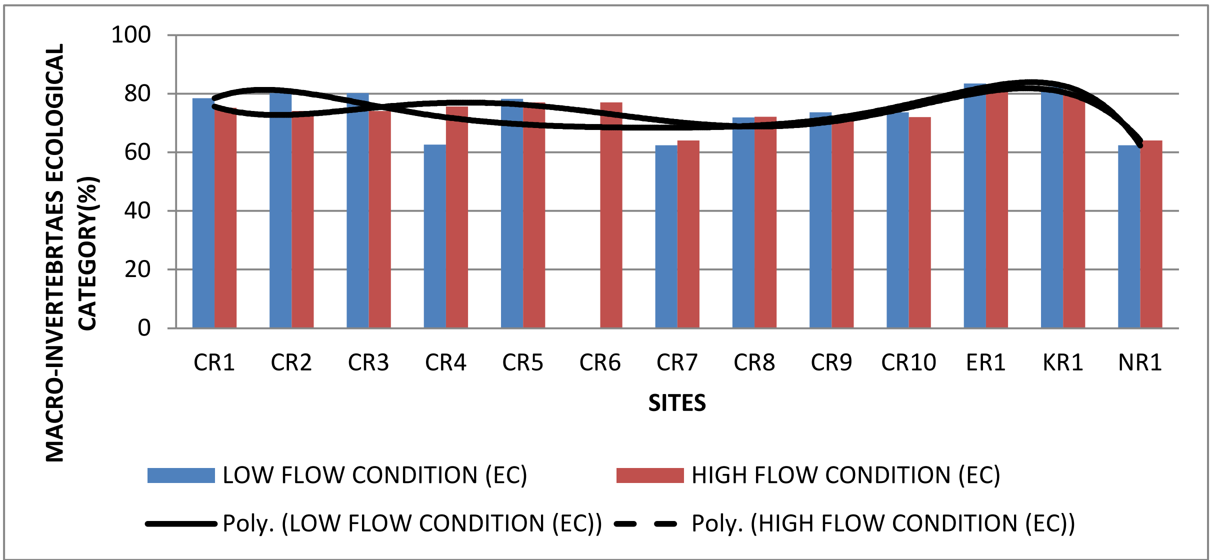


Figure 4.10: The Ecological Category (EC) for macro-invertebrates using the Macro-Invertebrates Assessment Response Index for the study sites in the Crocodile River and its tributaries.

Site CR4 had an ecological category C (moderately modified) for both flow conditions. According to Roux *et al*. (1999), this site was found to be in an ecological category B class (largely natural with few modifications), and this indicated that the site downstream has deteriorated due to flow modification and habitat change. The ecological category started to improve at sites CR5 and CR6 with an ecological category B/C (largely natural with little modification) indicating a recovery of the habitat further downstream. These sites CR5 and CR6 are upstream of the Nelspruit town and when compared with site CR7 downstream of the town the impact of the town on the Crocodile River was observed as the Ecological Category Class deteriorated from B/C to C (moderately modified). The deterioration of the Ecological Category Class was linked to the absence of sensitive macro-invertebrates families which was caused by the change of water quality due to sewage discharge, run-off from the urban area and agricultural activities. The absence or limited habitat preference which was caused by the construction of bridges across the river has also contributed to the deterioration.

When the ecological category C class (moderately modified) for sites CR7 in the current study was compared with the findings for Roux *et al*., (1999), which was in an ecological category D class (largely modified) a shift from largely modified to moderately modified condition was observed which indicated a small recovery in the habitat condition. The Crocodile River remains in an ecological category C class (moderately modified) from downstream of the Nelspruit town (sites CR7, CR8, CR9 CR10) until it confluence with the Komati river during both flow conditions although there was a slight change due to the activities that occurs along the river (agricultural activities, sewage discharge, littering and rural and urban run-offs). The change in habitat preference for certain macro-invertebrates species was also observed as a factor that lower the ecological category for the downstream sites of the river.

In reference to Figure 4.12, the Nels River site NR1 which is a tributary of the Crocodile River was in an ecological category C (moderately modified); the modification was linked to change in water quality and change in habitat at the site. The Elands (ER1) and the Kaap River (KR1) had an ecological category B class (largely natural with few modifications) and B/C class (largely natural with little modification) respectively. The few modifications on the Kaap River were associated with the change in water quality as there were abandoned mines in the catchment wich might pose a threat to the river through seepage of mining effluents.

4.4. Conclusion

The multivariate analysis of macro-invertebrates indicated that there was no significance difference between richness and abundance during both flow conditions and that the greatest macro-invertebrates abundance, richness and diversity occurs mostly during low flow condition. The high abundance, diversity and richness of macro-invertebrates at site CR2, NR1, CR9 and CR10 during high flow condition was not known. The macro-invertebrates assessment index further indicated that from the downstream of the Nelspruit town to downstream of the Malelane town the river is moderately modified with ecological category C class. The ecological modification of macro-invertebrates was attributed to change in water quality (e.g. sewage effluent, agricultural run-off, mining seepage and industrial effluent), habitat (river bank disturbance) and flow modification (weirs and impoundments). The lower reaches of the Crocodile River was concluded to have poor water quality compared to the middle and upper reaches and this was also confirmed by the presence of families such as the family Beatidae which dominated the macro-invertebrates in the upper reaches, while families Pleidea and Thiaridae dominated the macro-invertebrates communities at CR10 during both flow conditions. It was evident from this results that the anthropogenic activities that result in change in water quality, flow and habitat in the river had a negative impact on the macro-invertebrates abundance, diversity and richness especially during high flow condition.

CHAPTER 5

5. WATER QUALITY

5.1. Introduction

Degradation of water resources has long been a concern of human activity. According to Meybeck and Helmer (1989), region with dense human population were the earliest areas at risk, but water in isolated areas have also experienced degradation and the earliest anthropogenic threats to water resource were associated with human health. As population and their technology increase, impacts such as production of the domestic effluent, erosion following alteration of landscape by agriculture, urbanization and forestry, alteration of stream channels and lake margins through dams, proliferation of toxic chemicals from non-point source and point source are too diverse (Karr and Dudley, 1981; Karr *et al*., 1985).

The upper Crocodile River has good water quality; however trout farming have been identified as a source of eutrophication (Heath and Claassen, 1999). The deterioration of water quality in the Crocodile River remains a problem especially the downstream part of the river and these impacts occurs mostly during the winter months. In the middle of the Crocodile River, exotic afforestation of the Nels River causes an increase in manganese, an increase in sediment loads and decrease in pH values during logging operation. Agricultural runoff here is related to increases in electrical conductivity, trace elements and nutrients. The section from Nelspruit to the confluence with Kaap River is associated with domestic runoff, littering and an increase in nutrients. Industrial effluents from the Nelspruit cause an increase in manganese and boron concentration, while major sewage treatment works at the towns of Nelspruit, Matsulu and Kanyamazane are sources of high nutrients load in the river (Heath and Claassen, 1999). The lower Crocodile River downstream from the Kaap River confluence has poor water quality due to agricultural run offs associated with pesticides, increase in trace elements, nutrients and electrical conductivity. Hyacinth infestation is very common in this section and has been associated with fish mortalities. Mining activities in the Kaap River catchment have a high impact on water quality during low flows (Heath and Claassen, 1999) and the effectiveness of long term management of a river requires fundamental understanding of hydro-morphological, chemical and biological characteristics (Sthrestha and Kazama, 2007).

5.2. Materials and Methods

5.2.1. Field survey

Forty eight water quality samples were collected from twelve sites of the study area during September 2011 until August 2012. At site NR1 and LR1, water samples were not collected because the contract for a water quality service provider expired. Each site was visited four times during the survey and a polyethylene bottle was used to collect the water quality samples and insitu measurements of pH, TDS, Salinity, Temperature, and Conductivity were taken using an YSI Multi meter (HQ40d). Certain water quality variables such as Ammonium, Chlorine, Nitrate, Sulphates, Phosphate, Manganese and Sodium were analyzed by Mpumamanzi Laboratory in Nelspruit and Waterlab in Pretoria.

5.2.2. Data Analysis

Multivariate statistical techniques has been applied to characterize and evaluate surface and freshwater quality and it was used in verifying temporal and spatial variations caused by anthropogenic factors linked in seasonality for this study. Multivariate method such as cluster analysis (CA) and principal component analysis (PCA) were used to analyze the water quality data set including all the parameters at the study sites of the Crocodile River to obtain the spatial and temporal variation and to identify potential pollution source (Kazi *et al*., 2009). The application of different multivariate statistical techniques such as cluster analysis (CA), Principal component analysis (PCA), and factor analysis (FA) help in the interpretation of complex data matrices to better understand the water quality and ecological status of the study system, allowed the identification of possible factor source that influence water system and offer a valuable tool for reliable solution to problems to (Lee *et al.,* 2001; Reghunath *et al.,* 2002; Vega *et al*., 1998; Wunderlin *et al*., 2001).

5.3. Results and Discussion

Water quality constituents results such as temperature, salinity and total dissolved solid were analyzed only for winter and summer month. The water quality parameters results for the sites in the upstream of the Crocodile River before confluence with the Elands River was measured and found to have high concentration of nutrients. High concentrations of phosphate and ammonium at site CR2 were found with a mean value of 2 mg/L and 0.2 mg/L respectively was measured and calculated in winter and high concentration of nitrate was measured at site CR3 with a mean value of 7.5 mg/L in autumn. The presence of sulphate with a mean value of 88.1 mg/l and magnesium 40 mg/L at site CR1 was also measured and when site CR4 which was downstream of the Kwena dam was compared with its counterpart sites in the upper reaches, a high concentration of Chloride with a mean values of 14.5 mg/L and 6.3 mg/L was measured and calculated in autumn and spring. According to Kleynhans (1999), the Crocodile River had good water quality in the upstream reaches but susceptible to eutrophication due to trout farming in the area. The elevated concentrations of these water quality constituents in the upper reaches of the Crocodile River was due to fertilizer application for agricultural purpose and cattle feed.

Water quality parameters such as total dissolved solids, electrical conductivity and salinity in the Crocodile River increase with the increase in river distance (Table 5.1-5.4). The high concentration of these parameters in the river was measured in summer months and their presence was associated with agricultural and urban run-off and industrial effluents coming from the Nelspruit town. At site ER1 a high concentrations of total dissolved solids with a mean value of 528 mg/L and Salinity with a mean value of 0.358 (ppt) were measured in summer (Table 5.3); while magnesium with a mean value of 21.6 mg/L in spring, chloride with a mean value of 47.3 mg/L were measured in winter. Conductivity with mean value of 57.96 $mS{\cdot}m^{-1}$

was measured and calculated in winter. The high concentration of water quality constituents such as Chloride were due to industrial effluent from a paper mill upstream of the site, agricultural return flows in the upstream catchment of the area and sewage treatment effluents.

Table 5.1: Mean values of water quality results sampled in the Crocodile River and its tributaries during Spring season (September-November 2011).

Physico-chemical variables	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**KR1**
Cl^- (mg/l)	2.7	3.4	2.3	14.5	17.7	16.4	14.5	16.8	22.3	142.8	44.6	13.8
EC ($mS{\cdot}m$-^{1})	5.6	13.2	12.9	22.3	19.6	23.9	22.3	24	27.6	113.9	47.2	41
Mg (mg/l)	2.7	9.2	8.6	9.9	11.6	10.9	9.9	10.4	25.9	43.2	18.7	24.9
Na (mg/l)	1.9	3.6	3.7	13.1	10.8	14.1	13.1	15.3	25.0	1183	39.2	18.3
NH_4 (mg/l)	0.03	0.025	0.025	0.03	0.025	0.025	0.04	0.025	0.025	0.025	0.025	0.03
NO_3 (mg/l)	0.1	0.2	0.025	0.11	0.04	0.2	0.11	0.4	0.5	5.4	0.1	0.5
pH	7.4	7.7	7.9	7.8	7.9	7.9	7.8	7.8	7.8	8.3	8.2	8.0
PO_4 (mg/l)	0.1	0.005	0.1	0.1	0.005	0.03	0.1	0.04	0.05	0.4	0.005	0.03
SO_4 (mg/l)	3.5	6.3	1.5	15.4	27.2	19.1	15.4	24.6	38.7	30.3	75.7	46.3

Table 5.2: Mean values of water quality results sampled in the Crocodile River and its tributaries during Autumn season (April-June 2012).

Physico-chemical variables	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**KR1**
Cl (mg/l)	0.9	2.4	2.4	6.3	18.2	14.8	12.0	14.5	23.7	29.3	34.9	14.4
EC ($mS{\cdot}m^{-1}$)	6.2	12.7	12.7	13.7	27.0	25.0	21.6	27.0	43.6	37.6	40.6	37.2
Mg (mg/l)	9.2	8.4	4.7	6.9	9.9	9.3	8.1	9.0	19.33	21.2	16.5	24.7
Na (mg/l)	2	2	0.025	2.7	16.0	14.1	10.1	15.1	25.9	28.0	30.4	20.1
NH_4 (mg/l)	0.025	0.025	0.2	0.025	0.07	0.025	0.08	0.07	0.09	0.025	0.025	0.06
NO_3 (mg/l)	0.025	0.24	7.5	0,025	0.025	0.025	0.05	0.2	0.025	0.2	0.1	0.6
PH	7.6	7.5	7.8	7.9	7.8	7.6	7.5	7.6	7.6	7.9	8.2	8.2
PO_4 (mg/l)	0.04	0.03	0.04	0.01	0.04	0.08	0.04	0.06	0.04	0.009	0.005	0.06
SO_4 (mg/l)	1.5	2.9	1.5	2.7	26.92	24.9	19.4	23.6	41.1	34.8	59.4	37.7

Table 5.3: Mean values for Water Quality results sampled in the Crocodile River and its tributaries during Summer season (December-March 2012).

Physico-chemical variables	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**KR1**
Cl (mg/l)	1.7	3.2	1.5	2.0	17.1	13.1	11.9	11.2	39.9	104.9	52.6	11.7
EC (mS/m^{-1})	5.8	11.4	11.4	12.6	23.9	19.8	18.2	16.9	33.5	84.1	55.7	35.5
Mg (mg/l)	4.0	9.7	5.5	7.6	10.9	5.3	10.8	6.3	22.1	27.5	21.6	19.3
Na (mg/l)	2	2.8)	1.9	4.0	16.2	12.9	11.5	11.6	22.9	86.5	46.4	15.0
NH_4 (mg/l)	0.025	0.025	0.05	0.025	0.025	0.04	0.025	0.025	0.025	0.0025	0.0025	0.025
NO_3 (mg/l)	0.025	0.08	2.4	0.025	0.025	0.2	0.025	0.19	0.43	4.1	0.0025	0.5
pH	7.8	7.5	7.3	7.7	8.1	7.8	7.8	7.6	8.1	7.9	8.1	8.2
PO_4 (mg/l)	0.005	0.005	0.005	0.005	0.005	0.008	0.009	0.01	0.02	0.4	0.007	0.02
SO_4 (mg/l)	1.5(0)	1.5	1.5	2.2	27.1	19.4	18.8	16.2	38.4	25.4	85.8	36.2
Salinity (ppt)	0.0428	0.0656	0.0705	0.06.6	0.138	0.152	0.127	0.139	0.227	0.299	0.358	0.315
TDS (mg/l)	60.5	96.9	104	93.9	205	228	192	206	335	438	528	460
Temperature (˚C)	16.9	16.6	17	14.1	17.2	15.2	19.6	20	21.8	21.9	16.4	20.2

Table 5.4: Mean values for water quality results sampled in the Crocodile River and its tributaries during Winter Season (June-August 2012)

Physico-chemical variables	**CR1**	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**	**ER1**	**KR1**
Cl (mg/l)	-	6	5	3.9	17	-	-	19	26	38.3	47.3	17.9
EC (mS/m^{-1})	8.8	9.6	13.1	14.7	22.9	24.7	21.5	17.1	42.9	48.4	57.96	58.8
Mg (mg/l)	40.0	5.0	5.0	4.4	-	10	5.0	0.025	16.7	20	15.5	27.5
Na (mg/l)	2	5.3	-	-	-	-	-	-	-	-	45.1	30.8
NH_4 (mg/l)	-	0.025	0.025	0.1	0.025	0.1	-	-	0.1	0.1	0.025	0.06
NO_3 (mg/l)	-	0.025	7.3	0.1	0.025	0.2	-	-	-	0.1	0.6	-
PH	7.7	7.7	7.7	7.8	7.6	7.5	7.7	7.6	7.9	8.0	8.1	8.2
PO_4-P (mg/l)	0.1	0.2)	0.1	0.1	0.2	0.1	0.7	0.2	0.1	0.1	0.03	0.04
SO_4 (mg/l)	88.1	0.1	88.1	3.3	20	105	19	-	39	103	82.9	52.5
Salinity (ppt)	0.0362	0.0603	0.0648	0.065	0.065	0.132	0.118	0.124	0.192	0.321	0.318	0.284
TDS (mg/l)	52.5	87.9	95.5	98.5	98.5	196	175	184	284	327	476	422
Temperature (˚C)	13.2	17	15.9	11.8	11.8	19.3	20.1	18.9	20	20.6	12.3	17.4

The middle part of the Crocodile River system was characterised by the presence of littering and industrial effluent or run off, sewage discharge and domestic run off and these activities can cause an increase in nutrients as was observed during the study. The high concentration of magnesium at site CR7 downstream of the Nelspruit town during summer with a mean value of 10.8 mg/l was measure and calculated (Table 5.3), while in spring, autumn and winter the magnesium concentration was low with a mean values of 9.9 mg/l, 8.1 mg/l and 5.0 mg/l respectively. Phosphate concentration at this site during winter months had a mean value of 0.7 mg/l. Although high concentration of sulphate was measured at site CR6 during winter but the concentration was low at site CR7 in same month. At site CR6 magnesium was higher during spring when compared to the other seasons and the higher concentration of magnesium at this site was associated with the brewing of beer where by magnesium sulphate was mostly used. Salinity was higher in the middle reaches compared to the upper reaches of the Crocodile River and was associated with the different activities taking place in this area. At site CR8 Chloride concentration was higher with a mean value of 19 mg/l during winter (Table 4).

At site KR1 high concentration of salinity with a mean value of 0.315 (ppt), total dissolved solids with a mean value of 460 mg/l was recorded during summer month (Table 5.3). A high level of electrical conductivity with a mean value of 58.8 mS/m^{-1} was measured during winter month. The high concentration of these water quality constituents at this site was associated with the run-offs from agricultural activities and abandoned mines upstream of the site. This was also found by a study conducted by Heath and Claassen (1999), which stated that the Kaap River has poor water quality due to agricultural activities with associated pesticides, nutrients and electrical conductivity. Agricultural, urban and rural run-offs, industrial effluent at Malelane town played a huge role in the increase of water quality constituents such as chloride, magnesium, electrical conductivity and total dissolved solids (TDS) concentration during summer months as higher amount of sediment were deposited to the river.

The Bray-Curtis similarity for water quality for both high and low flow conditions (Figure 5.1), indicated that although there were similarities within sites in the Crocodile River and its tributaries, disimilarities were also observed from the cluster analysis at different percentages. This was linked to the presence of different physico-chemical or water quality parameters and different water use activities within the river which contributes different discharge.

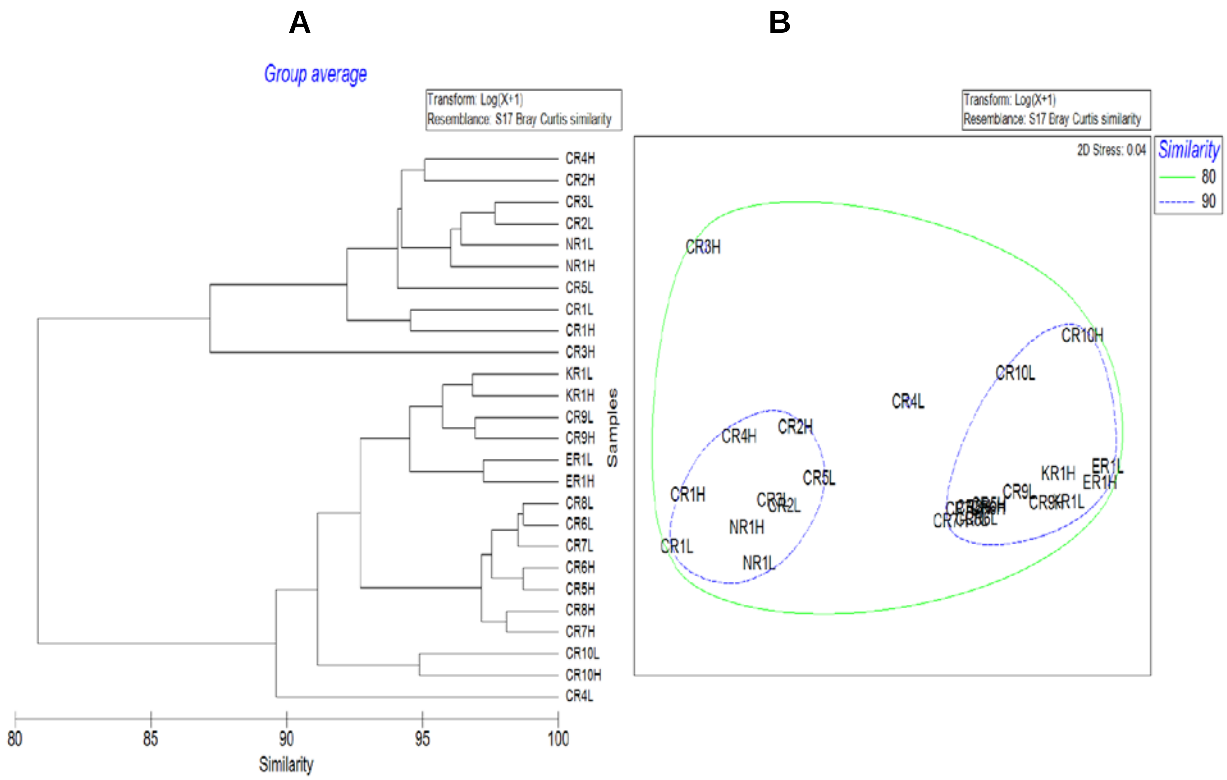


Figure 5.1: Bray-Curtis similarity matrix-based on cluster analysis (A) and two dimensional representation of the NMDS ordination (B) of the physico-chemical sites sampled in the Crocodile Rivers and tributaries during both flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.04.

The cluster analysis indicated similarity of sites within the Crocodile River and its tributaries at similarity value of 80% where a group formation of all the sites was observed. Another site grouping was observed at similarity of approximately 95%. The formation of site grouping at different percentages was an indication that water quality within the Crocodile River and its tributaries might share similar amount of concentrations of water quality constituents at certain stage during both flow conditions.

The NMDS ordination for all the sites sampled during both high and low flow conditions (Figure 5.2) indicated the same group formation as the Bray-Curtis Cluster analysis and the generated stress for the NMDS was 0.04. The NMDS ordination further indicated that during high and low flow condition. Sites CR3 and CR4 formed group formation with less than one site per group which was indication that they were separated from the rest of the sites due to differences in physico-chemical parameter concentrations which was linked to the utilization of fertilizer in the upper reaches of the Crocodile River.

The Cluster analysis and NMDS ordination for low flow condition in the Crocodile River and its tributaries (Figure 5.3 A and B), indicated that during low flow condition there were two group formation of sites. Site CR5 and NR1 were the only sites from the middle and lower reaches of the Crocodile River which formed a grouping with upper reaches sites of the Crocodile River especially upstream of the Kwena dam. Sites KR1 and ER1 were the only tributaries sites that formed a group formation with sites from the middle and lower reaches of the Crocodile River. Similarity was observed approximately at 80 and 90% and this was an indication that the physico-chemical parameter sampled within the Crocodile River and its tributaries might have some kind of uniform concentrations. The NMDS ordination for all the sites sampled during low flow condition (Figure 5.3), indicated same group formation with the Bray-Curtis cluster analysis and the stress generated for the NMDS was 0.01.The NMDS ordination indicated that at similarity at approximately 80% all the sites were grouped together indicating some level of similarity between the sites.

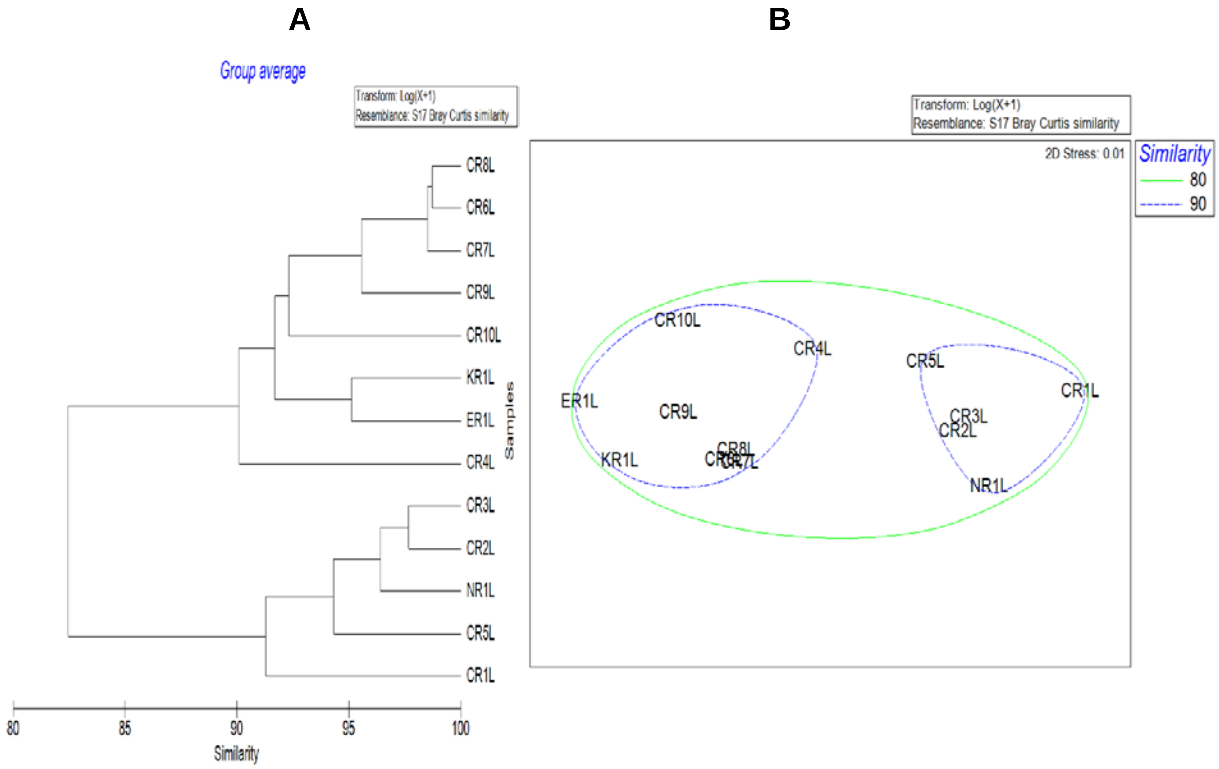


Figure 5.2: Bray-Curtis similarity matrix-based on cluster analysis (A) and Two dimensional representation of the NMDS ordination (B) of the physico-chemical sites sampled in the Crocodile Rivers and tributaries during low flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.01**.**

Although similarity was observed at 80%, dissimilarity amongst the sites was also observed indicating that the physico-chemical constituents within a specific site might differ. A further similarity was observed at similarity approximately 90% where two groups formation of sites were observed. The similarity within sites can be linked with sites having similar water quality constituents.

The Bray-Curtis similarity for high flow condition in the Crocodile River and its tributaries Figure 5.3A, indicated that site CR3 was the only site separated from the group formation at approximately 90% similarity. The separation was as a result of the high influx of nutrients concentration coming from a small tributary upstream and run offs from agricultural activities.

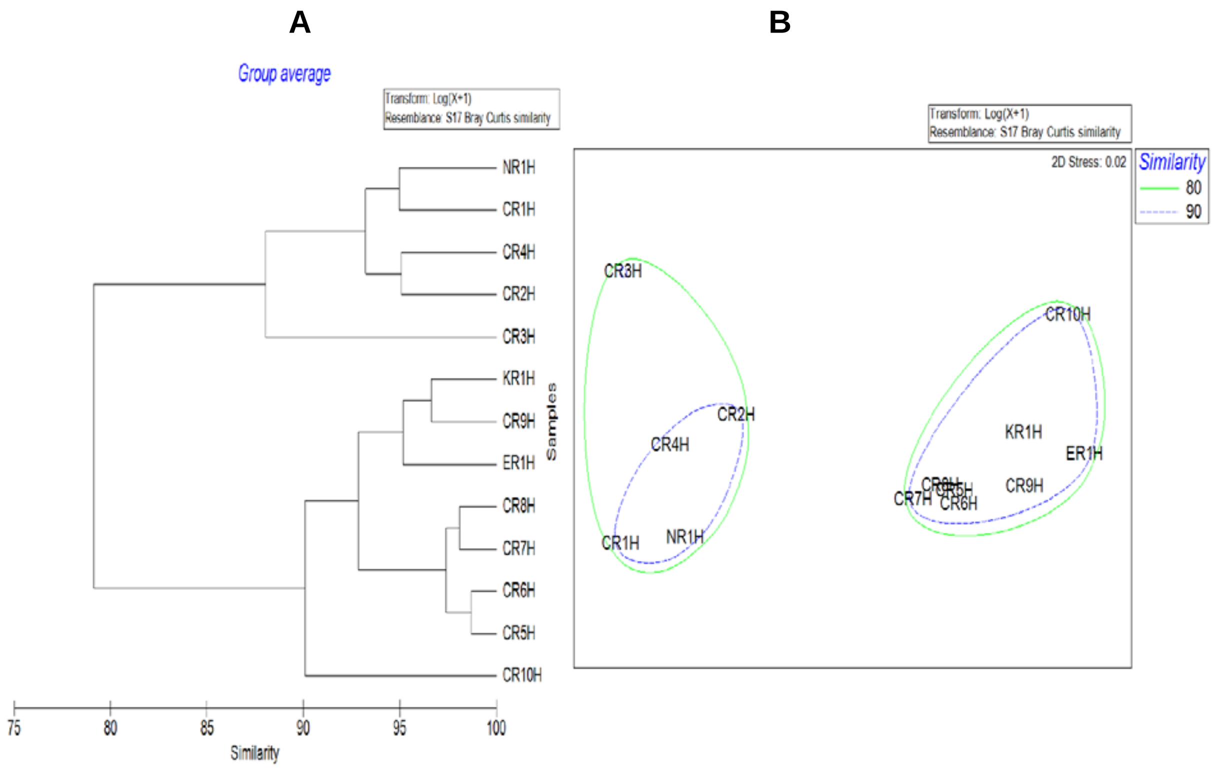


Figure 5.3: Bray-Curtis similarity matrix-based on cluster analysis (A) and Two dimensional representation of the NMDS ordination (B) of the physico-chemical sites sampled in the Crocodile Rivers and tributaries during low flow conditions. The NMDS ordination was completed with 30 iterations and showed a stress of 0.02.

There was a two group formation of sites at approximately similarity of 80%. Site NR1 on a tributary of the Crocodile River formed a grouping of sites at the upper reaches of the Crocodile River. This was linked to the similarity of water use activity found in the vicinity of these sites as they might have similar impact to the sites. The NMDS ordination (Figure 5.3B) for all the sites sampled during high flow condition indicated the same trend as in the Bray-Curtis similarity. The generated stress value for the NMDS was observed to be 0.02. The separation of sites within the Crocodile River can be due to the different water use activities as this river is used for different purposes and they produce different concentrations of contaminants to the river.

A Principal Component Analysis (PCA) was used to analyse water quality data set including all the parameters at the study sites of the Crocodile River to obtain the spatial and temporal variation and to identify potential pollution sources. In a PCA bi-plot the length of the lines approximates the variance of the variable and the longer the line the higher the variance. The distance between two points approximates the Euclidean distance between two observations in the multivariate space. Thus, observation that are far away from each other have a high Euclidean distance and vice versa.

In reference to Figure 5.4 sodium was by far the highest impact parameter in the bi-plot followed by ammonium and nitrate, while magnesium and phosphate variables had the lowest. A correlation between a group of sites and water quality parameters such as chlorine, total dissolved solids (TDS), electrical conductivity, sulphate and pH was observed.

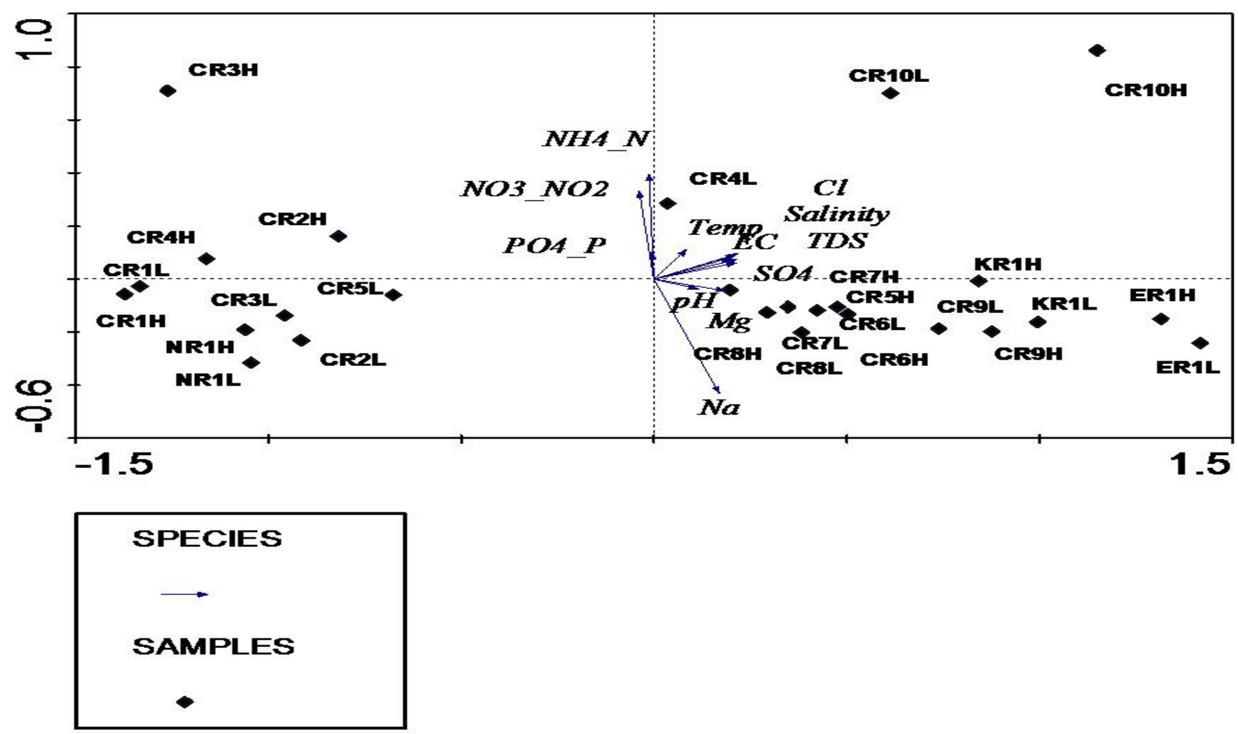


Figure 5.4: PCA bi-plot illustrating the similarities between the various sites during different seasons based on the physico-chemical characteristics of the water with the physico-chemical variables superimposed. The bi-plot describes 90.6% of the variation with 80.5% described on the first axis and 9.1% on the second axis.

Clustering of sites especially in the middle and lower reaches of the Crocodile River and its tributaries was observed. The clustering of site ER1 and KR1 was observed to be linked to the high content of sodium at the sites (Figure 5.4), while a clustering of sites such as CR1, CR2, CR3, CR4, CR5 and NR1 were associated with the high levels of ammonium, nitrate and phosphate which were linked to fertilizer containing these chemical being used for agricultural purposes. A separation of sites (indicating dissimilarity) especially between the upper reaches and some of the middle and lower reaches of the Crocodile River was observed. A high Euclidean distance was observed between site CR3 and CR10 (both flow conditions), indicating that these sites were receiving different or had different amounts of contaminants as they are situated in different vicinity of the river.

5.4. Conclusion

It is clear that the water quality of the river in the Catchment has deteriorated if compared with previous studies. Possible sources of pollution have been identified as the activities along the river that release or discharge effluents into the river. Some of these activities provide diffuse source of pollution as runoffs into the river. When determining the possible sources of pollution, the Crocodile River was categorised in to upper (CR1-CR4), middle (CR5-CR8) and lower (CR9-CR10) reaches. The results obtained indicated that total dissolved solids, electrical conductivity and salinity increase with the increase in river distance downstream during all the seasons. High concentration of nutrients namely: nitrate, phosphate and ammonium were measured from the upper reaches (CR1-CR4) of the Crocodile River during winter and autumn seasons while high concentrations of Chloride was measured during spring and autumn downstream of the Kwena dam. The high concentrations of phosphate and Chloride in the middle reach of the Crocodile River were linked to the discharge of sewage treatments and agricultural activity from the vicinity of the reach. The tributaries such Kaap (site KR1) and Elands River (site ER1) had high values of salinity and total dissolved solids during summer months while high electrical conductivity were observed to occur during winter months. The lower reaches of the Crocodile River were found to contain high concentration of chloride, magnesium, high electrical conductivity, total dissolved solids and salinity during summer months as more sediment were deposited in the river from different water use activities along the lower reaches. The presence of agricultural activities in the upper reaches and the presence of the combination of domestic, industrial and agricultural activities in the middle and lower reaches of the Crocodile River has a negative impact on water quality of the Crocodile River.

CHAPTER 6

6.1. General Conclusion

The multivariable statistical methods used indicated that richness and evenness of fish and macro-invertebrates in the Crocodile River and its tributaries increased longitudinally with the increase in river flow distance. This was linked to the presence of habitat complexity in the middle and lower reaches of the river. The statistical analysis further indicated that water temperature was one of the environmental variable for the formation of site grouping of macro-invertebrates and fish. The ecological category for both fish and macro-assemblage in the Crocodile River and its tributaries is better during low flow condition than high flow condition.

Macro-Invertebrates Response Assessment Index showed that during low flow condition the Crocodile River is mostly at ecological category class B (largely natural with few modification) above the Kwena dam, but from downstream of the dam the ecological category was in C class (moderately modified), while its tributaries were in ecological category B class (largely natural with few modification). The Fish Response Assessment Index showed that the ecological category for fish was mostly at C class (moderately modified) in the Crocodile River. The deterioration of the ecological category of the Crocodile River when compared with other studies was mostly believed to be associated with change of water quality resulting from agricultural run-offs, industrial and sewage effluent and mining seepage especially in the Kaap River. Habitat modification due to flow regulation also contributed to the modification of the fauna.

Total dissolved solids, electrical conductivity and salinity were found to increase with the increase in river flow distance during all seasons. The high concentration of these water quality constituents indicated that the Crocodile River is more pollutant in the downstream reaches. The upper reaches of the Crocodile River had higher levels of nutrients such as nitrate, phosphate and ammonium during winter and autumn season. The high level of this water quality parameters were associated with agricultural return flow in the upper reaches. In the middle reaches of the Crocodile River, Chloride and phosphate was found to be high in winter and magnesium was found to be high in summer. The high levels of Chloride and phosphate can be associated with sewage treatment effluent and agricultural return flows or run-offs from the Mbombela, Matsulu and Kanyamazane towns.

The lower reaches of the Crocodile River was found to have poor water quality compared to the upper and the middle reaches. This was also confirmed by the K-dominance curve which indicated that during low flow condition, macro-invertebrates communities at sites CR3 and CR4 were dominated by a single species namely Beatidae while site CR10 was dominated by Pleidae and Thiaridae. The presence of Pleidea and Thiaridae families at site CR10 indicated that the site was prone to change in water quality as these families are tolerant to such condition. The K-dominance curve for fish communities indicated that during low flow condition sites CR3, CR2 and CR 4 were dominated by single species at the same percentage namely *Chiloglanis pretoriae* while site CR10 was dominated by *Barbus viviparus*. The presence of *Chiloglanis pretoriae* at sites CR2, CR3 and CR4 indicated that the upper reaches contain good water quality during low flow condition, while the lower reaches contained poor water quality. The change in water quality in the lower reaches was associated with agricultural, industrial, mining and sewage treatment effluent in the lower reaches of the river. These activities were also believed to be the source of pollution in the Crocodile River and its tributaries.

The results obtained from this study has highlighted the poor ecological and water quality status of the Crocodile River and its tributaries. It also made a significant contribution to the understanding of the impact of anthropogenic activities such as agricultural, industrial and mining activities on a river system. The finding of the study add to the importance of conserving our fresh water ecosystem and to Eco-classification and reserve study of the Crocodile River catchment.

6.2. Recommendations

The following recommendations are made based on the results of this study;

- The use of both biological indicators and physico-chemical water quality in assessing the health of the Crocodile River is a key in solving the issues associated with the river.

- Programs such as Adopt a River and environmental awareness must be initiated in the Crocodile River.

- An eco-status approach must be followed when assessing the impact of the anthropogenic activities in the rivers as it gives an overview of what is happening in the catchment.

- Continuous monitoring in the Crocodile River and its tributaries using both water quality and biological indicators is of prime importance in identifying the causes of pollution in the river.

- The results of the study have also highlighted an opportunity for future research. A brief description is as follows;

    - ✓ A study focusing on the impact of mining effluent on selected macro-invertebrates and fish species in the Kaap River.
    - ✓ The impact of selected metals such as boron and manganese on selected fish species in the Crocodile River and the impact of paper mill effluent on macro-invertebrates in the Elands River.
    - ✓ Determining the reasons for the absence of *Chiloglanis bifurcus* species in the upper reaches of the Crocodile River.
    - ✓ Investigate how does water temperature play a role in macro-invertebrates and fish assemblages?

CHAPTER 7

7.1. References

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