MACROINVERTEBRATE AND DIATOM ASSEMBLAGE RESPONSES TO POLLUTION, WITH EMPHASIS ON SALINITY, IN THE KAT RIVER, EASTERN CAPE SOUTH AFRICA

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By

Ntombekhaya Mgaba

Institute for water research

Grahamstown

South Africa

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ABSTRACT

Salinity has been implicated as one of the major contributors to deteriorating water quality of freshwater ecosystems around the globe. In South Africa, anthropogenic activities such as mining, agriculture, industry and wastewater treatment works (WWTWs) are the major sources of increasing salinity levels of freshwater resources. The main focus of this study was to assess the impact of salinity on water quality of the Kat River using macroinvertebrates and diatoms as bioindicators. Biomonitoring using macroinvertebrates and diatom communities and concurrent sampling of water physicochemical variables were conducted bi-monthly from December 2015 to November 2016.This period covered summer and winter, and the study was conducted at five selected sites (Sites 1, 2, 3, 4 and 5) along the length of the Kat River. For macroinvertebrates biomonitoring, the South African Scoring System version 5 (SASS5) and Macroinvertebrate Response Assessment Index (MIRAI) were applied to collect and analyse data, while the Taylor et al (2006) protocol for collecting and analysing diatom assemblages was modified and used for diatom collection and analysis. Water physicochemical variables, including hydrogen ion concentration (pH), electrical conductivity (EC), dissolved oxygen (DO), temperature, turbidity and stream flow were determined *in situ* using appropriate multiprobe meter and/or techniques. Nutrients (NO_3-N, NO_2-N, NH_4-N and PO_4-P) were analysed in the laboratory using appropriate analytical methods. All data were subjected to appropriate statistical analyses and statistical decisions were made at an alpha value of 0.05. Particularly, multivariate analyses of both macroinvertebrates and diatoms assemblages were conducted using canonical correspondence analysis and Bray-Curtis similarity analysis, while indicator species analysis was used to determine which species is/are more significant with respect to biomonitoring in the Kat River. Biotic diversity indices were also measured and used to discriminate between least and most impacted sites. The Kat River water quality was found to have experienced a varying degree of modification compared to Generic Resources Water Quality Objectives limits. Change in DO, stream flow, EC, nutrients and turbidity exerted the greatest influenced on the macroinvertebrates assemblage structure, with organisms at Sites 4 and 5 (downstream sites) showing more significant negative impact compared to organisms at Sites 1, 2 and 3 (upstream sites).

Analysis of the diatom biomonitoring showed more negative impact at Sites 2, 4 and 5 compared to Sites 1 and 3. Fort Beaufort Wastewater Treatment Works and small-scale farming activities, as well as leaking of pipes carrying sewage, were found to be the likely major sources of anthropogenic activities responsible for the observed increased salinity and other pollutants in the Kat River. Overall, this study found macroinvertebrates (identified up to the family level) as good for biomonitoring to assess or predict water quality of the Kat River, while diatoms were found to be most suitable for biomonitoring to assess salinity in the Kat River.

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DEDICATION

I dedicate this thesis to one strong woman who risked her life working hard for us to have a better life, my mom Nothembile Mgaba.

ABBREVIATIONS AND ACRONYMS

AMD	Acid Mine Drainage
CCA	Canonical Correspondence Analysis
DEA	Department of Environmental Affairs
DO	Dissolved Oxygen
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
ER	Ecological Reserve
EWFD	European Water Framework Directive
EWQ	Environment Water Quality
FB	Fort Beaufort
FFHA	Fish Flow Habitat Assessment
FIFHA	Fish Invertebrates Flow Habitat Assessment
FRAI	Fish Response Assessment Index
GSM	Gravel, Sand and Mud
HFSR	Habitat Flow Stressor-Response
IHAS	Integrated Habitat Assessment System
IWRM	Integrated Water Resource Management
KRVC	Kat River Valley Catchment
MIRAI	Macroinvertebrates Responses Assessment index
NAEHMP	National Aquatic Ecosystem Health Monitoring Program
NWA	National Water Act
NWRS	National Water Resource Strategy
RDM	Resource Direct Measures
REMP	River Eco-Status Monitoring Program

RHAMM	Rapid Habitat Assessment Method and Model
RHP	River Health Program
RQOs	Resources Quality Objectives
SADI	South African Diatom Index
SDC	Source Directed
TDS	Total Dissolve Salts
TDS	Total Dissolved Solids
WRC	Water Research Commission
WSA	Water Service Act
WWTWs	Waste Water Treatment Works

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Rationale

The current global trends in urbanisation, industrialisation and agriculture, especially in developing countries, coupled with improved global healthcare have caused the human population to increase in recent years (Thornton, 2010). These trends are exerting tremendous pressure on freshwater resources around the world, leading to changes in the quantity and quality of freshwater resources. Poor water quality adversely affects human and ecosystem health (UN-water, 2012). It also has a negative effect on the economy through high water and wastewater treatment costs (Oberholster and Ashton, 2008; O'Keeffe, 2009; DWS, 2013). South Africa is a water-stressed country and needs to protect its available stressed freshwater resources for the benefits of the current and future generations (DWA, 2013). Human activities such as agriculture, landuse, discharge of wastewater and abstraction of water have progressively worsened both the quantity and quality of South Africa's water resources (Oelofse and Strydom, 2010). Apart from pollution, water abstraction also negatively influence water resources through flow alteration, thereby impacting the dilution capacity of in-stream hydrology.

Salinity has been identified as one of the major water stressors in South Africa. According to Stander (1987), historically, increase in salinity has been linked to deteriorating freshwater resources in South Africa. Increasing salinity has been experienced in a semi-arid, South-Eastern and South-Western part of South Africa (van Rensburg et al., 2011). Salinity increases have been caused by natural conditions and anthropogenic impacts. Naturally, water from different resources (rivers, streams, wetland, aquifer and pans) contains some amount of salts. A natural condition that promotes salinity in many rivers of the country is geology of the catchment, river flow, high evapotranspiration and low rainfall. In some cases, salinity enters river through sea spray in coastal areas. However, major sources of freshwater salinisation of South African rivers are from anthropogenic impacts such as domestic wastewater, agriculture, mining and industrial activities. These conditions increase salts concentration in rivers. The salinity of the Kat River increased tremendously in the early 1980's due to drought and land use (Lerotholi et al., 2004). Since the salinity concentration in a river depends on the amount

of stream flow to dilute the salts, the river had low stream flow because of the drought which resulted in high in-stream salinity.

The Kat River Valley Catchment (KRVC) is situated in a rocky geological land, with weathered igneous rocks responsible for the natural formation of calcium, sodium and magnesium (Dallas and day, 2004), resulting in high natural river salinity. Consequently, the main focus of this study is to investigate the impacts of anthropogenic activities on salinity in the Kat River using physicochemical analysis as well as macroinvertebrate and diatom-based biomonitoring. The rest of the chapter is devoted to a detailed review of freshwater salinisation, water resources management in South Africa, the approaches used in this study and the research aim and objectives.

1.2 Anthropogenic activities and salinity in South Africa's freshwater resources

South Africa experiences low annual rainfall and high evapotranspiration compared to many other countries in sub- Sahara Africa (Mzezewa et al, 2010). Low rainfall condition has been made worse in the last few years, especially in 2015-2016, whereby the country experienced an extreme drought condition. This has led to reducing water quantity in most of the country's rivers and reservoirs (e.g. dams), resulting in increased concentrations of total dissolved solids in freshwater resources (DWS, 2013). Although the drought situation is attributed to the *El Nino* weather phenomenon, which is a natural occurrence, anthropogenic activities such as mining, agriculture and discharges from Waste Water Treatment Works (WWTWs) are exacerbating the situation by increasing salinity levels of freshwater resources in the following ways:

- Ageing wastewater treatment plants are not able to remove all the pollutants, which end up discharged with the final effluent to receiving freshwater resources (Ellis, 2006).
- Mine effluents such as acid mine drainage (AMD) contains high loads of salts, which end up discharged with the final effluent to receiving freshwater resources (Adler et al., 2007).
- Agricultural activities are responsible for increasing salinity of freshwater resources through non-point source pollution, as well as by removing and replacing of natural plants with agricultural crops and using fertilisers, pesticides and herbicides that contain chemical ions (de Klerk et al., 2010; Mensah et al., 2015).

As is evidenced above, anthropogenic activities tend to increase the salinity of freshwater resources and contribute to the phenomenon of freshwater salinisation.

1.2.1 Salinisation of freshwater resources

Salinity is the 'saltiness' or dissolved salts, usually sodium chloride, magnesium sulphate, potassium nitrate and sodium bicarbonate, contained in a body of water (Sa and Boyd, 2015). Salinity has also been described as the property of water that results from the combination of various mineral salts (USEPA, 2011). The most common ions that contribute to salinity are Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, CO_3^{2-}, SO_4^{2-} and Cl^{-}. The sources of these ions include wastewater treatment works, industry, mining and agriculture. Also, all kinds of water from different sources such as rivers, sea, springs and aquifers contain some dissolved salts in different quantities. Mountain streams, which are known as unmodified freshwater systems, contain a natural level of dissolved salts (Lerotholi et al, 2004). Globally, the impact or increases of salinity on freshwater resources (i.e., freshwater salinisation) is a growing threat to the sustainability of freshwater resources (Dunlop et al., 2005). Freshwater salinisation can present toxic micro-environment to aquatic flora and fauna when dissolved salts reach certain levels beyond tolerable thresholds of the in-stream organisms (Dunlop et al., 2005). Depending on the source or origin of the salt, freshwater salinisation could be described as primary (naturally influenced) or secondary (anthropogenically influenced) phenomenon.

1.2.2 Primary salinisation

Primary salinisation occurs naturally in soil and water, influenced by geology and climate of the area. Primary salinisation contributes to increasing salt content of freshwater resources through geology and climate of the area (Lerotholi et al., 2004). There are also aquatic ecosystems that are naturally saline, including salt pans, salt marshes, salt lakes and lagoons. Geologically, salts that dissolved millions of years ago are transported by water to the nearby water resource or deposited through dust and evaporation (Hanson et al., 2006), maked geology the main cause of salts in rivers, in most cases (Lerotholi, 2005). The contribution of geology to river salinity increases when there is a reduced dilution due to low rainfall and the river receives water from the base flow (Cornish, 2003). Since the geology of an area and surrounding environments impacts salinity, an area covered by dolerites will likely have low total dissolved salts (TDS) levels because these rocks are more resistant to weathering and erosion. In an area where there is limestone bedrock, for example, dolomite and other calcareous sedimentary rocks, the salt content of the water will be high because they are

composed of calcium carbonate and magnesium and easily break down through weathering (Interlandi and Crockett, 2003).

The Kat River is vulnerable to salinisation due to its geological structures. Its bedrock consists of mudstone, limestone and shalestone that are identified by having high salts. Annandale et al (2007) opined that decomposed shales release a high concentration of sodium, chloride and sulphate ions and decomposed dolerites release calcium, magnesium and carbonate ions, all of which contribute to increasing salinity levels.

Climate change also plays a major role in salinisation of rivers as it affects processes such as evaporation and precipitation (Lerotholi, 2005). Studies have shown that extreme weather conditions such as floods and drought experience by different countries in recent years were due to climate change (Anderson et al., 2008). When rainfall rate is much higher than the rate of evaporation, salts are flushed out through the landscape and carried back to the ocean by surface and groundwater flow and do not accumulate. Drought worsens salinisation of water resource because of a shortage of water for dilution of excess salts. Countries such as Australia experiences excess evaporation of shallow groundwater and low rainfall in many areas, which results in salt accumulation. Malan and Day (2003) stated that high salinity conditions are usually experienced in the dry period. For example, in summer, shallow groundwater resources have greater potential for salts accumulation due to increase evaporation rates. Therefore, since arid and semi-arid areas receive less rainfall, which is exacerbated by climate change, the soil profiles of such areas have high levels of salt build ups (Smedema and Shaiti, 2002). The Kat River Valley Catchment climate is situated in sub-humid to semi-arid; therefore, the river has high salinity threat. Erosion and degradation of the land may cause rain to wash salt loads into freshwater bodies, thereby increasing salinity. Furthermore, water resources near the coast experience increased salinity caused by the intrusion of seawater, which is also worsened by climate change (Kumar, 2012).

1.2.3 Secondary salinisation

Secondary salinisation is caused by human-induced activities such as mining, WWTWs and agricultural farmlands (Cañedo-Arguelles et al., 2013). Mining is a major source of salinity that contributes to increasing salt content of freshwater resources in South Africa (Davis and Day, 1998).

South Africa's diverse mining sectors generate mine water commonly referred to as acid mine drainage (AMD) and Alkaline mine drainage. Gold and coal mines are associated with environmental contamination such as acid mine drainage (AMD) (Manders et al., 2009). Mine water may be carried off the mine by rainwater or surface drainage and deposited into nearby streams, rivers, lakes and ground waters (SDWF, 2011).

AMD can affect a freshwater resource even if it is away downstream of the mine (Dallas and Day, 2004) and make the freshwater less fit for use as well as harmful to the aquatic ecosystem. In addition, ions produced by the mine add to the salinity of freshwater. For example, pyretic formation due to coal mining can be oxidised into sulphuric acid and iron sulphate (Brown, 2005; Annandale et al, 2007), which add to the salinity of freshwater systems.

Wastewater effluent discharges contribute to freshwater salinisation by increasing the concentrations of dissolved solids. Wastewater effluent is made up of industrial wastewater, domestic wastewater, storm wastewater and seepage entering municipal sewage systems. Sometimes, the effluent is not properly treated due to faulty machines or technical problem, allowing salts contained in such effluent to be discharged into receiving water systems. Increase in human population and development of industrial hubs exert pressure on wastewater treatment works, consequently resulting in discharges of the large volume of saline wastewater into receiving waters (Zhao et al., 2016). Furthermore, most available technologies for municipal wastewater treatment works can remove organic matters and nutrients (N and P) but less efficient in removing salts (Ellis 2006; Nikhil et al., 2016) and thus, increase the potential of discharging effluents with high salts content into the receiving water resource.

Agriculture is one of the main non-point sources of pollution of freshwater resources. Common factors that lead to secondary salinisation caused by agricultural activities include irrigated land, which cause groundwater quality to become saline and water table to rise; clearing vegetation and change in land use, which also lead to rise in groundwater level and increase amount of salts accumulating in the soil; and irrigation return flow, which tends to increase salt loads of freshwater systems. Irrigation can reduce soil fertility by building up salts in soil (van Ransburg et al., 2011). Excess salts remain deposited in the soil after the evaporation of water. This leads to an accumulation of salts in agricultural land, which can be washed off to surrounding water resources (Smedema and Shaiti, 2002; van Ransburg et al., 2011). Vegetated agricultural land in arid regions has high evaporation rate and low rainfall to leach salts from

the soil (WWF-SA, 2013). Therefore, salts found in water used to irrigate crops in these areas become concentrated in the land (Smedema and Shaiti, 2002; Ransburg et al., 2011).

Return flows from irrigated agricultural land transports these salts to water resources (Smedema and Shaiti, 2002). Approximately, up to 260000 ha of irrigated land in South Africa is affected by salinity and 15000 ha are limited to the choice of crops because of its salt-tolerant (WWF-SA, 2013). It has been argued that agricultural activities are major contributors to increasing salinity of water resources in semi-arid and arid parts of the world (Williams, 1999; Williams et al 2001; Erickson et al, 2003; de Klerk et al. 2010). This is why the phenomenon of freshwater salinisation has become a growing concern in countries such as South Africa and Australia (Dunlop et al., 2005; Goga and Pegram, 2014). Increase in salinity is a persistent water quality problem in the coastal and arid western regions of South Africa. These regions are dominated by sodium ions and chloride ions (Adler et al., 2010). The Northern Highveld regions are dominated by calcium, magnesium and bicarbonate ions (Dallas and Day, 1993; Geotsch and Palmer, 1997). The river's catchment is a hub of small and large-scale farming activities that involve irrigation of farmlands. This puts the Kat River at a risk of increasing salinity due to impacts it receives from the farming activities.

1.3 Effect of salinity on freshwater ecosystems

Freshwater salinisation can have a significant negative impact on biological, chemical and physical components of freshwater. The most critical disruption caused by salinity is its effect on osmotic balance, which may cause the death of sensitive organisms. Salinity effects on aquatic biota can both be direct and indirect. Indirect effects may occur where an increase in salinity may result in loss of habitat and alteration of the natural assemblage structure. Direct effects are caused by an increase in salt which affects the long-term viability of undeveloped eggs of macroinvertebrate and seeds of aquatic plants (Nielsen et al 2003).

Some organisms are adapted to live in freshwater and others salty water. Salinity decreases species richness and growth of freshwater organisms (Hart et al., 1991; Nielsen et al., 2003). According to Hart et al (1991) and Nielsen et al (2003), available data suggest that aquatic biota will be seriously affected if salinity exceeds 1000 mg/L in freshwater ecosystems. Species of aquatic fauna and flora may be differentially sensitive to salt, including their life stages. For example, the upper limit of salinity tolerated by most freshwater aquatic plants

appears to be 4000 mg/L. However, the effects of salinity on aquatic plant start to show when the salinity is above 1000 mg/L (NWU, 2014). Visible effects include reduced growth rate as well as reduced development of roots and leaves.

The above discourse shows that there is a need to measure the salinity of aquatic ecosystems to manage it effectively. Therefore, sustainable freshwater resource management requires measurements of water quality parameters such as electrical conductivity (EC) or total dissolved solids (TDS) to understand salinity concentrations.

1.4 Measurements of salinity

Two methods frequently used to determine salt content of water are the measurement of total dissolved solids (TDS) and electrical conductivity (EC). Practically, TDS is measured by evaporating some amount of water to dryness and weighing the solids that remain. The unit of measurement is usually milligrams per litre (mg/L) or parts per million (ppm). Conversely, EC is practically measured by passing electrical current between metal plates electrodes in the sample and current flow between the plates is recorded. As the concentration of dissolved salts increases, the flow of current also increases and a higher EC is recorded. The unit of measurement of EC is usually millisiemens per metre (mS/m) or microsiemens per centimetres (uS/cm). TDS and EC are closely related, whereby TDS concentration is directly proportional to EC. Thus EC (in mS/m) may be converted to TDS (in mg/L) by multiplying the EC with an experimental value at a specific temperature. In South Africa, there is a common approximation for TDS concentration from EC by multiplying EC by a factor of 6.5 at 25^{o}C (i.e. TDS (mg/L) = EC (mS/m at 25° C) x 6.5) (DWAF, 1996; Brown, 2005; Lerotholi, 2005). In this study EC in mS/m were used to measure salinity of Kat River.

1.5 Water resource management in South Africa

1.5.1 Legislation and policies used in South Africa to manage freshwater resources

Sustainable water resource management and the provision of water-related services in South Africa are underpinned by two pieces of legislation: the Water Service Act (WSA) (Act No. 108 of 1997) and the National Water Act (NWA) (Act No. 36 of 1998). The WSA deals with the way drinking water-related services are delivered and managed by the government (DWAF, 1997). It also covers rules and regulations on how municipalities and other organisations

should provide water services, and clarifies the institutional arrangements pertaining to water service provisioning.

On the other hand, the NWA aims to ensure that water resources are protected, developed, used, conserved, managed and controlled in a way that takes equity and fairness to human and the ecosystems into account. It emphasises the provision of water for basic human needs, referred to as the human needs Reserve, and aquatic ecosystems need, referred to as the Ecological Reserve. The South African national Department of Water and Sanitation (DWS) is the custodian of the nation's water resources and is charged with the responsibility of achieving the three central principles of the Act, which are equity, efficiency and sustainability. Consequently, the NWA mandates the Minister of Water and Sanitation to develop a National Water Resource Strategy (NWRS) that clearly defines the steps, strategies and guidelines to achieve the overarching objectives of the NWA. The first edition of the NWRS was published in 2004 and the second and current edition in 2013 (DWAF, 2004; DWA, 2013). To achieve the principles of equity, sustainability and efficiency that are enshrined in the NWA, the NWRS contains two complementary strategies; the Resource Direct Measures (RDM) and the Source Direct Controls (SDC) that guide the implementation of the provisions of the Act.

The RDMs are designed to protect water resources by managing the quality and quantity of water resources needed for both humans and the aquatic environment (DWAF, 2004). RDMs consist of the national classification system for water resources, classification of every significant water resource, the determination of the Reserve and the setting of Resource Quality Objectives (RQOs) (DWAF, 2003). The classification system provides guidelines and procedures for determining different classes of water resources (DWA, 2003). Palmer et al. (2004) indicated that the classification system is the central action in balancing resource use and resource protection. The classification system sets out the procedure to follow when classifying every significant water resource, whereas the classification is sets of actions that lead to classifying water resources into three management classes. A Class I water resource is minimally used and its overall ecological condition has minimal changes compared to pre-development conditions; a Class II water resource is moderately used and its overall ecological condition has moderate changes compared to pre-development condition; while a Class III water resource is heavily used and its overall ecological condition has serious changes compared to pre-development condition (DWA, 2011).

The Reserve provides water requirements, in terms of quality and quantity, to satisfy basic human needs and protection of aquatic ecosystems. The human needs Reserve provides water for essential needs of individuals such as drinking and other domestic consumption, while the ecological Reserve provides for the quality and quantity of water required to protect aquatic ecosystems (Palmer et al., 2007).

The RQOs are numerical and narrative descriptors of conditions that need to be met in order to achieve the required management scenario (DWAF, 2003). They promote goals that balance the need to protect and use water resources sustainably. They also cover all demands of the water resources such as the requirement of the Reserve (DWAF, 2003). Resource classification, the Reserve and RQOs together provide tools to assess the water resources status and plan for its preferred condition (DWAF, 2003).

The SDCs are measures aimed at controlling and managing water use activities, including abstraction and discharges of waste into the environment. They provide for the use of regulatory measures such as permits, licenses, authorization and registration to control water use activities. SDCs provide guidelines for issuing water use licenses and authorisations, and a continuous process of monitoring and evaluating them. Licensing is aimed at minimizing the impact of water use activities on the resource (DWA, 2004).

RDM and SDC are therefore two of the most important strategies to manage water resources and to provide for different tools and approaches for monitoring surface water quality in South Africa. One of these approaches within RDM is environmental water quality (EWQ), which combines physicochemical, biomonitoring and ecotoxicological measurements (Palmer et al., 2004).

1.6 Environmental Water Quality (EWQ) as an approach for managing water resources in South Africa

Environmental water quality is a triad-based integrated approach, which combines water chemistry (physicochemical variables), biomonitoring and ecotoxicology with the aim of managing water quality (Palmer et al., 2004). Each aspect of the EWQ triad helps to provide data that are useful for quantifying requirements of water quality, which are also important parts of integrated water resource management (IWRM) (Palmer, 1999; Scherman et al., 2003).

Water chemistry provides information about the chemical and physical conditions of water resources; biomonitoring uses responses of living organisms to provide integrated information about the health of the aquatic ecosystems, while ecotoxicology provides links between water chemistry and specific biota responses to establish the chemical concentration that affects the biota (Palmer et al., 2004). Of these three aspects of EWQ, this study focuses on water physicochemistry and biomonitoring.

1.6.1 Water physicochemistry

In South Africa, water quality is traditionally assessed using the measurements of physicochemical variables such as nitrate (NO_3), nitrite (NO_2), electrical conductivity (EC), dissolved oxygen (DO), pH, temperature, turbidity, orthophosphate (PO_4), ammonium (NH_4), free chlorine (Cl) and metals (Huizenga et al., 2013). Elevated concentrations of some physicochemical variables such as metals and electrical conductivity may endanger in-stream biota (DWAF, 2008). High loads of nutrients in the freshwater ecosystem may contribute to eutrophication. Eutrophication can cause excessive algal growth, reduce water clarity and increase pH concentration, with its attendant consequences on overall water quality and decrease dissolved oxygen. Furthermore, it can alter ecosystem structures and function by causing the disappearance of some plant and animal species within the aquatic ecosystems. Excessive nutrient and organic input can also affect the aesthetic value of water resources because of obnoxious odour resulting from the decomposition of organic materials and increased likelihood of harmful microorganisms (Conley et al., 2009). In addition, algal bloom and organic matter input may lead to the production of toxic substances, and depletion of in-stream dissolved oxygen concentration (Conley et al., 2009; Conley, 2012). The resulting depletion of oxygen concentrations can lead to the death of aquatic life (Conley, 2012). Physicochemical monitoring of water resources is an important approach to ensuring the control of the concentrations of pollutants with a view to developing appropriate strategies for preventing same from reaching levels detrimental to aquatic ecosystems. Although water physicochemical variables are still widely used and form an important aspect of managing water quality, the approach has a number of setbacks:

- It is impossible to measure and analyse all chemicals suspended and dissolved in water (Arimoro et al., 2015).

- It does not provide an integrative picture of water quality because samples represent conditions of the time and location at which they were taken (Arimoro et al., 2015).
- It does not provide information on the ecological effects of pollutants on aquatic ecosystems (Bonada et al., 2006).

Therefore, to provide an integrative picture of river health and ecological conditions of the Kat River, physicochemical measurements were used in combination with biomonitoring in this study.

1.6.2 Biomonitoring

Biomonitoring has been used in South Africa as an approach for sustainable freshwater resource management since 1994 (Palmer et al., 2004). Biomonitoring (also referred to as biological monitoring) uses resident biota such as plants, animals and microorganisms to provide an integrative picture of aquatic ecosystem health (van Rensburg, 2011). Freshwater biomonitoring uses aquatic living organisms to evaluate changes in water quality and determine river health conditions (Holt and Miller, 2010). It can also be used to assess the ecological state of an aquatic ecosystem, diagnose impacts on emerging water pollutants, and predict water quality changes in ecosystems (Magoba, 2005; Navarro-Ortega et al., 2015). The approach provides useful information to solving environmental problems and can contribute to information needed for classifying water resources for different user sectors (e.g. aquatic ecosystems, agriculture and industrial sectors) (Hughes et al., 2012). Different bioindicators such as fish, macroinvertebrates, algae, diatoms and macrophytes are used in biomonitoring to assess the ecological state of water resources (Barbour et al., 1999; Stevenson and Rollings, 2017). Of these bioindicators, macroinvertebrates are among the most widely used in biomonitoring of freshwater resources (Charvet et al., 2000; Bonada et al., 2006; Odume et al., 2012). In this study, macroinvertebrates and diatoms were used as bioindicators to monitor the water quality (ecological state, the degree of pollution and impact of salinity) of the Kat River.

1.6.2.1 Biomonitoring using macroinvertebrates

Aquatic macroinvertebrates can easily be seen with the naked eyes and they spend part of or their entire life cycle in the aquatic environment. They live in different biotopes such as vegetation, stones, gravel, sand and mud (Dickens and Graham, 2002; Bonada et al., 2006).

They are also an important component of the aquatic food web and pathway for energy transfer, from primary producers to fish and birds.
Macroinvertebrates are relatively sedentary; thus, they give an indication of water quality with respect to their specific location. Their frequent application in biomonitoring could be attributed to their ability to provide an indication of ecosystem health because they are responsive to pollution. Their bioaccumulation potential also provides an opportunity for a chemical analysis of their tissue to reveal bioaccumulated pollutants, which would have otherwise remained undetected because of their low concentrations in the water resource.

The National River Health Programme (RHP) which was initiated and implemented by the Department of Water Affairs and Forestry (DWAF), now the Department of Water and Sanitation (DWS), used biomonitoring to contribute information to the sustainable management of South Africa's freshwater resources (DWAF, 2008). The main purpose of this programme was to serve as a source of information regarding the overall ecological status of South Africa's riverine system to support their management (Maseti, 2005; DWAF 2006). The RHP made use of different biological indicators such as fish, vegetation and macroinvertebrates to assess effects of deteriorating water quality on aquatic ecosystems (DWAF, 2008). The RHP was replaced in 2016 by the River Eco-Status Monitoring Programme (REMP).

The REMP is a component of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP). Thus, the aim of REMP is to monitor the ecological state of the riverine ecosystem in South Africa as they are reflected by the system drivers and biological responses in both in-stream and riparian environments (DWS, 2016). REMP establishes relative reference condition (i.e., a natural or close to natural conditions) by evaluating the status of physicochemical variables and macroinvertebrate assemblages.

In considering a reference condition, the characteristics of abiotic drivers of the system, including hydrology, physicochemical parameters and geomorphology, which define habitat for in-stream, as well as the characteristics of instream biota as a response to the system drivers, are taken into account. REMP is based upon existing approved eco-status models such as:

- River Data Integration (RIVDINT): A quaternary level instream and riparian assessment of fish, vegetation and invertebrate conditions.

- Rapid Habitat Assessment Method and Model (RHAMM): Site level assessment, incorporating Fish Response Assessment Index (FRAI), Macroinvertebrate Response Assessment Index (MIRAI), Vegetation Response Assessment Index (VEGRAI) and integrated eco-status).
- Fish Invertebrate Flow Habitat Assessment (FIFHA) (originally Fish Flow Habitat Assessment FFHA): use data generated by the Habitat Flow Stressor-Response (HFSR) model, for example, hydrology and Habitat-Flow Simulation (HABFLO) for the assessment.

REMP includes monitoring of ecological and specific biological components that have been established and approved as Resource Quality Objectives (RQOs) (DWS, 2016). Different biological indices contribute information that is fed into models used in REMP. For example, the South African Scoring System version 5 (Dickens and Graham 2002) feeds information into Macroinvertebrate Response Assessment Index (MIRAI). Separately, these biotic indices are also used to provide rapid river health conditions of riverine ecosystems. In South Africa, the single biotic index score approach such as the South African scoring system (SASS) have been developed and applied for biomonitoring. The SASS is a recommended rapid bioassessment tool for monitoring water quality and river health condition in South Africa. Currently, SASS is now in its fifth version (i.e. SASS5) and is widely used for biomonitoring freshwater ecosystems (Dickens and Graham, 2002). SASS5 is based on the presence or absence of aquatic macroinvertebrate taxa mostly at the family level and their sensitivity to deteriorating water quality (Dickens and Graham, 2002; Dallas, 2007). In the SASS5, each macroinvertebrate family is awarded a score based on its sensitivity to pollution, ranging between 1-15. A score of 1 represents the least sensitive and 15 represent the most sensitive. The final calculated results of SASS5 are expressed both as a SASS5 score and as an Average Score per Taxon (ASPT) value. The SASS5 score is calculated by summing the scores of all recorded taxa, whereas ASPT is obtained by dividing the SASS5 score by the numbers of the recorded families (Dickens and Graham, 2002; Dallas, 2007). The SASS5 as a rapid bioassessment tool has gained popularity because it is easy and simple to operate, yielding easily interpretable results. It is an affordable tool requiring no expensive sampling equipment (Dickens and Graham, 2002).

Although SASS5 is widely used to assess the river health of freshwater ecosystems in South Africa, it has the following limitations:

- It is a single biotic index and it masks taxonomic information from below the level of the family.
- It reduces complex biotic information into simple scores, thereby masking responses of an individual group of macroinvertebrate families to pollution.
- SASS score is habitat affected, biodiversity decreases due to un-availability of biotopes

In addition to using macroinvertebrates, diatoms were also employed in this study because diatoms are thought to be sensitive to increasing salts in freshwater ecosystems (Potapova, 2011).

1.6.2.2 Biomonitoring using diatoms

Diatoms are a large group of single-celled algae that are encased in a silica cell wall and inhabit most aquatic habitats. They have a short lifespan of up to six days and as a result, they respond very quickly to environmental changes. They contribute to more than half of the primary production in many aquatic ecosystems (Smol, 2001; Stroemer and Smol 2004).

They are a very diverse group, and are known for their interesting physical structures (Martin et al., 2012). Morphologically-based identification of individual species is primarily based on size, shape, a number of striae and sculpturing of cell wall as shown in (Figure 1.1). Different taxa have different environmental optima where assemblages can be used to reconstruct environmental conditions (Smol, 2001). For example, the use of environmental optima and tolerances of diatom species to reconstruct climate trends has become proxy for long-term climatic data (Smol, 2001). In terms of environmental water quality, diatoms have been used to study the impact of atmospheric pollution on surface water (Rhodes et al., 2017), as well as the impact of salinity and eutrophication on surface water (Watts and Bradbury 1982; Fritz et al 1991; Rhodes et al., 2017).

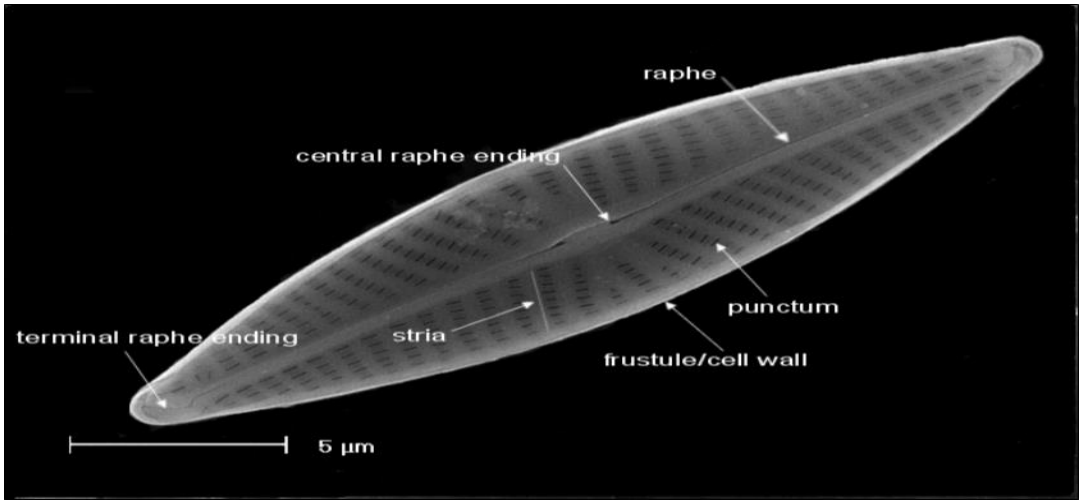

Figure 1.1: General features of pennate diatoms (Source: Taylor et al., 2007)

Many countries in Europe, Asia and South America use diatoms as a core component of their biomonitoring programmes (Lobo et al., 1996; Prygiel et al., 1999; John, 2000). Diatoms as proxy indicators were integrated into the European Water framework directive during the 1990s (Kelly et al., 2009). Diatoms are used in New Zealand as an indicator of Acid Mine Drainage (AMD) (Schowe et al., 2012). In South Africa, diatom versatility has been identified for monitoring of aquatic ecosystem health (Harding et al., 2004; Dalu et al., 2014). Many diatom species are sub-cosmopolitan, supporting the global use of generic indices.

Local (endemic) characteristics, which have been built into the South African Diatom Index (SADI), increase the power of the index for specific local and regional use. Diatoms have been described as versatile and important biomonitoring indicator organisms for aquatic environments because:

- They are abundant, diverse and part of the base of the aquatic food web.
- Benthic diatoms attach to their environment and do not easily give in to wash out or changes that affect planktonic indicators (Harding et al., 2004; Dalu et al., 2014) and are therefore able to reflect the condition of their environments.
- They are easy to sample and are usually equally represented across all surfaces at a single location.
- They are easy to identify to the species level although identification requires specialist training and microscope.
- They are good indicators of water chemistry especially salinity because of their biochemical composition.

Diatoms species that develop in an area depend on different environmental factors, including salinity, temperature, pH, water velocity, shade, depth and availability of substrate for growth and attachment (Martin et al., 2012). It has been suggested that diatom species abundance and richness decrease with increasing salinity (Dorgham, 2011). Therefore, in this study, the relationship between the Kat River water salinity and diatoms species abundance, distribution and richness were investigated.

1.7 Problem statement

Excess salinity may reach freshwater resources through non-point and point sources. Like many South African rivers, the Kat River is facing a deterioration of its water quality due to anthropogenic activities (DWA, 2011). Its catchment is a hub of major agriculture activities such as irrigation of citrus fruits and livestock farming in the upper, middle and lower reaches. In the early 1980's, commercial farming of citrus in the Kat River Valley Catchment (KRVC) was threatened by high salinity due to decreased water in the river (Lerotholi et al., 2004). In addition, the 2011 census by Statistics South Africa suggested a population increase in the KRVC compared to historical population records.

By extension, activities around the KRVC have increased since 1981, which have negatively impacted the water quality of the river. To sustainably manage the Kat River water quality, it is important to monitor its physicochemical variables, ecosystem health and understand the impact of anthropogenic activities on the aquatic ecosystem to recommend and initiate appropriate remedial actions. To this end, this study deploys the combined use of physicochemical measures, as well as macroinvertebrates and diatom bioassessment as a tool for assessing the effect of anthropogenic activities on the Kat River.

1.8 Research questions

Based on the above problem statement, this study addressed the following research questions

- What are the impacts of anthropogenic activities on the salinity of the Kat River?
- How do macroinvertebrates and diatoms assemblage structures respond to the deteriorating water quality in the Kat River?
- Can macroinvertebrates and diatom assemblage structure be reliably used to evaluate the impact of anthropogenic activities in the Kat River system and what are the comparative strengths and weaknesses of the two bioindicator organism group in the context of the Kat River assessment?

1.9 Aim

The aim of this research was to evaluate the impacts of anthropogenic activities on salinity and biological assemblages of Kat River, with a view to proposing appropriate salinity management strategy for the catchment.

1.10 Specific objectives

The following specific objectives were formulated as a means to achieve the aim of this study:

- To measure selected physicochemical variables at 5 selected sites along the Kat River.
- To undertake biomonitoring of macroinvertebrate and diatoms at the selected sites and to undertake a comparative analysis of the strength and weakness of the two bioindicator groups in the context of the Kat River assessment.
- To evaluate the relationship between salinity and the biomonitoring results.
- To evaluate salinity risks to the river ecosystem in relation to anthropogenic activities in the Kat River.

1.11 Thesis structure

This thesis consists of five chapters. Each chapter represents a major section of the study with various sub-sections in a chronological sequence; each chapter building on the preceding one.

Chapter 1: This chapter is a general introduction to the study. It provides brief background information on pollution of freshwater resources and reviews their management in South Africa. The chapter then reviews biomonitoring, specifically focusing on macroinvertebrates and diatoms as bioindicators. The chapter ends with the problem statement, research questions, aim, specific objectives, and structure of the thesis.

Chapter 2: This chapter is a description of the study area, including catchment characteristics and the selected sampling sites. Methods for sampling selected water physicochemical variables, diatoms and macroinvertebrates are described. The chapter ends with a discussion on statistical data analysis methods used in this thesis.

Chapter 3: This chapter presents macroinvertebrate-based biomonitoring and physicochemical study results. It begins with a presentation of water physicochemical characteristics, and then SASS5-based river health assessment and Macroinvertebrate Response Assessment Index (MIRAI). The chapter ends with a discussion and conclusion of each of the results presented.

Chapter 4: This chapter presents results of this study on diatoms. It begins with information on diatoms abundance and diversity and followed by Indicator species analysis on diatom community. Physicochemical variables in relation to diatoms for the five sampling sites are presented. The chapter ends with a discussion and conclusion of the results presented.

Chapter 5: In this chapter, comparative analyses of macroinvertebrates and diatoms responses are given. Then, discussion in relation to the overall aim, specific objectives and research questions set out to address in this study. The chapter ends with suggestions on the application and implementation of the findings, including recommendations for further studies to address specific issues encountered through the course of this study.

CHAPTER 2: STUDY AREA DESCRIPTION, MATERIALS AND METHODS

2.1 Introduction

This chapter provides a description of the study area as well as the general materials and methods used in this study. These include sampling for biomonitoring (macroinvertebrates and diatoms), physicochemical sampling, equipment used, preparation of solutions, preparation and analysis of water physicochemical variables, preparation and analysis of biomonitoring samples, stream flow measurements and discourse about the different statistical analysis methods used in this study.

2.2 Study area description

The Kat River is located in Nkonkobe Municipality in the Amatole District, Eastern Cape Province, South Africa. The Nkonkobe Municipality is located along the Southern slopes of the Winterberg Mountain and has an area of 3725 km^2 (Stats SA, 2011). The Kat River Valley Catchment (KRVC) has a (covers area) of 1715km^2 with a length of 80km (Holtzhausen, 2006). Kat River Valley Catchment (KRVC) is described as sub-humid in the north to semi-arid in the South. The KRVC receives a mean maximum annual rainfall of 1200 mm and a mean minimum annual rainfall of 400 mm, respectively. Thus, the catchment receives a mean annual rainfall of 502 mm, with mean an annual runoff of only 42 mm. Maximum mean daily temperature varies from 30^oC in February to 21^oC in July, while minimum mean daily temperature varies from 17^oC in January to 8^oC in July (Motteux, 2002).

The Kat River is situated between Seymour and Fort Beaufort and starts from Hogsback Mountains in the North East and Katberg in the North West. It is a tributary of the Great Fish River and is about 150 km in length. The River is a social-economic asset to the Nkonkobe Municipality because it supplies water for agricultural activities, including irrigation and livestock farming. It also provides grounds for spiritual activities and for domestic use such as drinking, bathing and laundry. Five sampling sites in the Kat River were selected for this study. These sites were named Sites 1- 5, (Figure 2.1). The study was carried out between December 2015 and November 2016, capturing all the seasons in South Africa and covering one hydrological year.

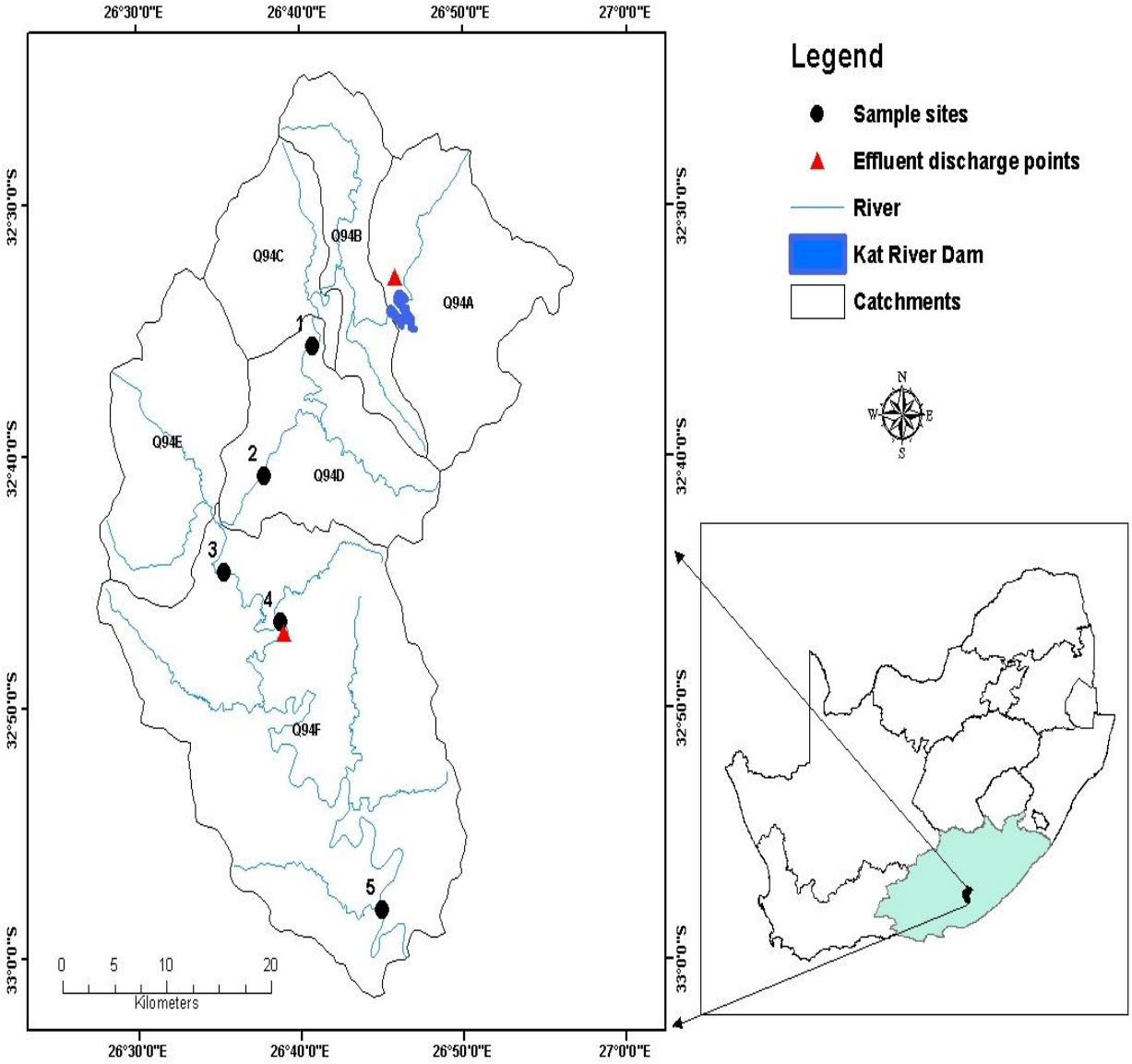

Figure 2.1: The Kat River, showing sampling sites and geographical setting of the Kat River Valley Catchment within Eastern Cape Province, South Africa.

2.2.1 Geology

Geology is the main driver of primary salinity in rivers (Allanson, 1995; Lerotholi, 2005). There is evidence that the natural geology of the Kat River is influenced by substantial quantities of rocks shales, mudstones and sandstones. (Lerotholi, 2005). The sandstones, found at the northern edge of the Katberg, sometimes are intruded by dykes and dolerite sills, which are igneous in origin (Vegter, 2000). Dolerite has been found downstream of the Kat Dam, alluvial deposits are commonly encountered along the Kat River, while mudstones and shales underlay the southern tip of the catchment (Woodford and Chavellier, 2002; Lerotholi, 2005). All these geological features contribute ions, and by extension provide the base salinity, to the Kat River.

2.2.2 Land use

The Kat River Valley Catchment (KRVC) is characterised by a variety of land use activities, ranging from export-oriented citrus farming, commercially oriented rangeland livestock farming, to small-scale vegetable/crop production and livestock farming. Citrus farming is mostly practised in the middle part of the catchment, while game and small-scale farming are practised mostly at the lower and upper part of the catchment (Holtzhausen, 2006). Other land use practices include forestry, wildlife management, grazing and instream laundry (Motteux and McMaster, 2002; Naidoo, 2008). Also, construction of informal settlement, weirs, and the Kat River Dam has influenced the flow regimes. The land use has a negative impact on water resources, affecting both quality and quantity (DWAF, 1997). For example, any land-use activity that reduces the infiltration rate of rainwater may increase the potential of flooding. Abstraction of water for irrigation, and reduce the amount of flow into rivers (Soviti, 2002). Land use involving agricultural and livestock are not the only major use of river water but they affect riverbank conditions by enhancing erosion and impacting the ecology of the Kat River (Naidoo, 2008).

2.2.3 Sampling sites

The study was conducted at five sampling sites (sites 1, 2, 3, 4 and 5) (Figure 2.1). The sites were selected according to the following criteria:

- All selected sites were within the same ecoregion
- All selected sites had the following features: stones (stone in current and stones out of current), vegetation (marginal and aquatic) and gravel, sand and mud (GSM)
- All selected sites were likely impacted by human activities (e.g., wastewater treatment works and agricultural farmlands) due to their location
- All selected sites were part of the river system

Site 1 (S32.59399° E026.67916°) is situated under the Kat River bridge across the main road that links Fort Beaufort and Queenstown (i.e., R67). This site is located upstream of all other sampling sites. There are human settlements around this site, with the mainland use being farming (small-scale, commercial and livestock grazing). It is likely that runoffs carrying residues of livestock dung silt from land erosion as results of overgrazing by livestock and small-scale crop farming may have negative impacts on the ecology of the site. However, all macroinvertebrates sampling biotopes, including stone, vegetation and GSM (gravel, sand and mud) required by SASS5 sampling protocol were adequately represented at this site (Figure 2.2).

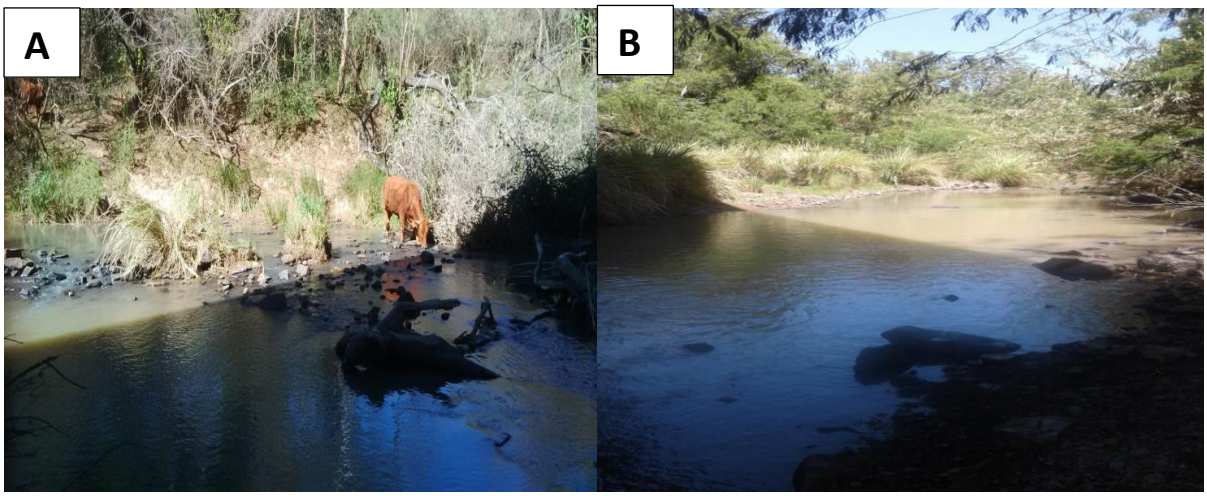

Figure 2.2: Site 1, showing sampling biotopes during summer (A) and in winter (B).

Site 2 (S 32.669162˚ E026.61260˚) is situated about 12.82 km downstream of Site 1. Like Site 1, this site is also surrounded by human settlements and there is a gravel road for vehicles across the site. The main land use is farming (small scale, commercial, and livestock grazing). Laundry and spiritual activities (cultural activities) also occur at this site. Runoffs carry residues of livestock dungs, silt from land erosion due to land clearing for farming and overgrazing, as well as chemicals from the laundry are expected to impact on the site's ecology. However, all macroinvertebrates sampling biotopes, including stone, vegetation and GSM (gravel, sand and mud) required by SASS5 sampling protocol were adequately represented at this site (Figure 2.3).

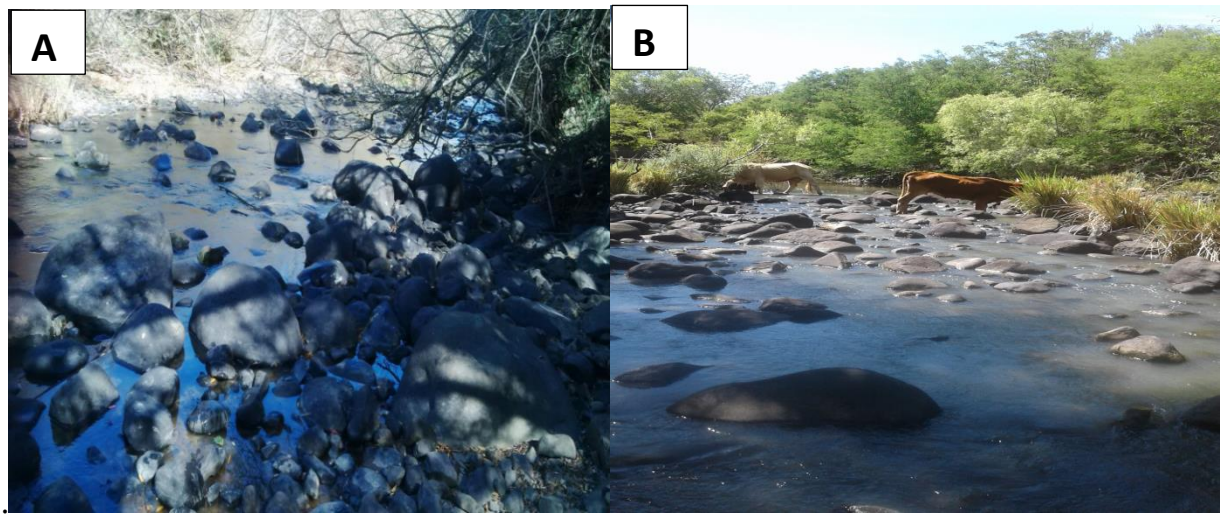

Figure 2.3: Site 2, showing sampling biotopes during summer (A) and in winter (B).

Site 3 (S32.74398˚ E026.58864˚) is located within a hub of commercial farming activities (largely citrus) and is situated about 6.38 km downstream Site 2. This part of the river's reach, including upstream and downstream of the site, provide means of irrigation for the commercial farming activities. Runoffs from the use of agrochemicals such as pesticides and fertilisers, as well as soil erosion due to land clearing and other farming activities, are likely to impact the site's ecology. All macroinvertebrates sampling biotopes, including stone, vegetation and GSM (gravel, sand and mud) required by SASS5 sampling protocol were represented at this site (Figure 2.4).

Figure 2.4: Site 3, showing sampling biotopes during summer (A) and in winter (B).

Site 4 (S32.77743° E026.64594°), is situated about 6.58 km downstream of Site 3 at Fort Beaufort, an urban settlement that suffers from a failing municipal infrastructure, including a number of leaking sewer pipes. Wastewater from the leaks flows down stormwater drains directly into the Kat River. However, it is not only wastewater that may likely impact the river's ecology but rubbish from the community and runoffs that carry residues of dungs from livestock farming, and silts from soil erosion due to livestock overgrazing and sand mining may contribute to pollution. All macroinvertebrates sampling biotopes, including stone, vegetation and GSM (gravel, sand and mud) required by SASS5 sampling protocol were represented at this site (Figure 2.5).

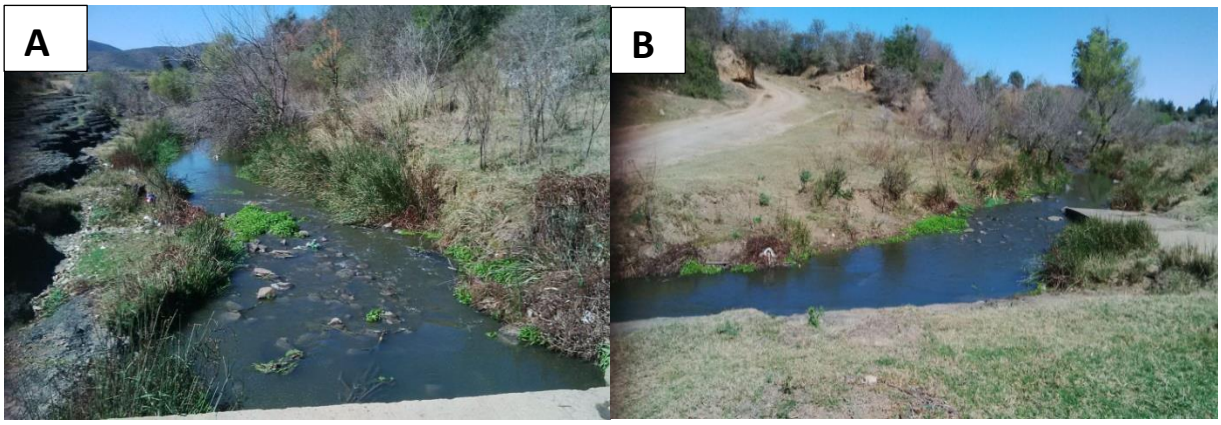

Figure 2.5: Site 4, showing sampling biotopes during summer (A) and in winter (B).

Site 5 (S32.96877° ES026.74992°), situated at the Great Fish Game Reserve, is the most downstream site and it was about 23.63 km downstream Site 4 (Figure 2.6). Site 5 located just a few kilometres from the confluence of the Kat River and Fish River. The site is likely to be impacted by wildlife activities such as defaecation, as well as accumulation of pollutants from the upstream sites. For Site 5 all macroinvertebrates sampling biotopes, including stone, vegetation and GSM (gravel, sand and mud) required by SASS5 sampling protocol were represented (Figure 2.6).

Land use and google earth maps of all the sites are shown in Figure 2.7. These maps show the anthropogenic activities that may impact each site.

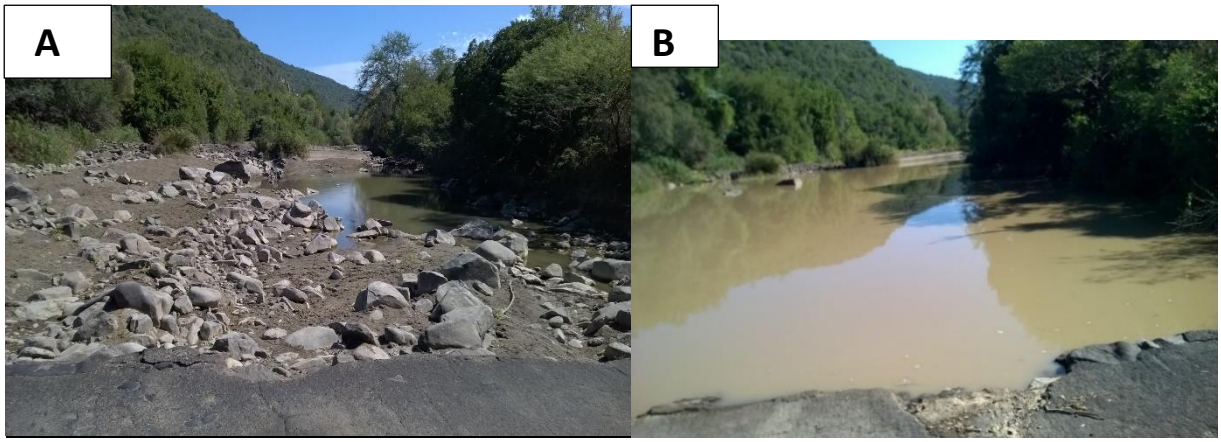

Figure 2.6: Site 5, showing sampling biotopes during summer (A) and in winter (B).

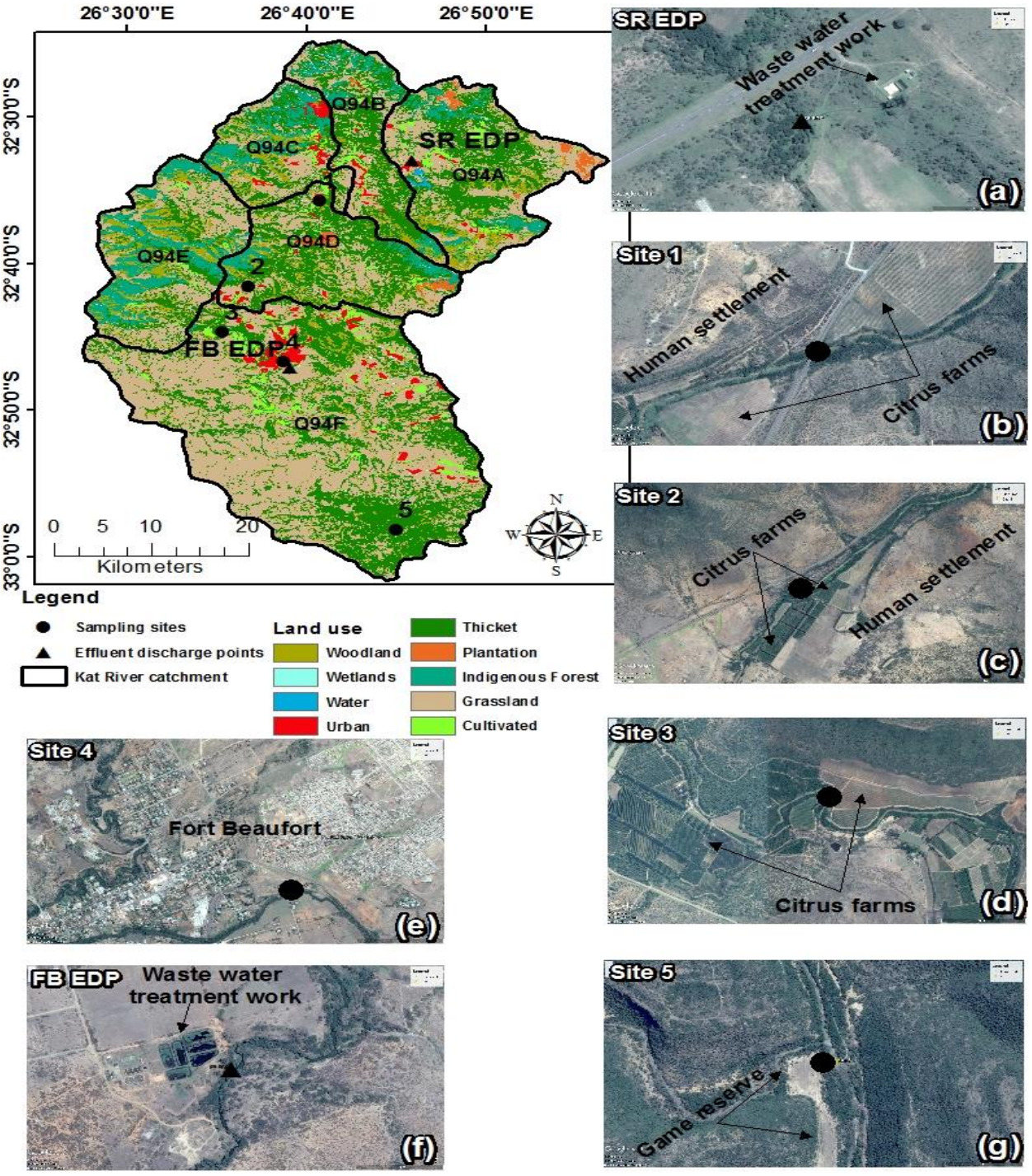

Figure 2.7: Land use and google earth maps the sampling sites on Kat River catchment. Land use maps are modified from the Department of Environmental Affairs (DEA, 2013-2014) (© GEOTERRAIMAGE - 2014). (a) represents Seymour effluent discharge point; (b) Site 1 and surrounding citrus farms; (c) Site 2 citrus farm and human settlement; (d) Site 3 and citrus farms; (e) Site 4 and Fort Beaufort urban area; (f) Fort Beaufort (FB) effluent discharge point and wastewater treatment works; (g) Site 5 situated at the Great Fish Game Reserve.

2.3 Sampling for environmental data

2.3.1 Water sampling and physiochemical analysis

Six field trips were undertaken to the various sites in the Kat River for one hydrological year; from December 2015 to November 2016. For each trip at all five (5) sites over six sampling period (October, December, February, April, June and August), electrical conductivity (EC), dissolved oxygen (DO) and pH were measured on-site using Hanna multiprobe meter. The temperature was measured using mercury-in-glass thermometer and turbidity was measured using a portable turbidity Orbeco-Hellige meter, respectively. Water samples were collected using a 500-mL acid washed bottles facing upstream to avoid sediments entering. Collected samples were then transported to the Institute for Water Research (IWR) water quality laboratory at Rhodes University and preserved in a refrigerator at a temperature of 4°C for analysis (UNEP/WHO, 1996). Nutrients analyses were undertaken within 24 hours of sample collection. Before analysis, samples were allowed to equilibrate to room temperature. Samples were analysed for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-Nitrogen (NH_4-N) and orthophosphate-phosphorus (PO_4-P). PO_4-P and NH_4-N were analysed using Merck spectroquant® phosphate and ammonium concentration test kits; catalogue numbers 1.14752.0001 and 1.14848.0001, respectively, according to the manufacturer's instructions. Nitrite-nitrogen was analysed according to APHA et al. (1971), while nitrate-nitrogen was analysed according to Ondrus (1996). Both nitrite-nitrogen and nitrate-nitrogen were analysed with the aid of a Biotech microplate reader at 540 nm. Total inorganic nitrogen (TIN) concentration was calculated by adding the concentrations of nitrate-nitrogen, nitrite-nitrogen and ammonium-nitrogen (Palmer et al., 2004).

2.3.2 Assessment of water quality variables in relation to Resource Water Quality Objectives

Physicochemical Variables for the Kat River sampled through the study period of December 2015- November 2016 were compared to South African generic Resource Water Quality Objectives (RWQOs). The RWQOs are defined as expressive instream water quality objectives that set a finer resolution than RQOs by providing details based on the management of water quality of the resource (DWAF, 1996).

The RWQOs represent water quality component of the Resource Quality Objectives. It is a useful tool in planning water user's compliance requirements based on water quality contaminants entering the water resource and requirements of the aquatic ecosystem. To understand the Kat River water quality status quo, the generic RWQOs were used since there are no RWQOs in place for this river. The generic RWQOs were obtained from the South African Water Quality Guidelines (SAWQGS) (DWAF, 1996). The SAWQGs have been developed as discrete values for water quality that represent the changes from one to another category of fitness (DWAF, 1996). The SAWQGs are used to establish generic water quality limits for different water user categories. The SAWQGs use Target Water Quality Range (TWQR) as a management category, which is used to set benchmark categories ranges for each water quality variable. The TWQR, Chronic Effect Value (CEV) and Acute Effect Value (AEV) in the SAWQGs are used to determine the fitness for use category. The TWQR is a national management objective used to set specific ideal concentration values for a particular water variable such that it remains within no effect range. The CEV represents the concentration of specific variable at which it is possible to cause chronic effects, while the AEV represents a concentration of a specific variable at which it is possible to cause acute effects. The SAWQGs define "fitness for use" and RWQOs describe "management actions that are required" for a water resource. The fitness for use of water is a decision of how appropriate the water quality is for its intended purpose. The following fitness for uses categories (Ideal, Acceptable, Tolerance and Unacceptable) were linked to the SAWQGs as shown in Table 2.1: The SAWQGs reflect TWQR, which may be considered as the ideal water quality. To interpret the physicochemical variables in terms of ecological perspective, the fitness for use categories were translated to the ecological category as stated in DWAF (1996), shown in Table 2.2.

Table 2.1: Generic Resources Water Quality Objectives limits derived from for selected physicochemical variables DWAF (1996)

Variables	**Ideal**	**Acceptable**	**Tolerable**	**Unacceptable**
EC uS/cm	30	50	85	120
DO mg/l	120	90	40	40
pH	7.25	8.2	0	8.4
PO_4-P mg/l	0.005	0.015	0.025	>0.025
NO_3-N mg/l	6	10	20	>20
NH_4-N mg/l	0.015	0.044	0.073	>0.073
TIN mg/l	0.5	2.5	10	>10

Table 2.2: Rules to derive fitness for use category, and translating the fitness for use into ecological categories.

SAWQGs User category and effect	**Water fitness for use category**	**Ecological health category**
Upper limit of the TWQR	Ideal -100% fit for use by all users at all times	A – Natural
Average of TWR and CEV	Acceptable - slight problem encountered for limited period	B – slightly modified
		C – Moderately modified
Upper limit of CEV	Tolerable - moderate to severe problem for limited period	D – Seriously modified
Above the CEV	Unacceptable: unfit for intended use at all times	E/F – Critically modified

2.3.3 Streamflow measurements

"Streamflow is a volume of water that moves over a designated point over a fixed period of time" (Richards et al, 1997). It is affected by seasonal rainfall and amount of dissolved solids. Streamflow is an important environmental factor because it impacts water quality and therefore, the ecology of the system by affecting DO, temperature and concentration of various substances in the water (Munson et al, 2008). Human activities such as water abstraction for irrigation affect the rate of stream flow. In this study, stream flow measurements were taken during water sampling from December 2015 to October 2016. The following materials, including stopwatch, floating ball, surveyor's tape measure, depth stick and data recording were used to measure streamflow according to the following steps:

Step 1: A transects, i.e. a stretch along the stream not less than 6 m were demarcated.

Step 2: Time (T) that it took the orange to travel the length of transect was recorded.

Step 3: The cross-sectional area at one point of the transect (A1) was determined, where A1 = total width (m) x average depth (m) = m^2

Step 4: The cross-sectional area at another point of the transect (A2) was determined, where A2 = total width (m) x average depth (m) = m^2

Step 5: The average cross-sectional of transect (A) was calculated as follows: A = A1 + A2 = m^2

Step 6: The stream flow (F) across transect was determined as follows: $F = \frac{ALC}{T} = m^3/s$, where F = stream flow; A = cross-sectional area of transect; L = length of transect; T = time in seconds (s) for the floating ball to travel the length of transect; C = coefficient or correction factor (0.8 for rocky bottom streams or 0.9 muddy bottom stream). Figure 2.7 is a diagrammatic presentation of stream flow measurement.

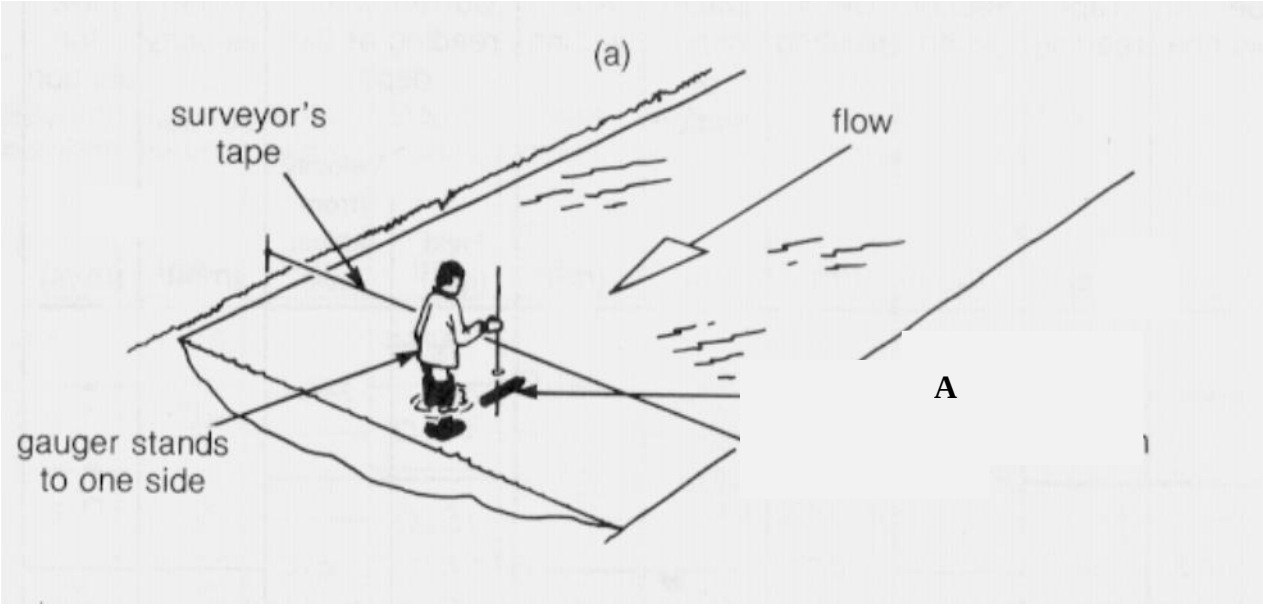

Figure 2 8: A diagrammatic representation of how the flow is measured (Source: Brassington, 1990).

2.3.4 Integrated Habitat Assessment System

Integrated Habitat Assessment System (IHAS) is a method used to assess the physical habitat integrity of streams and rivers. This method was developed to be used with SASS5 in biomonitoring programmes based on habitat availability (McMillan, 1998). IHAS is based on sampling habitat and general stream characteristics such as natural and anthropogenic impacts (McMillan, 1998), with the aim of assessing the physical characteristics and condition of the stream. Practically, the IHAS scoring is based on 100% points, divided into two sections: sampling habitat conditions (55 points) and stream conditions (45 points). The sampling habitat section is divided into three subsections: stones in current (SIC) (20 points), vegetation (15 points) and other habitats (20 points). Higher percentage scores are indicative of better habitat and stream conditions for macroinvertebrates communities. The ideal condition is not based on the ultimate unmodified stream, but on the representation of all habitats adequately and in reasonable conditions for macroinvertebrates. In this study, IHAS was conducted at each of the five sampling sites by completing an IHAS form, which involved scoring habitat and stream conditions. The obtained IHAS data was used as complementary aids to interpret the SASS5 results.

2.4 Sampling for biotic data

2.4.1 Macroinvertebrate sampling using South African scoring system version 5

Macroinvertebrates samples were collected any other month between sampling period of (October, December) 2015 and (February, April, June and August) 2016 at each of the five sampling sites using the South African Scoring System version 5 (SASS5) protocol (Dickens and Graham, 2002). Briefly, macroinvertebrates sampling was conducted using a SASS5 kick net (frame: 30 x 30 with mesh size 1 mm) held immediately downstream of the area to be sampled. Macroinvertebrates samples were collected from three biotope groups: vegetation (marginal and aquatic), stones (stones-out-of-current, stones-in-current), and sediment (gravel, sand and mud (GSM)). Three replicate samples were taken for each biotope, i.e. stone, vegetation and GSM, to make up nine samples collected at each site. The first collected sample from each biotope was tipped into a SASS5 tray filled with water, and macroinvertebrates were identified by the riverside. The identified families were recorded on a SASS5 sheet and abundance was estimated using the rank abundance method. After completing the SASS5

exercise, the collected macroinvertebrate samples were preserved in a solution of 70% ethanol, transported to the laboratory for sorting, identification and abundance counts.

Macroinvertebrates were identified using the keys described by Gerber and Gabriel (2002). After abundance count and sorting, individuals of each family were stored in 10-mL pill vials with a solution of 70% ethanol to prevent them from decomposing, so they could be used for further study if need be. Macroinvertebrates collected in the same biotope at the same site were kept in the same pill vial in the same honey jar.

2.4.2 River health assessment using the South African scoring system version 5 and Macroinvertebrate Response Assessment Index

The South Africa Scoring System version 5 (SASS5) is suitable for evaluation of river health (Dickens and Graham, 2002). It is the most widely used biomonitoring tool in South Africa to assess ecological health of rivers (Dickens and Graham, 1998: Palmer et al, 2004; Odume and Mgaba, 2016). To gain a deeper understanding of the SASS5 data, they were entered into Macroinvertebrate Response Assessment Index model (MIRAI). The MIRAI is designed to be able to give an insight into the causes and sources of any disturbance of macroinvertebrates community structure from natural condition to present ecological state (Kleynhans et al., 2005).

MIRAI was developed to be used specifically for Ecological Water Requirements (EWR) determinations but is recommended for use in REMP assessment too. Since none of the sites could be used as a reference site, a reference condition was constructed for the Kat River using macroinvertebrate information from minimally impacted sites from Rivers within the same ecoregion level II as the Kat River. This was made possible by information retrieved from the South African River Health Database as well as other historical data sources and the potential reference taxa recovered by the MIRAI model. Therefore, the MIRAI model was used in this study to derive the extent that the sampled sites changed from a reference condition. Briefly, the MIRAI model uses five metrics (flow modification, habitat modification, water quality, system connectivity and seasonality) to evaluate the present ecological state (PES) based on macroinvertebrate assemblages (Thirion, 2008). Each metric contributes to determining the ecological category of invertebrate assemblages for the selected site. These metrics were ranked and weighted according to which metric was considered to be the most important in determining the present ecological state (PES) of the invertebrate community.

For this study, data were entered into three MIRAI metrics (habitat modification, flow modification and water quality modification) to derive ecological category for aquatic invertebrate for the Kat River. Each metric was ranked according to its importance in determining the present ecological state of macroinvertebrates. Weightings between 0 and 5 were assigned to each of the metrics depending on how much they were expected to contribute to the overall score (Table 2.3). Where 0 means no modification and 5 means extreme modification (Table 2.4) shows percent habitat integrity assessment ratings. These were used to assess the changes caused to the habitat due to pollution with. descriptions of the changes experienced or caused by pollution.

Table 2.3: Ranking of MIRAI metrics modified from (Kleynhans, 2008)

Description	**Generic guidelines**	**Impact compared to reference site**
The modification has no impact on habitat quality, diversity, size and variability.	0	No
Few modifications on habitat quality, diversity, size and variability are very small.	1	Small
Moderate modifications in habitat quality, diversity, size and variability are limited.	2	Moderate
Large modification of habitat quality, diversity, size and variability. Large areas are not influenced.	3	Large
Only small areas are not influenced.	4	Serious
Almost the whole of the defined section is influenced adversely.	5	extreme

Table 2.4: Ecological Category conditions and description modified from (Kleynhans 1996)

Ecological Category conditions	**Rating %**	**Description**
A	90-100	Unmodified, natural reference condition
B	80-89	largely natural with few modifications
C	60-79	Moderately modified
D	40-59	Largely modified
E	20-39	Seriously modified

F	<20	Critically/extremely modified

2.4.3 Diversity indices analysis

Diversity indices are mostly used as tools for comparing communities. They are used in community ecology to show increase or decrease in diversity in order to indicate changes in habitat and ecosystem health (Clarke and Warwick 2001). Useful indices respond to pollution-induced changes and should differentiate such changes from natural variations (Ravera, 2001). Richness, Evenness, Shannon and Simpson indices were used to analyse macroinvertebrate community structure for the Kat River to assess pollution impact. Richness and evenness are the main factors that are taken to account when measuring diversity. Richness is a measure of different kinds of organisms that are present in a site or area, which represent a number of species available, while Evenness measure the relative abundance of different species found in a particular site. Simpson index weighs towards the abundance of commonest families but Shannon index takes account of the contribution of individual taxa to the diversity while assigning greater weight to most dominant taxa (Ogbeibu, 2005). Interpretation of diversity indices should be done with care since important information may get lost if care is not taken. For example, some species are sensitive/tolerant to pollution and they give different information about the in-stream water quality condition. Such vital information may get lost if community data is reduced to a single index without any proper caution or interpretation. Furthermore, ecologically unique communities are not necessarily diverse and would be lost if conservation decisions were made on the basis of diversity alone. Thus, in this study, other biotic index like MIRAI was used together with the diversity indices to ensure more informed analyses.

2.4.4 Diatoms sampling

Diatoms samples were collected at the five sites any other month for one hydrological year (October, December) 2015 and (February, April, June and August) 2016. Diatoms were collected using the protocol described by Taylor et al (2006). Briefly, rocks and cobbles were collected randomly from the river bed and transferred to a small white tray. The upper surface of the cobbles and rocks were vigorously scrubbed using a toothbrush and then washed with water approximately same volume as the sampling bottle. The scrubbed material-in-water was transferred into the sampling bottle and labelled. When rocks were not available, five stems of macrophytes were collected and transferred into a zipper plastic bag.

A small quantity of water, approximately the same volume as the sampling bottle, was added to the plastic bag and vigorously agitated to wash the plant inside the bag for a minute. The sample was then transferred to the sampling bottle and appropriately labelled. Similarly, where macrophytes stems were not available, five plant stems were collected by cutting the stems below the water line and just above the sediment level. The stems were transferred into a white sampling tray and water approximately the same volume as the sampling bottle was added to the tray. The stems were scrubbed with a toothbrush, transferred into a sampling bottle and appropriately labelled. The collected samples from rocks, macrophytes or plant stems were transferred to the laboratory and preserved in 99 percent ethanol until samples were prepared and analysed for slide mounting, identification, measurements and counting using Olympus SC30, and following a protocol developed by Taylor et al (2006).

2.5 Data analysis and statistics

Different statistical methods were used to examine the water physicochemical variables, macroinvertebrates and diatoms data in order to make relevant statistical comparisons between sites and season. The data were subjected to relevant univariate and /or multivariate statistical analyses to measure statistical differences and/or similarities and to elucidate trends, relationship and patterns. Data were recorded in Excel (2013) and copied to relevant statistic packages. This was done using Statistica version 13.3 and R version 3.3.3 software using different packages. All statistical significant decisions were made at alpha ≤ 0.05.

2.5.1 Box plot (Box and whiskers) graphs

Box plot (Box and whiskers) displays statistic such as medians quartiles, extremes, non-outlier ranges and outlier values. These summary statistics were plotted to allow the visualisation of physicochemical variables variation between sites as recommended by Baptista et al., (2007) and Odume et al., (2012). Box plots were used to test the discriminatory potential of selected physicochemical variables and biotic indices in Chapters 3 and 4. Box and whiskers graphs were performed using Statistica version 13.3.

2.5.2 Cluster analysis

Hierarchical clustering analysis is a multivariate method which aims to classify sites or samples that have same measured variables into a similar group and the variables have different measures placed in different groups**.**

In this case, hierarchical agglomerative clustering was used to group sites for each sampling season based on macroinvertebrate community. The cluster analysis demonstrates similarities and dissimilarities between sampling sites in terms of macroinvertebrates community structure in (Chapter 3). Results of hierarchical clustering are represented using dendrogram diagram, with the x-axis representing sites and samples and the y-axis the level of similarities of samples. Bray-Curtis similarity matrix was used since it permits all species to undergo the analysis to contribute to the final level of similarity although assigning greater weight to the common species (Clarke and Warwick, 1994). Hierarchical agglomerative clustering was performed using R package 2.0.5.

2.5.3 Ordination

Ordination shows similarities and dissimilarities of samples in the form of a map, usually in two or three dimensions. Distances between samples indicate differences in community composition, while the proximal samples indicate similarities between community compositions (Clarke and Warwick, 2001). Canonical correspondence analysis (CCA) is a multivariate method to elucidate the relationships between environmental variables and a biological community. CCA is widely used in the aquatic sciences. (Braak and Verdonschot, 1995). In this study, CCA was used to relate macroinvertebrate and diatom communities with the measured water physicochemical variables to elucidate variables influencing the observed community patterns between the sampling sites (Chapter 3 and 4). CCA was performed using R package vegan 2.4-4.

2.5.4 Indicator species analysis

Indicator species analysis was used in this study in Chapter 4, using package “Indicspecies” 1.7.6 within R version 3.3.3 software to conduct relationship between diatoms species and groups of sites. Indicator species can help to reflect environmental changes, habitat conditions

and ecological indicators for a community (De Cáceres et al., 2010). This analysis helps to determine the occurrence or abundance of indicator species (Dufrene and Legendre 1997).

The Indicator species analysis uses a function called indicator value (IndVal), which determines the most important species that can be used as an indicator of a particular site or habitat (Dufrêne and Legendre, 1997; De Cáceres and Legendre 2009). The IndVal analysis also gives a measure of the relationship between a group of sites and species and selects the group that has the highest association value (Dufrene and Legendre, 1997). A permutation test was used to test the statistical significance of the relationship.

CHAPTER 3: RESPONSE OF MACROINVERTEBRATE ASSEMBLAGE STRUCTURE TO WATER QUALITY IN THE KAT RIVER

3.1 Introduction

This chapter provides the results on macroinvertebrate assemblage in the Kat River. Chapter 2, which was on general materials and methods, contain details of the macroinvertebrates-based biomonitoring conducted to assess the impact of anthropogenic activities on the ecology and water quality of the Kat River. In this chapter, an overview of the materials and methods for sampling and analysis of physicochemical variables as well as macroinvertebrates biomonitoring are presented. This is followed by a detailed presentation of results for water physicochemical parameters, then SASS5-based river health assessment and Macroinvertebrate Response Assessment Index (MIRAI). Physicochemical variables in relation to macroinvertebrates assemblages at the five sampling sites are analysed and presented; followed by similarities analyses between the five sampling sites. The chapter ends with a discussion and conclusion, which was made in the context of aims and objectives of this chapter.

3.2 A recap of the materials and methods

Physicochemical variables, including total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), hydrogen ion concentration (pH), electrical conductivity (EC), dissolved oxygen (DO), temperature, turbidity and streamflow were measured over the study period as described in Chapter 2 section 2.3. Physicochemical variables sampled at Kat River over the study period were analysed and compared to derived generic RWQOs follow DWAF, (1996) in order to assess the river's ecological conditions according to the different ecological categories as presented in Table 2.3 (Chapter 2). Normality of environmental variables was performed using log (x+1) in order to assign all variables equal weight irrespective of their scale of measurement and macroinvertebrates biological data were transformed using log (x+1) and (Clarke and Warwick, 1994). The mmacroinvertebrate Response Assessment Index (MIRAI) model was used to compute macroinvertebrate community data in order to assess each sampling site's ecological status. Bray-Curtis analysis was used to show similarities or dissimilarities of macroinvertebrate community assemblage between sampling sites and seasons. Four taxonomic metrics belonging to measures of diversity (Shannon, Simpson, Evenness and Margalef) indices were evaluated for their discriminatory potential between the sites. Metrics were used as measurable units of a biological system that change in value

according to the level of pollution in streams. Ordination with CCA was used to explain the connection between the measured water quality variables and macroinvertebrate families in the Kat River collected during the study period.

Macroinvertebrates biological data were transformed using log (x+1) and normality of environmental variables was performed using log (x+1) in order to assign all variables equal weight irrespective of their scale of measurement (Clarke and Warwick, 1994).

3.3 Results

3.3.1 Physicochemical variables

The physicochemical variables results for the Kat River from December 2015 to November 2016 are shown in Table 3.1. The results are reported as means with their standard deviations and ranges. The results of physicochemical variables measured over the study period in all five sampling sites showed that EC and turbidity at Sites 4 and 5 were higher than the rest of the sites. The mean values for DO and streamflow showed that Sites 4 and 5 had lower DO concentrations and lower stream flows compared with Sites 1, 2 and 3. Sites 4 and 5 had high turbidity, which was different from upstream sites. Nitrate-nitrogen (NO_3-N) and nitrite-nitrogen (NO2-N) concentrations were high at Site 4 compared with Sites 1, 2, 3 and 5. Orthophosphate-phosphorus (PO_4-P) concentration was highest at Site 4 and lowest at Site 1. Ammonia-nitrogen concentration was higher at Site 5 compared to the other sites. Consequently, the TIN concentration at Sites 4 and 5 was significantly higher compared to Sites 1, 2 and 3.

Table 3.1: Physiochemical variable values at the five sampling sites over the study period (December 2015 – November 2016) in the Kat River: Mean ± Standard deviation. Values in brackets are ranges.

Variables	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PH	7.23±1.09 (6.70-7.90)	7.95 ± 0.32 (7.63-8.00)	7.82±0.66 (6.28-8.70)	7.62±0.68 (6.81-8.68)	7.63±0.86 (6.93-9.55)
Electrical Conductivity (mS/m	12.92±1.08 (10.90-13.90)	18.91±4.88 (19.00-21.90)	20.35±4.88 (11.80-20.30)	58.95±24.68 (20.3-95.90)	36.65±19.94 (16.70-61.40)
Dissolved Oxygen (mg/L)	8.97 ± 1.69 (6.07-10.10)	9.30±1.52 (7.50-11.40)	9.58 ±3.33 (6.51-16.00)	4.88±1.21 (2.55-5.74)	6.02±0.65 (5.08-6.90)
Turbidity (NTU)	29.49± 17.00 (4.94-34.40)	34.47±18.51 (9.36-49.80)	25.78±8.34 (12.50-33.00)	36.53±36.53 (12.72-106.0)	50.79±29.40 (10.50-100.0)
Temperature℃	20.45 ± 6.21 (8.11-23.47)	19.93 ± 5.88 (8.92-23.50)	20.5 ± 5.3 (10.78-25.60)	24.09 ± 7.28 (12.69-33.15)	21.5 ± 6.99 (8.72-29.50)
Nitrate- Nitrogen (mg/L)	1.14 ± 1.42 (0.18-3.94)	1.81±1.56 (0.28-2.36)	1.05±0.38 (0.38-1.42)	6.99±6.55 (0.87-1.42)	0.74±0.68 (0.01 -0.87)
Nitrite- Nitrogen (mg/L)	0.02 ± 0.02 (0.00-0.03)	0.02 ± 0.01 (0.01-0.02)	0.01 ±0.01 (0.00-0.03)	0.15 ±0.14 (0.01-0.26)	0.045± 0.04 (0.01-0.06)
Ammonia-Nitrogen (mg/L)	0.00 ± 0.01 (0.01-0.02)	0.01±0.00 (0.00-0.01)	0.01±0.00 (0.01-0.01)	0.37±0.21 (0.01-0.58)	0.44±0.67 (0.01-1.41)
Orthophosphate-Phosphorus (mg/L)	0.01 ± 0.00 (0.01-0.01)	0.17±0.18 (0.01-0.4)	0.06±0.14 (0.01-0.34)	0.55±0.67 (0.01-1.51)	0.11±0.15 (0.01-0.39)
Total Inorganic Nitrogen (TIN) (mg/L)	0.28 ± 0.32 (0.07-0.90)	0.52 ± 0.32 (0.13-1.06)	0.25± 0.096 (0.08-0.25)	1.95 ± 1.62 (0.85-4.93)	0.53 ± 0.64 (0.02-1.50)
Flow m^3/s	0.55±0.39 (0.27-0.58)	0.49±0.54 (0.15-0.54)	0.15±0.09 (0.06-0.31)	0.15±0.09 (0.07-0.31)	0.08±0.12 (0.00-0.26)

3.3.2 Seasonal variations of physicochemical variables

Seasonal comparison of the physicochemical variables between summer (October, December and February) and winter (April, June and August) showed varied results.

The two downstream sampling sites (Sites 4 and 5) had higher EC and turbidity values for both seasons compared to the three upstream sites (Sites 1, 2 and 3) (Figure 3.1). Both winter and summer recorded higher EC and turbidity concentrations for Site 4 compared to Sites 1, 2, 3 and 5. DO and flow were consistently higher at Sites 1, 2 and 3 compared to downstream sites (Sites 4 and 5) in summer but drastically dropped in winter (Figure 3.1). Sites 3, 4 and 5 showed higher pH results in summer compared to Sites 1 and 2, but only Sites 4 and 5 had higher levels of pH in winter compared with the rest of the sites. Orthophosphate results for both seasons were higher at Site 4 compared to Sites 1, 2, 3 and 5. Ammonium concentrations were less than 0.03 mg/l for all sites except Sites 4 and 5 in winter (Figure 3.3). Nitrate and nitrite results showed higher concentration at Site 4 compared to the other sites in summer compared, but concentration of nitrate for Sites 1 and 2 was slightly higher in summer than Sites 3, 4 and 5, while Site 4 and 5 showed slightly higher nitrite concentrations in winter than upstream sites (Figure 3.2). TIN concentration showed high results at Site 4 in summer and high results of Site 5 in winter. Generally, results of most of the physicochemical variables were higher in summer compared to winter, especially results at Sites 4 and 5.

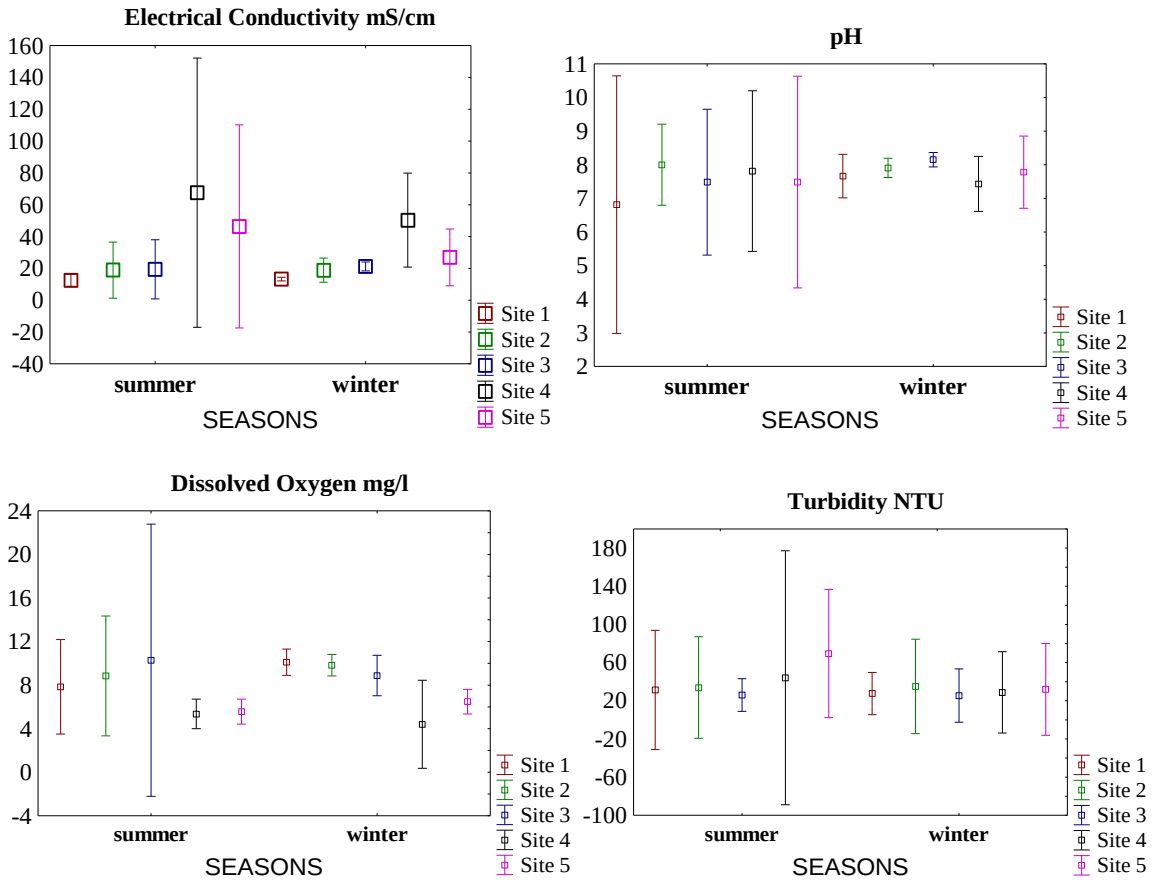

Figure 3.1: Seasonal variability of electrical conductivity (EC), pH, dissolved oxygen (DO) and Turbidity values between the five sampling sites in the Kat River through the study period (December 2015–November 2016)

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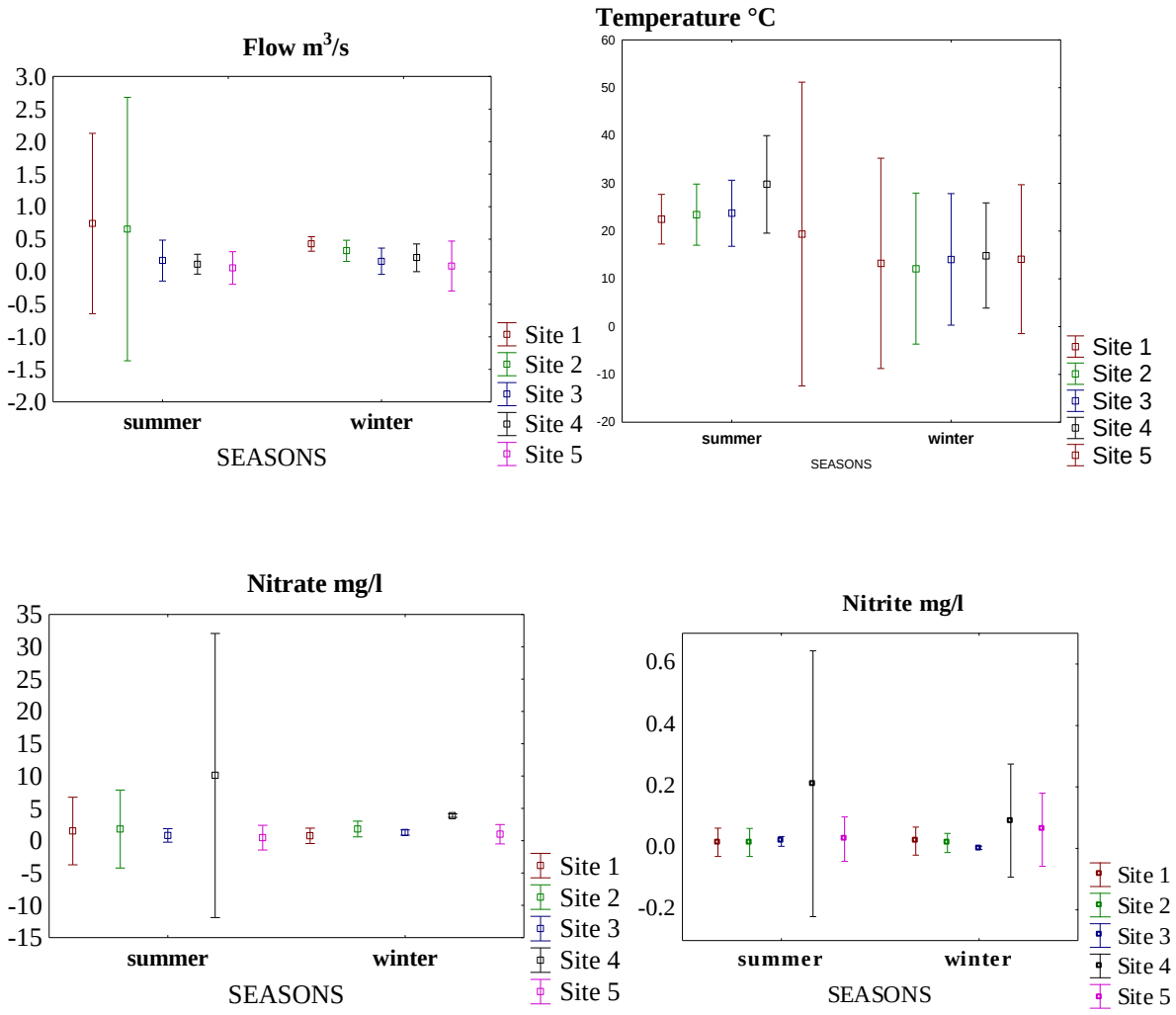


Figure 3.2: Seasonal variability of stream flow, temperature, nitrate (NO3) and nitrite (NO2) values between the five sampling sites in the Kat River over the study period (December 2015-November 2016).

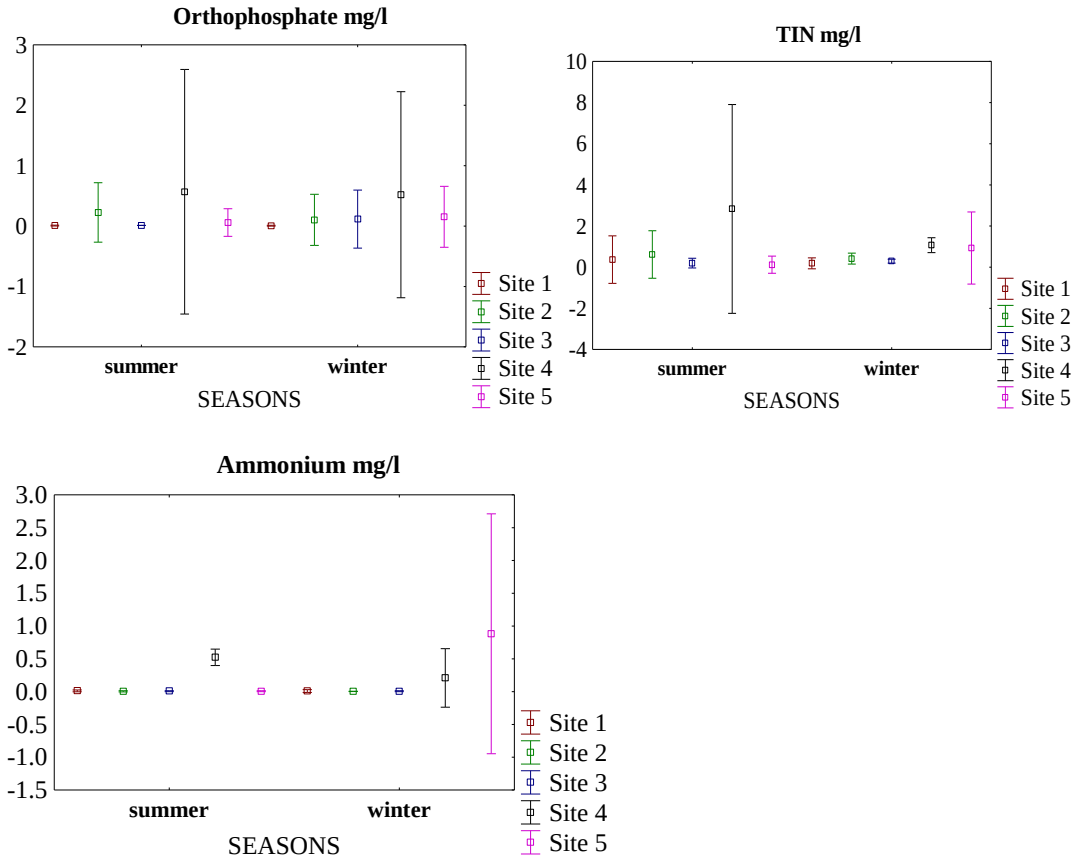
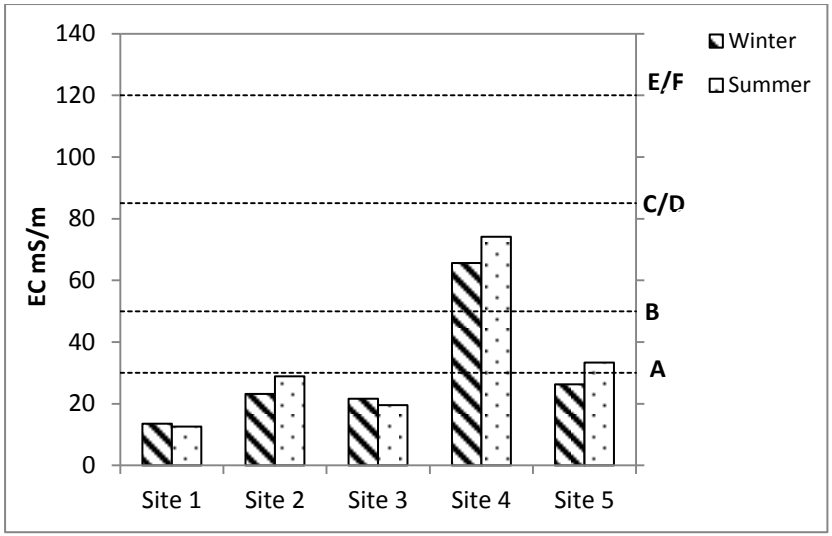


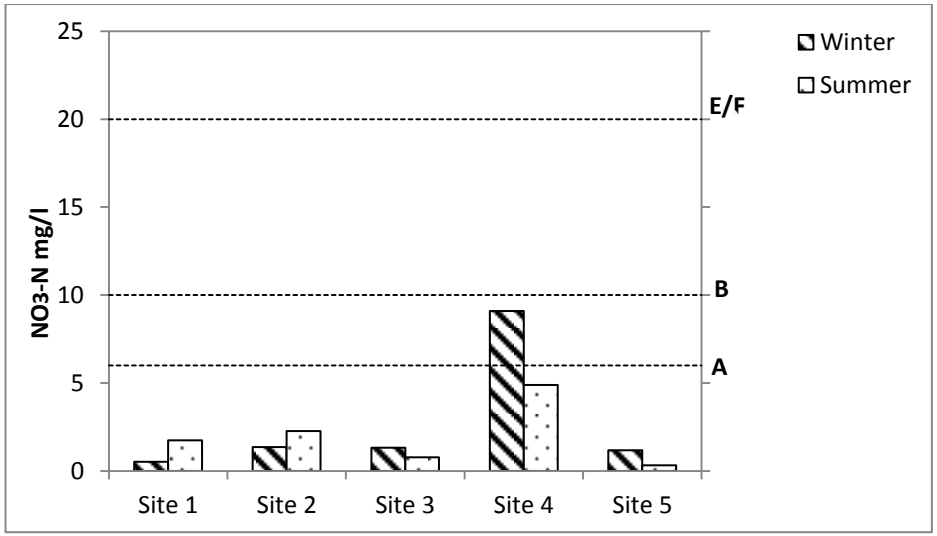
Figure 3.3: Seasonal variability of Orthophosphate (PO4), total inorganic Nitrogen (TIN) and Ammonium (NH4) values between the five sampling sites in the Kat River for the duration of the study period (December 2015– November 2016).

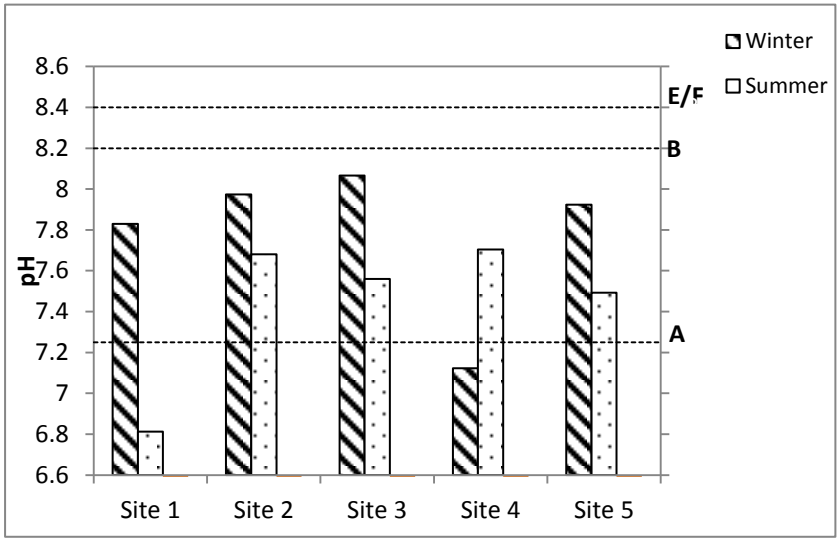
3.3.3 Comparison of the values of physicochemical variables with Resource Water Quality Objectives

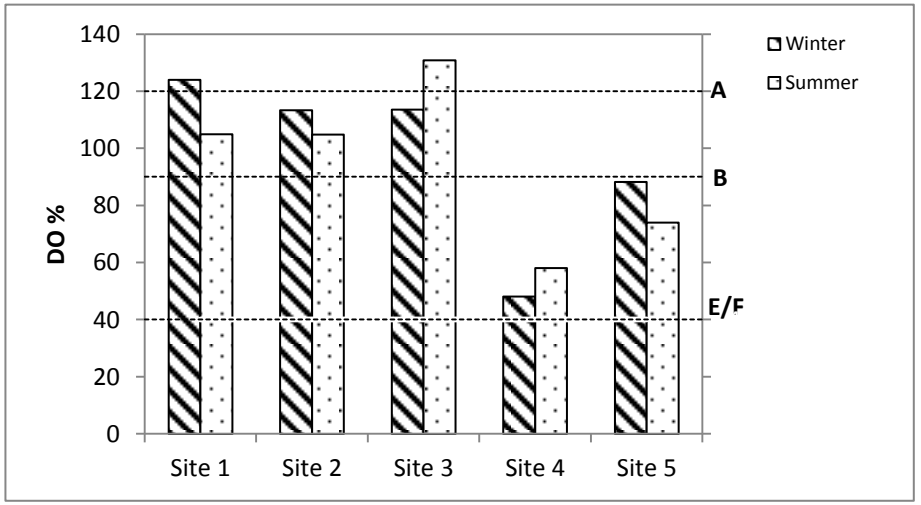
Physicochemical variables for the Kat River sampled through the study period of December 2015-November 2016 were compared to generic Resource Water Quality Objectives (RWQOs) physicochemical variables benchmark for ecological reserve determination for rivers in South Africa (Table 2.1, Chapter 2). According to the generic RWQOs for the selected physicochemical for the five sampling sites, pH results showed ecological category B for all sites (Sites 1, 2, 3, 4 and 5) (Figure 3.4). DO concentration for Sites 1 and 3 were natural with category A for summer and winter, Site 2 showed slightly modified with category B and Site 5 revealed seriously modified with category D for both seasons. DO concentration for Site 4 was critically modified with category E/F for summer and winter. TIN concentration revealed that Sites 1, 2 and 3 were natural with category A for both summer and winter.

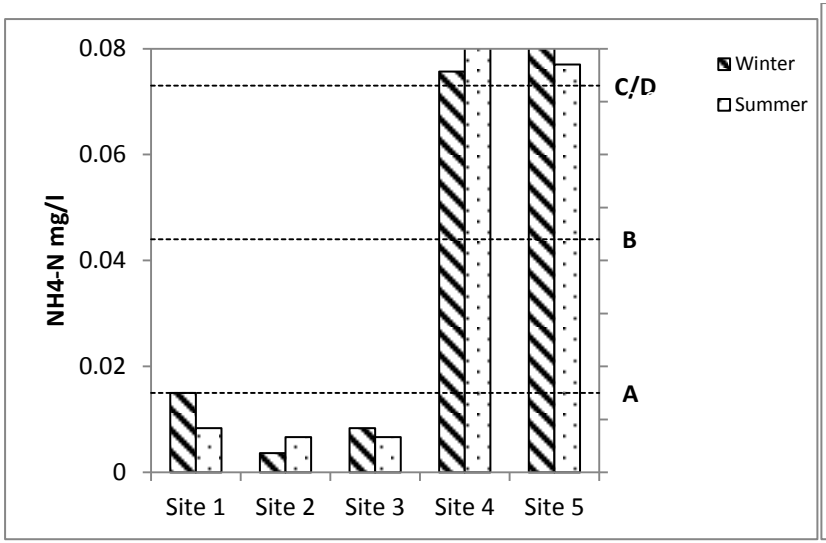
TIN results for Site 5 revealed category A in summer but dropped to category B with slightly modified in winter. Site 4 was critically modified with category C/D for winter and B for summer. Orthophosphate results for Site 1, 2 and 3 for both seasons had category A with the natural condition. Sites 5 orthophosphate results showed slightly moderate with category B for winter and moderately modified with category C for summer, while Site 4 recorded category E/F for both summer and winter with seriously modified. Electrical Conductivity (EC) for the five selected sites during the study period showed that Sites 1, 2 and 3 had EC concentrations within the Ideal limits that correspond with ecological category A, even though Site 2 seemed to have slightly high EC concentrations compared to the other two sites. At downstream, Site 4 had high EC concentration that was over the acceptable limit for both seasons with category C, while Site 5 recorded category A in winter with natural and B in summer with slightly modified ecological condition. Nitrate concentration results revealed that Sites 1, 2, 3 and 5 are natural with category A, Site 4 in summer were natural with category A, in winter moderately modified with category B. Ammonium concentration showed that Sites 1, 2 and 3 were natural with category A, downstream Sites 4 and 5 were critical modified with category E/F. These results indicate that upstream sites 1, 2 and 3 for the seven-selected physicochemical variable were within the generic ecological categories limits.

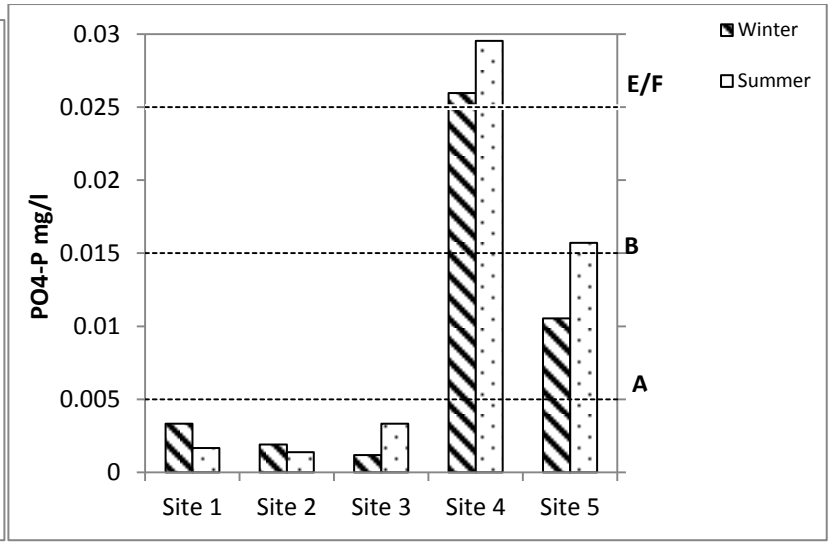

EC mS/m
140
120
100
80
60
40
20
0
E/F
C/D
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5


NO3-N mg/l
25
20
15
10
5
0
E/F
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5


pH
8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
6.8
6.6
E/F
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5


DO %
140
120
100
80
60
40
20
0
A
B
E/F
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5


0.08
0.06
0.04
0.02
0
NH4-N mg/l
C/D
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5


0.03
0.025
0.02
0.015
0.01
0.005
0
PO4-P mg/l
E/F
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5

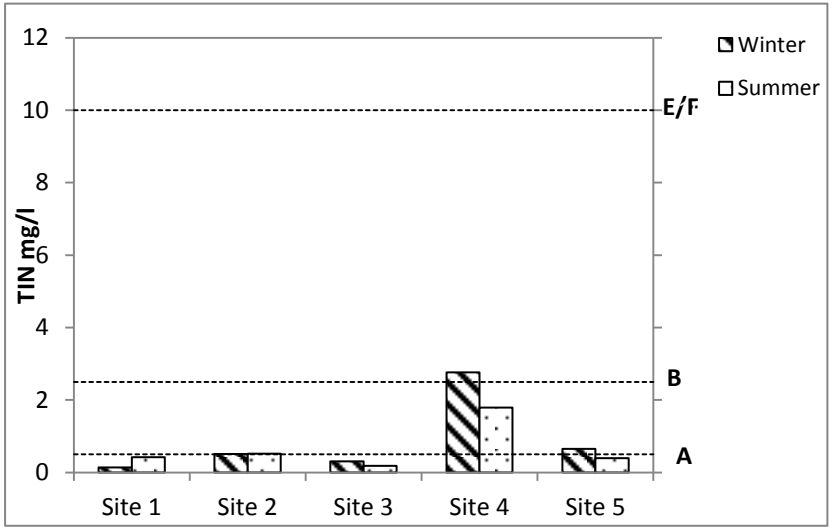

12
10
8
6
4
2
0
TIN mg/l
E/F
B
A
Winter
Summer
Site 1
Site 2
Site 3
Site 4
Site 5

Figure 3.4: Seasonal variability of electrical conductivity (EC), nitrate (NO3-N), pH, dissolved oxygen (DO), Ammonium (NH4-N), Orthophosphate (PO4-P) and total inorganic nitrogen (TIN) concentrations between the five sampling sites in the Kat River during the study period December 2015 –November 2016.

3.3.4 Biomonitoring river health using Macroinvertebrate Response Assessment Index

The river health condition of the Kat River was assessed using the South African Scoring System version 5 (SASS5). SASS5 data for each site during each assessment month included (SASSS Score, Number of Taxa and ASPT) represented on (Appendix A and B). Macroinvertebrate response assessment index (MIRAI) was used to analyse the data throughout the study period (December 2015 – November 2016). Unfortunately, no site was found good enough on the Kat River to serve as a reference site because of pollution. Therefore, a reference condition was constructed using a range of data sources, including those in the River Health Database, MIRAI's predicted reference taxa and other historical data sources. Tables 3.2-3.11 represent ecological categories descriptions for all the sampling sites for summer and winter. The tables also show scores which each site achieved for each metric for summer and winter based on Table 2.4 in Chapter 2. Site 1 for summer scored 65.7 and 64.9 for winter, which corresponded to category C with fair condition (Tables 3.2 and 3.3); Site 2 for summer scored 68.2 and drop in winter to 63.1, which corresponded to ecological category C with fair condition (Tables 3.4 and 3.5); Site 3 for summer scored 67.2 but dropped to 62.2 in winter, which corresponded to category C with fair condition for both seasons (Tables 3.6 and 3.7); Site 4 scored 37.5 in summer but dropped to 22.0 in winter scored, which reflected ecological category E with a critically modified condition (Tables 3.8 and 3.9); and Site 5 scored of 41.1 in summer, which corresponded to category D with poor condition, but it scored 32.3 in winter with ecological category E, representing a critically modified condition (Table 3.10 and 3.11). Once again, the results for both seasons showed that Kat River water quality was not in a natural condition and the results for all sites showed a decline in winter with an ecological category for Site 5 ranging from poor to critical. This may be caused by the load of anthropogenic pollution that ends up at this site.

Table 3.2: MIRAI for Site 1 data collected during summer. Note: Ecological category conditions A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	65.3	*0.345*	22.5088	1	100
Habitat	**H**	50.1	*0.310*	15.5433	2	90
Water Quality	**WQ**	80.1	*0.345*	27.6328	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				65.685		290
Invertebrate EC Category				**C**		

Table 3.3: MIRAI for Site 1 data collected during winter. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	65.3	*0.345*	22.5088	1	100
Habitat	**H**	49.3	*0.310*	15.2902	2	90
Water Quality	**WQ**	78.7	*0.345*	27.1491	1	100
Connectivity & Seasonality	**Cs**	100.0	*0.000*	0	3	0
Invertebrate EC				64.9481		290
Invertebrate EC Category				**C**		

Table 3.4: MIRAI for Site 2 data collected during summer: MIRAI for Site 2 data collected during summer. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	%Weight For Metric Group
Flow Modification	**FM**	63.6	*0.345*	21.9175	1	100
Habitat	**H**	67.5	*0.310*	20.961	2	90
Water Quality	**WQ**	73.5	*0.345*	25.3574	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
						290
Invertebrate EC				68.2359		
Invertebrate EC Category				**C**		

Table 3.5: MIRAI for Site 2 generated from SASS data collected during winter. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	%Weight For Metric Group
Flow Modification	**FM**	52.6	*0.345*	18.1488	1	100
Habitat	**H**	56.0	*0.310*	17.3651	2	90
Water Quality	**WQ**	80.1	*0.345*	27.6328	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
						290
Invertebrate EC				63.1467		
Invertebrate EC Category				**C**		

Table 3.6: MIRAI for Site 3 data collected during summer. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	%Weight For Metric Group
Flow Modification	**FM**	60.6	*0.345*	20.915	1	100
Habitat	**H**	67.5	*0.310*	20.961	2	90
Water Quality	**WQ**	73.5	*0.345*	25.354	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				67.235		290
Invertebrate EC Category				C		

Table 3.7: MIRAI for Site 3 data collected during winter. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	RANK OF METRIC GROUP	%WEIGHT FOR METRIC GROUP
Flow Modification	**FM**	54.2	*0.345*	18.6933	1	100
Habitat	**H**	54.4	*0.310*	16.8676	2	90
Water Quality	**WQ**	77.3	*0.345*	26.6542	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				62.2151		290
Invertebrate EC Category				C		

Table 3.8: MIRAI for Site 4 data collected during summer. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Of Score Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	44.3	*0.345*	15.2884	1	100
Habitat	**H**	32.9	*0.310*	10.2009	2	90
Water Quality	**WQ**	34.7	*0.345*	11.9782	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				37.4675		290
Invertebrate EC Category				**E**		

Table 3.9: MIRAI for Site 4 data collected in winter. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	15.3	*0.345*	5.26008	1	100
Habitat	**H**	17.8	*0.310*	5.53588	2	90
Water Quality	**WQ**	32.6	*0.345*	11.2444	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				22.0403		290
Invertebrate EC Category				**E**		

Table 3.10: MIRAI for Site 5 data collected during summer. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	50.6	*0.345*	17.4513	1	100
Habitat	**H**	35.1	*0.310*	10.9025	2	90
Water Quality	**WQ**	37.0	*0.345*	12.7495	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				41.1034		290
Invertebrate EC Category				**D**		

Table 3.11: MIRAI for Site 5 data collected during winter. Note: Ecological category A =natural, B =good, C =fair, D =poor, and E/F =critically modified.

Invertebrate EC Metric Group		Metric Group Calculated Score	Calculated Weight	Weighted Score Of Group	Rank Of Metric Group	% Weight For Metric Group
Flow Modification	**FM**	37.4	*0.345*	12.9013	1	100
Habitat	**H**	20.8	*0.310*	6.44112	2	90
Water Quality	**WQ**	37.7	*0.345*	12.9935	1	100
Connectivity & Seasonality	**CS**	100.0	*0.000*	0	3	0
Invertebrate EC				32.3359		290
Invertebrate EC Category				**E**		

3.3.5 Analyses of macroinvertebrate assemblages in the Kat River

Macroinvertebrates were sampled at the five sampling sites during the study period and analysed using Bray-Curtis to investigate the similarities and/or dissimilarities between sites and seasons. Macroinvertebrate communities were mostly cluster by sites (Figure 3.5). Cluster 1 was made of Sites 1 and 2 for both seasons. These sites are situated at the upstream of Kat River; they represent sites that mostly get impact from agricultural land. The highest Bray-Curtis similarity for this study showed 60% level of similarity between these sites and seasons. Cluster 2 consisted of Site 3 sampled in both summer and winter; this site also received impact from agriculture activities. Cluster 3 was made of samples collected from Site 4; this site was mostly impacted by the Fort Beaufort Wastewater Treatment Works. Cluster 4 was comprised of Site 5; this site received impact from all the other four sites (Figure 3.5).

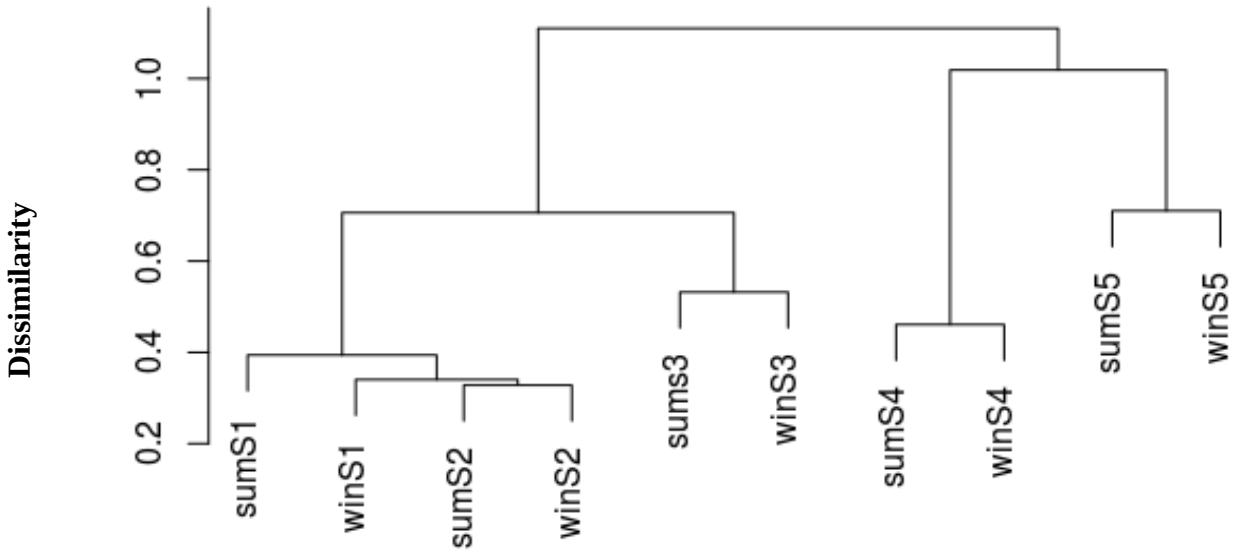


Figure 3.5: Bray – Curtis similarity dendrogram analysis of macroinvertebrate abundance sampled over summer and winter at the five sampling sites in the Kat River.

Y-axis represents proportions of dissimilarity between sampling sites and seasons. Abbreviation format: sites are represented by S =site, number = site number, (S1=site 1, S2=Site2, S3=Site 3, S4=Site4 and S5=Site5), seasons (sum=summer, win=winter).

3.3.6 Macroinvertebrate diversity indices

Boxplot with whiskers graphs was used to compare diversity indices results obtained during the study period. Selected diversity indices were Shannon, Simpson, Evenness and Margalef indices (Figure 3.6). The results revealed that Shannon index, Simpson index, Margalef index and Evenness were all low at Site 4, enabling the discrimination of Site 4 from other sites (i.e. Sites 1, 2, 3 and 5). Shannon and Margalef index results for Site 1, 2 and 3 and 5 were almost in the same range; Site 4 had the lowest values. Simpson index for Site 3 was the lowest; those of Sites 1, 2 and 5 were higher in the same range, while Site 4 index was higher than Site 3. Evenness results revealed that Sites 1, 3, 4 and 5 have the same value lower than that of Site 2(Figure 3.6). Simpson and Evenness indices could not discriminate between Site 4 and other sites (i.e. Sites 1, 2, 3 and 5). The fluctuating of diversity indices results for this study may be caused by the intense differences in the anthropogenic impacts received by this river. Based on the criteria in sub-section 2.5.1 in Chapter 2, three metrics; Margalef, Simpson and Evenness indices in the diversity category could not able the discrimination of downstream Sites 4 and 5 from Sites 1, 2 and 3.

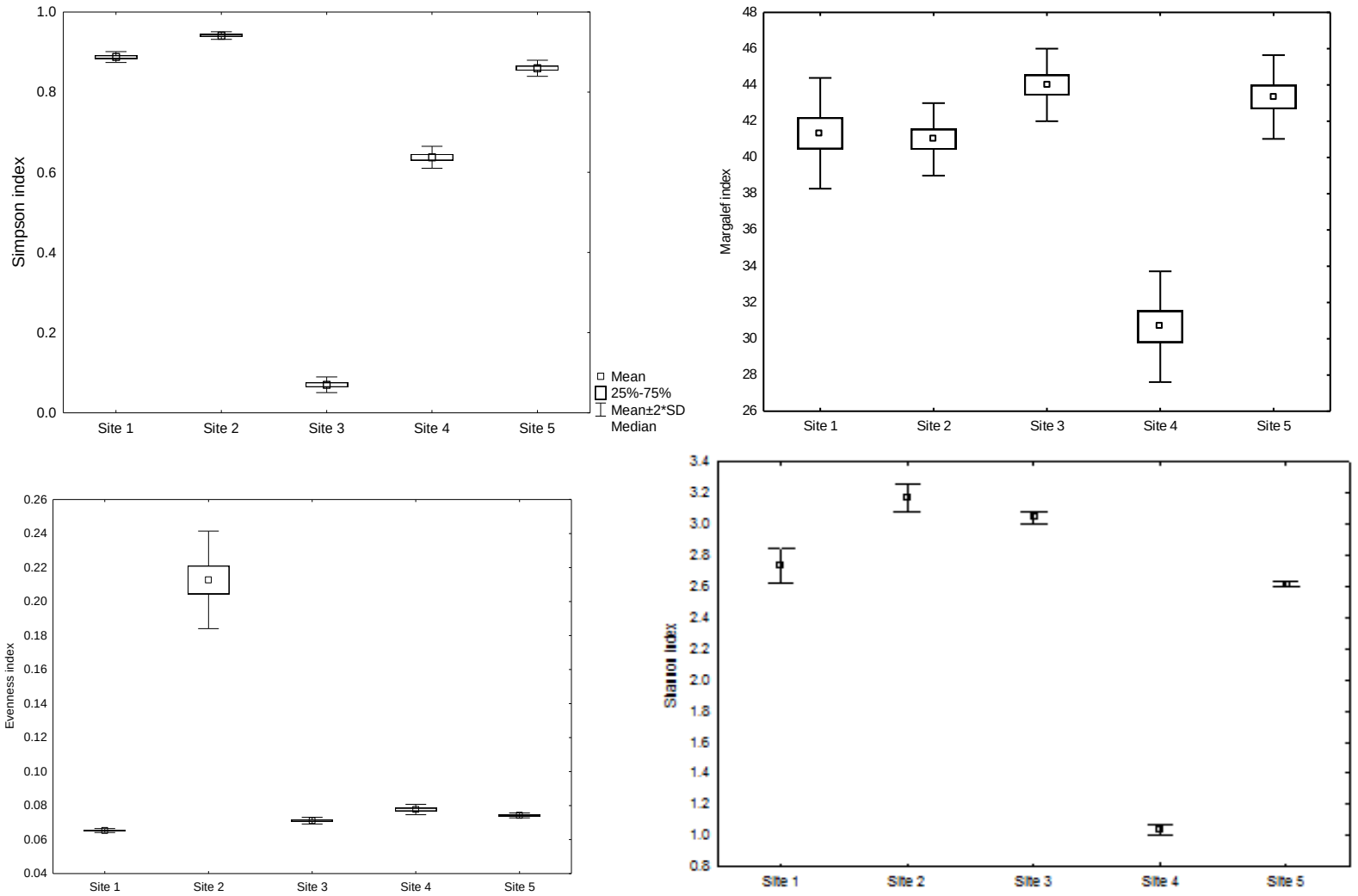


Figure 3.6: Box plot showing the median, 25-75 percentile distribution, non-outlier range for metrics in diversity category at the five sampling in the Kat River during sampling in summer.

The diversity indices used to test water quality changes in the sampling sites included Shannon index, Simpson index, Margalef index and Evenness (Figure 3.7). The selected macroinvertebrates-based indices indicated effects of deteriorating water quality on abundance in the Kat River. The results revealed that the recorded Simpson, Shannon and Margalef indices were all lower at Site 4, enabling the discrimination of Site 4 from sites (i.e. Sites 1, 2, 3 and 5). Evenness index results were lower at upstream sites compared to downstream sites, with Site 5 being the highest. Based on the criteria in sub-section 2.5.1 in Chapter 2, the three metrics; Shannon, Simpson and Margalef indices in the diversity category enabled discrimination of Site 4 from Sites 1, 2, 3 and 5. Only two metrics (Evenness index and Margalef index) enabled the discrimination of Site 4 and 5 from upstream sites (1, 2 and 3).

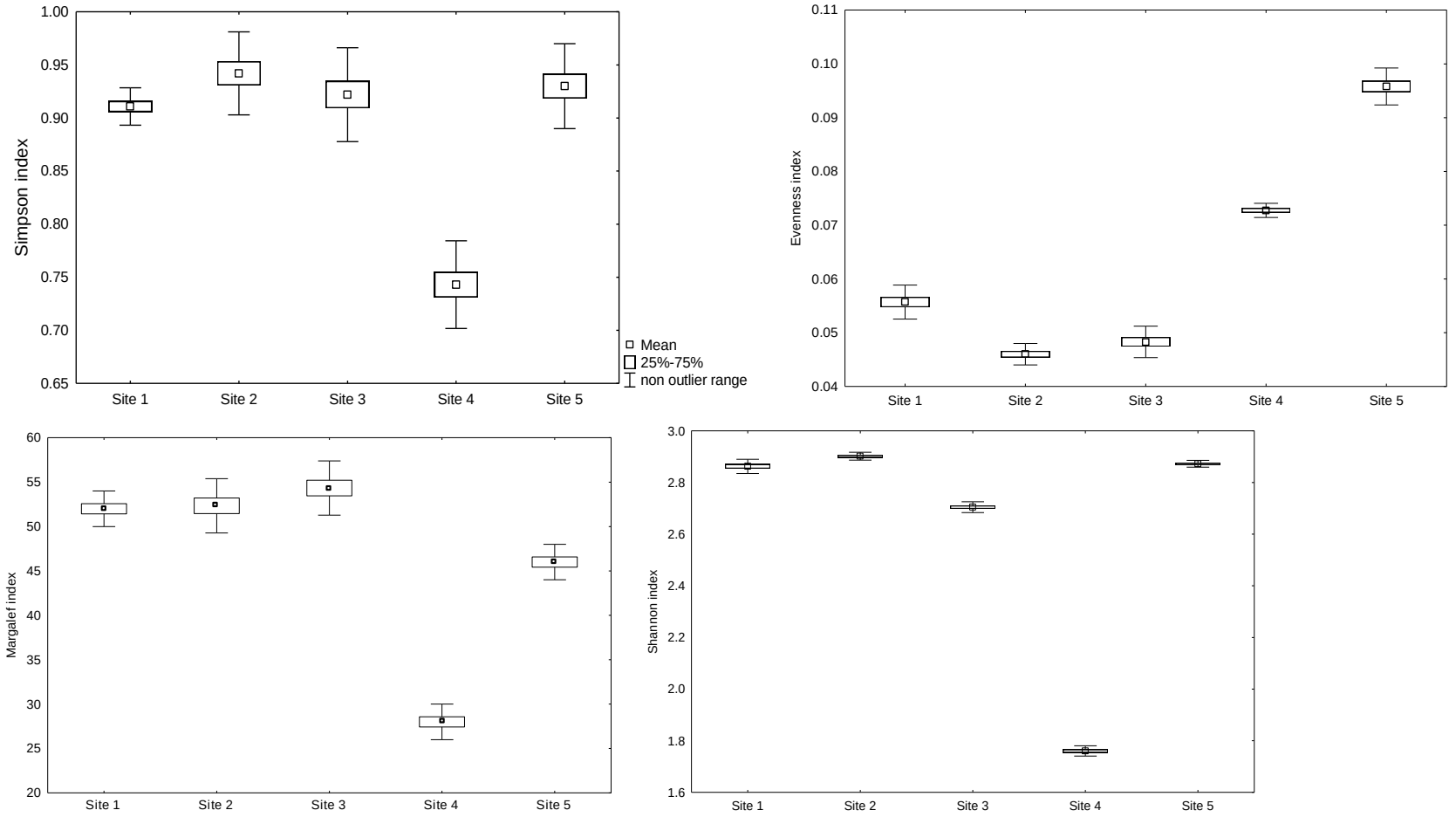


Figure 3.7: Box plot showing the median, 25-75 percentile distribution, non-outlier range for metrics in diversity category at the five sampling in the Kat River sampled in winter.

3.3.7 Relating macroinvertebrate assemblages and physicochemical variables

The CCA results for summer are presented in Figure 3.8. Nine variables were used for the final CCA ordination (i.e. pH, DO, EC, PO_4-P, turbidity, temperature and NO_3-N, NO_2-N and NH_4-N). The first axis of the CCA with Eigenvalue 0.617 explained 25.25 % of the total variance. The three axes of the ordination explained a combined cumulative variance of 48.15 % ordination between the families and the physicochemical variables (Table 3.12). The Monte-Carlo permutation test (100 permutations) revealed that the Eigenvalue of the first axis was significant. The Pearson's correlation test indicated strong correlations between the physicochemical variables and the macroinvertebrate families on the three CCA axes. The CCA ordination results indicated that high DO concentration and macroinvertebrate families such as Corbiculidae, Heptageniidae, Psephenidae, Leptophlebiidae, Baetidae and Perlidae were strongly positively correlated. These families were strongly negatively correlated with increased concentration of EC, PO_4-P, NO_3-N, NO_2-N, NH_4-N, temperature and pH. Increase in EC is strongly positively correlated with macroinvertebrate families such as Hydrophilidae, Chironomidae and Muscidae. Increase in pH value positively correlated with families such as Belostomatidae, Notonectidae, Lymnaeidae, Gerridae and Leptoceridae. These later families were less abundant at sites with a high concentration of DO (i.e., Sites 1, 2 and 3). Macroinvertebrates families such as Oligochaeta, Psychodidae, Culicidae and Hirudinea were correlated with an increase in EC and PO_4-P. Increase in NO_3-N, NO_2-N and NH_4-N concentrations was positively correlated with macroinvertebrate families such as Corixidae. Increase in turbidity positively correlated with macroinvertebrate families such as Nepidae, Chlorolestidae, Naucoridae and hydraenidae (Figure 3.8). Of the nine physicochemical variables included in the CCA, correlations between temperature, PO4-P and the macroinvertebrate families were less strong compared with other variables (Table 3.12).

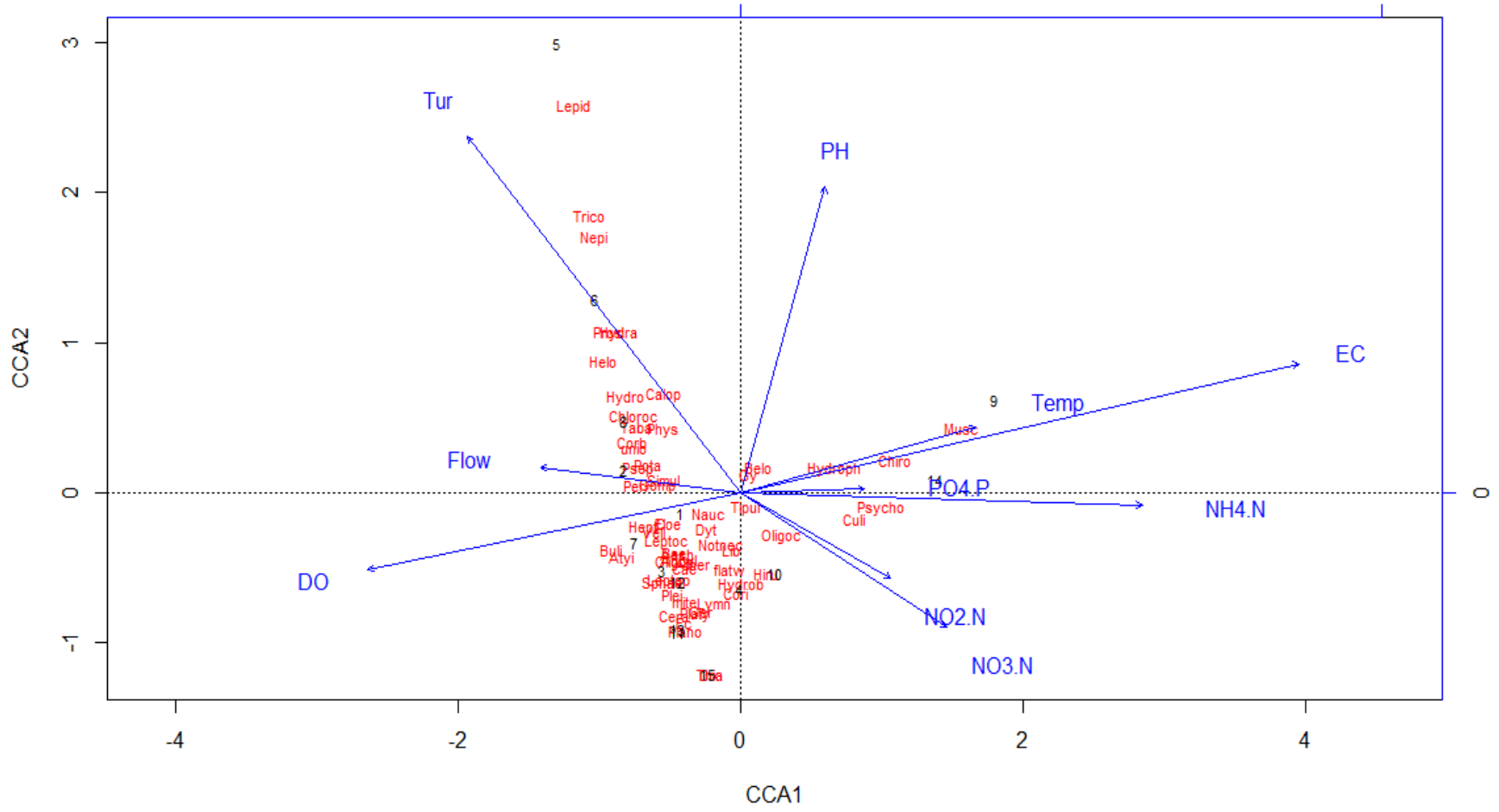


Figure 3.8: Canonical correspondence analysis (CCA) for macroinvertebrate communities and physicochemical variables at five sampling sites at Kat River for summer.

Families' abbreviations: Culi-Culicidae, Syph- Syphidae, Simul- Simuliidae, Musc- Muscidae, Chiro- Chironomidae, Cera- Ceratopogonidae, Helo- Helodidae, Hydroph- Hydrophilidae, Dyt-Dytiscidae, Hydra- Hydraenidae, Gy- Gyrinidae, El- Elmidae, Ph- Philopotamidae, Pi-Pisuliidae, Ec –Ecnomidae, Leptoc- Leptoceridae, Hydrops- Hydropsychidae >2sp, Chl-Chlorolestidae, Pl –Platycnemidae, Go- Gomphidae, Co- Coenagrionidae, Li- Libellulidae, Ae-Aeshnidae, Ca- Caenidae, Leptop- Leptophlebiidae, Bae- Baetidae>2sp, Ta- Tabanidae, Ath-Atheridae, Tip- Tipulidae, Syrp-Syrphidae, Di- Dixidae, Ps- Psychodidae, Po- Potamonautidae, Co- Corixidae, Na- Naucoridae, Notonec- Notonectidae, Ve- Veliidae, Ge- Gerridae, Anc-Ancylidae, Phy- Physidae, Lym- Lymnaeidae, Not –Notonemouridae, Tu- Turbellaria, Mi- water mites, Oli- Oligochaeta, Hi- Hirudinea, Pl- Planorbinae, Thi- Thiaridae.Sites and sampling period abbreviations: S= site, number before site = sampling period, number after site are site numbers

Table 3.12: Canonical correspondence analysis (CCA) properties for the correlation between macroinvertebrate and physicochemical variables in the Kat River

CCA properties	**Axis**		
	1	2	3
Canonical Eigen value	0.431	0.279	0.221
Percent variance explained	25.251	13.590	9.313
Percent cumulative variance explained	10.412	7.221	5.653
Pearson correlation of families and environmental Scores	0.019	0.053	0.189

Similarly, CCA ordination tri-plot was used to elucidate the correlations between the measured water quality variables and the macroinvertebrate families in the Kat River recorded in winter for this study. The CCA results are presented in Figure 3.9. Nine variables were used for the final CCA ordination (i.e. pH, DO, EC, PO4-P, turbidity, temperature and NO3-N, NO2-N and NH4-N). The first axis of the CCA with Eigenvalue 0.414 explained 25.97 % of the total variance. The three axes of the ordination explained a combined cumulative variance of 49.53 % between the macroinvertebrates and water physicochemical variables (Table 3.13). The Monte-Carlo permutation test (100 permutations) revealed that the Eigenvalue of the first axis was significant. The Pearson's correlation test indicated slightly strong correlations between the water physicochemical variables and the macroinvertebrate families on the three CCA axes.

The CCA ordination results indicated that increase in DO concentration and macroinvertebrate families such as Baetidae, Heptageniidae were strongly positively correlated. Increase in pH value positively correlated with families such as Gyrinidae and Leptoceridae. Families such as Oligochaeta, Psychodidae, Culicidae and Hirudinea were correlated with an increase in EC and PO4-P. Increase in NO_3-N, NO_2-N and NH_4-N concentration was positively correlated with macroinvertebrate families such as Coenagrionidae and Chironomidae. Increase in turbidity positively correlated with macroinvertebrate families Physidae, Libellulidae and Notonectidae (Figure 3.8).

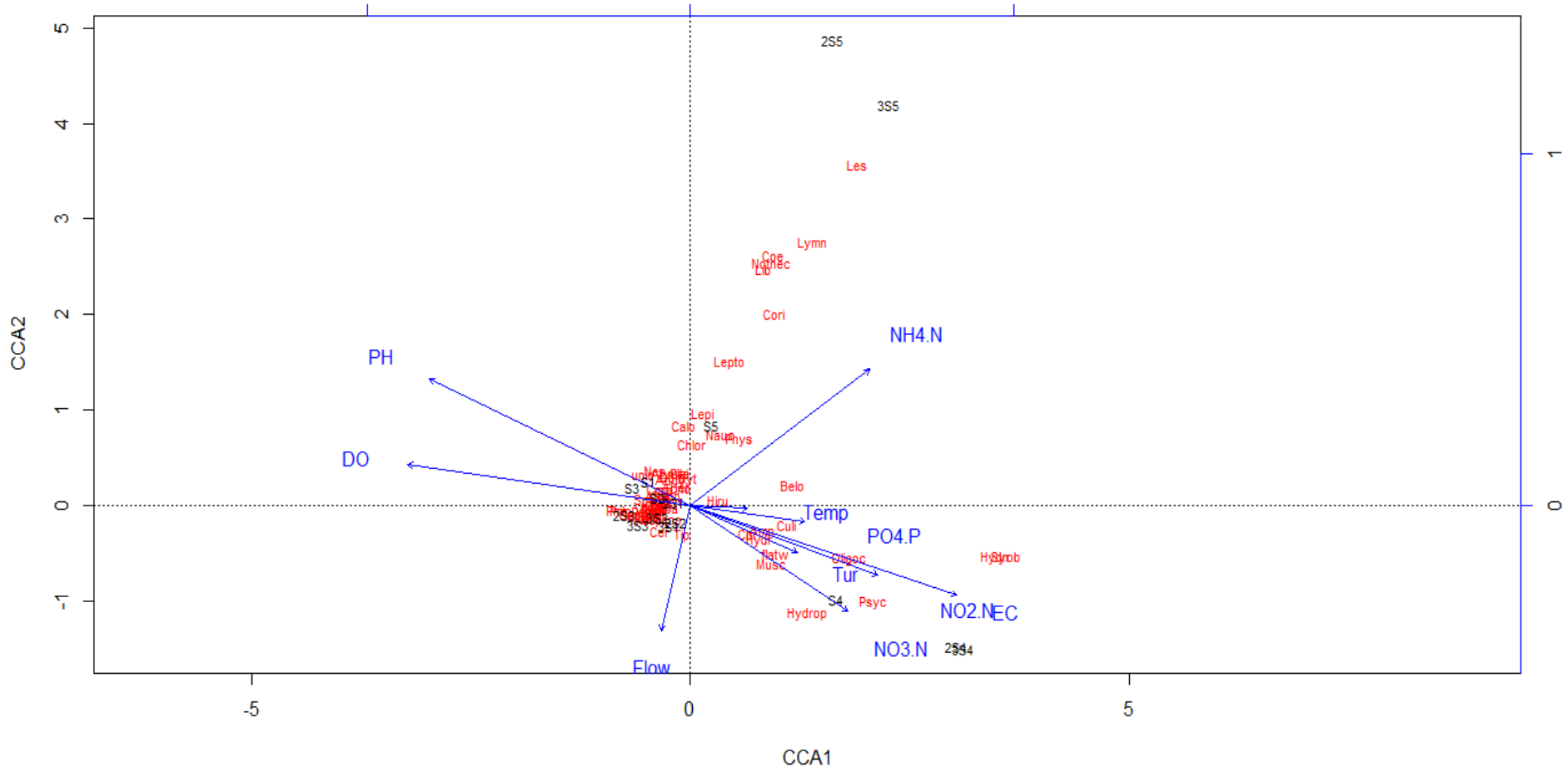


Figure 3.9: Canonical correspondence analysis (CCA) for macroinvertebrate communities and physicochemical variables at five sampling sites at Kat River for winter.

Families' abbreviations: Culi-Culicidae, Syph- Syphidae, Simul- c- Muscidae, Chiro- Chironomidae, Cera- Ceratopogonidae, Helo- Helodidae, Hydroph- Hydrophilidae, Dyt- Dytiscidae, Hydra- Hydraenidae, Gy- Gyrinidae, El- Elmidae, Ph- Philopotamidae, Pi- Pisuliidae, Ec –Ecnomidae, Leptoc- Leptoceridae, Hydrops- Hydropsychidae >2sp, Chl- Chlorolestidae, Pl –Platycnemidae, Go- Gomphidae, Co- Coenagrionidae, Li- Libellulidae, Ae- Aeshnidae, Ca- Caenidae, Leptop- Leptophlebiidae, Bae- Baetidae>2sp, Ta- Tabanidae, Ath- Atheridae, Tip- Tipulidae, Syrp-Syrphidae, Di- Dixidae, Ps- Psychodidae, Po- Potamounatidae, Co- Corixidae, Na- Naucoridae, Notonec- Notonectidae, Ve- Veliidae, Ge- Gerridae, Anc- Ancylidae, Phy- Physidae, Lym- Lymnaeidae, Not –Notonemouridae, Fl- flatworms, Mi- water mites, Oli- Oligochaeta, Hi- Hirudinea, Pl- Planorbinae, Thi- Thiaridae.Sites and sampling period abbreviations: S= site, number before site = sampling period, number after site are site numbers.

Table 3.13: Canonical correspondence analysis (CCA) properties for the correlation between macroinvertebrate and physicochemical variables in the Kat River

CCA properties	**Axis**		
	1	2	3
Canonical Eigen value	0.724	0.501	0.429
Percent variance explained	25.97	13.52	10.04
Percent cumulative variance explained	18.62	5.64	4.25
Pearson correlation of families and environmental Scores	0.087	0.327	0.393

3.3.8 Integrated Habitat Assessment System

Results of the Integrated Habitat Assessment System (IHAS) of the Kat River obtained during the study period (December 2015- November 2016) showed marginal differences between Sites 4 and 5 (Figure 3.10). The IHAS results for Sites 1, 2 and 3 were also similar. Sites 1 and 3 had the highest overall mean IHAS value compared to the other sites. The IHAS values were generally lower in winter compared to summer (Figure 3.10).

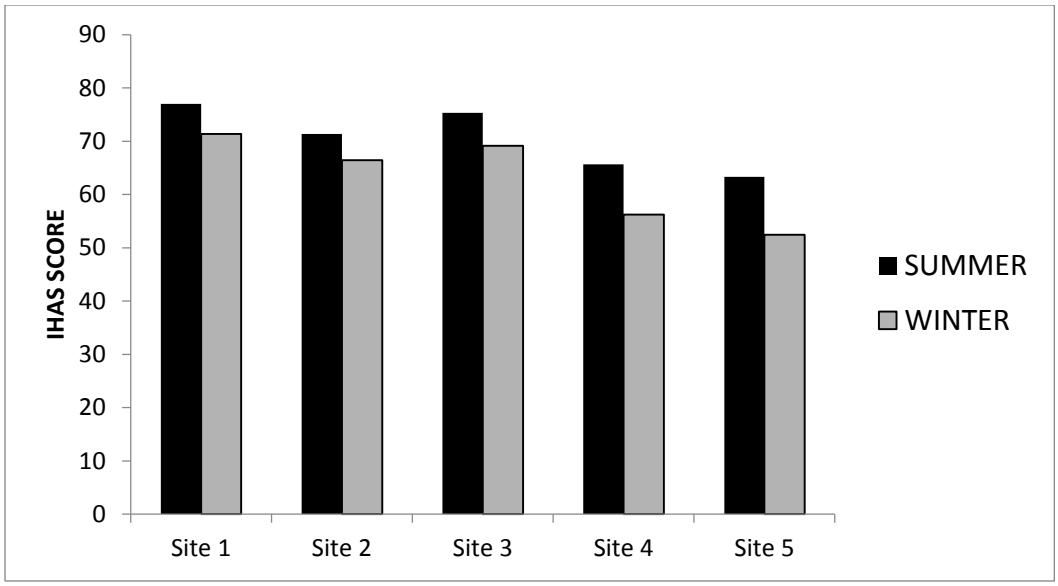


Figure 3.10: Integrated Habitat Assessment System (IHAS) scores at the five sampling sites in the Kat River through the study period (December 2015- November 2016)

3.4 Discussion

3.4.1 Status of the physicochemical variables of the Kat River

Non-point and point source impacts from agricultural lands, run-offs from informal settlements, and wastewater effluent discharges from the Kat River WWTW are the main contributors to the observed poor water quality in Kat River, particularly at the downstream sites (Lerotholi et al., 2004). Physicochemical variables measured during the study period revealed an evidence of deteriorating water quality, especially with increase in EC, nutrients and low DO values at sites that receive impact from farmlands, informal settlement and WWTWs. Physicochemical variables are the main factors that control structure of aquatic ecosystem (Hulyal and Kaliwal, 2009). Changes in water physicochemical variables have an effect on aquatic organisms that live in the river (Ramesh et al 2015). In this study, the impacts of most of the physicochemical variables were higher in summer compared to winter, especially at downstream sites (4 and 5) (Figure 3.1-3.3). The pH value for Kat river upstream Sites 1, 2 and 3 ranged between 6.1-8.0 and downstream Sites 4 and 5 ranged 6.9-7.8 which are an acceptable condition, aquatic organisms become stressed when pH of water is too low or too high (Tucker and D'Abramo, 2008).

The pH of Kat River was within the acceptable limit of the generic ecological category benchmarks. Therefore, pH did not have an impact on the Kat River ecosystem. This outcome supports a previous study by Lerotholi et al (2004) at the Kat River whereby pH values were also found to be within an acceptable limit. The pH concentrations for all sites were found to be above 7 in winter. It has been reported that increase in water salinity had a slightly small impact on pH depending on which salts are in solution (Sereshti and Aliakbarzadeh, 2013).

Dissolved oxygen is important for aquatic life (Odume and Mgaba, 2016). In this study, dissolved oxygen (DO) for upstream Sites 1, 2 and 3 ranged between 6.1-11 mg/L, while downstream ranged between 2.6-6.9 mg/L. Site 3 had the highest DO concentration of 11 mg/L for both summer and winter, while Site 4 had the lowest concentration of 3.5 mg/L at winter. In a previous study by Lerotholi (2005), DO range between 5-11mg/L, but the minimum for this current study was even lower. This means that the DO concentration fell since the study by Lerotholi (2005). Decreased DO concentration at downstream sites indicates increased pollution loads. These results agree with Odume and Mgaba (2016) who reported that upstream sites on the Kowie River, located few kilometres from the Kat River, had high DO concentrations but low at downstream impacted sites. There are several factors that can affect DO such as high temperature, increase pressure and water salinity. Also, runoffs from informal settlements and wastewater can result in low DO values (Odume and Mgaba, 2016). All these factors played a role in the decreasing DO at the downstream sites.

Electrical Conductivity (EC) concentration of upstream Sites 1, 2 and 3 ranged between 10.9 – 26 mS/m, while downstream Sites 4 and 5 ranged 16.9-65.5 mS/m. The highest concentration value was recorded at Site 4 as 95.9 mS/m in summer, while the lowest concentration was 10.9 mS/m in summer at Site 1. Compared to previous studies done on the Kowie River and Buffalo River, the present EC of the Kat River was low (Maseti, 2005; Dalu and Fronman, 2016; Odume and Mgaba, 2016). Compared to these studies, Kat River EC concentration was shown to be moderately impacted. Work done by Soviti (2002) on the Kat River reported EC values in the same range as this study. Increase or decrease in EC of freshwater indicates pollution impact. Moss (2008) intimated that sewage leaks and agricultural runoffs increase the concentration of EC by adding nitrates and phosphates ions into the waterbody.

Kat River is closely situated to agricultural farmlands, it is, therefore, highly possible that the river receives agriculture runoffs. Thus, wastewater treatment works and agricultural farmland are possibly the main sources for human impact in this catchment. It could be suggested that the high EC concentration recorded at Site 4 could be attributed to the sewage leaks and inadequate treatment of wastewater by the Fort Beaufort WWTW.

Total Inorganic Nitrogen (TIN) concentration for upstream Sites 1, 2, and 3 ranged between 0.07-1.06 mg/L, while that for downstream sites ranged from 0.02-4.95 mg/L. The highest concentration was recorded at Site 4 (4.95 mg/L) in summer, while the lowest concentration was recorded at Site 5 (0.02 mg/L) in summer. The high TIN concentration at Site 4 could be due to sewage leakages, inadequate treatment of wastewater by the Fort Beaufort WWTWs and runoffs from informal settlements. Odume and Mgaba (2016) previously reported that runoffs from farms, informal settlements and effluent discharge from WWTWs are among the major sources that contribute to increasing nutrients values in South African streams. Abdel-Raoef et al (2012) and Wassie (2008) have both indicated that TIN concentration greater than 0.3 mg/L could result in abundant algal growth. In this study, the recorded TIN concentrations showed that upstream sites had good water quality status, while downstream sites had acceptable water quality condition.

Streamflow can be affected by anthropogenic impact such as nutrients, increase in salts loads and changes in the hydrology of the stream (Bates et al., 2002). The stream flow recorded over the study period showed that upstream Sites 1, 2 and 3 ranged between 0.15-0.58 m^3/s, while downstream sites ranged between 0-0.31 m^3/s. The highest stream flow was recorded at Site 3 (0.58m^3/s), while the lowest was recorded at Site 5 (0 m^3/s) in summer. Flow regulation is one of the features ascribed to salinisation (Lerotholi, 2005). Flow has an effect on water quality, whereby low flow leads to high concentration of chemicals but high flow can provide high dilution effect, resulting in lower chemical concentration (Palmer et al., 2004). The average streamflow results in this study were in an agreement with those reported by Dalu et al (2014) for the Kowie River. Previous research results for stream flow of Kat River ranged between 0.01-0.12 m^3/s in summer and 0.01-0.30 m^3/s in winter (Lerotholi, 2005). For both summer and winter, stream flow results for this study improved compared to Lerotholi (2005).

Overall, physicochemical variables measured through the study period showed that the Kat River water quality is deteriorating with high concentrations of EC, turbidity, TIN and low DO for sites situated close to the informal settlement and WWTW (i.e. Sites 4 and 5) for both seasons. The results showed evidence of pollution at the sampling sites when compared with ecological reserve determination benchmarks (DWA, 2007; Roussow, 2011). Generally, the results appeared to represent fair conditions at Sites 1, 2 and 3 with high DO and low EC, TIN, PO_4-P and turbidity (Figure 3.1-3.4). Site 4 had a DO, TIN, PO_4-P result which represents largely modified condition for both summer and winter (Figure 3.4). TIN and PO_4-P concentrations at Sites 4 and 5 for both summer and winter showed a condition above the generic ecological benchmarks (Table 2.1, Chapter 2). Site 2 was surrounded by citrus and livestock farming activities. It was a site close to an informal settlement. As such, traditional rituals and cultural activities were performed there on regular basis. All these factors might be responsible for the increased in the TIN and EC concentrations found over the sampling period. Site 5 is situated in the Great Fish River game reserve and downstream of all the other four sites. As such, it had impacts from all the upstream sites.

In terms of salinity, the findings suggest that the Kat River receives salts from different sources, including wastewater effluent, untreated sewage, runoffs from the informal settlement and agricultural lands. Through the monitoring period, increasing levels of EC, which represent high salinity, was measured at Sites 4 and 5. Site 4, for both seasons recorded high salinity that could be caused by combined effects from the inadequate treatment wastewater from Fort Beaufort WWTWs, leaking sewage pipes and runoffs from the surrounding informal settlements. For Site 5, salinity increased in summer and could be attributed to the low stream flow experienced at this site. This observation is in agreement with Bates et al. (2002) who reported that change in stream hydrology, especially the flow regime, impact salinity.

The findings from this study also suggest that citrus farming in the Kat River catchment had little impact on salinity, which agrees with a previous study by Lerotholi (2005). However, Site 2 which is located between sites 1 and 3 showed an increase in salinity concentration compared to sites 1 and 3. These results could be attributed to impact from small scale farmers, informal settlement and geology of the site.

3.4.2 Ecological health assessment of the Kat River

In this study, the Macroinvertebrate Response Assessment Index (MIRAI) model was used to compute macroinvertebrates community assemblage data to assess present ecological category status for the Kat River. MIRAI results revealed that the Kat River water quality was largely impacted. The samples results showed that upstream Sites (1, 2 and 3) were impacted approximately equally by anthropogenic impacts. Out of the five sites, Sites 1, 2 and 3 achieved ecological category C, which corresponds to fair ecological condition for both summer and winter. These sites were situated nearby farmlands and sensitive macroinvertebrate families such as Baetidae, Leptophlebiidae and Notonemouridae and Perlidae were abundant in these sites. According to Clunie et al (2002) and Dunlop et al (2005), these families are sensitive to increase in salinity, which might account for their presence due to unimpacted water quality. MIRAI results showed that Site 4 water quality was largely impacted, resulting in an ecological category E with critical ecological condition for both seasons. This site was situated close to the Fort Beaufort WWTW and received untreated sewage leakages and run-offs from informal settlements. Dominant taxa found at Site 4 were Chironomidae, Simuliidae, Ceratopogonidae, Muscidae and Culicidae, which are all relatively tolerant to salinity. Previous studies by Wassie (2008), Odume and Mgaba (2016) and Sirisinthuwanich et al. (2017) reported that these families are highly tolerant to water quality stresses. That might be the reason for the disappearance of sensitive families in this site and left with only tolerant families. The MIRAI results for Site 5 revealed that Kat River water quality is improving slightly. Site 5 achieved ecological category D, corresponding to poor ecological condition in summer and ecological category E with critical ecological condition in winter.

The slight improvement of the ecological condition in summer of Site 5 is possibly resulting of water from the tributaries that join the Kat River after Site 4 and before Site 5. Similar observations were made by Odume and Mgaba (2016) who worked in the Bloukrans River (also located some few kilometres from the Kat River).

3.4.3 Macroinvertebrates assemblage response to physicochemical variables based on multivariate analysis

Canonical correspondence analysis (CCA) was used to evaluate the relationship between the measured physicochemical variables and macroinvertebrate data among the five selected sites. At Sites 4 and 5, some of the macroinvertebrates that were found in the upstream sites disappeared or their abundances reduced. The abundance of macroinvertebrate families declined in winter for all five sites. CCA results showed that increased EC, nutrients correlated positively with tolerant macroinvertebrates such as Chironomidae, Culicidae, Planorbinae, Naucoridae and Turbellaria. Conversely, less tolerant families such as Perlidae, Oligoneuridae, Leptophlebiidae and Notonemouridae correlated positively with high DO in the present study. Baetidae, Leptophlebiidae and Notonemouridae showed high preference to high DO and better habitat (Rawi et al., 2014). For the freshwater macroinvertebrate families, they obtain oxygen by using dissolved oxygen in the water. Thus, a decrease in oxygen concentration affects the diversity and abundance of the families. Accordingly, in this study, most sensitive families were found at upstream Sites 1, 2 and 3, whereby the DO concentrations were high. When EC concentration is high, abundance and biodiversity of sensitive families decline or disappear (Dunlop, 2005). Site 4 EC concentrations were high compared to all other sites and most sensitive families Perlidae, Leptophlebiidae and Notonemouridae where were not found on this site. The most abundant macroinvertebrates found at Site 4 were Chironomidae and Oligochaeta. Elias et al. (2014) suggested that Chironomidae and Oligochaeta can be unaffected by high organic pollution and low DO concentration because they have high haemoglobin affinity for oxygen and this may account for their large numbers at Site 4.

Increase in human land use along the river continued to worsen the water quality and caused changes in macroinvertebrates composition, from sensitive families such as Heptageniidae, Perlidae, Baetidae and Leptophlebiidae to tolerant families such as Ceratopogonidae, Oligochaeta, Chironomidae and Muscidae. Although seasonal variation can cause a catastrophic shift and washed away macroinvertebrate assemblages, anthropogenic impact contributes to water quality degradation (Peter and Maybeck, 2000). Results of this study compared to the

previous study in the Kat River by Lerotholi et al (2004) and Soviti (2002) showed that macroinvertebrate assemblage has declined drastically at the downstream sites.

In terms of salinity, macroinvertebrates are known to have different sensitivity and their response to salinity will differ due to the level of tolerance (Dunlop et al., 2005). James et al, (2003) and Kefford (2016) pointed out that leeches, Oligochaeta and flatworms can be tolerant to elevated salinity and other pollutants levels. These families were abundant at Site 4 that had high salinity and other pollutants. Increase in salinity can result in loss of biodiversity and poor water quality of the water resource (Land and Water Australia, 2002). Some authors have reported that macroinvertebrates are tolerant to increased salinity up to 1000 mg/L (Nielson et al., 2003). For this study, none of the sites and seasons experienced salinity that was higher than 1000 mg/L. The highest salinity recorded was 620.75 mg/L at Site 4. According to Nielson et al (2003), macroinvertebrates are less affected by salinity at this level of concentration. However, macroinvertebrates such as Leptophlebiidae and Notonemouridae Perlidae and Tricorythidae were not present at Site 4, probably because these families are sensitive to even a minor increase in salinity and other pollutants (Hart et al, 1991). Macroinvertebrates such as Baetidae, Leptophlebiidae, Notonemouridae and Perlidae were abundant at Site 3. Physidae, Lymnaidae and Planobinae were abundant at Site 1. According to Clunie et al (2002) and Dunlop et al (2005), these families are not sensitive to increase in pollution and salinity, which might account for their presence in Site 1 that recorded low EC levels. Dominated families found at Site 4 were Chironomidae, Simuliidae, Ceratopogonidae, Muscidae, Culicidae and Dytiscidae, which are all relatively tolerant to salinity.

3.5 Conclusion

The study found anthropogenic impacts such as farming activities, the Fort Beaufort WWTWs, rubbish dumping and runoffs from informal settlements to be the main contributors to the observed poor water quality and macroinvertebrate diversity in Kat River. The studies also found the downstream sites to be most impacted and therefore showing poorer river health conditions.

In terms of seasonal variation, the anthropogenic impact on the Kat River's water quality and macroinvertebrate communities as found to be worse in winter than in summer. Furthermore, the entire country was experiencing a very severe drought during the sampling period, which also affected the Kat River and its catchment. Droughts have been reported to influence the water quality, habitat and hydrology of river (Chang et al., 2017). Therefore, it was likely that the drought situation impacted significantly this study in the Kat River.

CHAPTER 4: DIATOM ASSEMBLAGE STRUCTURE IN RESPONSE TO WATER QUALITY IN THE KAT RIVER

4.1 Introduction

This chapter starts with a brief recap of the materials and methods employed in using diatoms for biomonitoring in the Kat River. Chapter 2, which was on materials and methods, contains details of the diatom-based biomonitoring conducted to assess impacts of anthropogenic activities on the water quality of the Kat River. After the brief recap of the materials and methods, results of the diatom biomonitoring and water quality assessments are presented. Results of abundance and diversity of diatoms at each of the five sampling sites were used to assess the ecological health of the river. This was followed by indicator species analysis of the diatom community at all sites. For water quality assessment, physicochemical variables in relation to diatoms assemblage for the five sampling sites are presented. The river's ecological health and water quality assessments are then discussed vis-à-vis appropriate literature and conclusions drawn from the discussions.

4.2 A recap of materials and methods

Physicochemical data collection was explained in details under materials and methods in Chapter 2. For species abundance, total number of sampled diatom species were grouped as either being abundant (A) (i.e., a species that forms over 40% of the total number of sampled species), common (C) (i.e., a species that form less than 40% but greater than 20% of the total number of sampled species), occasional (O) (i.e. a species that form less than 20% but greater than 10% of the total number of sampled species) or rare (R) (i.e., a species that form less than 10% of the total number of sampled species) (Table 4.1). Diatoms biological data were transformed using square root transformation. These groupings were based on pre-analysis of the data and researcher's best own judgement. Boxplot analysis was employed to assess discrimination between sites and diatoms species for summer and winter. Indicator species analysis was employed in this study, using package "Indicspecies" in R. This analysis helps to determine the occurrence or abundance of indicator species and reflect environmental conditions for each group of selected sites. Indicspecies chooses a species that is exclusively faithful to a group (Dufrene & Legendre 1997). This method is useful in long-term environmental monitoring for conservation and ecological management (De Cáceres., 2010).

Characteristics for an indicator species include the following: (i) species should reflect the biotic or abiotic state of the environment (ii) species should provide proof of evidence for environmental change impact, and (iii) species should be able to predict information about the diversity of all species within the area. The analysis was performed in three ways for this study: using separate season's (summer and winter) as well as combined data for both seasons. Sampling sites of this study were grouped into two according to the pollution impact they are subjected to. Group 1 (Sites 1, 2 and 3) consisted of the sites that were moderately polluted, while Group 2 (Sites 4 and 5) was made up of the critically modified sites. Canonical Correspondence Analysis (CCA) ordination tri-plot was used to elucidate the correlations between the measured water quality variables and the diatoms species in the Kat River recorded over the study period.

4.3 Results

4.3.1 Diatoms abundance and diversity

During the sampling period from December 2015 to November 2016, 28 families, 43 genera and 156 species were found at all five sampling sites (Table 4.1). For both seasons, Sites 2 and 4 had the lowest diatom diversity compared to Sites 1, 3 and 5. Winter results showed more species abundance at the five sites compared to summer. In summer 78 species were recorded and 91 species recorded in winter. Thirty-three (33) species that were present in summer did not appear in winter. These species include *Cocconeis placentula var. lineate, Navicula radiso, N. symmetrica Patrick, N. schroeteri Meister, N. capitatoradiata, N. riediana, N. recens, N. rosttellata, N. radiso, Nitzschia linearis var-subtilis, N. calida Grunow, N. gracilis Hantzsch* and *N. droveillensis coste*. In summer, 49 species did not appear, including *Cocconeis Placentula var euylypta, Navicula Tenelloids hustedt, N. Tripunctata, N. Trivialis, N. Cryptotenelloides, Nitzschia Etoshensis, N. Agnita, N. Calida Grunow* and *N. Aurariae Cholnoky*.

Abundance and biodiversity results at Site 1 for summer revealed that none of the species was abundant (A), 3 were common (C), 13 were occasional (O) and 33 species were rare (R). At Site 1 in winter, C and R dropped to 2 and 29 species, respectively. Compared to summer, O increased to 21 species and none of the species was abundant. At Site 2 in summer, there were 19 R, 15 O, 1 C and none of the species was abundant. In winter, there were 18 R and 19 O species but no species was in abundant. At Site 3, there were 2 C, 14 O, 23 R, and none of the species was in abundant in summer. For winter, 1A, 1 C, 27 O and 17 R species were found. At Site 4 in summer, results showed 2 A, 1 C, 14 O and 14 R, species. For winter, 1 A, no C, 13 O and 21 R, and species were found. At Site 5, 1A, 3 C, 11 O and 25 R and species were recorded in summer. For winter, 1A, no C, 17 O and 23 R, were recorded.

Table 4.1: Kat River diatoms abundance for summer and winter over the sampling period (December 2015 - November 2016)

			SUMMER					**WINTER**				
FAMILY	**GENERA**	**SPECIES**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
AULACOSEIRACEAE												
	Aulacoseira	*A. granulata*		R	O	O	O	O		O	O	
		A. Muzza	R									
STEPHANODISCACEAE												
	Cyclotella	*C.meneghiniana*	O	O	O		O	O	O	O	O	O
		C. ocellata										
		C.stelligera								R		
MELOSIRACEAE												
	Melosira	*M.Varians*	O		O		O	O	O	O	O	
EUNOTIACEAE												
	Eunotia	*E. exigua*										R
		E. incisa		O		O						O
		E. Formica				O						
		E. minor		O								O
TABELLARIACEAE												
	Diatoma	*D. Vulgaris Bory*	O	R	O		O	O	O	O	O	O
FRAGILARIACEAE												
	Fragilaria	*F. biceps*	R						R			O
		F. ulna	O	R	O			O	O	O	O	O
		F. rumpens	O	A	O		O		R	O	O	O
		F. ungeriana						O				

		F ulna var						R				R
		F var. vaucheriae						R	R			
		F. Crotonensis	R						R			
STAUROSIRACEAE												
	Staurosira	*S. elliptica*	R						R			R
		S. construens					O			O		
ULNARIACEAE												
	Tabularia	*T. Fasciculata*			O				O			R
ACHNANTHIDIACEAE												
	Achnanthidium	*A. eutrophilum*	O									
		A. crassum		R								
		A. biasole								R		
		A. affine								R		
		A. minutissimum		O		R		O		O		
		A. standeri Cholnoky		R								R
COCCONEIDACEAE												
	Cocconeis	*C.engelbrechtii*	R					R				
		C. var. lineata			R							
		C. var euglypta					R					O
		C. Ehrenberg	C	O	O	O	R	C	A	C	A	R
ACHNANTHIDIACEAE												
	Lemnicola	*L.hungarica*	O	O		O	O	R			R	A
	Planothidium	*P. rostratum*								R		
PLEUROSIGMATACEAE												
	Pleurosigma	*P. salinarum*						R				
		P. elongatum										R
STAURONEIDACEAE												
	Craticula	*C.accomoda*							O	R	R	O
		C.Halophila	O	O	O	O	R	O	O	O	O	O
		C.buderi	O	O	O	O	O	O	O	O	O	O
PINNULARIACEAE												
	Pinnularia	*P. borealis*	R				R					
	Reimeria	*R. nuata*	R	R	O	O	R					
		R. uniseriata Sala,										
CYMBELLACEAE												
	Navicymbula	*N. pusilla*						R				
	Cymbella	*C. turgidula*	R				R		O	O		
		C. kappi			O			R		O	O	
		C. tumida		R	R			O		O	O	R
		C. Aspera								R		
		C. neocistula									R	

CATENULACEAE												
	Amphora	*A. coffeaeformis*				R			R			R
		A. copulata	R									
		A. normannii									R	
DIPLONEIDACEAE												
	Diploneis	*D.subovalis Cleve*		O	R	R	O	R				
		D.puella					R					
SURIRELLACEAE												
	Cymatopleura	*C. solea var. apiculate*			R							
		C. solea								R		
	Surirella	*S. angusta*								R		
	Stauroneis	*S. phoenicenteron*				R						
RHOICOSPHENIACEAE												
	Rhoicosphenia	*R. abbreviate*	O				R				R	R
	Stenopterobia	*S. delicatissima*				R						
BACILLARIACEAE												
	Nitzschia	*N. sigma*	R	O	R	R		O	O			
		N. etoshensis Cholnoky							R	R	O	
		N. agnita Hustet								R		
		N. acicularis					O	R				R
		N. linearis var. subtilis	R									
		N. calida								R		
		N. aurariae										
		N. nana		O	R			O		R		R
		N. debilis						R		R	R	
		N. elegantula			R					R		
		N. communis					C	O				
		N. reversa				R						
		N. capitellata					C	R				O
		N. gracilis	R									
		N. draveillensis	R									
		N. pura	R									
		N. recta	R	R	R	O		R	R			
		N. microcephala		O	R	R	R					
		N. Filiformis	R			R		O			R	
		N. iremissa						R				
		N. palea	O	O	O	C	A	O	O	O	O	O
		N. Fonticola							R			
		N. linearis	O	R	R	O	O	R	R	R		R
		N. Frustulum	R					R		R		
		N. amphibia	R			O		R				
		N. clausii	C						R			

		N. pusilla				R		R				
	Tryblionella	*T. hungarica*	O	R		R					R	
		T. apiculate	R	O	O		R	R				
		T. levidensis		R	O	R	R					
		T.coarctata		R								
		T. calida							O			
	Denticula	*D. sundayensis Archibald*					R					
		D. kuetzingii Grunow		R				R		R		
		D. subtilis Grunow		R								
	Hantzschia	*H. amphioxys*			R				O	R	R	R
		H. distinctepunctata										R
GOMPHONEMATACEAE												
	Gomphonema	*G. minutum*	O	O	C	O	O O		O	O	O	O
		G. aff. Gracile										R
		G. parvulum var	R	R		R	R	O	O			
		G. acuminatum	R					R	R	A	R	
		G. Venusta	R		R			O	R	R	O	O
		G. italicum								R		R
		G. italicum										
		G. pseudoaugur									R	O
		G. clavatum				R						
		G. pumilum var	R	O	C	O	O	O	O	O	O	O
		G. insigne						R				
	Encyonema	*E. Mesianum*	R		R	O				O		
		E. caespitosum	R	R	R							
		E. minutum	R	O	O	O	O				R	
		E. silesiacum			R	O			O			R
		E. neogracile	R				R					
RHOPALODIACEAE												
	Rhopalodia	*R. operculata*		R				O				
		R. gibba	R					R				
AMPHIPLEURACEAE												
	Frustulia	*F.saxonica*							R			
DIADESMIDACEAE												
	Luticola	*L. goeppertiana*										R
		L. mutica									R	
	Diadesmis	*D.confervacea*								R		
SELLAPHORACEAE												
	Fallacia	*F.tenera*		O		R						
		F. monoculata	R									

NEIDIACEAE												
	Neidium	*N. productum*				R						
ANOMOEONEIDACEAE												
	Adlafia	*A. bryophila*			R						R	
NAVICULACEAE												
	Navicula s.s.	*N. radiosa*				O						
		N. zanonii	R			A	R	R	R	R	R	O
		N. rosttellata			R							
		N. symmetrica				R	R					
		N. schroeteri					R					
		N. veneta				R	R		R			
		N. angusta	R		R			O	O	O		
		N. tenelloides									R	
		N. erifuga							O			
		N. tripunctata										O
		N. trivialis						R				
		N. cryptotenelloides								O		
		N. capitatoradiata					R					
		N. riediana	R						R			
		N. cryptotenella			O							
		N. recens	C	C	O	A	C	C	O		O	O
	Navicula s.l.	*S. stroemii*					R					
		S. seminulum				O						
	Gyrosigma	*G. scalproides*	O	R				R	R			
		G. rautenbachiae									P	
		G. acuminatum	R		R		R	O	O	R	R	R
		G. attenuatum						R				
		G. parkerii						R				
	Caloneis	*C. silicula*						O				
		C. Schumanniana				R						
		C. bacillum								R		
		C. Molaris				R						
	Hippodonta	*H. capitata*				R						

Note: The full scientific names of the diatom species are provided in Appendix C

Diatoms diversity indices selected for Kat River in summer

The diversity indices used to test water quality changes in the sampling sites included Shannon index, Simpson index, Margalef index and Evenness (Figure 4.1). The results revealed that Shannon index and Simpson index could not differentiate between upstream sites (1, 2 and 3) and downstream sites (4 and 5). Evenness and Margalef indices enabled the discrimination of upstream sites from the downstream site (Figure 4.1). Based on the criteria in sub-section 2.5.1 of Chapter 2, the two metrics in the diversity category; Evenness index and Margalef index enabled the discrimination of upstream Sites 1, 2 and 3 from downstream Sites 4 and 5.

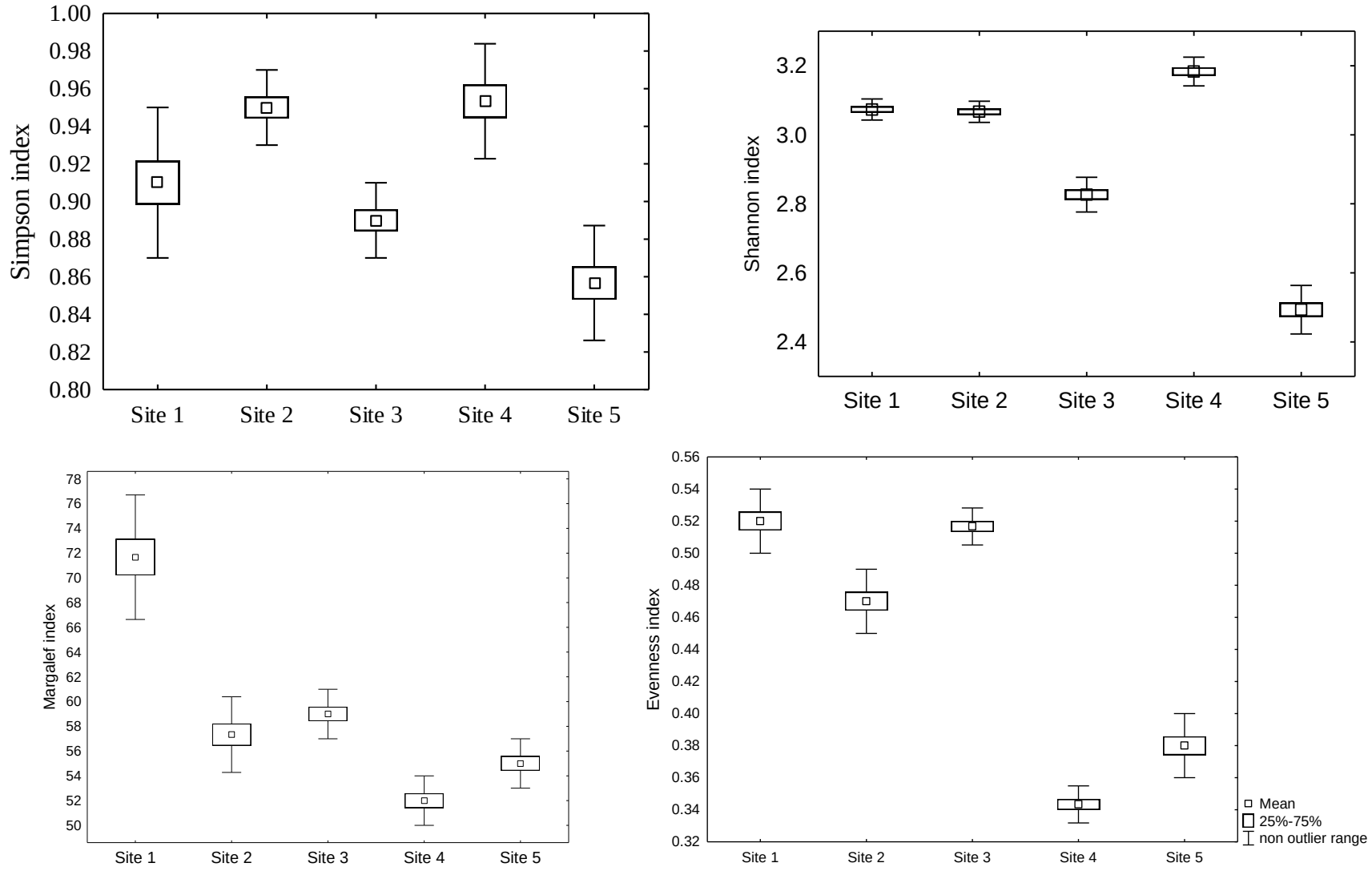


Figure 4.1: Box plot showing the median, 25-75 percentile distribution, non-outlier range for metrics in diversity category at the five sampling sites in the Kat River in summer

Diatoms diversity indices selected for Kat River in winter

The diversity indices used to test water quality changes in the Kat River in winter included Shannon index, Simpson index, Margalef index and Evenness (Figure 4.2). The results revealed that none of the four indices could differentiate between upstream and downstream sites (Figure 4.2). Shannon, Simpson and Margalef indices were higher at Site 1. Evenness and Shannon indices were higher at Site 5. Based on the criteria in sub-section 2.5.1 of Chapter 2, none of the selected metrics in the diversity category enabled the discrimination of Sites 1, 2 and 3 from Sites 4 and 5.

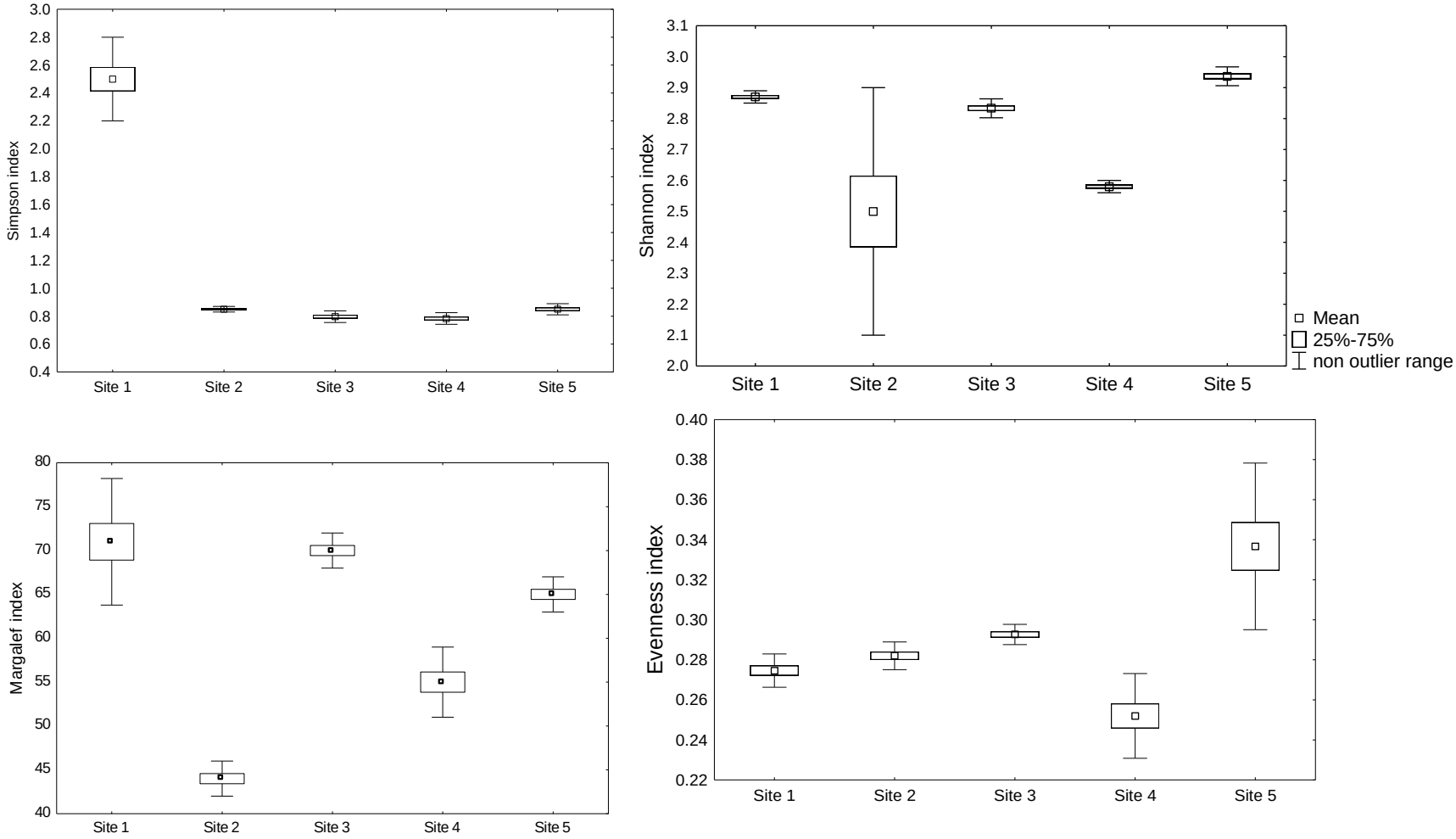


Figure 4.2: Box plot showing the median, 25-75 percentile distribution, non-outlier range for metrics in diversity category at the five sampling sites in the Kat River in winter.

4.3.2 Seasonal variation of indicator species analysis

Out of the 156 species recorded, 91 were present in winter, whereby Group 1 was represented by 54 species and Group 2 by 37 species. Diatoms species with p-values for both summer and winter are listed in Table 4.2. In winter, the analysis selected four indicator species. Two of the species (*Gomphonema minutum* and *Diatoma Vulgaris Bory*) were associated with Sites 1, 2 and 3. For Group 2, which was represented by Sites 4 and 5, the analysis associated two species to these sites (*Nitzschia palea* and *Gyrosigma acuminatum*) (Table 4.2).

Table 4.2: Diatom-indicator species for winter with corresponding p-values (***= high significant**= moderate significant and *=low significant)

WINTER	
Total number of species: 156	
Selected number of species: 91	
Species names	**p-Value**
Gomphonema minutum	0.006 **
Diatoma Vulgaris Bory	0.049 *
Fragilaria ulna	0.058.
Melosira varians Agardh	0.082.
Craticula accomoda	0.172
Gomophonema acuminatum	0.216
Cymbella Kappi	0.196
Cymbella tumida	0.26
Achnanthidium monutissimum	0.236
Nitzschia communis	0.487
Nitzschia recta Hantzsch	0.477
Nitzschia Filiforms	0.506
Cymbella turgidula Grunow	0.496
Staurosira elliptica	0.492
Gyrosigma scalproides	0.477
Denticula Kuetzingii Gronow	0.487
Navicula veneta kutzing	1
Navicula erifuga	1
Navicula riediana	1
Nitzschia agnita	1
Nitzschia elegantula Grunow	1

Nitzschia iremissa Cholnoky	1
Nitzschia Fonticola Grunow	1
Nitzschia cleusii Hantzsch	1
Nitzschia pusila Grunow	1
Fragilaria ungeriana	1
Fragilaria capucina var.vaucheriae	1
Gomophonema aff-gracile	1
Gomophonema Halicum	1
Gomphonema insigne Gregory	1
Encyonema Mesianum	1
Cymbella Aspera	1
Amphona normanii	1
Gyrosigma rautenbachiae	1
Gryrosigma porkerii	1
Tryblionella apiculate	1
Tryblionella levidensis	1
Cyclotella ocellata	1
Diphloneis suboralis cleve	1
Rhopalodia gibba	1
Caloneis silicula	1
Tubulana Fasciculate	1
Achananthes standeris cholnoky	1
Frastulia saxonica	1
Achnanthidium biasole	1
Hianum	1
Achananthidium affine	1
Planothidium rostratum	1
Discostella stellyera	1
Cymatopleura solea	1
Surirella angusta	1
Planothidium rostratum,	1
Coloneis Bacillum	1
Encyonopsis lea var	1
WINTER	
Group 2 (Sites 4 and 5) number of species 37	
Species names	**p-value**
Nitzschia palea	0.006 **
Gyrosigma acuminatum	0.003 **

Encoyonema minutum	0.057
Fragilaria ulna var.acus	0.101
Nitzschia linearis	0.236
Navicula radiso	0.275
Nitzschia Frustulum	0.138
Aulacoseira grunulata	0.314
Amphora Coffeformis	0.328
Cocconeis engelbrechtil	0.387
Cocconeis placentula var euylypta	0.416
Navicula tenelloids hustedt	0.411
Navicula tripunctata	0.416
Navicula trivialis Lange-Bertalot	0.387
Navicula cryptotenelloides	0.393
Nitzschia calida Grunow	0.393
Nitzschia aurariae Cholnoky	0.393
Nitzschia amphibia	0.387
Fragilaria Crotonensis	0.376
Encyonema caespitosum	0.411
Staurosira construens	0.393
Hantzschia distincepunctata	0.416
Eunotica exiga	0.411
Eunotia incisa Gregory	0.411
Eunotia minor	0.416
Pleurosigma salinarum	0.387
Pleurosigma elongatum W Smith	0.416
Tublionell calida	0.411
Navicymbula pusilla	0.387
Laticola mitica	0.411
Diadesmis confervcea	0.393
Cyclostephanos dubis	0.393
Luticola goeppertiana	0.411
Rhorcosphenia abbreviata	0.411
Gomephonema pseudoaugur Krammer	0.736
Fragilaria biceps	0.734
Nitzschia Capitellata Hustedt	0.744

Similarly, out of the 156 species analysed, 78 were selected to represent summer (Table 4.3), whereby Group 1, which was made up of Sites 1, 2 and 3 were presented by 45 species, while Group 2 (Sites 4 and 5) were represented by 33 species. In summer, only three significant species were selected to represent the sites. Two species (*Fragilaria ulna* and *Gyrosigma acuminatum*) were associated with Group 1, representing Sites 1, 2 and 3, while Group 2, representing Sites 4 and 5 had one species (*Cocconeis placentula var euylypta*) associated to it.

Table 4.3: Diatom-indicator species for summer with corresponding p-values (***= high significant**= moderate significant and *=low significant) Diatoms indicator species for summer, with p-value and statistical analysis

SUMMER	
Total number of species: 156	
Selected number of species: 78	
Group 1 (Site 1, 2 and 3) number of species. 45	
Species names	**p-value**
Fragilaria ulna	0.238*
Cyclotella meneghiniana	0.33
Encyonema caespitosum	0.222
Tribloinella apiculate	0.439
Gyrosigma rautenbachie	0.683*
Navicula angusta Grunow	0.488
Navicula riediana	0.472
Nitzschia nana Gronow	0.468
Gomphonema acuminatum	0.456
Cymbella kappi	0.466
Gyrosigma scalproides	0.496
Reimera uisenata sala	0.48
Encyonopsis lea var	0.512
Cocconeis engelbrechtil	1
Cocconeis placentula var. lineata	1
Navicula rosttellata	1
Navicula cryptotenella	1
Nitzschia linearis var-subtilis	1
Nitzschia elegantula Grunow	1
Nitzschia grcilis Hantzsch	1
Nitzschia droveillensis coste	1
Nitzschia pura hustedt	1

Nitzschia Frustulum	1
Nitzschia cleusii Hantzsch	1
Fragilaria bceps	1
Fragilaria Crotonesis	1
Gomophonema acuminatum	1
Cymbella turgidula Grunow	1
Staurosira elliptica	1
Aulacoseira Muzza	1
Amphora Capulata	1
Hantzschia amphioxys	1
Tryblionella coarctata	1
Eunotia minor	1
Caloneis silicula	1
Rhapalodia gibba	1
Denticula Kuetzingii Grunow	1
Denticula subtilis Grunow	1
Achananthidium	1
Actinela aeteoroaia	1
Adlafia navicular bryophila	1
Achannthes standeris Cholnoky	1
Fallacia monoculata	1
Achanthidium crassum	1
SUMMER	
Group 2 (Sites 4 and 5) number of species. 33	
Species names	**p-value**
Gomphonema parvulum var	0.296
Cocconeis placentula var euylypta	0.137*
Navicula symmetrica Patrick	0.128
Navicula veneta kutzing	0.143
Navicula zanonii	0.418
Rameria sinuata	0.307
Nitzschia amphibia	0.259
Navicula radiso	0.404
Navicula schroeteri Meister	0.394
Navicula capitatoradiata	0.394
Nitzschia acicularis	0.394
Nitzschia communis	0.402
Nitzschia reversa	0.404
Nitzschia Capitella Hustedt	0.402
Nitzschia pusila Grunow	0.39

Fragilaria ungeriana	0.404
Gomphonema claratum Ehre	0.39
Staurosira elliptica	0.402
Amphora Coffeformis	0.39
Eunotia formica Ehrenberg	0.39
Diploneis puella	0.394
Rhoplodia operculata	0.394
Sellaphora stroemii	0.394
Sellaphora seminulum	0.378
Denticula sundoyensis Archibold	0.388
Caloneis Schumanniana	0.39
Caloneis Molaris	0.39
Stauronas phoenix	0.404
Neidium prouctum	0.404
Stenopterobia delicattissima	0.404
Cymatopleura	0.404
Hippodonta capitata	0.39
Eunotia incisa Gregory	0.73

Indicator species analysis function (IndVal) was used to elucidate which of the species had significant indicator value for each of the seasons and sites. The analysis was based on percent frequency of species within a group. The analysis confirmed that species with the highest abundance and/or frequency within each group significantly represents the group (Table 4.4). *Diatoma Vulgaris Bory*, *Fragilaria ulna* and *Cocconeis placentula Ehrenberg* were the most frequent species that represented moderately polluted sites found at Sites 1 and 3 in winter (Table 4.4). Summer sampling showed that no species appeared frequently enough to be chosen to represent any of the five sampling sites (Table 4.4). *Navicula zanonii* was found at Site 4 in winter as the most frequently appearing species, representing polluted sites (Table 4.4). In winter, some of the sites, including Sites 2 and 5, were not represented by any species. For summer, no specific species represented any of the five sampling sites. In all, indicator species were mostly available in winter but not summer.

Table 4.4: results for Indicator species analysis (IndVal) for overall data sampled during study Period (December 2015-October 2016)

Season	**Sites**	**Species**	**IndVal**	**p-value**	**frequent**
Winter	1	*F. ulna*	0.52	0.027	18
Winter	1	*D. Vulgaris Bory*	0.45	0.002	23
Winter	3	*C. placentula Ehrenberg*	0.51	0.01	26
Winter	4	*N. zanonii*	0.51	0.023	8

According to the indicator species results for Kat River during the sampling period of December 2015 and November 2016, diatoms analyses showed that biodiversity improved in winter but decreased in summer. Also, the analysis discriminated between moderately modified sites (1 and 3) and seriously modified sites (2, 4 and 5). The following species were found to be more abundant during the sampling period: *Navicula zanonii, Cocconeis placentula Ehrenberg, Navicula recens, Nitzschia palea, Fragilaria capucina var rumpens, Gomphonema acuminatum, Lemnicola hungarica*. Some of the species were generalist species, which occured across various groups. Those species cannot be used as good indicators (Appendix D). Generally, indicator values range between 0-1 or expressed as a percentage. However, p-values of generalist species in this study were undeterminable in most case (represent as N/A in Appendix D). The reason for this is because those species are represented at all sites. Therefore, the association with the sites could not be statistically tested as there was no Group that they could be compared to.

4.4 Relating diatom assemblages and physicochemical variables

Canonical Correspondence Analysis (CCA) ordination tri-plot was used to elucidate the correlations between the measured water quality variables and the diatoms species in summer. The CCA results are presented in Figure 4.16. During initial exploration of the data, the majority of the physicochemical variables exhibited high multi-collinearity ($P< 0.05$) and subsequently, TIN was eliminated from the final CCA ordination. After elimination of this variable, the remaining nine variables that were used for the final CCA ordination (i.e. pH, DO, EC, PO_4-P, turbidity, temperature and NO_3-N, NO_2-N and NH_4-N).

The first axis of the CCA with Eigenvalue 0.634 explained 16.44% of the total variance. The three axes of the ordination explained a combined cumulative variance of 37.8% between the diatoms species and the physicochemical variables (Table 4.5). The Monte-Carlo permutation test (100 permutations) revealed that the Eigenvalue of the first axis was significant. The Pearson's correlation test indicated moderately correlations between the physicochemical variables on the three CCA axes.

The CCA ordination results indicated that increase in DO concentration and diatom species such as *Navicula radiso, Navicula symmetrica Patrick, Gomphonema minutum, Fragilaria ulna, Fragilaria ulna var. acus, Nitzschia sigma, Gomphonema pumilum var, Diatoma Vulgaris Bory*, and *Achnanthidium monutissimum* were strongly positively correlated. These families were negatively correlated with increased concentrations of EC. On the other hand, diatom species, including *Nitzschia etoshensis, Nitzschia palea* and *Lemnicola hungarica* were strongly positively correlated with increased concentrations of EC. These later families were less abundant at sites with a high concentration of DO (Sites 1, 2 and 3). Diatoms species such as *Cymbella kappi, Craticula buderi, Craticula Halophila* and *Encyonema caespitosum* were correlated with a decrease in temperature and PO4-P. Decreases in NO_3-N, NO_2-N and NH_4-N concentration were positively correlated with diatom species such as *Navicula angusta Grunow, Navicula recens, Cyclotella meneghiniana, Rhoplodia operculata* and *Rhopalodia gibba*. Increase in pH positively correlated with diatoms species such as *Encyonema Mesianum, Navicula radiso* and *Diploneis suboralis cleve,* and a decrease in turbidity revealed a positive correlation with *Gomphonema parvulum var* and *Cymbella tumida* (Figure 4.3).

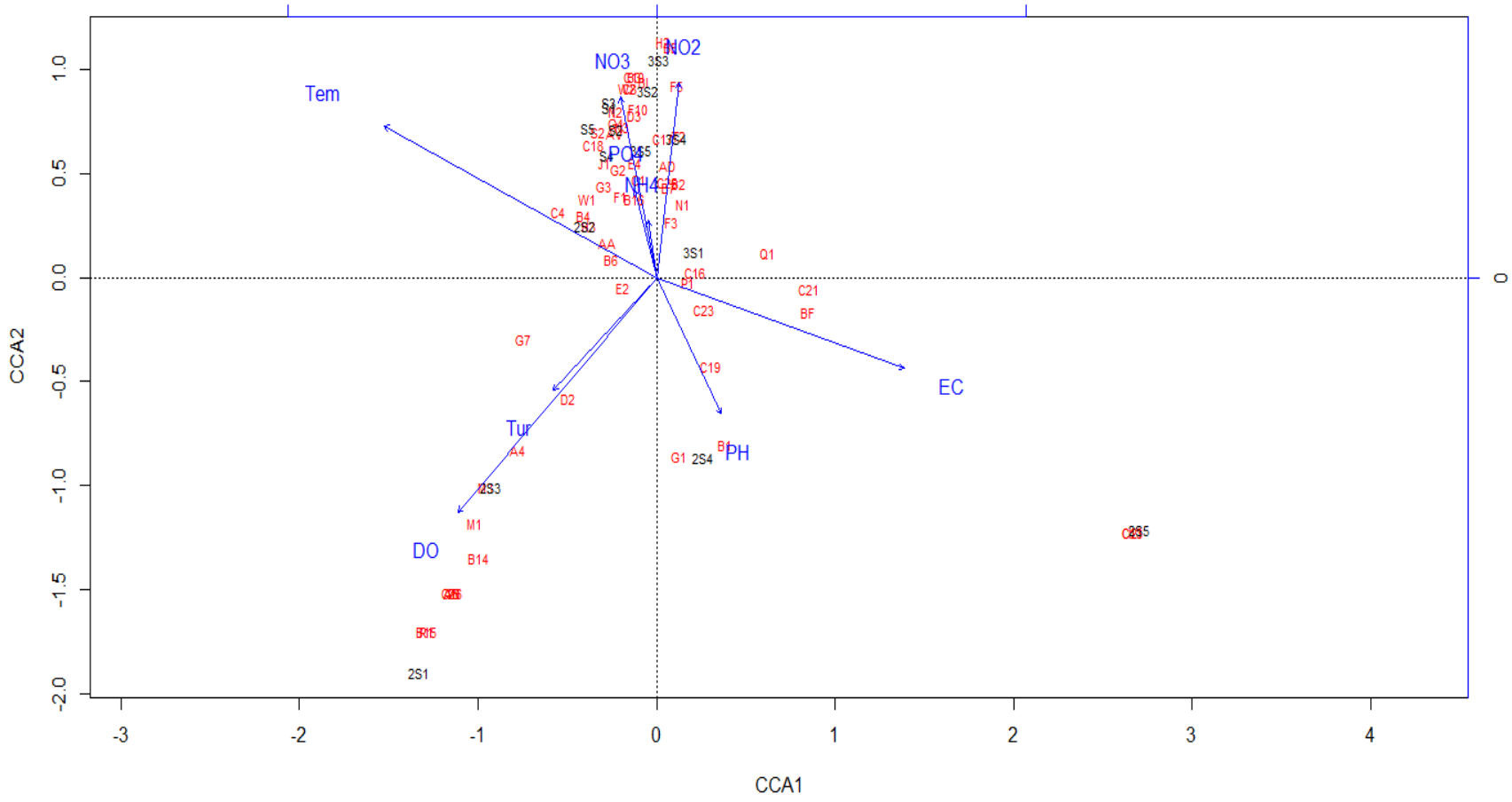


Figure 4.3: Canonical correspondence analysis (CCA) for diatoms and physicochemical variables at the five sampling sites at Kat River in summer.

Diatoms species abbreviations: A4-*Cocconeis placentula Ehrenberg*, B1-*Navicula radiso*, B2-*Naviculartzanonii*, B7-*Navicular angusta grunow*, B15-*Navicula crytotenella*, B16-*Navicula recens*, C1-*Nitzschia sigma*, C4-*Nitzschia acicularis*, C8-*Nitzschia nana grunow*, C11-*Nitzschia communis*, C13-*Nitzschia apitellata Hustedt*, C17-*Nitzschia recta Hantzsch*, C18-*Nitzschia microcephala*, C19-*Nitzschia Filiformis*, C21-*Nitzschia, palea*, C23-*Nitzschia linearis*, C25-*Nitzschia amphibia*, C26-*Nitzschia cleusii Hantzsch*, D2-*Fragileria capucina var rumpens*, E2-*Craticula halophila*, E3-*Craticula buderi*, F1-*Gomphonema minutum*, F3-*Gomphonema parvulum var*, F10-*Gomphonema pumilum var*, G1-*Encyonema mesianum*, G3-*Encyonema minutum*, H2-*Cymbella kappi*, H3-*Cymbella tumida*, I2-*Staurosira construens*, J1-*Aulacoseira grunulata*, M1-*Gyrosigma scalproides*, M3-*Gyrosigma scalproides*, N1-*trybloionella hungarica*, N2-*Tryblionella apiculate*, N3-*Tryblionella levidensis*, P1-*Cyclotella meneghiniana*, Q1-*Diploneis subovalis cleve*, T2-*Sellaphora seminulum*, W1-*Reimeria sinuate*, W2-*Reimeria uniseriata sala*, Z-*Rhoicosphenia abbreviate(agardh*) *Lange-Betalot*, AA-*Diatoma Vulgaris Bory*, AB-*Achnanthidium eutrophilum*, AC-*Melorira varians Agardh*, AN-*Fallacia monoculata*, AV-*Achnanthidium minutissium*, BF-*Lemnicola hungarica*, BI-*Fallacia tenera*

Table 4.5: Canonical correspondence analysis (CCA) properties for the correlation between diatom species and water physicochemical variables in the Kat River for summer

CCA properties	**Axis**		
	1	2	3
Canonical Eigen value	0.634	0.520	0.399
Percent variance explained	16.44	11.77	9.60
Percent cumulative variance explained	11.49	9.46	5.97
Pearson correlation of species and environmental Scores	0.190	0.190	0.327

Similarly, CCA ordination tri-plot was used to elucidate the correlations between the measured water quality variables and the diatoms species in winter. The first axis of the CCA with Eigenvalue 0.697 explained 18.28% of the total variance. The three axes of the ordination explained a combined cumulative variance of 42.13 % between the diatoms species and the physicochemical variables (Table 4.6). The Monte-Carlo permutation test (100 permutations) revealed that the Eigenvalue of the first axis was significant. The Pearson's correlation test indicated slightly strong correlations between the physicochemical variables and the diatoms species on the three CCA axes.

The CCA ordination results indicated that increase in DO concentration and diatom species such as *Cymbella kappi*, *Achnanthidium minutissium*, *Fragileria capucina var rumpens*, *Gomphonema italicum*, *Fragilaria ulna* and *Melosira varians Agardh* were strongly positively correlated. These families were negatively correlated with increased concentrations of EC. These later families were less abundant at sites with a high concentration of EC in Sites 4 and 5. On the other hand, diatoms species including *Navicula zanonii* was strongly positively correlated with increased concentrations of EC. Diatoms species such as *Navicula radiso, Amphora Coffeformis*, *Fragilaria biceps*, *Gyrosigma acuminatum*, *Gomphonema claratum Ehre*, *Gomphonema Venusta*, *Gyrosigma scalproides* and *Nitzschia recta Hantzsch* were correlated with a decrease in temperature and PO4-P. Increase in turbidity and TIN concentration positively correlated with diatom species such as *Nitzschia nana Gronow*, *Nitzschia palea*, *Nitzschia linearis* and *Craticula bucleri*. Increase in pH is strongly positively correlated with diatoms species such as *Fragileria capucina var rumpens*, *Achnanthidium monutissimum*, *Gomphonema italicum*, *Gomphonema minutum* and *Cymbella kappi* (Figure 4.4).

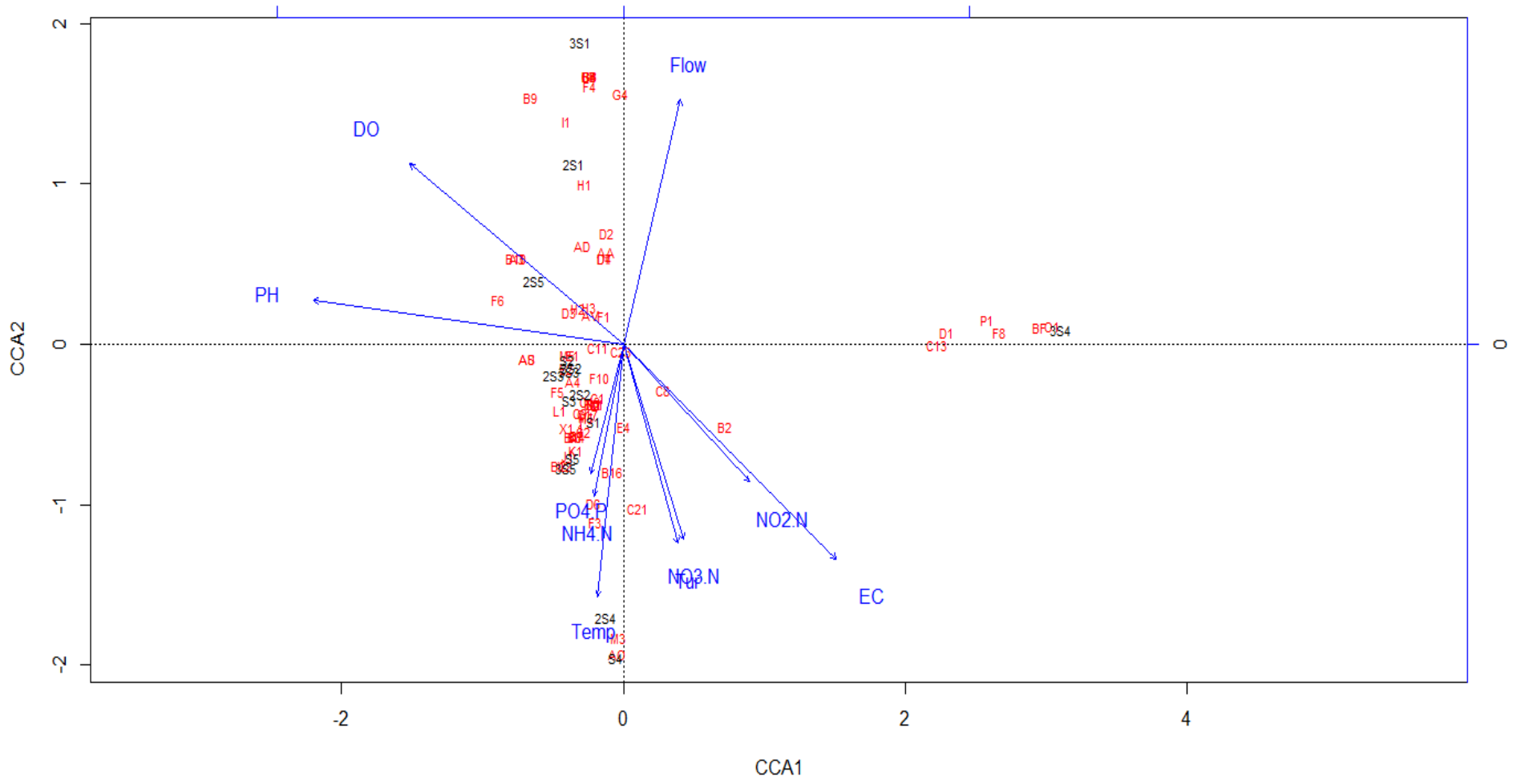


Figure 4.4: Canonical correspondence analysis (CCA) for diatoms and physicochemical variables at the five sampling sites at Kat River in winter.

Diatoms species abbreviations: A4-*Cocconeis placentula Ehrenberg*, B1-*Navicula radiso*, B2-*Naviculartzanonii*, B7-*Navicular angusta grunow*, B15-*Navicula crytotenella*, B16-*Navicula recens*, C1-*Nitzschia sigma*, C4-*Nitzschia acicularis*, C8-*Nitzschia nana grunow*, C11-*Nitzschia communis*, C13-*Nitzschia apitellata Hustedt*, C17-*Nitzschia recta Hantzsch*, C18-*Nitzschia microcephala*, C19-*Nitzschia Filiformis*, C21-*Nitzschia, palea*, C23-*Nitzschia linearis*, C25-*Nitzschia amphibia*, C26-*Nitzschia cleusii Hantzsch*, D2-*Fragileria capucina var rumpens*, E2-*Craticula halophila*, E3-*Craticula buderi*, F1-*Gomphonema minutum*, F3-*Gomphonema parvulum var*, F10-*Gomphonema pumilum var*, G1-*Encyonema mesianum*, G3-*Encyonema minutum*, H2-*Cymbella kappi*, H3-*Cymbella tumida*, I2-*Staurosira construens*, J1-*Aulacoseira grunulata*, M1-*Gyrosigma scalproides*, M3-*Gyrosigma scalproides*, N1-*trybloionella hungarica*, N2-*Tryblionella apiculate*, N3-*Tryblionella levidensis*, P1-*Cyclotella meneghiniana*, Q1-*Diploneis subovalis cleve*, T2-*Sellaphora seminulum*, W1-*Reimeria sinuate*, W2-*Reimeria uniseriata sala*, Z-*Rhoicosphenia abbreviate(agardh*) *Lange-Betalot*, AA-*Diatoma Vulgaris Bory*, AB-*Achnanthidium eutrophilum*, AC-*Melorira varians Agardh*, AN-*Fallacia monoculata*, AV-*Achnanthidium minutissium*, BF-*Lemnicola hungarica*, BI-*Fallacia tenera*.

Table 4.6: Canonical correspondence analysis (CCA) properties for the correlation between diatoms species and water physicochemical variables in winter

CCA property	**Axis**		
	1	2	3
Canonical Eigen value	0.7244	0.498	0.411
Percent variance explained	19.01	13.07	10.78
Percent cumulative variance explained	8.66	8.03	5.48
Pearson correlation of species and environmental Scores	0.087	0.327	0.393

4.5 Discussion

4.5.1 Diatoms community composition

Most of the diatoms species identified during the study were pollution and salinity tolerant, which include *Cocconeis placentula Ehrenberg, Navicula recens, Nitzschia Capitellata Hustedt, Nitzschia palea, Nitzschia cleusii Hantzsch* and *Nitzschia zanonii*. *Fragilaria ulna, Fragileria capucina var rumpens, Craticula Halophila, Craticula budeeri, Gomphonema minutum, Gomphonema acuminatum, Gomphonema pumilum var, Gyrosigma acuminatum, Diatoma Vulgaris Bory, Melosira varians Agardh*and *Lemnicola hungarica* (Taylor, 2006; Holmes and Taylor, 2015). Among these species, there are some that could represent indicator species for the Kat River (written in bold) as they had high indicator values between sites and seasons. ***Fragilaria alnu****, Fragileria capucina var rumpens,* ***Diatoma Vulgaris Bory*** and *Melosira varians Agardh* have a broad range of tolerance to chemical, hydrological and physical disturbances, including low to moderate tolerant to salinity and nutrients (Albany and Akcaalan, 2008). According to Taylor et al (2006), ***Cocconeis placentula Ehrenberg*** is highly tolerant to salinity and moderately tolerant to nutrients. However, in this study, ***Cocconeis placentula Ehrenberg*** was found mostly at the moderately modified sites (1 and 3) that had low EC concentration, implying it has low to medium tolerant to salinity and nutrients. This agrees with Salomoni et al. (2006) who also reported *Cocconeis placentula Ehrenberg*as, an indicator of less polluted conditions. Again, in this study, *Cocconeis placentula Ehrenberg* was mostly found at sites that are close to agriculture activities in summer, which collaborates Walsh and Wepener (2006) report that the species is likely to be found in summer. *Nitzschia Capitellata Hustedt, Nitzschia palea, Nitzschia cleusii* and ***Nitzschia zanonii*** were found to be highly tolerant to salinity and pollution but low tolerant to nutrients. In this study, these species were found in abundance at the downstream sites that were polluted by wastewater effluent, untreated wastewater leakages and runoffs from informal settlements. Bere et al. (2014) asserted that these species are indicators of hypereutrophic conditions. *Craticula Halophila* and *Craticula buderi*, which are high pollution tolerant species but salinity tolerant are moderate to high, were found in abundance at all sampling sites.

Gomphonema minutum, *Gomphonema acuminatum* and *Gomphonema pumilum var* were found to be moderately tolerant to increased salinity, high pollution and high nutrients. In fact, these species have been suggested to be a good indicator of high organic pollution (Holmes and Taylor, 2015), which this study has confirmed. *Gyrosigma acuminatum* has a broad range of tolerant such as high salinity, high pollution and high alkaline (Taylor et al., 2006). *Navicula recens* has also been reported to be tolerant to high salinity, high nutrients and high pollution (Bere et al., 2014). This is supported in this study, whereby *Navicula recens* was found in abundance at Site 4, the most polluted site, at summer. Physicochemical results for this site were mostly out of limit in summer compared to winter. Similarly, *Lemnicola hungarica* has been reported to be tolerant to moderate to high salinity, high pollution and low alkaline (Taylor et al., 2006). This assertion was supported by this study as *Lemnicola hungarica* was found in abundance in winter at Site 4, the most polluted and highest EC reported site.

Indicator species analyses technique has been used to determine the occurrence or abundance of indicator species and reflect environmental conditions of each site. Good indicator species are always present in a site of a given group and never occur in other sites or other groups. For summer the indicator species analyses selected 45 species as indicator species for group 1 and 33 species for group 2. Out of these, 2 species for group 1 (*Fragilaria ulna* and *Gyrosigma acuminatum*) and 2 species for Group 2 (*Cocconeis placentula var euylypta*) were zeroed in as the significant indicator species for summer. In winter indicator species analyses selected 54 species as indicator species for Group 1 and 37 species for Group 2. Out of these, 2 species for Group 1 (*Gomphonema minutum* and *Diatoma Vulgaris Bory*) and 2 species for Group 2 (*Nitzschia palea* and *Gyrosigma acuminatum)* where zeroed in as the most significant indicator species for winter.

The indicator value from indicator species analyses was used to find out which species were the most significant for each sampling based on sites. IndVal analyses revealed that *Diatoma Vulgaris Bory* and *Fragilaria ulna* were the most significant species for Site 1 in winter, *Cocconeis placentula Ehrenberg* was the most significant specie for Site 3 and *Navicula zanonii* was the most significant species for Site 4 in winter. All these indicator values were associated with winter. There was no significant indicator value for summer and Sites 2 and 5.

Indicator value results for this study did not give clear enough pictures of the sites and seasons, as only a few species were selected to represent the groups, sites and seasons (Table 4.2-4.4). In fact, there were no species selected for Sites 2 and 5 for both summer and winter. In indicator value analysis, species with the highest abundance and/or frequency within each group are chosen to represent the group or site (Dufrene & Legendre 1997; De Cáceres., 2010). The inability of the analysis in this study to select indicator species could be attributed to the pollution levels, which was almost the same for all the sites and resulting in same species availability at all sites in summer. For example, Sites 1, 2 and 3 for summer had a *Gyrosigma acuminatum,* which is highly tolerant to salinity and pollution as indicator species; the same species represented Sites 4 and 5 at winter.

4.5.2 Response of diatoms community with physicochemical variables

The Canonical correspondence analysis (CCA) was used to find a relationship between measured physicochemical variables and diatoms assemblages among the five sampling sites. Sites 2, 4 and 5 seemed to share most characteristics, including common species and were affected by pollution almost the same way. Most high salinity and pollution tolerant species were found at Sites 2, 4 and 5, although summer results were not able to clearly distinguish between high impacted sites and moderately modified sites. The CCA results showed that increase in the concentration of EC, nutrients, turbidity and PO_4-P correlated positively with the abundance of diatoms such as *Navicula zanonii, Navicula recens, Gyrosigma acuminatum, Fragilaria ulna var. acus, Navicula radiso, Cocconeis placentula var euylypta, Navicymbula pusilla* and *Gomphonema pumilum var*. Most pollution and salinity sensitive species were not found at Site 2, 4 and 5 probably because of the increased EC, nutrients, turbidity and PO_4-P due to domestic wastewater and runoffs. This finding agrees with Bere and Tundisi (2011) assertion that increase in nutrients and EC due to domestic wastewater and industry pollution impact sensitive species. Sites 1 and 3 were negatively correlated with high EC but associated with species such as *Cocconeis placentula Ehrenberg, Achnanthidium minutissimum, Diatoma Vulgaris Bory, Melosira varians Agardh, Navicula symmetrica Patrick, Nitzschia sigma* and *Caloneis silicula* as well as with high DO in the present study.

Comparatively, in this study, most pollution and salinity sensitive species were found at upstream Sites 1 and 3, which had high DO and moderate pH concentrations. In cases where high EC was recorded, abundance and biodiversity decline, and many of the sensitive species do not appear. Some diatom species such as *Nitzschia zanonii* that are tolerant to high pollution and salinity were dominant at downstream sites, which meant they were not easily affected by the high pollution and low DO at these sites.

Diatoms are known as good indicators of salinity (Fritz, 2013). They have different tolerances to salinity and their physiology can be affected directly or indirectly by other growth factors such as saline system and ion composition. Diatom assemblages can also be influenced by salinity. In some diatom species, low salinity can lead to decrease in the species cell dimensions (Adenan et al, 2013). *Gyrosigma rautenbachiae* is one of the species that are tolerant to salinity and pollution (Holmes and Taylor, 2015). This species was found in Site 4, where the highest EC concentration was recorded in this study. This was in agreement with the assertion by Holmes and Taylor (2015) that *Gyrosigma rautenbachiae* preferred brackish and polluted water. This study also found *Nitzschia frustulum* and *rhopalodia gibba* mostly at sites impacted by moderate salinity, which supports the assertion by Taylor et al. (2006) and Holmes and Taylor (2015) that the species are tolerant to moderate to high salinity.

4.6 Conclusion

The present study has shown that the Kat River water quality is impacted by anthropogenic activities that have led to increasing salinity and nutrient loads, which have in turn impacted the diatom assemblage in this river system. Furthermore, this study has shown that diatoms are good bioindicators for biomonitoring water quality, especially salinity in the Kat River. Diatoms could also be considered for wider integration in the River Eco-Status Monitoring Programme (REMP) and other routine monitoring programme of the country.

CHAPTER 5: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General Introduction

The effectiveness of diatoms and macroinvertebrates as bioindicators of environmental conditions in the Kat River was assessed in the previous two chapters. This chapter concentrates on the comparison between the suitability of macroinvertebrates and diatoms as bioindicators for biomonitoring in the Kat River. This comparison includes addressing questions such:

- Which physicochemical variables are more significant in explaining macroinvertebrate and diatom assemblage responses?
- Which biotic indices are sensitive to macroinvertebrate and diatom responses to pollution in the Kat River?
- Which statistical methods are more significant in explaining macroinvertebrate and diatom data?
- Which level of taxonomic resolution (family for macroinvertebrates or species for diatom) is more appropriate for explaining pollution effects in the Kat River?
- What are the strengths and weaknesses of using macroinvertebrates and/or diatoms in bioassessment of salinity and other pollutants in the Kat River?

Inferences were them made to decide the more sensitive bioindicators based on analysis of answers provided for the above questions. The outcomes were critically discussed in relation to the overall aim and specific objectives set out to be addressed in this study. Finally, general conclusions were made with respect to the overall aim of the study as well as recommendations for future study.

5.2 Comparison between macroinvertebrate and diatom sensitivity for biomonitoring in the Kat River

5.2.1 Using physicochemical variables to explain macroinvertebrate and diatom assemblage response to pollution in the Kat River

The Kat River water quality was assessed through the analyses of EC, DO, pH, flow, turbidity and nutrients (NO_3-N, NO_2-N, NH_4-N and PO_4-P). The analyses generally showed increased concentrations of NO_3-N, NO_2-N, NH_4-N, PO_4-P, EC and turbidity, as well as decreased values for DO and flow. Concurrently, bioassessment of macroinvertebrates and diatom were carried out to investigate their sensitivity/tolerant to anthropogenic impacts. For macroinvertebrates, the bioassessment revealed a decline in abundance of least pollution tolerant families such as Perlidae, Notonemouridae, Scirtidae, Baetidae, Leptophlebiidae, Heptageniidae and Tricorythidae, while pollution tolerant families such as Oligochaeta, Turbelleria, Hirudinea, Physidae, Chironomidae, Ceratopogonidae and Muscidae were favoured. Macroinvertebrates families such as Leptophlebiidae, Athericidae, Notonemouridae, Baetidae, Hydropsychidae, Helodidae, Tricorythidae, Veliidae, Perlidae, Heptageniidae and Leptoceridae correlated positively with high DO and stream flow as well as low nutrients and EC. Conversely, macroinvertebrates families such as Naucoridae, Lymnaeidae, Corixidae, Planorbinae, Belostomatidae, Chironomidae, Hirudinea, Gyrinidae, Culicidae, Turbellaria and Oligochaeta were strongly correlated positively with increased concentration of EC and nutrients as well as decreased DO and flow.

For the diatom assemblage, the bioassessment results revealed that less tolerant species such as *Fragilaria alnu, Fragilaria capucina var rumpens, Diatoma Vulgaris Bory* and *Melosira varians Agardh* correlated with medium-high flow, low-moderate nutrients and low-moderate EC. Conversely, highly tolerant species such as *Nitzschia Capitellata Hustedt, Nitzschia palea, Nitzschia cleusii* and *Nitzschia zanonii* were found to be highly tolerant to increased EC and decreased DO and flow. Increase and decrease of these variables could indicate pollution impact on water quality.

Out of the nine physicochemical variables (EC, DO, pH, temperature, TIN, PO_4-P, flow and turbidity) selected for this study, stream flow, nutrients and EC were found to be among the most important variables that structure diatom communities in this study. Physicochemical variables showed that high EC was correlated with diatoms species such as *Nitzschia amphibia*, *Craticula Halophila, Nitzschia linearis, Navicula riediana, Nitzschia pura hustedt, Gyrosigma scalproides, Gyrosigma acuminatum, Trybloionella hungarica, Nitzschia Filiformis,* and *Encyonema Mesianum.* Similarly, high flow correlated positively with species such *Cocconeis placentula Ehrenberg, Fragilaria ulna* and *Diatoma Vulgaris Bory*; and increased correlated positively with species such as *Encyonopsis lea var, Rameria sinuate, Craticula accomoda, Cymbella tumida* and *Navicula symmetrica Patrick. Navicula zanonii, Navicula recens, Gomphonema Venusta, Amphora Coffeformis, Gyrosigma acuminatum, Fragilaria ulna var.acus, Navicymbula pusilla, Navicula radiso, Cocconeis placentula var euylypta* and *Gomphonema pumilum var.*

5.2.2 Using biotic indices to explain macroinvertebrate and diatom data

The diversity indices, including Simpson, Shannon, Margalef and Evenness were used to discriminate upstream (moderately modified) sites from the downstream (most impacted) sites for both macroinvertebrates and diatoms. Simpson and Evenness indices for macroinvertebrates were unable to discriminate upstream sites from downstream sites for both summer and winter, but Margalef indices discriminated upstream sites from downstream sites for summer and winter. In that case, the biotic index that can be selected as indicative of responses to pollution with macroinvertebrates as bioindicators is Margalef index. For diatoms, out of the four indices, only Margalef and Evenness indices were able to discriminate upstream sites from downstream sites in summer. For diatoms, the diversity indices could not distinguish between moderately modified sites and most impacted sites in winter, which means that diatoms were poor bioindicators compared to macroinvertebrates. None of the selected diversity indices could be used to discriminate impacted site using diatoms.

5.2.3 Using statistical methods to explain macroinvertebrate and diatom data

Macroinvertebrates were sampled at the five sampling sites over the study period. Cluster analysis using Bray-Curtis similarity index effectively distinguished compositional similarity among the different sites into groups or clusters.

The analysis classified macroinvertebrates into 3 major groups according to similarities between sites and seasons. The 3 major groups were similarities between macroinvertebrates, between sites and sampling periods. Macroinvertebrate communities were mostly clustered by sampling period and sites. In summer, upstream sites showed a very close similarity of more than 70% macroinvertebrates abundance between sites. Generally, all the sites showed close similarities of macroinvertebrate abundance of 10%. Also, for both seasons, the Bray-Curtis similarity dendrogram separated the upstream sites (least polluted) from downstream sites (most polluted). In winter, upstream sites showed a very close similarity of more than 78% macroinvertebrates abundance between sites. Generally, all the sites showed close similarities of macroinvertebrate abundance of 19%. Furthermore, CCA of macroinvertebrates group upstream sites separate from the downstream sites. Generally, macroinvertebrates analysis for both Bray-Curtis similarity and CCA agreed that upstream sites shared the same characteristics and are polluted in almost the same way, while downstream sites were the most impacted sites and shared similar characteristics.

For diatoms, indicator species analysis was used to determine the occurrence and/or abundance of indicator diatom species and environmental conditions that influenced the species assemblage structure. Indicator species classified diatoms into two groups: the most polluted sites were separated from the least polluted sites. Indicator species selected 91 species from 156 recorded during the sample period to represent winter. The 91 species were divided into two groups: Group 1 (for least polluted sites) was represented by 54 species, while Group 2 (for most impacted sites) was represented by 37 species. In winter, indicator species analysis selected four indicator species. Two of the species (*Gomphonema minutum* and *Diatoma Vulgaris Bory*) were associated with the least polluted sites (Group 1), while the other two species (*Nitzschia palea* and *Gyrosigma acuminatum*) were associated with the most polluted sites (Group 2). Similarly, indicator species analysis selected 78 species out of 156 recorded to represent summer. The 78 species were divided into two groups: Group 1 (for least polluted sites) was represented by 45 species, while Group 2 (for most impacted sites) was represented by 33 species.

In summer, indicator species analysis selected three species (*Fragilaria ulna* and *Gyrosigma acuminatum*) for Group 1 and one species (*Cocconeis placentula var euylypta)* for Group 2. Indicator value analysis (IndVal) was used to find out the most significant indicator value among these species. The IndVal analysis showed that *Diatoma Vulgaris Bory* and *Fragilaria ulna* were the most significant species that represent least polluted sites in winter. Also, *Cocconeis placentula Ehrenberg* was found as the most significant species that represents moderately polluted sites, while *Navicula zanonii* was found to be the most significant species that represents most polluted sites. Furthermore, CCA analysis of diatoms separated species into salinity-sensitive associated with upstream sites and salinity-tolerant associated with downstream sites.

5.2.4 What is an appropriate taxonomic resolution for explaining pollution effect in the Kat River?

Macroinvertebrates families have different sensitivity to change in water quality and they respond differently to different impact, which could lead to a shift of diversity and abundance at the community level. Classification of the sensitivity/tolerance of macroinvertebrate orders have been given to include very sensitive (Emphemeroptera, Trichoptera and Plecoptera), sensitive (Coleptera, Hemiptera and Hydracarina), tolerant (Odonata, Hemiptera and Pelecypoda) and very tolerant (Diptera, Turbelleria and Annelida) (Taylor et al., 2006). In this study, macroinvertebrates at the family taxonomic level of resolution (belonging to the above orders) were used to assess water quality of the Kat River. Families such as Baetidae, Leptophlebiidae, Heptageniidae, Perlidae, Notonemouridae and Leptoceridae were associated with moderately modified sites; Gyrinidae, Gerridae, Corixidae, Notonectidae and Elmidae were associated with moderately impacted sites; while Muscidae, Chironomidae, Simuliidae and Oligochaeta were associated with the most impacted sites.

Conversely, in this study, diatoms were used up to species level of taxonomic resolution to examine ecosystem health of the Kat River. Diatoms are known to be influenced by pH, phosphate, salinity and nutrients (Gell, 1997; Bere and Tundisi, 2011; Dalu et al., 2014).

The outcome of the study showed that species such as *Melosira varians Argardh, Diatoma Vulgaris Bory* and *Fragilaria ulna* were associated with upstream sites, which were the moderately modified, while *Nitzschia palea, Cocconeis placentula Ehrenberg, Nitzschia palea, Gyrosigma acuminatum,* and *Cocconeis placentula var euylypta* were associated with downstream sites, which were the most polluted.

Although macroinvertebrates were not identified up to the species level in this study, previous studies by other researchers have shown that identifying the macroinvertebrates to the species-level has not proven to be sensitive in detecting changes water quality variables, especially salinity, as diatoms do (Belore et al., 2002; Wessie, 2008). In fact, they opined that there is no significant difference in species-level biomonitoring of macroinvertebrates compared to family-level biomonitoring of macroinvertebrates. Furthermore, with respect to assessing instream water quality, macroinvertebrates are very good at detecting nutrients and alkalinity, while diatoms have a strong affinity for detecting changes in electrical conductivity (Belore et al., 2002). It is also critical to note that species-level macroinvertebrates biomonitoring is very expensive in terms of cost and time, both of which the study lacked. Based on the above presumptions, especially the fact that this study main focus was bioassessment of salinity, the study team resolved to employ family-level taxonomic resolution for macroinvertebrate biomonitoring but the species-level taxonomic resolution for diatom biomonitoring.

5.2.3 Strengths and weaknesses of using macroinvertebrates or diatoms in bioassessment of salinity

From the above sub-sections, it is clear that macroinvertebrates and diatoms have different sensitivity to anthropogenic pollution as they respond differently to different pollution loads. Both groups have different sensitivities between the families, genera and species. They are easily identified to family (macroinvertebrates) and species level (diatoms). In most cases, the relative abundance and diversity of these aquatic biota are used to examine the pollution loads or state of river water quality. Table 5.1 presents strengths and weaknesses of macroinvertebrates and diatoms in bioassessment of aquatic ecosystem health in the context of the present study.

Table 5.1: Comparative Strengths and weaknesses of macroinvertebrates- and diatoms-based bioassessment of the Kat River (3 = Very strong, 2 = Strong, 1 = Weak, 0 = Very weak)

CHARACTERISTICS OF THE GROUP	**MACROINVERTEBRATES**	**DIATOMS**
Response to changes in water quality	3	3
Not affected by seasonal changes	0	2
Not affected by habitat changes	0	3
Not sensitive enough to detect small amount of salts	1	2
Convenience of sampling method	3	3
Sensitivity to various types of pollution	2	3
Discriminatory potential of impacted	2	3
Ease of identification to an appropriate level of taxonomic resolution	3	2
Rapidity of method	3	1
Ease of communication of results and retrieval of reliable indicator species	2	3
Detect nutrients and TIN	3	3
Detect electrical conductivity and salinity	2	3
Overall	**24/36 = 67%**	**31/36 = 86%**

5.2.4 Conclusion

For physicochemical variables, streamflow, nutrients, EC and DO were significant in explaining macroinvertebrate data, while streamflow, nutrients and EC were significant in explaining diatom data. For biotic indices, Margalef diversity index was significant in explaining macroinvertebrate data, but none of the diversity indices used in the study significantly explained diatom data. For statistical methods, Bray-Curtis similarity and CCA significantly explained macroinvertebrate data, while indicator species analysis and CCA significantly explained diatom data.

For the level of taxonomic resolution, families of macroinvertebrates were significant in explaining the generally observed poor water quality, but diatoms at the species level were significant in explaining the contribution of the individual physicochemical variable in the observed poor water quality. For strengths and weakness, the characteristics of macroinvertebrates scored 24/36 (67%) in terms of significance in bioassessment of salinity, while diatom score 31/36 (86%) in terms of significance in bioassessment of salinity.

5.3 General conclusions

This study investigated the impact of anthropogenic activities in the Kat River Valley on the river's ecological health. The findings showed that Kat River had been significantly impacted by anthropogenic activities such as wastewater leakages, wastewater treatment effluent and farming. High levels of salinity were recorded at sites associated with these human activities. Macroinvertebrates and diatoms are mostly used as bioindicators to assess river water and this was proven in this study. Physicochemical variables showed that water quality for this river was impacted by anthropogenic activities. Physicochemical variables evaluation indicated that the Kat River water quality is moderately to critically modified compared to the Generic Resources Water Quality Objectives limits. Since some of the field sites are associated with citrus production and other agricultural activities, some acknowledgement that unmeasured stressors (pesticides) could have influenced biological assemblages is warranted. Macroinvertebrates composition was found to be sensitive to organic pollution and nutrients. Macroinvertebrates composition is also affected by habitat availability and seasonal variability. Meanwhile, diatoms were found to be able to detect small increases in salinity and other pollutants; they are also less affected by habitat availability. Both macroinvertebrates and diatom bioassessment at the downstream sites were found to be affected by inadequate wastewater treatment and leakages. Overall, this study found both macroinvertebrates and diatom as good bioindicators for bioassessment of non-point and point sources of pollution in the Kat River, with diatoms having the added quality of assessing small changes in salinity.

5.4 Recommendation

- Continuous monitoring of the Kat River salinity is recommended to improve river water quality.
- Diatoms and macroinvertebrates analysis should be combined to serve in any future bioassessment task of the Kat River.
- This study was conducted during the drought period, a long-term study involving a period of wet and dry seasons will offer a more integrated picture of the river's health.
- It is recommended that improvements in treating the Fort Beaufort Wastewater Treatment Works processes are necessary to ensure that discharged effluent meets the required standards.
- Repair or change of leaking sewage pipes is recommended to prevent untreated influent leaking into the river.
- Appropriate management strategies of both runoffs and untreated effluent before discharge into the Kat River should be initiated to improve the river's ecological status and health.
- Approaches used in this study could be investigated in other river systems with similar impacts to determine their utility.

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APPENDICES

Appendix A

Table A1: South African Scoring System (SASS) data collected from Kat River in November

	November `				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					A
Perlidae		A			A
EPHEMEROPTERA					
Baetidae 1sp		A	A		
Baetidae 2sp	A			A	
Baetidae>2sp					A
Leptophlebiidae			1		
Tricorythidae		A	A		A
Heptageniidae		A	A		
Oligoneuridae					
ODONATA					
Aeshnidae					
Libellulidae	A				
Coenagrionidae	A	1		1	A
Gomphidae					1
Platycenemidae					
Calopterygidae					
Lestidae					
Chlorolestidae					
TRICOPTERA					
Hydropsychidae 1sp		A		A	A
Hydropsychidae 2sp					
Hydropsychidae >2sp					
Leptoceridae		A		A	A
Ecnomidae					
Pisuliidae					A
Philopotamidae					
COLEOPTERA					
Elmidae					
Gyrinidae	A	A	A	A	1

Hydraenidae					
Dytiscidae					
Hydrophilidae					
Helodidae					
DIPTERA					
Ceratopogonidae	1				1
Chironomidae	A	1		A	
Muscidae					
Simuliidae		A	A	A	
Syphidae					
Tabanidae	A		A		1
Athericidae					
Syrphidae					
Ephydridae					
Psychodidae					
CRUSTACEA					
Potamounatidae	A	1	A		
Atyidae					
Amphipoda					
HEMIPTERA					
Corixidae	A		A		
Naucoridae	A			A	A
Notonectidae					B
Pleidae			A		
Veliidae		1			
Gerridae					
GASTROPODA					
Ancylidae	A			A	
Physidae					A
Lymnaeidae					
Thiaridae					
Planorbinae					
ANNELIDA					
Hirudinea				1	
Oligochaeta			A	A	
HYDRACARINA					
water mites					
TURBELLARIA					
flatworms				A	
Sphaeriidae					
Corbiculidae					

Table A2: South African Scoring System (SASS) data collected from Kat River in February

	February				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					
Perlidae	A	A	A		
EPHEMEROPTERA					
Baetidae 1sp		1			A
Baetidae 2sp	A		A		
Baetidae>2sp					
Leptophlebiidae	A		A		
Tricorythidae	A		A		
Heptageniidae	A	A	A		
Oligoneuridae					
ODONATA					
Aeshnidae	1				
Libellulidae			A		A
Coenagrionidae	A		A		A
Gomphidae	1				
Platycenemidae					
Calopterygidae					
Lestidae					
Chlorolestidae					
TRICOPTERA					
Hydropsychidae 1sp					
Hydropsychidae 2sp			A		
Hydropsychidae >2sp					
Leptoceridae		1			
Ecnomidae					
Pisuliidae					
Philopotamidae					
COLEOPTERA					
Elmidae					
Gyrinidae	1	A	A		
Hydraenidae					
Dytiscidae			A		
Hydrophilidae					
Helodidae					

DIPTERA					
Ceratopogonidae					
Chironomidae			A	B	B
Muscidae				A	
Simuliidae	1	A			
Syphidae					
Tabanidae		1	1		
Athericidae			1		
Syrphidae					
Ephydridae					
Psychodidae					
CRUSTACEA					
Potamounatidae	A	A	A		
Atyidae					
Amphipoda					
HEMIPTERA			1		
Corixidae			A		A
Naucoridae					
Notonectidae	A	A			B
Pleidae	1				
Veliidae	A	A	A		
Gerridae		1			
GASTROPODA					
Ancylidae			A		
Physidae					
Lymnaeidae					
Thiaridae					
Planorbinae			1		
ANNELIDA					
Hirudinea			1	A	
Oligochaeta	1		A	A	
HYDRACARINA		1			
water mites					
TURBELLARIA					
flatworms					1
Sphaeriidae					
Corbiculidae			A		

Table A3: South African Scoring System (SASS) data collected from Kat River in April

	April				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					
Perlidae					
EPHEMEROPTERA					
Baetidae 1sp		A	1		
Baetidae 2sp					
Baetidae>2sp				A	
Leptophlebiidae	A	A	A		A
Tricorythidae					
Heptageniidae		A	A		
Oligoneuridae	A	A			A
ODONATA		A	A		
Aeshnidae					
Libellulidae					
Coenagrionidae					
Gomphidae					
Platycenemidae	1				
Calopterygidae					A
Lestidae					
Chlorolestidae	A	A			A
TRICOPTERA					
Hydropsychidae 1sp			1		
Hydropsychidae 2sp					
Hydropsychidae >2sp					
Leptoceridae					
Ecnomidae					
Pisuliidae		A		A	
Philopotamidae					
COLEOPTERA					
Elmidae			1		
Gyrinidae			1		
Hydraenidae					
Dytiscidae	A				
Hydrophilidae					
Helodidae					
DIPTERA					

Ceratopogonidae	A	1			
Chironomidae					
Muscidae		A			
Simuliidae					
Syphidae		1	A		
Tabanidae					
Athericidae					
Syrphidae		1			
Ephydridae	1	1		A	
Psychodidae					
CRUSTACEA		1	1	A	
Potamounatidae					
Atyidae				A	
Amphipoda	1				
HEMIPTERA					
Corixidae					
Naucoridae					
Notonectidae					
Pleidae					
Veliidae					
Gerridae					
GASTROPODA	A	A	A		
Ancylidae					
Physidae					
Lymnaeidae					
Thiaridae					1
Planorbinae			1	A	A
ANNELIDA		A			
Hirudinea			1		
Oligochaeta					
HYDRACARINA		1			
water mites					
TURBELLARIA					
flatworms					
Sphaeriidae					
Corbiculidae					

Table A4: South African Scoring System (SASS) data collected from Kat River in June

	June				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					
Perlidae					
EPHEMEROPTERA					
Baetidae 1sp			A		
Baetidae 2sp					
Baetidae>2sp			A		
Leptophlebiidae	A	A			
Tricorythidae					
Heptageniidae		A	A		A
Oligoneuridae	A	A			
ODONATA	A	A			
Aeshnidae					
Libellulidae					
Coenagrionidae	A	A			
Gomphidae					
Platycnemidae	A				
Calopterygidae					A
Lestidae	1	1		1	A
Chlorolestidae		1			
TRICOPTERA					
Hydropsychidae 1sp	1		A		A
Hydropsychidae 2sp					
Hydropsychidae >2sp					
Leptoceridae					
Ecnomidae					
Pisuliidae			A		
Philopotamidae	A	A			
COLEOPTERA					
Elmidae					A
Gyrinidae					
Hydraenidae					
Dytiscidae					
Hydrophilidae					
Helodidae					
DIPTERA					

Ceratopogonidae	A	1	A	A	
Chironomidae					
Muscidae		A			A
Simuliidae					
Syphidae					
Tabanidae					
Athericidae					
Syrphidae					
Ephydridae	1	A		1	A
Psychodidae					
CRUSTACEA	A	A	A		
Potamounatidae					
Atyidae		1		1	A
Amphipoda	A		A		
HEMIPTERA					
Corixidae					
Naucoridae					
Notonectidae					
Pleidae					
Veliidae					
Gerridae					
GASTROPODA	1	A			
Ancylidae					
Physidae					
Lymnaeidae					
Thiaridae		A		A	A
Planorbinae					
ANNELIDA		1			A
Hirudinea					
Oligochaeta					
HYDRACARINA					
water mites					A
TURBELLARIA					
flatworms					
Sphaeriidae	1				
Corbiculidae					

Table A5: South African Scoring System (SASS) data collected from Kat River in August

	August				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					
Perlidae					
EPHEMEROPTERA					
Baetidae 1sp					
Baetidae 2sp	A	A	A		
Baetidae>2sp	A				
Leptophlebiidae	A	A	A		
Tricorythidae	A	A			
Heptageniidae	A	A	A		
Oligoneuridae					
ODONATA					
Aeshnidae	1		A		
Libellulidae		1			1
Coenagrionidae			A		A
Gomphidae	1				1
Platycnemidae					
Calopterygidae					
Lestidae					1
Chlorolestidae					
TRICOPTERA					
Hydropsychidae 1sp		A	A		
Hydropsychidae 2sp					
Hydropsychidae >2sp					
Leptoceridae			1		A
Ecnomidae					
Pisuliidae					
Philopotamidae	1				
COLEOPTERA					
Elmidae		1			
Gyrinidae	A		A		
Hydraenidae					
Dytiscidae	1	A			A
Hydrophilidae					
Helodidae					
DIPTERA					

Ceratopogonidae					
Chironomidae	A	A	1	A	A
Muscidae					
Simuliidae	A	A			
Syphidae					
Tabanidae	1				
Athericidae			1		
Syrphidae					
Ephydridae					
Psychodidae					
CRUSTACEA					
Potamounatidae	1	1	A		
Atyidae					
Amphipoda					
HEMIPTERA					
Corixidae	A				1
Naucoridae		1			
Notonectidae					A
Pleidae					
Veliidae		1			
Gerridae					
GASTROPODA					
Ancylidae					1
Physidae					A
Lymnaeidae					
Thiaridae					
Planorbinae	1				
ANNELIDA					
Hirudinea					
Oligochaeta		1			A
HYDRACARINA					
water mites					
TURBELLARIA					
flatworms				A	
Sphaeriidae		A	1		
Corbiculidae	1				

Table A6: South African Scoring System (SASS) data collected from Kat River in October

	October				
Taxa	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
PLECOPTERA					
Notonemouridae					
Perlidae			A		
EPHEMEROPTERA					
Baetidae 1sp	1	A	A	A	
Baetidae 2sp		A			
Baetidae>2sp					
Leptophlebiidae		A	A		
Tricorythidae		A			
Heptageniidae	1	A			
Oligoneuridae					
ODONATA					
Aeshnidae			1		
Libellulidae	1		1		1
Coenagrionidae	A	1			A
Gomphidae	A				
Platycnemidae					1
Calopterygidae					
Lestidae					
Chlorolestidae					
TRICOPTERA					
Hydropsychidae 1sp		A	1		
Hydropsychidae 2sp					
Hydropsychidae >2sp					
Leptoceridae					A
Ecnomidae					
Pisuliidae					
Philopotamidae					
COLEOPTERA					
Elmidae					
Gyrinidae		A		1	
Hydraenidae					
Dytiscidae			1	1	
Hydrophilidae					
Helodidae					
DIPTERA					

Ceratopogonidae					
Chironomidae	1	A	A	A	
Muscidae					
Simuliidae		A	A		
Syphidae					
Tabanidae			1		
Athericidae					
Syrphidae					
Ephydridae					
Psychodidae					
CRUSTACEA					
Potamounatidae			1		
Atyidae					
Amphipoda					
HEMIPTERA					
Corixidae	A	B	1	A	A
Naucoridae		1	1		A
Notonectidae		1			A
Pleidae					
Veliidae	1		1		
Gerridae					
GASTROPODA					
Ancylidae					
Physidae				1	1
Lymnaeidae					
Thiaridae					
Planorbinae					A
ANNELIDA					
Hirudinea					
Oligochaeta		A	A	A	
HYDRACARINA					
water mites					
TURBELLARIA					
flatworms					
Sphaeriidae					
Corbiculidae	A	A			A

Appendix B

Table B1: SASSS Score data collected over the study period (December 2015-November 2016)

SASS SCORE					
Seasons	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
December	148	154	139	91	109
February	131	177	189	22	38
April	133	156	144	45	143
June	172	159	183	47	97
August	201	180	174	17	78
October	157	165	166	44	93

Table B2: ASPT data collected over the study period (December 2015-November 2016)

ASPT					
Seasons	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
December	5.92	6.42	6.32	4.33	3.4
February	5.96	6.81	6.09	4.4	3.16
April	6.65	6.5	6.26	3.46	5.3
June	6.37	5.68	6.3	3.35	4.85
August	6.48	6	6.44	2.53	4.11
October	6.27	6.28	6.28	3.61	4.16

Table B3: Number of Taxa data collected over the study period (December 2015-November 2016)

NO. of TAXA					
Seasons	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
December	25	24	22	21	32
February	22	26	31	5	12
April	20	24	23	13	27
June	27	28	29	14	20
August	29	30	27	7	19
October	24	26	26	12	22

Appendix C

Table C: The full scientific names of the diatom species sampled at Kat River over the study period (December 2015-November 2016).

DIATOMS SPECIES NAMES
Cocconeis engelbrechtil
Cocconeis placentula var. lineata
Cocconeis placentula var euylypta
Cocconeis placentula Ehrenberg
Navicula radiso
Navicula zanonii
Navicula rosttellata
Navicula symmetrica Patrick
Navicula schroeteri Meister
Navicula veneta kutzing
Navicula angusta Grunow
Navicula tenelloids hustedt
Navicula erifuga
Navicula tripunctata
Navicula trivialis Larnge-Bertalot
Navicula cryptotenelloides
Navicula capitatoradiata
Navicula riediana
Navicula cryptotenella
Navicula recens
Nitzschia sigma
Nitzschia etoshensis
Nitzschia agnita
Nitzshia acicularis
Nitzshia linearis var-subtilis

Nitzchia calida Grunow
Nitzschia aurariae Cholnoky
Nitzschia nana Gronow
Nitzschia debilis
Nitzschia elegantula Grunow
Nitzschia communis
Nitzschia reversa
Nitzschia Capitellata Hustedt
Nitzschia gracilis Hantzsch
Nitzschia droveillensis coste
Nitzschia pura hustedt
Nitzschia recta Hantzsch
Nitzschia microcephala
Nitzschia Filiformis
Nitzshia iremissa Cholnoky
Nitzschia palea
Nitzschia Fonticola Grunow
Nitzschia linearis
Nitzschia Frustulum
Nitzschia amphibia
Nitzschia cleusii Hantzsch
Nitzshia pusila Grunow
Fragilaria biceps
Fragilaria ulna
Fragileria capucina var rumpens
Fragilaria ungeriana
Fragilaria ulna var.acus
Fragilaria capucina var.vaucheriae
Fragilaria Crotonensis
Craticula accomoda
Craticula Halophila
Craticula bucleri
Gomphonema minutum

Gomphonema aff-gracile
Gomphonema parvulum var
Gomphonema acuminatum
Gomphonema Venusta
Gomphonema italicum
Gomphonema Halicum
Gomphonema pseudoaugur Krammer
Gomphonema claratum Ehre
Gomphonema pumilum var
Gomphonema insigne Gregory
Encyonema Mesianum
Encyonema caespitosum
Encoyonema minutum
Encyonema silesiacum
Encoyonema Neogracile
Cymbella turgidula Grunow
Cymbella kappi
Cymbella tumida
Cymbella Aspera
Cymbella noicistula Krammer
Staurosira elliptica
Staurosira construens
Aulacoseira grunulata
Aulacoseira Muzza
Amphora Coffeformis
Amphora Capulata
Amphona normannii
Hantzschia amphioxys
Hantzchia distinctepunctata
Gyrosigma scalproides
Gyrosigma rautenbachiae
Gyrosigma acuminatum
Gyrosigma attenuatum

Gyrosigma porkerii
Trybloionella hungarica
Tryblionella apiculate
Tryblionella levidensis
Tryblionella coarctata
Eunotica exiga
Eunotia incisa Gregory
Eunotia Formica Ehrenberg
Eunotia minor
Cyclotella meneghiniana
Cyclotella ocellata
Diploneis suboralis cleve
Diploneis puella
Tabularia Fasciculata
Rhoplodia operculata
Rhopalodia gibba
Sellaphora stroemii
Sellaphora seminulum
Denticula sundoyensis Archibold
Denticula Kuetzingii Gronow
Denticula subtilis Grunow
Pleurosigma salinarum
Pleurosigma elongatum W Smith
Rameria sinuate
Reimera unisenata sala
Caloneis silicula
Caloneis Schumanniana
Caloneis Molaris
Stauronas phoeni
Abbrevata
Diatoma Vulgaris Bory
Achnanthidium
Melosira varians Agardh

Nensis
Actinela aeteoroaia
Adlafia navicula bryophila
Tublionell calida
Tubulana Fasciculate
Neidium productum
Stenopterobia delicattissima
Cymatopleura
Achananthes standeri Cholnoky
Fallacia monoculata
Navicymbula pusilla
Laticola mutica
Frastulia saxonica
Achnanthidium biasole
Hianum
Achnanthidium affine
Planothidium rostratum
Achnanthidium monutissimum
Discostella stellyera
Diadesmis confervcea
Cyclostephanos dubis
Cymatopleura solea
Surirella angusta
Planothidium rostratum
Coloneis Bacillum
Encyonopsis lea var
Lemnicola hungarica
Luticola goeppertiana
Rhorcosphenia abbreviate
Fallacia tenera
Pinnularia borealis
Hippodonta capitate
Achanthidium crissum

Appendix D

Table D: Generalist species that occurred across various groups

Species	s.1	s.2	index	Stat	p-value
Tubulana Fasciculate	1	0	1	0.3333333	1.000
Cocconeis placentula var. lineata	0	1	2	0.4082483	0.422
Cocconeis placentula var euylypta	0	1	2	0.5773503	0.131
Cocconeis placentula Ehrenberg	1	0	1	0.9462545	0.006
Navicula radiso	1	0	1	0.3333333	1.000
Navicula zanonii	0	1	2	0.5630925	0.443
Navicula rosttellata	1	0	1	0.3333333	1.000
Navicula symmetrica Patrick	1	0	1	0.4714045	0.485
Navicula schroeteri Meister	0	1	2	0.4082483	0.392
Navicula veneta kutzing	**1**	**1**	**3**	**0.3651484**	**NA**
Navicula angusta Grunow	**1**	**1**	**3**	**0.3651484**	**NA**
Navicula capitatoradiata	0	1	2	0.4082483	0.392
Navicula riediana	**1**	**1**	**3**	**0.3651484**	**NA**
Navicula cryptotenella	1	0	1	0.3333333	1.000
Navicula recens	**1**	**1**	**3**	**0.8944272**	**NA**
Nitzschia sigma	**1**	**1**	**3**	**0.6831301**	**NA**
Nitzschia acicularis	0	1	2	0.4082483	0.392
Nitzschia linearis var-subtilis	0	1	2	0.4082483	0.411
Nitzschia nana Gronow	**1**	**1**	**3**	**0.3651484**	**NA**
Nitzschia elegantula Grunow	1	0	1	0.3333333	1.000
Nitzschia communis	0	1	2	0.4082483	0.396
Nitzschia reversa		0	1	0.3333333	1.000
Nitzschia Capitella Hustedt	0	1	2	0.4082483	0.396
Nitzschia grcilis Hantzsch	1	0	1	0.3333333	1.000
Nitzschia droveillensis coste	0	1	2	0.4082483	0.411
Nitzschia pura hustedt	0	1	2	0.4082483	0.411
Nitzschia recta Hantzsch	0	1	2	0.6940111	0.092
Nitzschia microcephala	**1**	**1**	**3**	**0.5163978**	**NA**
Nitzschia Filiforms	**1**	**1**	**3**	**0.3651484**	**NA**
Nitzschia palea	**1**	**1**	**3**	**0.9660918**	**NA**
Nitzschia linearis	**1**	**1**	**3**	**0.8164966**	**NA**
Nitzschia Frustulum	1	0	1	0.3333333	1.000
Nitzschia amphibia	**1**	**1**	**3**	**0.5163978**	**NA**
Nitzschia cleusii Hantzsch	1	0	1	0.3333333	1.000
Nitzschia pusila Grunow	1	0	1	0.3333333	1.000

Fragilaria bceps	1	0	1	0.3333333	1.000
Fragilaria ulna	**1**	**1**	**3**	**0.7302967**	**NA**
Fragileria capucina var rumpens	**1**	**1**	**3**	**0.7745967**	**NA**
Fragilaria ungeriana	1	0	1	0.3333333	1.000
Fragilaria capucina var.vaucheriae	1	0	1	0.3333333	1.000
Craticula Halophila	**1**	**1**	**3**	**0.8944272**	**NA**
Craticula bucleri	**1**	**1**	**3**	**0.9309493**	**NA**
Gomphonema minutum	**1**	**1**	**3**	**0.8944272**	**NA**
Gomphonema parvulum var	1	0	1	0.6117753	0.272
Gomophonema acuminatum	1	0	1	0.3333333	1.000
Gomphonema Venusta	0	1	2	0.5773503	0.164
Gomphonema claratum Ehre	1	0	1	0.3333333	1.000
Gomphonema pumilum var	**1**	**1**	**3**	**0.8164966**	**NA**
Encyonema Mesianum	1	0	1	0.6666667	0.200
Encyonema caespitosum	**1**	**1**	**3**	**0.4472136**	**NA**
Encoyonema minutum	**1**	**1**	**3**	**0.6324555**	**NA**
Encyonema silesiacum	1	0	1	0.4714045	0.494
Encoyonema Neogracile	**1**	**1**	**3**	**0.3651484**	**NA**
Cymbella turgidula Grunow	1	0	1	0.3333333	1.000
Cymbella Kappi	0	1	2	0.3741657	0.746
Cymbella tumida	1	0	1	0.6666667	0.110
Staurosira elliptica	1	0	1	0.3333333	1.000
Staurosira elliptica	0	1	2	0.4082483	0.396
Aulacoseira grunulata	1	0	1	0.6435382	0.562
Aulacoseira Muzza	1	0	1	0.3333333	1.000
Amphora Coffeformis	1	0	1	0.3333333	1.000
Amphora Capulata	1	0	1	0.3333333	1.000
Hantzschia amphioxys	1	0	1	0.3333333	1.000
Gyrosigma scalproides	1	0	1	0.4714045	0.486
Gyrosigma acuminatum	1	0	1	0.5416026	0.679
Trybloionella hungarica	0	1	2	0.7071068	0.041
Tryblionella apiculata	**1**	**1**	**3**	**0.5163978**	**NA**
Tryblionella levidensis	**1**	**1**	**3**	**0.6831301**	**NA**
Tryblionella coarctata	1	0	1	0.3333333	1.000
Eunotia incisa Gregory	1	0	1	0.4714045	0.475
Eunotia formica Ehrenberg	1	0	1	0.3333333	1.000
Eunotia minor	1	0	1	0.3333333	1.000
Cyclotella meneghiniana	**1**	**1**	**3**	**0.5773503**	**NA**
Diphloneis suboralis cleve	**1**	**1**	**3**	**0.5163978**	**NA**
Diploneis puella	0	1	2	0.4082483	0.392

Caloneis silicula	1	0	1	0.3333333	1.000
Rhoplodia operculata	0	1	2	0.4082483	0.392
Rhopalodia gibba	1	0	1	0.3333333	1.000
Sellaphora stroemii	0	1	2	0.4082483	0.392
Sellaphora seminulum	0	1	2	0.4082483	0.435
Denticula sundoyensis Archibold	1	0	1	0.3333333	1.000
Denticula Kuetzingii Gronow	1	0	1	0.3333333	1.000
Denticula subtilis Grunow	1	0	1	0.3333333	1.000
Rameria sinuata	1	0	1	0.5773503	0.231
Reimera unisenata sala	1	0	1	0.4714045	0.491
Caloneis Schumanniana	1	0	1	0.3333333	1.000
Caloneis Molaris	1	0	1	0.3333333	1.000
Stauronas phoenix	1	0	1	0.3333333	1.000
Achnanthidium	1	0	1	0.3333333	1.000
Melosira varians Agardh	**1**	**1**	**3**	**0.6324555**	**NA**
Adlafia navicula bryophila	1	0	1	0.3333333	1.000
Neidium prouctum	1	0	1	0.3333333	1.000
Stenopterobia delicattissima	1	0	1	0.3333333	1.000
Cymatopleura	1	0	1	0.3333333	1.000
Achananthes standeris cholnoky	1	0	1	0.3333333	1.000
Fallacia monoculata	1	0	1	0.3333333	1.000
Achnanthidium monutissimum	**1**	**1**	**3**	**0.3651484**	**NA**
Encyonopsis lea var	1	0	1	0.4714	0.468
Lemnicola hungarica	0	1	2	0.88607	0.007
Fallacia tenera	**1**	**1**	**3**	**0.36514**	**NA**
Pinnularia borealis	**1**	**1**	**3**	**0.36514**	**NA**
Hippodonta capitata	1	0	1	0.3333	1.000
Achanthidium crissum	1	0	1	0.3333	1.000