ASSESSMENT OF THE TYHUME RIVER HEALTH STATUS USING MACROINVERTEBRATES AS INDICATORS

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By

NOKONWABA NELLINETH FASI

Supervisor: Professor Daniel O. Okeyo

Department of Zoology and Entomology

University of Fort Hare

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DECLARATION

I declare that this dissertation is a presentation of my original research work, conducted under the supervision of Prof Daniel O. Okeyo. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions.

Signed ……

Nokonwaba Nellineth FASI

Date ……

ABSTRACT

Ten sampling sites were selected along the Tyhume River corresponding to the upper, mid and lower reaches of the River. In total 48 families were collected during the study. The study observed different types of macroinvertebrate taxa; very low tolerant to pollution (e.g Heptageniidae; Notonemouridae; Perlidae), moderately pollution-tolerant (e.g. Caenidae; Chlorolestidae; Tricorythidae) and very tolerant of polluted conditions (e.g. Chironomidae; Muscidae; Oligochaetae). Both univariate and multivariate analyses were done using PRIMER V6. Above Confluence (ABCON, Site 10) had the highest number (37) of species while Mtloko (MTLOK, Site 2) had the lowest number (24) (Chi-square = 0.87; $p < 0.05$). The highest number of individuals (4023) was recorded at Macfairlane (MACFA, Site 5) while the lowest number (1240) at Honeydale (HONEY, Site 8) (Chi-square = 0.00; $p < 0.05$). Margalef's index indicated Above Confluence (ABCON, Site 10; 4.762), had the highest values of species richness while Mtloko (MTLOK, Site 2; 3.227) had the lowest values (Chi-square = 1; $p < 0.05$). Pielou's evenness index indicated that Gqumashe (GQUMA, Site 7) had the highest values (0.7137), while Macfairlane (MACFA; Site 5) had the lowest (0.5109) (chi-square = 1; $p < 0.05$). Shannon-Wiener diversity indices, the highest diversity index values (2.573) of macroinvertebrates was recorded at Honeydale (HONEY, Site 8), and the lowest (1.684) at Macfairlane (MACFA, Site 5) (chi-square = 1; $p < 0.05$). The ASPT Scores did not differ significantly ($P > 0.05$) across sites. No significant difference was observed in the ASPT scores across the 10 sites ($F = 0.75$; $df = 9.60$; $P > 0.05$). Site 4 had the highest mean ASPT Scores with mean of 7.6), whereas the lowest. Three biotopes types, namely: stone, vegetation, gravel-sand-mud, stone and vegetation being the dominant biotopes at the 10 sites. The cluster analyses showed that macroinvertebrates were dependent on biotope preference while stone biotope showing greater macroinvertebrate densities. Euclidean distance of site classification with respect to physico-chemical parameters showed very low stress value (0.01) implying that physico-chemical parameters influenced species distribution within each sampling site. The results also showed that good health conditions existed at the most upstream sites than the lower reaches of the river, thus, indicating impacts of pollution within the river.

DEDICATION

This dissertation is dedicated to my late father, Cebo Fasi, who taught me that even the largest task can be accomplished if it is done one step at a time; and to my mother, Nokhaya Gloria Fasi, who taught me that the best things in life are worth fighting for; and lastly, to my beloved daughter, Buncwane Fasi, who made me stronger and more resilient towards achieving my dream.

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ACRONYMS

ANOVA	Analysis of Variance
AMD	Acid Mine Drainage
ANOSIM	Analysis of Similarity
ASPT	Average Score per Taxon
AUSRIVAS	Australian River Assessment System
BMWP	Biological Monitoring Working Party
CSIR	Council for Scientific and Industrial Research
DEAT	Department of Environmental Affairs and Tourism
DO	Dissolved Oxygen
DSS	Decision Support System
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
EIA	Environmental Impact Assessment
EPA	Environmental Protection Authority
EPT	Ephemeroptera, Plecoptera and Trichoptera
FAII	Fish Assemblage Integrity Index
FPOM	Fine Particulate Organic Matter
GPS	Global Positioning Systems
GSM	Gravel, Sand, and Mud

HII	Habitat Integrity Index
IHAS	Integrated Habitat Assessment System
MANOVA	Multivariate Analysis of Variance
MDG	Millennium Development Goals
MDS	Multidimensional Scaling
NEMA	National Environmental Management Act
NGO	Non-Governmental Organization
NWA	National Water Act
pH	Hydrogen Ion
RBP	Rapid Biomonitoring Protocol
RDP	Reconstruction and Development Programme
RHP	River Health Programme
RIVPACS	River Invertebrate Prediction and Classification
RVI	Riparian Vegetation Index
S	Stone biotope
SA	South Africa
SASS5	South African Scoring System version 5.0
SIMPER	Similarity Percentage
TDS	Total Dissolved Salts

UK	United Kingdom
UNCHE	United Nations Conference on the Human Environment
USA	United States of America
VEG	Vegetation Biotope
WCED	World Commission on Environment and Development
WQI	Water Quality Index
WRC	Water Research Commission
WSSD	World Summit for Sustainable Development

PREAMBLE

Human alteration and degradation of rivers worldwide are the primary reasons why biomonitoring programs are utilized (Department of Water Affairs & Forestry, DWAF, 1997). DWAF initiated the development of policies and legislation to guide protection, management and sustainable use of water resources through the establishment of the National Water Act No. 36 of 1998 (NWA, 1998). The main principle of NWA is that of ensuring sustainability of aquatic systems by monitoring aquatic ecosystems (Pollard & du Toit, 2005). Rivers are homes for small animals such as macroinvertebrates. The term macroinvertebrate describes animals without backbone, which can be seen with naked eye as well as be retained in a 0.25 mm mesh net (Dallas *et al*., 2004; Dickens & Grahams, 2002). Macroinvertebrates are used worldwide to provide an estimate of river health status; they provide a quantitative assessment of river health based on index scores (Kasangaki *et al.,* 2007). Since these organisms have different ranges of tolerance to pollution, they are regarded as the good indicators of pollution in rivers. The advantage of using macroinvertebrates is that they are exposed to all levels of water quality that affect them, including high and low concentrations (Dallas, 2002; Kleynhans, 2005). Thus families present in the riverine ecosystems provide both the historical and present state of water quality and allow realization of events that could have been missed by other more traditional physico- chemical analyses (Masese *et al*., 2009; Mason & Parr, 2003). Biological impairment of aquatic ecosystems is generally indicated by the absence of pollution intolerant macroinvertebrates families and the dominance of pollution tolerant families (Ollis *et al.,* 2010; Gerber & Gabriel, 2002a). Studies of pollution tolerance or resistance by macroinvertebrates also need to cover their habitat preference and abundances (Palmer & Taylor, 2004).

Thesis Structure: The study comprises 6 chapters.

Chapter 1: This chapter is the general literature review of the objectives of the study. Sustainability, water resources management and biomonitoring in general are briefly reviewed. River health, physic-chemical parameters used during data gathering, and their possible impacts on the aquatic ecosystem, are also described and reviewed. The chapter states the overall aim and the objectives of the study.

Chapter 2: In this chapter physical and biological indicators used during data gathering are described and critically reviewed. Procedures followed in selecting sites are described; data analysis methods are also described. The study starts from description of the study area. Procedures followed in selecting sites are described. Procedures followed in data collection along the river are also described.

Chapter 3: This chapter is the first data-based chapter. It focuses on the results gathered during the study.

Chapter 4: This chapter is the second data-based chapter. Chapter 4 focuses on discussion of results gathered during the study.

Chapter 5: This is a concluding chapter based on results obtained during this study, with recommendations on further biomonitoring of the same river.

Chapter 6: This chapter is the compilation of all the references in this study.

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Background

River resources are in danger of degradation, raising negative impacts for the community, environment and economy (Sulaiman, 2016). These resources are representatives of the most heavily exploited commodities in southern Africa and, therefore, require protection for sustainability (Sibanda *et al.*, 2014). The social and economic value of rivers can be expressed in terms of the goods and services provided by these aquatic ecosystems (Boon, 2000). Some of the goods and services that we get from healthy river systems include, tourism and recreation value (generated by aquatic habitats and species), domestic water value, consumptive water value, medicinal riparian plants value, building materials value, and cultural value (Palmer *et al.,* 2004a).

By 2008 over a billion of the world's people lacked safe drinking water, while over twice that number have no adequate sanitation (Sulaiman, 2016). Water is an essential ingredient to virtually all the generations, thus, having strong national commitment to improving the quality of life of current generations, while reinforcing the notion of development that aims for equity within and between generations (Department of Water Affairs and Forestry, DWAF, 2004a; Watson & Dallas, 2013). Managing the natural resource base of economic, social and environmental strategies, requires both integration between the different pillars, and decision making that promotes equity, both within the current generation, and among future generations (Dallas & Day, 2007). The South African government is faced with extremely limited and scarce water resources and, therefore, recognizes sustainability as fundamental (Palmer & Taylor, 2004). To achieve this, DWAF initiated the development of policies and legislation to guide protection, management and sustainable use of water resources, through the

establishment of the National Water Act No. 36 of 1998 (NWA, 1998). The main principle of NWA is that of ensuring sustainability of aquatic systems by monitoring aquatic ecosystems. The monitoring results can in turn, be used to support certain legal principles contained in the National Environmental Management Act (NEMA) No. 107 of 1998 (Palmer, 1999). The NWA gives DWAF the tools to gather the optimal protection, use and management of South Africa's water resources (DWAF, 2006). The main purpose of NEMA is to ensure present and future generations the right to an environment that is not harmful to health.

1.1.2 The National Water Act (NWA)

The main principle of National Water Act No. 36 of 1998 (NWA, 1998), is that of ensuring sustainability of aquatic systems. The NWA gives effect to the constitutional right of access to water, by aiming to achieve integrated water resource management ensuring equitable and sustainable use, and access to resources. NWA gives DWAF the tools to gather the optimal protection, use and management of the South Africa's water resources (DWAF, 1994).

The NWA is founded on three principles, which are derived from Fundamental Principles and Objectives for a New South African Water Law and the National Water Policy, for managing water resources (NWA, 1998). According the NWA (1998), the principles are as follows:

- *Equitable access to water*: that is equal access to water irrespective of race, gender and age;
- *Sustainable use and protection of water resources*: humankind and ecosystems are interdependent and there should be a balance between water resources utilisation, development and their protection. People have to be conscious of the

fact that their land-use activities may impact negatively on the quality and quantity of water in their catchments;

- *Efficient water use*: as South Africa is a water-scarce country with evaporation higher than rainfall, water has to be used efficiently whilst ensuring social and economic development.

1.1.3 The National Environmental Management Act (NEMA)

The National Environmental Management Act (NEMA) deals with natural systems and the physico-chemical, aesthetic and cultural properties of that system, which influence human health and well-being (Bredenhand, 2008). The main purpose of this Act is to refer people and future generations' right to an environment that is not harmful to health. Other laws formed prior are overruled by NEMA. Getting correct information is crucial to solving environmental problems, and NEMA guarantees people access to government information about the state of the environment, actual or future threats and hazardous waste, if reasonable. According to Roux *et al*. (1999), the Act can be applied to things that have taken place since its adoption, and can include:

- The violation of an environmental law;
- Government ignoring principles of NEMA;
- Government giving permission for a potentially harmful activity, or development needing permission by law, without requiring a proper environmental impact assessment (EIA) first; and
- Anyone, including government, causing significant pollution or environmental degradation through construction or service provision.

1.1.4 Biomonitoring Concepts

It is important to monitor and manage the health of river systems, as these are central to human welfare and economic development in many ways (Breu *et al*., 2008; Watson & Dallas 2013). This is done through an ecosystem monitoring programme called biomonitoring. Biomonitoring is the process of measuring biological aspects of aquatic ecosystem (Gerhartd, 2000), utilizing one or more living organism component (Kaaya *et al*., 2015; Rajele, 2004). Biomonitoring evaluates the impact change on river resources for the entire river continuum, utilizing one or more components of the biota (e.g. macroinvertebrates) (Dallas, 2007; Mangadze *et al*., 2016; Mason & Parr, 2003). Biomonitoring has been applied in numerous regions throughout the world (Rajele, 2004) and is in the state of continual refinement in order to improve precision and accuracy of identifying and assessing impacted aquatic resources. Historically, biomonitoring research in aquatic sciences has focused on fish, macroinvertebrates and algae; therefore, there are many biological indicators from which to choose (Dickens & Graham, 2002; Mangadze *et al*., 2016; Ollis *et al*., 2006a). Algae, fish, and macroinvertebrates are especially sensitive to changes in water quality and are, therefore, the living organisms most commonly used in monitoring water quality (Palmer *et al*., 2005). Of all organisms, macroinvertebrates appear to be the most widely used (Dallas, 2005; Mangadze *et al*., 2016; Ollis *et al*., 2006b). The assessment of biotic aquatic community health typically compares aquatic community assemblages found in a collection of sites varying in degree of human impacts (Silveira *et al*., 2005), from those observed in reference conditions (Azrina *et al*., 2006; Mangadze *et al*., 2016; Maseti, 2005).

1.1.5 River Health Programme (RHP)

South Africa has a history of biological assessment research in aquatic sciences starting more than 30 years ago (1972), although only becoming popular in 1994, after the formal design and implementation of the River Health Programme (RHP) (Chutter, 1998). South Africa uses what is called “rapid bioassessments”. This is not time-consuming, and is cost effective, far less than the more detailed bioassessments (Dallas, 2000; Watson & Dallas, 2013). The South African government has committed itself, through Section 24 of the constitution, to ensuring that all households have access to clean tap water (RHP, 2001). This was the initiative of DWAF, which is the custodian of South Africa’s water resources. The activities of the RHP are informed by legislation in the National Environmental Management Act (NEMA) No. 107 of 1998 and the National Water Act (NWA) No. 36 of 1998. The main purpose of RHP is to serve as a source of information regarding the overall ecological status of South Africa’s river systems, expanding this information in order to support their management (RHP, 2004). The NWA recognizes the best way to achieve the equitable, efficient, and sustainable use of water resources, would be to manage aquatic ecosystems (including rivers) at the catchment scale and through joint participation by all interested parties. The objectives of RHP (RHP, 2003, 2004) are to:

- measure, assess and report on the ecological state of aquatic ecosystems;
- detect and report on the spatial and temporal trends in the ecological state of aquatic ecosystems;
- identify and report on emerging problems regarding aquatic ecosystems;
- ensure that all reports provide scientifically and managerially relevant information for the national aquatic ecosystem management.

The RHP established comprehensive sampling indices that generate baseline data for biomonitoring (RHP, 2003). These indices include, the use of aquatic macroinvertebrates [e.g. South African Scoring System (SASS); Chutter, 1998], fish assemblages [Fish Assemblage Integrity Index (FAII); Kleynhans, 1999] and riparian vegetation [Riparian Vegetation Response Assessment Index (VEGRAI); Kleynhans *et al*., 2005]. Indices also include physical indicators, which are habitat [Habitat Integrity Index (HII); Kleynhans *et al*., 2005], geomorphology [Geomorphological Index (GI); Rowntree & Ziervogel, 1999], water quality [Water Quality Index (WQI); Eekhout *et al*., 1996) and water flow [Hydrological Index (HI); Eekhout *et al*., 1996)].

1.1.6 The use of macroinvertebrates in biomonitoring

The use of biota (e.g. macroinvertebrates, fish and vegetation) and abiotic components (e.g. water quality and geomorphology; Baron *et al*., 2003; Kamis *et al*., 2014; Ollis *et al*., 2006a; Weber *et al*., 2004), is recognized as a tool in providing a direct and integrated measure of the health of an aquatic system as a whole, and any changes that may occur (Dallas & Day, 2007; Rajele, 2004). The advantage of using macroinvertebrates is that, they are mostly sedentary, with different ranges of tolerance to pollution (Chang *et al*., 2014) and are exposed to all levels of water quality that affect them at high or low concentrations (Bailey *et al*., 2004; Dickens & Graham, 2002). Thus, macroinvertebrate families present in riverine ecosystems provide both the historical and present state of water quality (Parsons *et al*., 2003) and allow realization of events that could have been missed by other more traditional physico-chemical analyses (Masese *et al*., 2009). Macroinvertebrates are animals without backbones which are easily seen by naked eyes. Specifically, these include various groups of worms (flatworms, eelworms and segmented roundworms), molluscs (snails and bivalves),

crustaceans, mites, and above all, insects (Revenga *et al.,* 2000). Common physical and chemical (physico-chemical) measurements often taken simultaneously to a biological measurement include pH, temperature, salinity, turbidity, conductivity, nutrient levels and the amount of dissolved oxygen (DO) in the water (Maseti, 2005; Rajele, 2004; Statzner *et al*., 2001). These measurements are used because they provide details of environmental conditions at the moment a sample was taken. The major goal in biomonitoring is to evaluate the impact of human activities on biological resources (Mangadze *et al*., 2016; Nelson, 2011). Protocols for using macroinvertebrates to monitor water quality have been published and implemented in many countries.

The presence or absence of specific types of macroinvertebrates is just one way in which information can be obtained about environmental quality (Dallas, 2007; Tate & Husted, 2016). Other information can be obtained by using diversity, abundance, and community structure as tools to detect environmental quality (Azrina, *et al.*, 2006; Baron *et al*., 2003; Maseti, 2005). Drastic environmental changes may influence the biology of macroinvertebrates and such changes can be obtained through biomonitoring of physico-chemical variables (Weber *et al*., 2004).

1.1.7 The South African Scoring System version 5 (SASS5) Index

SASS was developed (Chutter, 1998) as relatively simple index, which is based on the families of riverine macroinvertebrates present at a site (Dickens & Graham, 2002; Maseti, 2005; Odume *et al.*, 2012). SASS monitoring forms the backbone for RHP under the custodianship of DWAF (RHP, 2004; Statzner *et al*., 2001). SASS is a technique based on BMWP that has been adopted for South African conditions (Chutter, 1998; Thirion *et al.,* 1998). SASS has been tested and has been proven scientifically reliable, robust technique and is now in the 5th version (SASS5; Dickens & Graham, 2002).

SASS5 protocol differs from other versions mainly in terms of sensitivity weighting for certain taxa (Dickens & Graham, 2002). It is widely used in South Africa as a tool for assessing water quality and river health (Dallas, 2005; Vos *et al*., 2002; Watson & Dallas, 2013). SASS5 is very economical, simple and being easy to use, provided that the person doing the sampling is able to identify macroinvertebrates in the field, to family level (Chutter, 1998; Dallas, 2004; Roach *et al.,* 2000). Although SASS5 has proved useful in assessing water quality and general river health, problems still exist in interpreting collected data using the instrument (Maseti, 2005; Ollis *et al*., 2006). For instance, if a site has a SASS5 score less than the expected as compared to a least impacted site, the problem arises in determining what such an expected SASS5 Score, and indeed, macroinvertebrate community might be (Dallas, 2004; Ollis *et al*., 2006a). Biotic differences such as geology, geomorphology, climate, and others, which are a response to regional differences, need to be taken into account when interpreting data. For this reason, a regional reference condition approach which incorporates spatial differences in invertebrate communities has been adopted in South Africa (Dallas, 2000). SASS5 also cannot provide information about the degradation of habitat, so habitat assessment indices (e.g. Integrated Habitat Assessment System (IHAS; McMillan, 1998), are routinely conducted with SASS5. Results from the IHAS are used to aid interpretation of the final SASS5 scores. IHAS results are used to moderate SASS assessment when biotope quality impaired (Dickens & Graham, 2002). It is important to point out, though, that a technique like SASS cannot be used as a replacement for all other types of monitoring. For instance, it cannot distinguish between the effects of different kinds of pollutants, or be used in very large, strongly flowing rivers; and is to some extent dependent on habitat availability. SASS5 is not accurate for lentic conditions; rivers recently exposed to floods, and should be used with caution in

ephemeral rivers (Dickens & Graham, 2002; Watson & Dallas, 2013). SASS5 is not destructive, in that the specimens are returned to the river after being identified.

SASS protocol has now been modified by other Africa countries, suiting their conditions, in order to develop their own aquatic macroinvertebrate indices. These biotic indices are the Namibian Scoring System (NASS) (Palmer & Taylor, 2004), the Okavango Assessment System (OKAS; Kaaya *et al*., 2015), and the Zambian Invertebrate Scoring System (ZISS) and the Tanzania River Scoring System (TARISS) (Kaaya *et al*., 2015).

PROBLEM STATEMENT

Degradation or any discreet event that damages abiotic properties of a riverine ecosystem, plays a role in structuring most ecological communities, particularly stream benthic communities.

RESEARCH QUESTIONS

Assessment of the taxonomic composition and sensitivity of macroinvertebrates associated with three types of biotopes (stones, vegetation and gravel-sand-mud, GSM) resulted in monitoring of effectiveness of using the SASS5 protocol as a tool for assessing the health status of the Tyhume River.

RESEARCH HYPOTHESIS

It was hypothesised that unimpaired streams have much diversity of macroinvertebrate taxa and are dominated by intolerant families whilst polluted streams have less macroinvertebrate diversity taxa and are dominated by tolerant species. Biotope availability influence macroinvertebrates assemblages on a site.

1.1.8 Aim

The aim of this study was to come up with baseline data of the health status of Tyhume River.

1.1.9 Objectives

The study identified five objectives:

- To determine the characteristics of habitat types at study sites on Tyhume River;
- To determine the diversity of macroinvertebrates inhabiting Tyhume River at each site;
- To determine the ecological state of each site;
- To evaluate the effect of habitat preference in macroinvertebrate distribution;
- To determine the effects of physicochemical parameters on macroinvertebrates distribution

1.2 LITERATURE REVIEW

1.2.1. Determining the characteristics of habitat types

1.2.1.1 The SASS5 Biotopes

SASS5 requires collections of macroinvertebrates from a full range of biotopes available at each site (Dallas, 2007; Odume *et al.*, 2012). Most sites must include some of the following biotopes: stones in current (SIC), stones out of current (SOOC), sediment (e.g. sand, mud or a combination of these), instream aquatic vegetation (AQV), marginal vegetation (MV) along the river banks, and gravel (Brown, 2001). The presence of a diversity of biotopes would indicate high SASS5 scores under natural flow and water quality conditions (Dallas, 2000; Maseti, 2005). Therefore, availability of biotopes for sampling may affect SASS scores (Odume *et al.*, 2012). These biotopes and their sampling protocols are described in detail by Dickens & Graham (2002). Macroinvertebrates encountered from each biotope are recorded on a SASS sheet, with their abundance being noted on the sheet. The total SASS5 score is greatly affected by the number of biotopes sampled (Dallas, 2004; Diedericks, 2011).

1.2.1.2 The SASS5 Sensitivity Scores

SASS5 is a scoring system based on riverine macroinvertebrates, whereby each taxon is allocated a sensitivity/tolerance scores according to the water quality conditions it is known to tolerate (Bird *et al*., 2014, Diedericks, 2011). There are three categories where each taxon of macroinvertebrates from South African rivers has been allocated a score. The higher the score, the greater the organism's sensitivity and the lower is its tolerance (Chang *et al*., 2014; Tripole *et al.*, 2008)**.** According to RHP standards, tolerance scores range from 1 to 15 (Table 1.1; Dallas, 2005; Maseti, 2005; Vos *et al*., 2002). Organisms with low values (1-5) are considered to be highly tolerant to pollution; organisms with medium values (6-10) are considered moderately tolerant to pollution; organisms with high tolerance values (11-15) are considered to have very low tolerance to pollution (Dickens & Graham, 2002; Gerber & Gabriel, 2002a). Examples of high-scoring, pollution sensitive macroinvertebrate taxa include Baetidae and Perlidae; examples of low-scoring, pollution intolerant macroinvertebrate taxa include Simullidae (Gerber & Gabriel, 2002a).

Table 1.1 Macroinvertebrate tolerance scores (Gerber & Gabriel, 2002a).

Class	Water quality preference	Sensitivity weighting
A	Highly tolerant to pollution	1-5
B	Moderately tolerant to pollution	6-10
C	Very low tolerance to pollution	11-15

1.2.2 Determining the macroinvertebrate families inhabiting the river

1.2.2.1 Macroinvertebrate Identification

Benthic macroinvertebrate assemblages have been used to assess the biological integrity of stream ecosystems, throughout the world, with relatively good success (Dallas, 2004; Kaaya *et al*., 2015). The use of the indices gives an indication of the quality of the riverine environment, since some of the organisms are regarded as good indicators of the existence of pollution in rivers (Leigh *et al*., 2016). Macroinvertebrates population tells us something about the trend in river health (Mangadze *et al*., 2016). The composition of the macroinvertebrate population typically includes immature forms of aquatic insects, aquatic worms, crustaceans, and molluscs (Weber *et al.,* 2004). These animals are mostly benthic (bottom dwellers) and are associated with bottom substrates such as rocks, logs, sediment, debris, and submerged plants (Odhiambo & Mwangi, 2014; Turley *et al*., 2016). Macroinvertebrates are invertebrates, large enough to be seen with the naked eye, that inhabit rivers, streams, lakes, and ponds (Riens *et al*., 2013).

They are affected by the physical, chemical, and biological conditions of the stream; they can't escape pollution, thus, show the effects of short and long-term pollution events. Some are very intolerant of pollution, may show the cumulative impacts of pollution (Dickens, 2000; Weber *et al*., 2004), and their ability to reflect the environment in which they live through the scores of all the individual taxa sampled at a site (Dallas, 2004; Farrell, 2014). These are summed and/or averaged to provide a value by which the integrity of the biotic community at the site can be gauged (Kasangaki *et al*., 2006). Biological impairment of aquatic ecosystems is generally indicated by the absence of pollution intolerant macroinvertebrates families, such as mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddiesflies (Trichoptera) (Ross-Gillespie *et al*., 2018), and

the dominance of pollution tolerant families, such as Chironomid and Oligochaetae taxa (Al-shami *et al*., 2010; Gerber & Gabriel, 2002a). The presence or absence of specific types of macroinvertebrates is just one way in which information can be obtained about environmental quality and the river condition (Buss *et al*., 2015; Scherman *et al*., 2006). Biomonitoring metrics are based on the idea that unimpaired streams and rivers have many macroinvertebrate taxa and are dominated by intolerant families (Morse *et al*., 2007). Conversely, polluted streams have fewer numbers of macroinvertebrate taxa and are dominated by tolerant species (Buss *et al*., 2015; Riens *et al*., 2013).

1.2.2.2 Macroinvertebrate Diversity in Aquatic Systems

Macroinvertebrate diversity and distribution is a reflection of the stream health conditions (Romero *et al*., 2013; Buss *et al*., 2015), and macroinvertebrates sampled in healthy streams are different from those found in impaired water systems (Odhiambo & Mwangi, 2014; Odume *et al.*, 2012); macroinvertebrate diversity is highly great in the healthy than the impaired water systems (Bowd *et al*., 2006). Study conducted by Masese *et al*. (2009), indicated that Heptageniidae and Oligoneuridae families dominated the healthy water systems, due to their high sensitivity rate to the environmental stresses occurring in fresh water system. Masese *et al.* (2009) also found that balance of life in water systems was affected due to the greater numbers of few species of the Baetidae and the Chironomidea families observed, which are known to be pollution tolerant, and their presence in a river is an indication of pollution.

In a healthy river, there will be diversity in macroinvertebrate community and will include a variety of pollution-sensitive/intolerant macroinvertebrates (Diedericks, 2011; Malherbe & Vuren, 2010). It is expected that in an unhealthy river, there may be only a few types of macroinvertebrates present and dominated by pollution-tolerant

macroinvertebrates, such as midges (e.g. Chironomidae) (Al-shami *et al*., 2011; Ashton, 2007; Azrina *et al*., 2006; Mbikwana *et al*., 2010). Several researchers have investigated the effects of pollution on macroinvertebrates and found reduced diversity in pollution impacted areas and community shifts from intolerant to tolerant taxa (Azrina *et al*., 2006; Bredenhand & Samways, 2009; Dallas, 2007b; Diedericks, 2011). Many Ephemeroptera, Plecoptera and Trichoptera species are highly sensitive to Acid Mine Drainage (AMD) and will almost be eliminated in impacted streams (Brown, 2001; de Moor *et al*., 2003). The species diversity is an inconsistent measure of pollution levels, since, although diversity can be high in clean water and low in polluted, it may be as high or as low at intermediate levels of pollution (Bailey *et al*., 2004; Watson & Dallas, 2013). Diversity indices alone can and have been used as evidence of stream perturbation, but some authors recommend the use of additional evidence, such as species composition and biomass. This is because variations in stream physico-chemical conditions are a particular site may not be significant but may create variable diversity in even relatively unpolluted streams (Ollis *et al.,* 2006a; Statzner *et al*., 2001).

Mayflies (Ephemeroptera)

Mayfly nymphs are characterized by an elongated body, large head, well-developed mouthparts and stout legs (Gerber & Gabriel, 2002a). Paired gills vary greatly - they can be typically leaf-like, oval or even fringed. Present are three long filaments at the end of their abdomen, except in the family Baetidae, where some species only have two tails and one set of wing pads (Gerber & Gabriel, 2002a; Malherbe *&* Vuren, 2010). These animals are usually found on logs, branches, barks, leaves, mostly under stones and submerged rocks (Buss & Salles, 2007). Most mayflies are sensitive to pollution and require high to medium oxygen levels (de Moor *et al*., 2003). The most unusual feature of mayflies is that the adults only live a few hours and never eat (Sharma *et al*., 2009).

Stoneflies (Plecoptera)

Most times, stoneflies can be distinguished from mayflies by their streamlined body for crawling on rocks, and the presence of only two tail filaments and two sets of wing pads (Gerber & Gabriel, 2002a; Mangadze *et al*., 2016). Some have gills on their thorax, but others just obtain dissolved oxygen (DO) all over their body (Bredenhand, 2008). These animals are restricted to cool, fast flowing rivers where the substrate consists of large rocks and stones (Gerber & Gabriel, 2002b; Nelson, 2011). These animals also have different types of feeding habits. Some feed on plant material, either by shredding dead leaves and other large pieces of detritus, while others are predators (Callisto *et al*., 2001). Larvae breathe DO (Gerber & Gabriel, 2002b). Almost all of the stoneflies are sensitive to pollution and require high DO levels (Al-shami *et al*., 2010). The most unusual feature of this group is that, some kinds are programmed to emerge only during the coldest months; hence, they are called the winter-stoneflies (Gerber & Gabriel, 2002b; Riens *et al*., 2013).

Caddiesflies (Trichoptera)

Larvae of different caddiesflies have elongated soft bodies and can be divided in two categories, namely: cased-caddiesflies, where the animal stays inside the case from larval stages through to pupal stages, or caseless-caddiesflies (Gerber & Gabriel, 2002a; Riens *et al*., 2013). Both these larvae have fingerlike gills on the abdomen and anal appendages that make them assume their type of sedentary life. They breathe DO by means of gills and their overall body surface (Callisto *et al*., 2001; Riens *et al*., 2013). Caddisflies build cases of heavy material (rocks), uses grass and plants to make cases and spin silk out of their lower lip (Bredenhand, 2008; Gerber & Gabriel, 2002b). The latter material is used to glue together stones or pieces of vegetation into a covering, for their protection during the larva and pupa stages (Kemp *et al.*, 2014).

Chironomidae (Diptera)

Chironomid larvae are small delicate and somewhat mosquito larvae-like in appearance. However, they are not mosquitoes of any sort; and the term sandflies generally refers to various species of biting flies unrelated to the Chironomidae (Lee *et al*., 2006). As adults, they lack scales on the wings and do not have a long proboscis (Gerber & Gabriel, 2002b), often occur in huge swamps, usually in the evening. Larvae of some species are bright red in color due to a hemoglobin analog; these are often known as bloodworms (Odume & Muller, 2011; Al-shami *et al*., 2010). They are often associated with degraded or low-biodiversity ecosystems because some species have adapted to virtually anoxic conditions and are dominant in polluted waters (Al-shami *et al*., 2010). They swim by means of characteristic whipping movements of the body. The larvae are often and are an important food item for many freshwater fish and other aquatic animals. Riens *et al.* (2013) in their study confirmed that Chironomidae larvae are less affected by water quality because of their ability to use atmospheric oxygen. Their ability to capture oxygen is further increased by their making undulating movements (Riens *et al.,* 2013).

1.2.3 Determination of the ecological state of each site

Interpretation of the status of the site being assessed is based on three calculated values, namely: the number of taxa, the SASS Score (which is the sum of the scores for taxa present at a site), and Average Score Per Taxon (ASPT) (Chutter, 1998; Thirion *et al.,* 1998; Tripole *et al.*, 2008). A high ASPT score indicates high ecological status, and low values indicate degraded ecological status (Dickens & Graham, 2002; Odume *et al.*, 2012). ASPT scores are the least variable of the scores (Dallas, 2004) and these provide the most reliable measure of a natural class of river health (Dallas, 2004; Diedericks, 2011). An ecological condition determination entails a comparison between the present

state of the ecosystem and its natural state. ASPT values can only be compared at a site-level and not a habitat-level (Maseti, 2005; Palmer *et al*., 2004a). ASPT values are considered more reliable than SASS total scores, in that, they account for the number of taxa constituting a sample, which aids in standardizing scores across sites with different habitat diversity. [Total SASS scores can increase with increasing habitat diversity (Dickens & Graham, 2002; Diedericks, 2011)]. Both SASS and ASPT are tools, for biological monitoring. In healthy rivers, ASPT scores give more reliable results; while in polluted, unhealthy rivers SASS Score may be more reliable (Diedericks, 2011). In the absence of any real reference sites, default ASPT boundaries are adopted to assign ecological classes at different sites (DWAF, 2004b).

Table 1.2 The default benchmark category boundaries for the biotic index SASS5 (Palmer *et al*., 2004b).

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Class Boundary	Range of ASPT Scores
Natural (N)	7
Good (G)	6
Fair (F)	5
Poor (P)	<5

1.2.4 The effect of habitat preference on macroinvertebrate distribution

The diversity of habitats and microhabitats (biotopes) in lotic ecosystems is due to high diversity of substrates and the heterogeneity of the sediment (Callisto *et al*., 2001). The generic term “habitat” also known as biotope as is the case in this study, is used to describe the physical and chemical components of the river, which provide the ideal

environment for biota colonization (Parsons *et al.*, 2003). In a river, habitat for macroinvertebrates includes the rocks and sediments of the stream bottom, the plants in and around the stream, leaf litter and other decomposing organic material that falls into the river, and submerged logs, sticks, and woody debris (Al-shami *et al*., 2011; Farrell, 2014; Nelson, 2011). Substrate is a major factor governing the distribution and diversity of aquatic macroinvertebrates (Azrina *et al*., 2006; Brooks *et al*., 2005). Most species are restricted or more abundant in few (two or three) rather than several kinds of substrate (Gerber & Gabriel, 2002b; Bailey *et al*., 2001). Mayfly larvae are found attached under stones and in gravel substrates (Arimoro & Muller, 2010; Gerber & Gabriel, 2002b). The stonefly nymph (Plecoptera) is sluggish and can be found in unpolluted waters within leafy/woody debris and or under stones (Houghton, 2006). Caddiesflies larvae (Trichoptera) are unique in that they typically build a casing around themselves for protection from aquatic predators (Ross-Gillespie *et al*., 2018). The casing is made from a wide range of materials, depending on what is available: bits of leaves, twigs, grass, sand, gravel, seeds, mollusc shells and a variety of other debris (Gerber & Gabriel, 2002b). Caddiesflies larvae without casings can be found in rapid running waters. Those with casings can be found in all parts of the stream. On the whole, the larvae are omnivorous (scavengers) and feed on algae, fungi, detritus, and sometimes very small invertebrates (Callisto *et al*., 2001; Mangadze *et al*., 2016; Riens *et al*., 2013).

1.2.5 Physico-chemical parameters and their impact on macroinvertebrate distribution

River water is a complex mixture of chemicals. The river is affected by the composition of rain water, the geology of the river basin itself, animals and plants in and outside the water and by human activities. It is recommended that physico-chemical parameters at

each site be monitored during each biomonitoring period (Palmer *et al*., 2004a). Studies have shown that physico-chemical parameters in aquatic systems have strong impact on the biota (Palmer *et al*., 2004a). Monitoring for physico-chemical parameters such as hydrogen concentration (pH), temperature, electrical conductivity (EC) and low dissolved oxygen (DO) concentration, help identify which pollutants are responsible for impacts on a river (Farrell, 2014; Lent *et al*., 2000). Physico-chemical conditions may contribute to the reduction in the number of all the macroinvertebrates, the elimination of sensitive families and the dominance of tolerant families in the study area (Buss *et al*., 2002; Mbikwana *et al*., 2010). Organisms tend to congregate in large numbers where conditions are favorable for them (Nelson, 2011). Extreme levels of physico-chemical parameters (e.g. water temperature, hydrogen ion concentration (pH), electrical conductivity (EC), and chemical parameter (e.g. dissolved oxygen (DO) in the river also have adverse effect on the macroinvertebrates (Dallas, 2007). The pollution intolerant organisms will be eliminated and pollution tolerant organisms increased in areas where there is high pollution rate (Leigh *et a*l., 2016). Monitoring for physico-chemical parameters helps identify which pollutants are responsible for impacts to a river (Ollis *et al.*, 2006a). Because macroinvertebrates are stationary and are sensitive to different degrees of pollution, changes in their abundance and variety vividly illustrates the impact pollution has on a river (Nelson, 2011).

1.2.5.1 Temperature

The intensity of heat available of a substance is measured in units of Degrees Celsius (˚C). Temperature is the key physical variable that directly affects many of the physical, biological and chemical factors influencing aquatic organisms (Dallas & Rivers-Moore, 2014; Farrell, 2014; Londagin *et al*., 2007; Ross-Gillespie *et al*., 2018). If temperatures are outside the range of tolerance for organisms for extended periods of time they can

become stressed and die, resulting in a change in the types of organisms inhabiting the stream (Bredenhand & Samways, 2009). Temperature can be modified by various factors such as weather, removal of riparian vegetation, turbidity (Farrell, 2014; Walters *et al*., 2009). All aquatic organisms have preferred temperature in which they can survive; preferred range for majority of macroinvertebrates is between 15 ˚C - 30 ˚C. The abundance and diversity of aquatic organisms is influenced by temperature (Fengqing *et al*., 2012). High water temperature stress aquatic ecosystems by reducing the ability of water to hold essential dissolved gases and demonstrate increased productivity due to a suite of environmental and ecological factors including: increased temperature (Londagin *et al*., 2007; Parsons *et al*., 2003), increased habitat heterogeneity (Farrell, 2014), and an increased abundance of macroinvertebrate prey (Callisto *et al*., 2001; Mangadze *et al*., 2016). The solubility of gases such as oxygen and carbon dioxide increases as temperature decreases (Farrell, 2014; Walters *et al*., 2009). Warm water contains less DO than cold water (Kefford *et al*., 2002; Londagin *et al*., 2007). Inversely the solubility of most minerals increases with increasing temperature (Kefford *et al*., 2002). Growth rates are related to temperature (Walters *et al*., 2009), with higher growth rates and shorter lifecycles associated with warmer weather. Higher temperature release of heated effluents causes reduction in community richness (Bonada *et al*., 2006; Bredenhand & Samways, 2009). Industrial discharges or storm water runoff from hot surfaces (e.g. roads and car parks), could increase the temperature quickly and some macroinvertebrates like mayflies and midges, such as chironomids, might be lost (Lent *et al*., 2000). Some macroinvertebrates might be able to tolerate slight increases in temperature (Buss & Salles, 2007). Increased solar radiation may also raise surface water temperatures, further affecting the number and diversity of macroinvertebrates (Fengqing, 2012; Londagin *et al*., 2007). In addition, as

water warms, the level of DO falls and eventually stresses aquatic animals (Bredenhand & Samways, 2009; Farrell, 2014; Leigh *et a*l., 2016). Sensitive macroinvertebrates such as stoneflies, which are restricted to cool, fast flowing waterbodies, cannot cope with such changes (Farrell, 2014; Gerber & Gabriel., 2002a).

1.2.5.2 Hydrogen ion (pH)

Acidity of the water is measured on a scale of zero to 14 pH units. pH less than 7 is regarded acidic; at 7 is regarded neutral, and above 7 is regarded as basic (Farrell, 2014; Tripole *et al*., 2008). pH of less than 5 and greater than 9.0 are considered extreme conditions can be toxic to most aquatic life (Hussain & Pandit, 2012). Higher temperatures, due to high levels of pollution in water result in increase in pH levels (Kasangaki *et al*., 2008). Lower pH rate of stream water (less than a pH of 6) can trigger the release of heavy metals, which are toxic to benthic macroinvertebrates (Buss & Salles, 2007). South African rivers are seldom naturally very alkaline (Dallas & Day, 2004). The typical pH range for most surface water in South Africa is 6 to 8 (Dallas & Day, 2004). For example, according to Gerber & Gabriel (2002a), cased caddiesflies (macroinvertebrate family Leptoceridae) and marsh beetles (macroinvertebrate family Helodidae), prefer any stream with low pH.

1.2.5.3 Conductivity

Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water, and measured in units, microSiemens per centimeter (µS/cm) (Dallas & Day, 2004; Farrell, 2014). EC is regarded as one of the salinity indicators (Chessman, 2003) and several authors have studied changes in macroinvertebrate communities along the salinity gradient in streams and rivers (Bredenhand, 2008; Farrell, 2014; Kefford, 2007; Maseti, 2005). EC of most freshwater

ecosystems ranges from 10 µS/cm to 1000 µS/cm and suffer little ecological stress when the EC is below 1500 µS/cm (Kefford, 2007). High salinity rates can lead to changing macroinvertebrate communities by eliminating sensitive organisms (Bredenhand & Samways, 2009; Kay *et al*., 2001).

1.2.6 Data Analysis

Data analysis for this study was undertaken using a computer Programme called Plymouth Routines in Multivariate Ecological Research version 6 (PRIMER v6; Clarke & Warwick, 2001; Clarke & Gorley, 2006) developed in Plymouth Marine Laboratory in the United Kingdom.

1.2.6.1 Univariate measure

Many diversity indices have been developed to describe responses of a community to environment variation, combining the three components of community structure, namely richness (e.g. Margalef Index (Margalef, 1951), evenness (uniformity in the distribution of individuals among the species) and abundance (total number of individuals present) (Shannon-Wiener Index (Shannon & Weaver, 1949). Richness is an adjusted form attempting to allow for differing numbers of individuals such that a site with more species than the other is more diverse. Equitability expresses how evenly individuals are distributed among different species often in terms of evenness. The number of species (S) and abundance (N) within a sampling site are usually measured. However, these measures are not dimensionless quantities and thus tend to be less informative.

Diversity indices such as richness of site in terms of number of species for a given number of individuals in the site divided up among the different species. Diversity indices reduce the multivariate (multispecies) complexity of assemblage data into single index evaluated for each sampling site. The assumption is that undisturbed environments are

characterized by high diversity or richness, an even distribution of individuals among the species, and moderate to high counts of individuals. The best use of diversity-related indices in river and stream monitoring is probably as an indicator of changes in species composition when comparing impacted and reference assemblages (Shi *et al*, 2017). Many criticisms have been made against the usefulness of diversity indices when employed separately in assessment of river systems (Metcalfe, 1989) and now these indices are preferred to be used together with other metrics. Chi-square test was used to test if there were any statistically ($p < 0.05$) significant differences existed among these metrics (Clarke & Gorley, 2006).

Analysis of variance (ANOVA)

Analysis of Variance (ANOVA) has two variables, a dependent variable and an independent variable. ANOVA tests to determine the significance of differences among sites, habitats. The difference is considered to be significant when $p < 0.05$ (Clarke & Warwick, 2001). When measuring biological data, units are not always the same, and therefore, transforming the data is necessary. Moreover, individuals are not always evenly distributed resulting in different abundances. This can result in abundant species dominating the rarer species. Transformation is suggested prior to assessment of community similarity (Clarke & Warwick, 2001), as similarities calculated on original abundance data values can be over dominated by a small number of highly abundant species or families, thereby failing to reflect similarity of the overall community composition. Transformation techniques range from no transformation, square root transformation, fourth root transformation, log (x + 1) and ultimately the presence/absence transformation (Clarke & Warwick, 2001). Log transformation is generally preferred as it scales down abundant species so that rare species are not overshadowed by dominant species, resulting in biased conclusions. Root-root or

square root transformation is recommended for use with Bray-Curtis similarity coefficient (S) as it is not affected by joint absences (Clarke & Warwick, 2001). The Bray Curtis (abundance data) coefficient is used to measure community similarity. This coefficient focuses on taxa presence, rather than common taxa absences. The Bray-Curtis similarity coefficient (*S*) is one of the most reliable similarity coefficients (Clarke & Warwick, 2001) where *S* lies in the range 0 to 100. Tests for normality include the Chi square test which is frequently used, ranges from zero to one, with low p-values (smaller than 0.05) resulting in the rejection of the hypothesis of normality. Values that are greater than 0.05 suggest that there is insufficient evidence to reject the null hypothesis, and lead to the assumption that the data are indeed from a normal distribution (Clarke & Warwick, 2001).

1.2.6.2 Multivariate measure

Multivariate analysis for this study was undertaken using a computer Programme called PRIMER v5 (2001) (Plymouth Routines in Multivariate Ecological Research version 6) developed in Plymouth Marine Laboratory in the United Kingdom. Multivariate analyses was performed on log (x + 1) transformed data. These included cluster analysis and non metric multidimensional scaling (MDS).

Cluster analysis

Cluster analysis defines groups of cases based on the similarity of multiple variables measured for each case; the results of which are presented in a dendrogram. The clusters in a dendrogram reflect underlying natural divisions in a population (Clarke & Warwick, 1994). Similarity is often measured in terms of distance, which has a very general meaning in cluster analysis. For example, the number of species common to 2 stream sites could be a measure of distance between the sites. Cluster analysis returns

to you a dendritic tree, or dendogram, that shows how sites were grouped (or split) first, which next, and so on, until the number of clusters you initially specified is obtained. It is so important at first to identify the clusters. This should be followed immediately by understanding why the clusters occur so that factors other than those used in constructing the dendrogram can be identified (Clarke & Warwick, 1994). In order to determine statistically significant ($P < 0.05$) clusters, a Similarity profile (SIMPROF) test needs to be performed to determine which species contributed to each cluster. Dallas (2007); Maseti (2005) and Rajele (2004) used cluster analysis to group stream sites according to the invertebrate species present at each site. They used least impaired sites to ensure that clusters were related to natural species distributions rather than human disturbance. They found that the site clusters were best explained in terms of the ecoregion in which they were located.

Ordination

This is a map of sites usually in two dimensions reflecting the similarities in their biological communities. Nearby sites have very similar communities; sites far apart have few species in common or the same species at different levels of abundance. Multidimensional scaling (MDS) is an effective and widely applicable method available. It constructs a map of sites in a specified number of dimensions which attempts to satisfy all conditions imposed by similarity matrix. The adequacy of MDS depends on the stress value. Ordination is considered to be useful in presenting a similarity relationship when stress levels of ordination are low. A stress value of < 0.05 gives excellent presentation with no prospect of misinterpretation. A stress value of < 0.1 provides good ordination and is unlikely to give misinterpretation. A stress value of < 0.2 gives a two dimensional picture although conclusions should not only be based on ordination, but should also be drawn from cluster analysis (Clarke & Warwick, 1994).

Principal Component Analysis (PCA)

The PCA technique takes multiple variables and defines a smaller number of new variables by constructing linear combinations of the original variables. The new variables are combined in such a way to separate the cases as much as possible. It assumes linear relationships between variables (Clarke & Warwick, 2001). Thus, this technique is inappropriate for species data analysis, but acceptable for water quality data. In addition, PCA is appropriate when using variables that are not measured with units in the same order of magnitude (e.g. pH, temperature, conductivity). PCA has the drawback of not maintaining the among-sample distance (Clarke & Warwick, 1994). The diverse array of Multidimensional scaling is easier to understand in terms of the number of dependent variables on one side of the equation and the number of independent variables on the other. They are intended to preserve the rank order of similarities in the relative separation of the corresponding sample in a 2-dimensional plot (Clarke & Warwick, 1994). The ordination results in a scatter plot where each sample is represented by a point. The greater the distance between them, the more dissimilar are the samples.

Draftsman's Rank Correlation

The Draftsman's Rank Correlation is a non-parametric coefficient of rank correlation between two variables (X, Y) used to determine whether or not an association exists between the two variables (Clarke & Warwick, 1994). However, a possible third variable Z may be responsible for the correlation between X and Y and thus its effects need to be removed so that the degree of correlation between the two can be quantified (Clarke & Warwick, 2001). Draftsman's Rank Correlation can be understood to be a linear correlation coefficient computed on the ranks of the data.

Analysis of similarity (ANOSIM)

ANOSIM is the equivalent of ANOVA in univariate analysis and is designed for non-normally distributed data (Clarke & Warwick, 1994). It reflects differences in average rank similarity between and within samples (Clarke & Warwick, 1994). A one-way ANOSIM allows a statistical test of the null hypothesis that there are no family or species differences between groups of samples sites. The significance level is determined by the number of permutations (Clarke & Warwick, 2001). ANOSIM has a test statistic, Global R (R), which lies in the range -1 to 1 (Clarke & Warwick, 1994). R = 0, indicates similarities between and within sites will be the same on average, and R = 1, indicates that samples from the same site are more similar to each other than any samples from different sites. ANOSIM does not depend on Bray-Curtis similarities or similarities calculated from species abundance data compared to other multivariate analysis methods such as Multivariate Analysis of Variance (MANOVA).

Similarity percentage

Similarity percentage (SIMPER) a non- parametric multivariate analysis designed to identify species that are most important in creating the observed pattern of similarity between species (Clarke & Warwick, 1994). SIMPER uses the Bray-Curtis measure of similarity, to compare samples and therefore the mean similarity between can be obtained for each species.

CHAPTER 2: MATERIALS & METHODS

2.1 DETERMINING THE CHARACTERISTICS OF HABITAT TYPES

2.1.1 The study area

The study area [Figure (Fig.) 2.1] was located along the Tyhume River between coordinates, 26º - 27º E and 32º - 33º S. Tyhume River is a major tributary of the Keiskamma River. The study area, which is in the upper reaches of Amatole Mountains, is characterized by a humid climate and intense precipitations, from January to May (Mayekiso, 1994). The local climate is known to have rainy summers and dry winters of 600 and 1200 mean annual rainfall, respectively (Mayekiso, 1994). The air temperature varies around the year from a minimum of 18 ºC to a maximum of 27 ºC.

2.1.1.1 Study site selection

According to SASS5 standards (Dickens & Graham, 2002), the potential site must measure up to the criteria required for SASS5 biomonitoring. According to Kleyhans *et al*. (2005) and Dickens & Graham (2002), SASS5 monitoring sites would be those that:

- have a good perennial flow;
- have diversity of biotopes (at least two biotopes) such as stones, marginal vegetation and/or sediment); and
- have biotopes within 20 m section of the river.

SASS5 required determination of biotopes for sampling (Watson & Dallas, 2013). The process for determination was done following the method by Dallas & Day (2007).

Sampling points were selected as close to the SASS5 requirements according to procedures of Dickens & Graham (2002) and referred as study site in this thesis (Fig. 2.1). Photographs were taken to show the site characteristics. Global Positioning Systems (GPS) was used for recording exact locality details (altitude, longitude and latitude as well as date and time) of study sites.

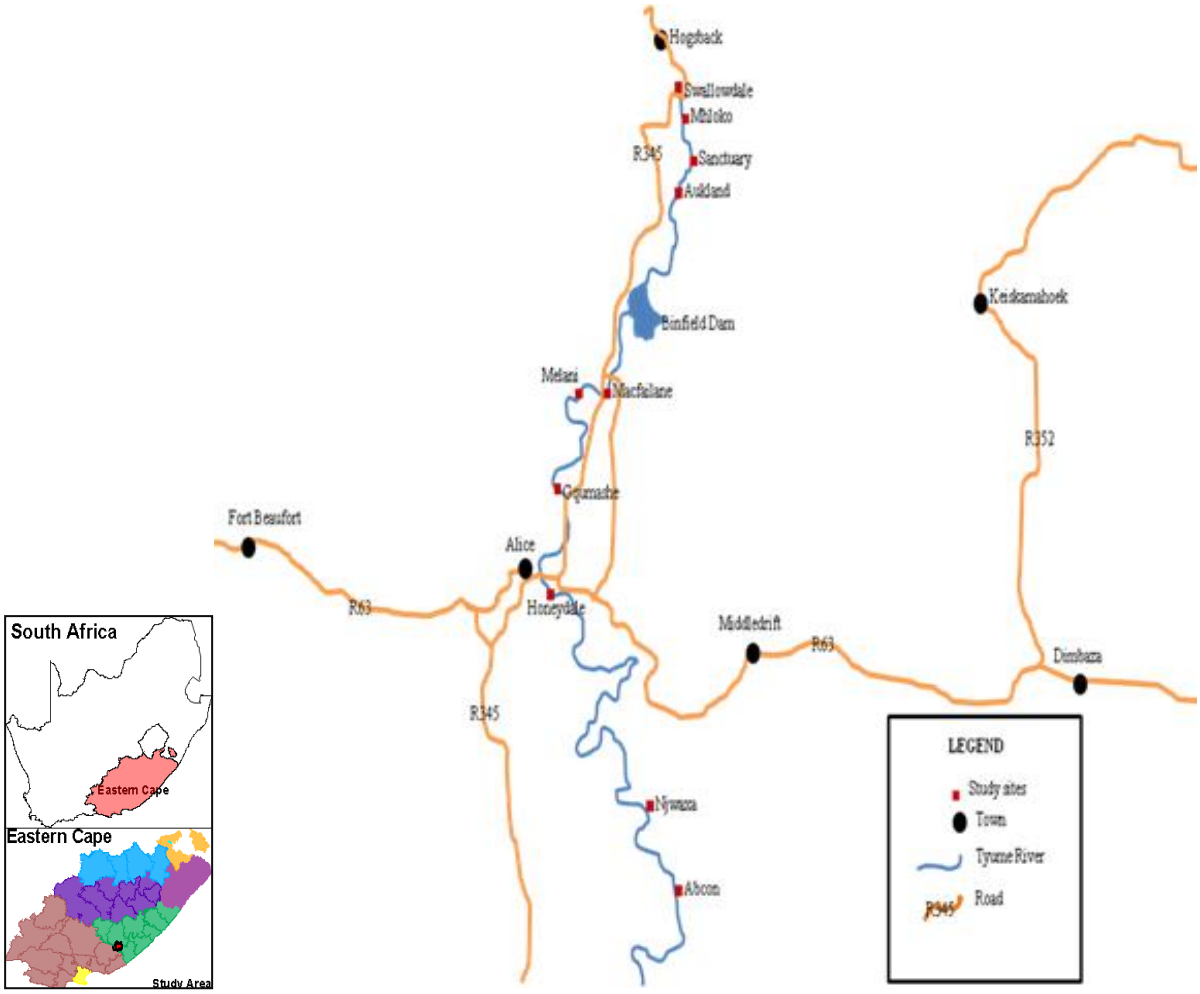

Fig. 2.1 Map of the Tyhume River study area and the study sites

2.1.1.2 THE STUDY SITE CHARACTERISTICS

Ten sampling sites (1. Swallowtail (SWALL)**,** 2. Mtloko (MTLOK), 3. Sanctuary (SANCT), 4. Auckland (AUCKL), 5. Macfairlane (MACFA), 6. Melani (MELAN), 7. Gqumashe (GQUMA), 8. Honeydale (HONEY), 9. Njwaxa (NJWAX), and 10. Above Confluence (ABCON), were selected from the upper to the lower catchment areas of Tyhume River for assessment of river characteristics. The upper catchment area was defined as the section along the river above Binfield Park Dam; the area below Binfield Park Dam was defined as the lower catchment.

2.1.1.3 Photographic characteristic and Geographic Position System (GPS)

Site 1 Swallowtail (SWALL)

Site 1 (Fig. 2.1; Fig. 3.1) was selected as a potential reference site due to the perceived absence of least impacted sites on the Tyhume River, and due to its location on the mountain head waters and low exposure to human impact. This site showed a range of biotopes for macroinvertebrates sampling. Access by car to this site was limited being located in forested areas. Small waterfalls were seen upstream and the banks were mostly vegetated by a mixture of exotic and indigenous trees and small patches of aquatic vegetation. Fallen trees and logs provided extra habitat for macroinvertebrate colonization. Pebbles, cobbles and boulders characterized the substrate.

Fig. 2.2 Study Site 1: Swallowtail (32 ^{0}35' 758'' S, 26 ^{0} 56' 830'' E) showing fallen trees and logs as well as boulders characterize the site

Site 2 Mtloko (MTLOK)

Site 2 (Fig. 2.1; Fig. 2.2) had limited range of biotopes. The river banks (both left and right hand sides) were unstable at this site and were encroached by alien vegetation. Marginal vegetation was minimal. There were pools and the substrate was mostly bedrock and boulders with small riffle areas, resulting in limitation in multiple macroinvertebrate habitats.

Fig. 2.3 Study Site 2: Mtloko (32^0 36' 649'' S, 26^0 54' 564'' E) showing boulders and small riffle areas are seen

Site 3 Sanctuary (SANCT)

Site 3 (Fig. 2.1; Fig. 2.4) harbored marginal and very sparse vegetation dominated by trees. Exotic trees and indigenous grass dominated the narrow strip of closed and open canopy. This site was also adjacent to rural residential areas. Cobbles and pebbles characterized the riffles which reflected good habitat for macroinvertebrates. Pools were deep.

Fig. 2.4 Study Site 3: Sanctuary (32^0 37' 509'' S, 26^0 55' 959'' E) showing exotic trees and indigenous grasses dominate the narrow strip of closed and open canopy

Site 4 Auckland (AUCKL)

Site 4 (Fig. 2.1; Fig. 2.5) was immediately above Binfield Park Dam. The site had substrate of mostly bedrock and large boulders with riffles present in small patches. Marginal and fringing vegetation was approximately two metres tall. Aquatic and the marginal vegetation present were mostly out of current. Indigenous *Acacia* bushes that characterized the riverbanks were mostly chopped for firewood by surrounding communities. Pools were deep with cobble–boulder substrate upstream and downstream of the riffle area. A low bridge used for cattle and human crossing, crosses over at this site; there was evidence of the bridge flooding during high rains.

Fig. 2.5 Study Site 4: Auckland (32^0 38' 370 ''S, 26^0 56' 167'' E) showing cobble – boulder substrate upstream and downstream at the riffle area

Site 5 Macfairlane (MACFA)

Site 5 (Fig. 2.1; Fig. 2.6) was at a site immediately located downstream of Binfield Park Dam; so one could establish the quality of the outflow of the Binfield Park Dam water into parts of the Tyhume River below Dam. Marginal vegetation was very sparse and dominated by trees, especially on one side of the banks. This site was on open system

with no canopy cover, more so on one of the banks. The substrate consisted of stones, gravel and sand, and a little vegetation hanging into the water. Sedges and reeds also dominated aquatic and marginal vegetation.

Fig. 2.6 Macfairlane, Study Site 5 (32^0 42' 677 "S, 26^0 53' 126" E) showing little overhanging aquatic vegetation

Site 6 Melani (MELAN)

Site 6 (Fig. 2.1; Fig. 2.7) also located below the Binfield Park Dam received runoff from a lumber mill positioned above the site and surrounding areas. Macroinvertebrate habitat was limited as the substrate was mostly boulders with small riffle areas. Marginal vegetation was partially minimal or thick and found in pools. There was a low-water bridge at this site that was used for cattle and human crossing.

Fig. 2.7 Study Site 6: Melani (32^0 43’ 224" S, 26^0 51’ 660" E) showing marginal vegetation either minimal or thick in parts of the river

Site 7 Gqumashe (GQUMA)

Site 7 (Fig. 2.1; Fig. 2.8) was located below Ntselamanzi Location and was also exposed to sources of impacts, such as farmlands, cattle grazing area and a garbage dumping site. In this site available biotopes were dominated by stones of a wide range, (from pebbles to boulders) supporting variable macroinvertebrate biotopes. Substrate was predominantly bedrock and cobbles; marginal vegetation minimal.

Fig. 2.8 Study Site 7: Gqumashe (32^0 45’ 477" S, 26^0 51’ 98" E) showing anthropogenic influence evident by a modern bridge

Site 8 Honeydale (HONEY)

Site 8 (Fig. 2.1; Fig. 2.9) had indigenous vegetation characterizing the riverbanks and there was good availability of a wide range of macroinvertebrate biotopes. Marginal vegetation was minimal, comprising predominantly sedges, grasses and trees. The substrate varied from sand to mud and bedrock. Boulders and bedrock dominated the substrate although cobbles and gravel were present. Slabs of concrete from a broken weir lay within the site.

Fig. 2.9 Study Site 8: Honeydale (32^0 48’ 157" S, 26^0 51’ 607" E) showing typical characteristics of good availability of a wide range of macroinvertebrate biotopes

Site 9 Njwaxa (NJWAX)

Site 9 (Fig. 2.1; Fig. 2.10) presented good availability of a wide range of macroinvertebrate biotopes. This site was located within the rural area. Macroinvertebrate habitat was limited as the substrate was mostly bedrock and with deep pools. Marginal vegetation was minimal and found in pools, predominantly reeds, sedges and grasses occupied upstream of the site.

Fig. 2.10 Study Site 9: Njwaxa (32^0 52’ 538’’ S, 26^0 53’ 531’’ E) showing a ridge of Cemented dam across the entire width of the river

Site 10 Above confluence (ABCON)

Site 10 (Fig. 2.1; Fig. 2.11) was located above the confluence of the Tyhume and Keiskamma Rivers. This site had indigenous vegetation characterizing the riverbanks and there was good availability of a wide range of macroinvertebrate biotopes. Boulders and bedrock dominated the substrate; although cobbles and gravel were also present. Riparian vegetation was very sparse and dominated by trees.

Fig. 2.11 Study Site 10: Above Confluence (32^0 54' 811'' S, 26^0 56' 222'' E) showing typical characteristics of good availability of a wide range of macroinvertebrate biotopes

2.3 DETERMINING THE DIVERSITY OF MACROINVERTEBRATE FAMILIES INHABITING THE TYHUME RIVER

2.3.1 Macroinvertebrate sampling

Macroinvertebrate collection required determination of biotopes at each study site (see section 2.1.1.1); the process following Dallas & Day (2007). Macroinvertebrates were collected from all available biotopes per site following SASS protocol (Dickens & Graham, 2002). Three replicates were taken at each site. The replicate samples were taken to assess whether single samples taken at a particular biotope at a particular time was a true reflection of all the macroinvertebrate families present at that biotope. Most sites included the following biotopes: stones, vegetation and sediment (e.g. gravel, sand, mud) such as also described in Madikizela *et al.* (2001). Surveys were undertaken: March to September 2006. Reason for only 3 surveys was because of heavy floods in summer time.

2.3.1.1 Sampling the stones biotope

Stones (also referred to as stones-in-current, SIC) and bedrock, were sampled for macroinvertebrates, by holding a standard SASS5 net (950 μm mesh-size) in the current, and then kicking the stones just upstream of the net for two minutes if stones are loose; maximum five minutes if stones immovable (too large to move) - while ranging across the river to cover a number of different biotopes (Dickens & Graham, 2002). Stones were also lifted, held under water in front of the net and macroinvertebrates were picked up with forefingers or forceps into the net. The cleaned rocks were discarded outside the sampling area. Stones (also referred to as stones-out-of-current, SOC), were sampled for one minute using the same procedure for SIC. Samples collected from both in and out of current were combined into a single 'Stone' biotope sample.

2.3.1.2 Sampling vegetation biotope

Marginal and aquatic vegetation were sampled following the methods by Dickens & Graham (2002); by disturbing a two-metre long strip of the submerged part of the vegetation with the SASS5 net held just below the water surface.

2.3.1.3 Sampling the gravel biotope

Sampling of Gravel, Sand & Mud (GSM) followed Dallas (2004); stirring and sweeping GSM for one minute in total. Samples collected in and out of current were combined into single GSM biotope sample.

2.3.2 Macroinvertebrates Field Identification

Macroinvertebrates were sampled using the SASS5 (Dickens & Graham, 2002). Identification of the organisms was made to family level using Gerber & Gabriel (2002b), Graham & Dickens (2001) and Kasangaki *et al*. (2008); and recorded on a standard SASS5 data sheet (Appendix I). The identification of different macroinvertebrate families was also done using Field Guide On Aquatic Macroinvertebrates of South Africa by Gerber & Gabriel (2002b). Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera) were scored according to the number of species present based on the SASS5 standard. A hand lens (10 X magnification) was used to examine small organisms.

2.3.2.1 Macroinvertebrates diversity in Tyhume River

The macroinvertebrates diversity by site was determined to show the condition of the river. Macroinvertebrates were counted individually per taxon from samples per site and the numbers were subjected to the following univariate diversity indices:

- Shannon-Wiener diversity index (H') given as:

$$H' = \sum i \; pi \ln (pi)$$

where pi = proportion of total count arising from i^{th} species (Shannon & Wiener, 1949). For highly polluted, Wilhm & Dorris (1968) set diversity index as < 1, polluted, -3 for moderately polluted, and > 4 for unpolluted water bodies.

- Margalef's species richness (d) which incorporates the total number of individuals (N) to measure number of species present for a given number of individuals and is given as:

$$d = S\text{-}1)/\log N$$

where S is the number of species, and N is the total number of individuals in the sample

- Pielou's evenness index (J') given as:

$$J' = H'/H'max = H'/logs$$

where H'max is the maximum possible value of Shannon diversity that is achieved if all species were equally abundant (i.e. logS).

2.3 DETERMINATION OF THE ECOLOGICAL STATE OF EACH SITE

Metrics were calculated with the taxa lists of macroinvertebrate samples and indices SASS5 score, Average score per taxon (ASPT); and number of Taxa, were generated and correlated against the selected physico-chemical parameters. The SASS5 scores from the SASS5 score sheets were calculated by adding the sensitivity weightings of each taxonomic group at each site and analyzed. ASPT was then calculated using the following formula:

$$ASPT = \frac{\text{SASS5 scores from each site}}{\text{Number of taxa from each site}}$$

The ASPT scores were then used to interpret the ecological status of the river. ASPT score = 7 (N); ASPT score = 6 (G); ASPT score = 5 (F); ASPT score < 5 (P).

2.4 THE EFFECT OF HABITAT PREFERENCE ON MACROINVERTEBRATE DISTRIBUTION

Macroinvertebrates were enumerated individually per site and the numbers were subjected to three multivariate analyses, namely: Cluster analysis, Ordination and Similarity percentage. In order to down weight, the importance of the highly abundant families (henceforth referred to as species), data was log (x + 1) transformed. This enabled similarity between sampling sites to depend not only on high values, but also on the values of less common or mid-range species (rare species not overshadowed by dominant species, resulting in biased conclusions).

2.4.1 Cluster analysis

Cluster analysis was done using the programme CLUSTER on Bray-Curtis similarity resemblence matrix that showed similarity (percentage of sampling site based on their group averages. The Bray–Curtis coefficient was selected to calculate distances between variables and % similarities between sites. In order to determine statistically significant ($P < 0.05$) clusters, a Similarity profile (SIMPROF) test was performed to determine which species contributed to each cluster.

2.4.2 Ordination

To represent the data in a low dimensional (2-D) space, a non-metric multidimensional scaling (MDS) was done. MDS were used to gain an understanding of patterns in macroinvertebrate assemblages with biotope, and to determine whether differences among biotopes were greater than differences among sites. Sampling sites that are

close together represent sampling sites that are very similar in terms of faunal composition and those that are far apart correspond to very different values of the variable set.

2.4.3 Similarity percentage (SIMPER)

SIMPER was performed to assess whether there were any significant differences existed between biotopes, as far as faunal composition is concerned and was performed with a cut cumulative percentage set at 90 %. SIMPER was undertaken to assess which families were responsible for rejection or acceptance of the null hypothesis differences between sites and biotopes.

2.5 DATA COLLECTION FOR PHYSICOCHEMCAL PARAMETERS

Measurements of physicochemcal parameters of water were taken on each day of the biomonitoring survey. Water temperature, pH, and conductivity were measured at each site.

2.5.1 Temperature

Temperature measurements were collected from each SASS5 study-site site during the sampling surveys. A calibrated temperature meter (Hanna Model HI 98129-HI 98130 Water Proof) affixed to a temperature probe, was used in the study; the temperature probe was inserted into the flowing river water. Temperature measurements were recorded according to procedures from Chutter (1998); initially letting the probe sits in the water until stable. The results were recorded on South African Scoring System (SASS5) data sheets (Appendix I).

2.5.2 pH

pH measurements were collected from each SASS5 study-site site during the sampling surveys. A calibrated pH meter (Hanna Model HI 98129-HI 98130 Water Proof) affixed to a pH probe, was used in the study; the pH probe was inserted into the flowing river water. pH measurements were recorded according to procedures from Chutter (1998); after letting the probe to sit in the water until stable. The results were then recorded on SASS5 data sheets (Appendix I).

2.5.3 Conductivity

Conductivity measurements were collected during the sampling surveys. A calibrated conductivity meter (Hanna Model HI 98129-HI 98130 Water Proof) affixed to a conductivity probe, was used in the study; the conductivity probe was inserted into the flowing river water. Conductivity measurements were recorded according to procedures from Chutter (1998); after letting the probe sit in the water until stable.

2.5.4 Evaluation of the influence of physico-chemical parameters on macroinvertebrate distribution

Mean values of physico-chemical parameters measured over the biomonitoring period were used for statistical analysis. Following Draftsman correlation, physico-chemical (Temperature, pH and Electrical Conductivity) data of the sampling sites were log(x + 1) transformed and normalized since the variables were measured in different units (Clarke & Warwick, 2001). A resemblance triangular matrix was then developed from the transformed normalized data by Euclidean distance. A Multidimensional scaling MDS and Principal Component Analysis (PCA) ordinations of the sampling sites were plotted. Vector length on the ordination reflects the importance of that variable's contribution to the two PC axes in relation to all other possible PCs. This was compared with MDS for

species data in order to see the influence of physico-chemical parameters on species distribution within sampling sites (Clarke & Warwick, 2001). Stress value on the MDS ordination indicated how faithfully the high-dimensional relationships among sampling sites are represented in the ordination plot.

CHAPTER 3: RESULTS

3.1.2 Characteristics of the biotopes at the study sites along Tyhume River

Three biotopes types (e.g. stone (S), vegetation (V), gravel, sand and mud (GSM)), were determined from the ten study sites.

3.1.2.1 The stones biotope (S)

The stones biotope consisted of movable stones of at least cobble size (3 cm diameter) to approximately 20 cm in diameter, within the fast and slow flowing sections of the river.

3.1.2.2 Vegetation biotope (V)

Vegetation biotope included overhanging grasses, bushes, twigs, reeds and filamentous algae on the edge of the stream.

3.1.2.3 Gravel, Sand and Mud (GSM) biotope

Gravel, Sand and Mud (Gravel-Sand-Mud, GSM) biotope was a combination of the three biotopes together.

- ***Gravel (G)***: typically consisted of smaller stones (2 mm - 3 mm up to 3 cm in diameter);
- ***Sand (S)***: included sandbanks within the river, small patches of sand in hollows at the side of the river or sand between stones;
- ***Mud (M)***: consisted of very fine particles, usually as dark-coloured sediment.

All study sites were wadable; and each site had a defined channel. These study localities were accessible and matched the description required for South African Scoring Service (SASS) monitoring sites. Out of the 10 study sites four (Sites 3, 6, 7

and 10) consisted of all the three biotope types (S, V, GSM), while six (Sites 1, 2, 4, 5, 8 and 9) consisted of only two biotope types (S, V) (Table 3.1).

Table 3.1 Biotopes found at each study site along the Tyhume River. S = stone; V = vegetation; GSM = gravel, sand and mud

Site No	Site name (abbreviation)	Biotopes present
1	Swallowtail (SWALL)	S, V
2	Mtloko (MTLOK)	S, V
3	Sanctuary (SANCT)	S, V, GSM
4	Auckland (AUCKL)	S, V
5	Macfairlane (MACFA)	S, V
6	Melani (MELAN)	S, V, GSM
7	Gqumashe (GQUMA)	S, V, GSM
8	Honeydale (HONEY)	V, GSM
9	Njwaxa (NJWAX)	S, V
10	Above confluence (ABCON)	S, V, GSM

3.2 IDENTIFIED MACROINVERTEBRATES IN TYHUME RIVER

3.2.1 Macroinvertebrate diversity

Forty-eight distinct macroinvertebrate families inhabited the 10 study sites along the Tyhume River (Table 3.2; Appendix II). The majority of macroinvertebrate taxa belonged to members of Order Diptera (totaling eight macroinvertebrate families; Table 3.2) and mostly with the lowest sensitivity scores; this was followed by Orders

Ephemeroptera and Odonata (each with seven families). The highest sensitivity scores were shown by the families in the Order Plecoptera.

Table 3.2 Checklist of macroinvertebrate families collected from Tyhume River; (numbers included in parentheses represent sensitivity scores)

Macroinvertebrates **Order & family**	**Sensitivity scores**	**Study sites where found**
EPHEMEROPTERA		
1. Baetidae1sp	(4)	3, 4, 5, 7, 10
2. Baetidae 2sp	(6)	All 10
3. Baetidae >2sp	(12)	All 10
4. Caenidae	(6)	1, 2, 3, 7, 10
5. Heptaginiidae	(13)	All 10
6. Leptophlebiidae	(9)	2
7. Tricorythidae	(9)	1, 2, 3, 4, 5, 6, 7, 10
PLECOPTERA		
8. Perlidae	(12)	1, 2, 3, 4, 5, 6, 7, 10
9. Notonemouridae	(14)	1, 4, 5, 6, 7, 10
TRICHOPTERA		
10. Ecnomidae	(8)	1, 2, 4, 6, 10
11. Hydropsychidae 1sp	(4)	9
12. Hydropsychidae 2sp	(6)	5, 9, 10
13. Psychomyiidae	(8)	All 10
14. Leptoceriidae	(6)	1, 4, 6, 8, 10
COLEOPTERA		
15. Dytiscidae		

	(5)	6, 7, 8, 9, 10
16. Gyrinidae	(5)	1, 2, 4, 5, 6, 8
17. Elmidae	(8)	4, 5, 6, 7, 8, 9, 10
18. Psephenidae	(10)	1, 2, 3, 4, 5, 6, 7
ODONATA		
19. Lestidae	(8)	1
20. Chlorolestidae	(8)	1, 3, 6, 8
21. Aeshnidae	(8)	All 10
22. Coenagrionidae	(6)	1, 3, 4, 5, 6, 7, 8, 9, 10
23. Corduliidae	(8)	2
24. Gomphidae	(6)	3, 5, 6, 7, 10
25. Libellulidae	(4)	3, 4, 10
HEMIPTERA		
26. Belostomatidae	(3)	3, 6, 7, 8, 9, 10
27. Corixidae	(3)	3, 4, 6, 7, 8, 9, 10
28. Gerridae	(5)	4, 5, 6, 7, 8, 10
29. Nepidae	(3)	6, 8
30. Naucoridae	(7)	6, 8
31. Veliidae	(5)	1, 2, 6
DIPTERA		
32. Athericidae	(10)	1,10
33. Culicidae	(1)	6
34. Muscidae	(1)	All 10
35. Chironomidae	(3)	All 10
36. Simuliidae	(5)	1, 5, 6, 7, 8, 9, 10

37. Syrphidae	(1)	3, 4, 5
38. Tabanidae	(5)	1, 2, 3, 4, 8, 9
39. Tipuliidae	(5)	1, 8, 9, 10
GASTROPODA		
40. Planorbidae	(3)	3, 6, 8, 9, 10
41. Physidae	(3)	1, 7
42. Thiaridae	(3)	1, 3, 9, 10
ANNELIDA		
43. Leeches	(3)	2, 8, 9, 10
44. Oligochaetae	(1)	1, 2, 4, 5, 6, 7, 8, 9, 10
PELECYPODA		
45. Corbiculidae	(5)	1
46. Sphaeriidae	(3)	5, 6
AMPHIPODA		
47. Potamonautidae	(3)	All 10

3.2.1.1 Macroinvertebrates diversity by study sites

ABCON (Site 10) supported the highest number (37) of macroinvertebrate families while MTLOK (Site 2) had the lowest number (24) (Table 3.3). Chi-square test showed no significant difference (Chi-square = 0.87; $p < 0.05$). The highest number of individuals (4023) was recorded at MACFA (Site 5) while the lowest number (1240) at HONEY (Site 8) (Table 3.3); thus, indicating the range of abundance was between 1240 and 4023. Chi-square test showed highly significant difference (Chi-square = 0.00; $p < 0.05$). Following Margalef's Index Indication, ABCON (Site 10) had the highest values (4.762) of species richness while MTLOK (Site 2) had the lowest values (3.227) (Table 3.3);

thus, indicating the range of richness was between 4.762 and 3.227. Chi-square test showed no significant difference (Chi-square = 1; $p < 0.05$). Pielou's Evenness Index indicated that GQUMA (Site 7) had the highest values (0.7137), while MACFA (Site 5) had the lowest (0.5109) (Table 3.3), thus, indicating the range of evenness was between 0.7137 and 0.5109. Chi-square test showed no significant difference (Chi-square = 1; $p < 0.05$). According to Shannon-Wiener Diversity Indices for all sampling sites, the highest diversity index values (2.573) of macroinvertebrates were recorded at HONEY (Site 8), and the lowest (1.684) at MACFA (Site 5) (Table 3.3), thus, indicating the diversity ranged between 2.573 and 1.684. Chi-square test showed no significant difference (Chi-square = 1; $p < 0.05$).

Table 3.3 Diversity indices of macroinvertebrates within sampling sites along Tyhume River

Site No	Site name	Number of species (S)	Total number of individuals (N)	Margalef's Index (d)	Pielou's Evenness Index (J')	Shannon-Wiener Index (H')
1	SWALL	31	3188	3.719	0.6011	2.064
2	MTLOK	24	1246	3.227	0.5962	1.895
3	SANCT	28	1730	3.621	0.6948	2.315
4	AUCKL	29	2127	3.654	0.6470	2.179
5	MACFA	27	2774	3.279	0.5109	1.684
6	MELAN	35	4023	4.096	0.5940	2.112
7	GQUMA	31	1854	3.987	0.7137	2.451
8	HONEY	28	1240	3.791	0.7723	2.573
9	NJWAX	33	1244	4.491	0.7031	2.458
10	ABCON	37	1920	4.762	0.6647	2.400
	Chi square test	**0.87**	**0.00**	**1.0**	**1.0**	**1.0**

3.3. THE DETERMINATION OF THE ECOLOGICAL STATUS OF THE SITES.

3.3.1 Ecological class boundaries

The state of the aquatic ecosystem varied among the majority of study sites. Sixty percent of the study sites retained their 'NATURAL' state (N; Sites 1 - 4, 7 and 10) (Table 3.4; Appendix III), followed by 20 % in 'GOOD' (Sites 5 and 6) and 'FAIR' (Sites 8 and 9) states, respectively. Most of the sites with natural state were located above the University of Fort Hare and Alice Town. The four sites which indicated unnatural conditions were all located in the lower catchment area (below the Binfield Dam) of Tyhume River.

Table 3.4 Ecological classes (based on ASPT scores) at different study sites (N = natural state; G = Good state and F = Fair state)

Site No	*Site name*	*Present status*
1	SWALL	N
2	MTLOK	N
3	SANCT	N
4	AUCKL	N
5	MACFA	G
6	MELAN	G
7	GQUMA	N
8	HONEY	F
9	NJWAX	F
10	ABCON	N

3.3.2 Mean ASPT scores of macroinvertebrates for all sites

ASPT scores at all study sites ranged between 5.3 and 7.6 (Fig. 3.1). Tests for mean differences in ASPT among sites were nonparametric. Sanct (Sanctuary Site 2), Auckl (Auckland Site 4) and Abcon (Above Confluence Site 10), had the highest ASPT (mean of 7.6), whereas the lowest ASPT score (5.3) was recorded for Njwax (Njwaxa Site 9) (Fig. 3.1). ASPT scores did not significantly differ across all study sites (Chi square = 0.999; $p < 0.05$). According to the results from this study, therefore, none of the 10 study sites had a range of ASPT scores of < 5; THUS, indicating no sites with poor (P) class boundary.

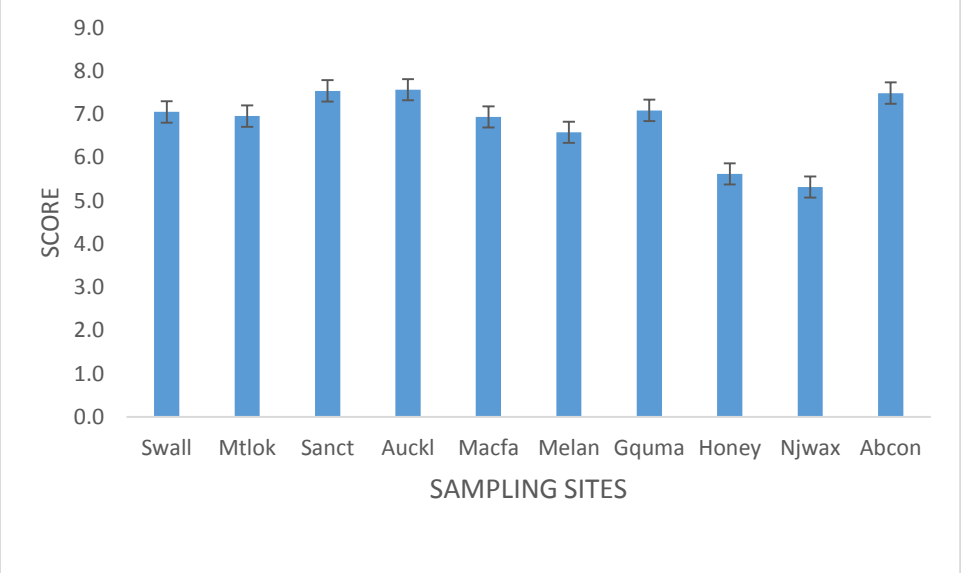

Fig. 3.1 Mean ASPT scores recorded at the 10 Study Sites along Tyhume River

3.4 THE EFFECT OF HABITAT PREFERENCE ON MACROINVERTEBRATE DISTRIBUTION.

From the hierarchical cluster analysis based on the Bray-Curtis similarity, four main clusters were observed at a Similarity Percentage of 61 % (Fig. 3.2). MTLOK (Mtloko Site 2) and SANCT (Site 3 Sanctuary) formed a cluster; HONEY (Honeydale Site 8) and

NJWAX (Njwaxa Site 9) formed another cluster; ABCON (Above Confluence Site 10), MACFA (Mcfairlane Site 6) and MELAN (Melani Site 5) and last cluster made up of SWALL (Swallowtail Site 1), AUCKL (Auckland Site 4) and GQUMA (Gqumashe Site 7). However, Similarity Profile (SIMPROF) did not find any statistically significant difference between these clusters ($P < 0.05$).

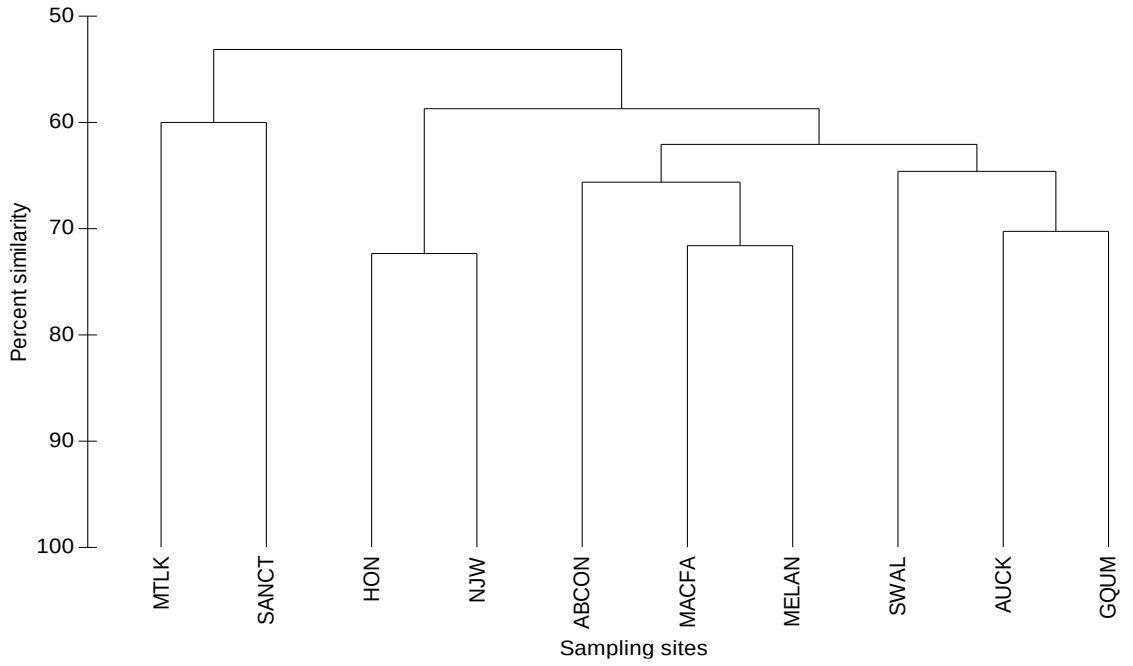

Fig. 3.2 Dendrogram from hierarchical clustering of ten sampling sites along Tyhume River indicated by macroinvertebrate distribution

The non-metric Multidimensional Scaling (MDS) Ordination for all the sampling sites clearly indicated the four clusters (Fig. 3.3). The low stress value of 0.07 implied that the MDS is an excellent representation with no prospects of misinterpretation. Sites close together on this map-like demonstration have similar resemblance in community structure as opposed to sites further apart. The agreement between the clustering and the MDS plot gives confidence in both as an approximation to the high dimensional pattern found in the data.

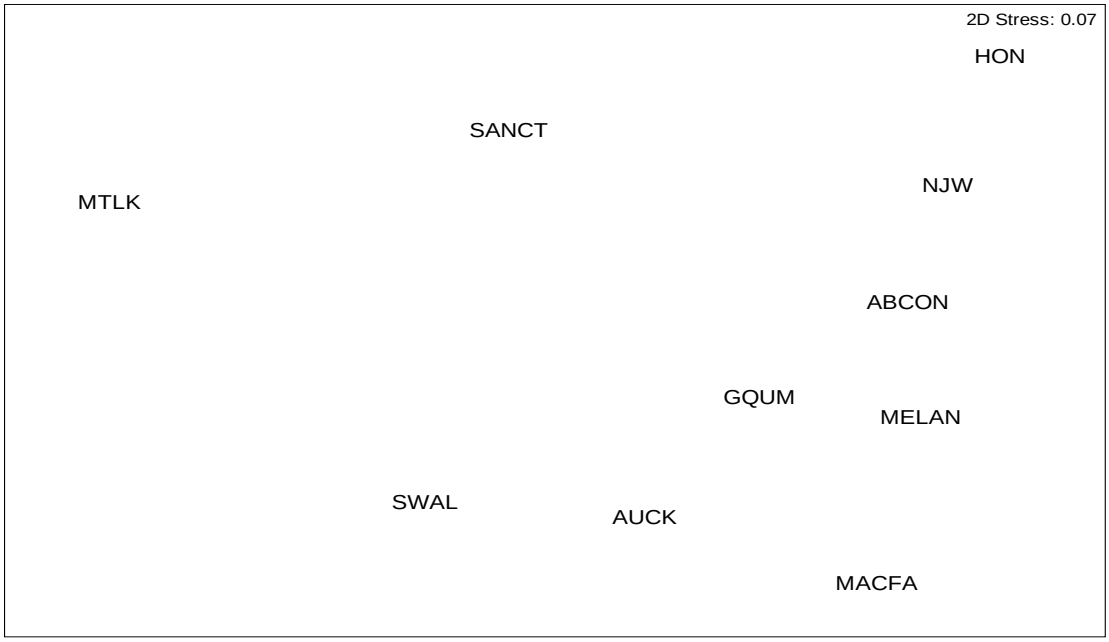

Fig. 3.3 MDS ordination showing the classification of sites with respect to macroinvertebrate distribution

3.4.1 Site similarities and differences based on macroinvertebrate biotope preference

Cluster analysis of all biotopes within the sampling sites following a Bray-Curtis Similarity Index and Similarity Profile test, gave a dendrogram with three clusters corresponding to the three biotopes of each sampling site. Three main clusters (Stone, S; Vegetation, V; and gravel-sand-mud, GSM), were observed at a percentage similarity of 40 % (Fig. 3.4). SIMPROF test showed that these clusters were statistically significant ($p<0.05$).

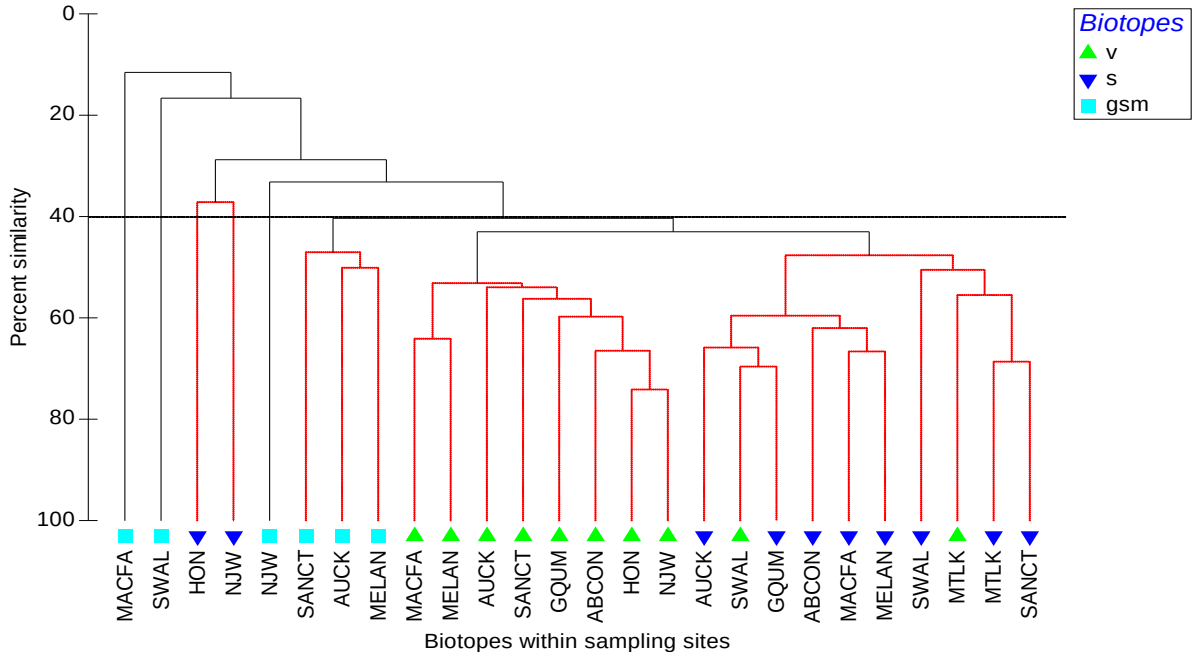

Fig. 3.4 Dendrogram from hierarchical clustering of biotopes within all sampling sites along Tyhume River as indicated by macroinvertebrate distribution

3.4.1.1 Stone

Following Similarity Percentage (SIMPER) Analysis of the biotope clusters, S biotopes cluster had an average similarity of 61.83 **%** (Fig. 3.4). Macroinvertebrate families such as Baetidae >2sp, Psychodidae, Tricorythidae, Heptageniidae, Perlidae and Baetidae 2sp, in that hierarchical order of value, contributed the most to the similarity percentage of this cluster (Table 3.5).

Table 3.5 Percentage similarity for the stone biotope cluster; Sim/SD = Similarity/Dissimilarity percentage; Contrib% = Contribution percentage and Cum.% = Cumulative percentage

Species	Average Abundance	Average Similarity	Sim/SD	Contrib%	Cum.%
Baetidae > 2 sp	6.07	9.70	11.11	15.68	15.68
Psychodidae	4.74	6.86	3.72	11.1	26.78
Tricorythidae	4.02	5.78	4.02	9.34	36.12
Heptageniidae	4.17	5.67	2.48	9.17	45.29
Perlidae	3.34	4.90	7.36	7.92	53.21
Baetidae 2 sp	3.80	4.76	1.35	7.70	60.92
Aeshnidae	2.83	3.19	2.15	5.16	66.08
Athericidae	2.72	3.19	1.35	5.15	71.23
Notonemouridae	2.29	3.02	3.02	4.88	76.12
Potamonautidae	220	2.91	2.17	4.70	80.82
Psephenidae	1.52	1.54	1.22	2.49	83.30
Oligochaeta	1.60	1.51	1.24	2.44	85.74
Syrphidae	1.40	1.45	1.13	2.35	88.09
Diptera	1.54	1.32	1.12	2.14	90.23

3.4.1.2 Vegetation

Following Similarity Percentage (SIMPER) Analysis of the biotope clusters, V biotopes cluster had an average similarity of 55.95 **%** (Fig. 3.4). Macroinvertebrate families such as Baetidae >2sp, Baetidae 2sp, Psychodidae, Leptoceridae, and Athericidae, in that hierarchical order of value, contributed the most to the similarity percentage of this cluster (Table 3.6).

Table 3.6 Percentage similarity for the vegetation biotope cluster; Sim/SD = Similarity/Dissimilarity percentage; Contrib% = Contribution percentage and Cum.% = Cumulative percentage

Species	Average Abundance	Average Similarity	Sim/SD	Contrib%	Cum.%
Baetidae > 2 sp	5.23	8.47	9.96	15.13	15.13
Baetidae 2 sp	4.83	7.68	5.87	13.73	28.86
Psychodidae	4.17	6.38	4.96	11.41	40.27
Leptoceridae	3.64	5.38	8.37	9.61	49.88
Athericidae	3.07	3.76	1.49	6.71	56.59
Coenagrionidae	2.83	3.44	1.99	6.15	62.74
Gyrinidae	2.91	3.42	1.41	6.12	68.86
Heptageniidae	2.72	2.80	1.25	5.01	73.87
Veliidae	2.01	1.89	1.13	3.38	77.25
Dytiscidae	1.84	1.76	1.28	3.14	80.39
Corixidae	1.51	1.10	0.71	1.97	82.36
Zygoptera juvs	1.35	1.00	0.61	1.78	84.14
Notonectidae	1.51	0.99	0.60	1.78	85.92
Potamonautidae	1.30	0.79	0.51	1.41	87.33
Tipulidae	1.01	0.78	0.61	1.40	88.72
Belostomatidae	1.30	0.73	0.59	1.31	90.03

3.4.1.3 GSM

Following Similarity Percentage (SIMPER) Analysis of the biotope clusters, GSM biotopes cluster had an average similarity of 48.04 **%** (Fig. 3.4). Macroinvertebrate families such as Baetidae >2sp, Athericidae, Aeshnidae and Heptageniidae, in that hierarchical order of value, contributed the most to the similarity percentage of this cluster (Table 3.7).

Table 3.7 Percentage similarity for the Gravel-Sand-Mud biotope cluster; Sim/SD = Similarity/Dissimilarity percentage; Contrib% = Contribution percentage and Cum.% = Cumulative percentage

Species	Average Abundance	Average Similarity	Sim/SD	Contrib%	Cum.%
Baetidae > 2 sp,	4.86	12.63	7.32	26.30	26.30
Athericidae	3.66	8.79	13.43	18.30	44.60
Aeshnidae	3.02	7.27	4.48	15.13	59.73
Heptageniidae	2.82	4.65	2.72	9.68	69.41
Corixidae	2.05	3.77	19.66	7.85	77.26
Gomphidae	2.69	3.13	0.58	6.52	83.77
Potamonautidae	1.73	2.24	0.58	4.66	88.43
Oligochaeta	1.38	1.70	0.58	3.54	91.97

3.5 CHARACTERISTICS OF SELECTED PHYSICO-CHEMICAL PARAMETERS

Three selected physico-chemical parameters (temperature, Hydrogen Concentrations (pH) and Electrical Conductivity (EC)), were measured at different sampling sites, between the months of March and September 2006.

3.5.1 Temperature

Mean temperature at all the 10 study sites ranged between 12.3 ˚C and 18.1 ˚C. The lowest mean temperature of 12.3 ˚C was recorded at study Site 2 (Mtlok), while the highest mean temperature of 18.1 ˚C was recorded from study Site 10 (Abcon) (Fig. 3.1). Moderate mean temperature of 15.5 ˚C and 15.7 ˚C was recorded from Site 5 (Macfa) and Site 6 (Melan), respectively, in that order. Eight out of the 10 study sites recorded between the mean temperature range of between 15 ˚C and 30 ˚C, which is preferred for the survival of majority of macroinvertebrates.

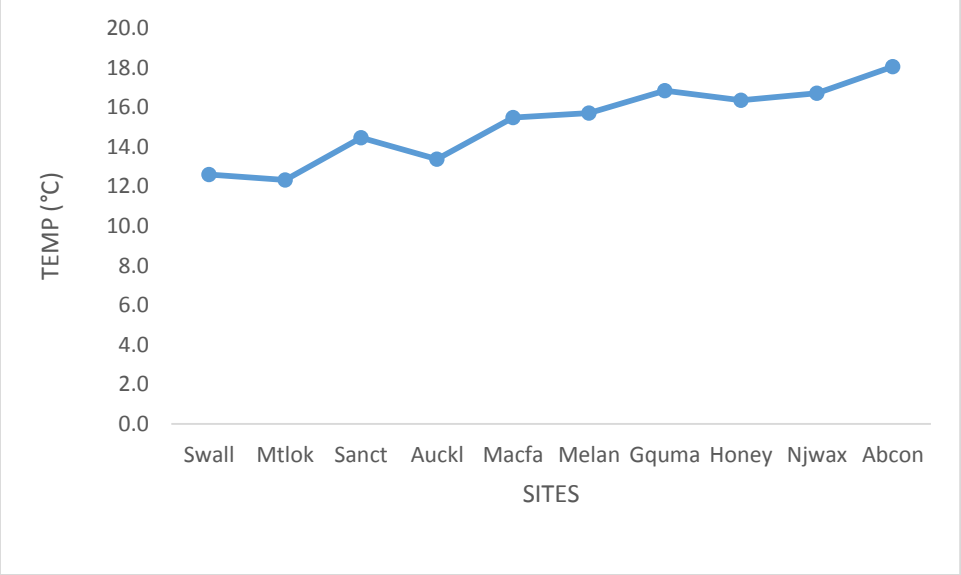

Fig. 3.5 Mean temperature at study sites in Tyhume River during 2006

3.5.2 pH

The river water was slightly acidic. Mean pH recorded from the 10 study sites was close to neutral (ranging from pH of between 6.60 and 7.14) (Fig. 3.2). Study Site 3 (Sanct) had the lowest mean pH (pH 6.52), while Site 10 (Abcon) had the highest mean pH (pH 7.14). Such environmental conditions favoured optimum requirements for macroinvertebrates life; the typical pH range for most surface water in South Africa

is 6 to 8. All 10 study Sites recorded between the mean pH range (pH 6 – pH 8) typical for most waters in South Africa.

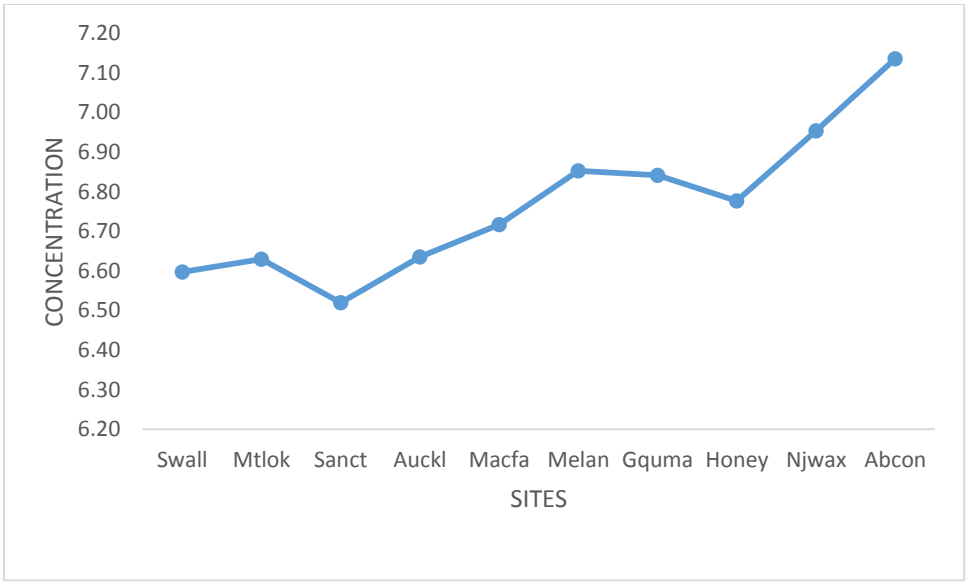

Fig. 3.6 Mean pH at the study sites in Tyhume River during 2006

3.5.3 Electrical conductivity (EC)

EC levels at the 10 study sites ranged between 16 µs/cm and 157 (Fig. 3.3). Site 2 (Mtlok) and Site 4 (Auckl), both located above Binfield Dam, recorded the lowest mean EC (16 µs/cm each), while Site 8 (Honey) and Site 10 (Abcon), both located below Binfield Dam, had the highest mean EC (157 µs/cm each) recorded during the study. The first seven out of the 10 study sites recorded between the mean EC range of 16 µs/cm and 100 µs/cm (Fig. 3.3), typical of most freshwater ecosystems; the last three study sites also recorded mean EC below stressful threshold (150 µs/cm) except Site 10 (157 µs/cm).

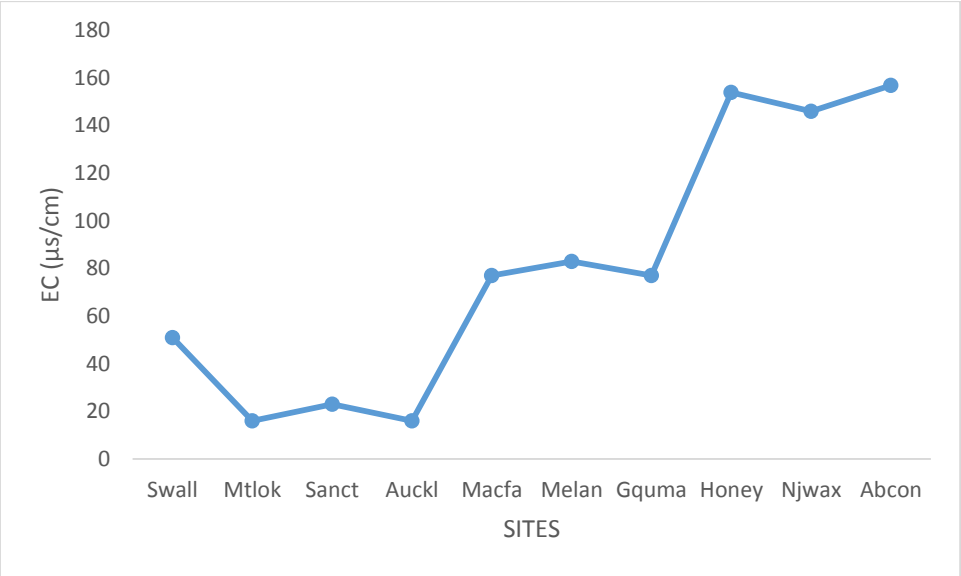

Fig. 3.7 Mean conductivity measurements at study sites in Tyhume River during 2006

3.5.4 The effect of physico-chemical parameters on the macroinvertebrate distribution

Physico-chemical variables are represented by arrows that approximately point towards the factor direction of maximum variation (Fig. 3.8). The results show that the 1st Principal Component (PC1) had highest contributions from pH and the first axis increasing from right to left due to the negative sign (Fig. 3.8). PC2 had equally weighted combination of the variables conductivity and temperature (Fig. 3.8; Appendix IV). The main contribution of conductivity was to the second axes in the left direction while the main contribution of temperature was to the second axes increasing from left to right.

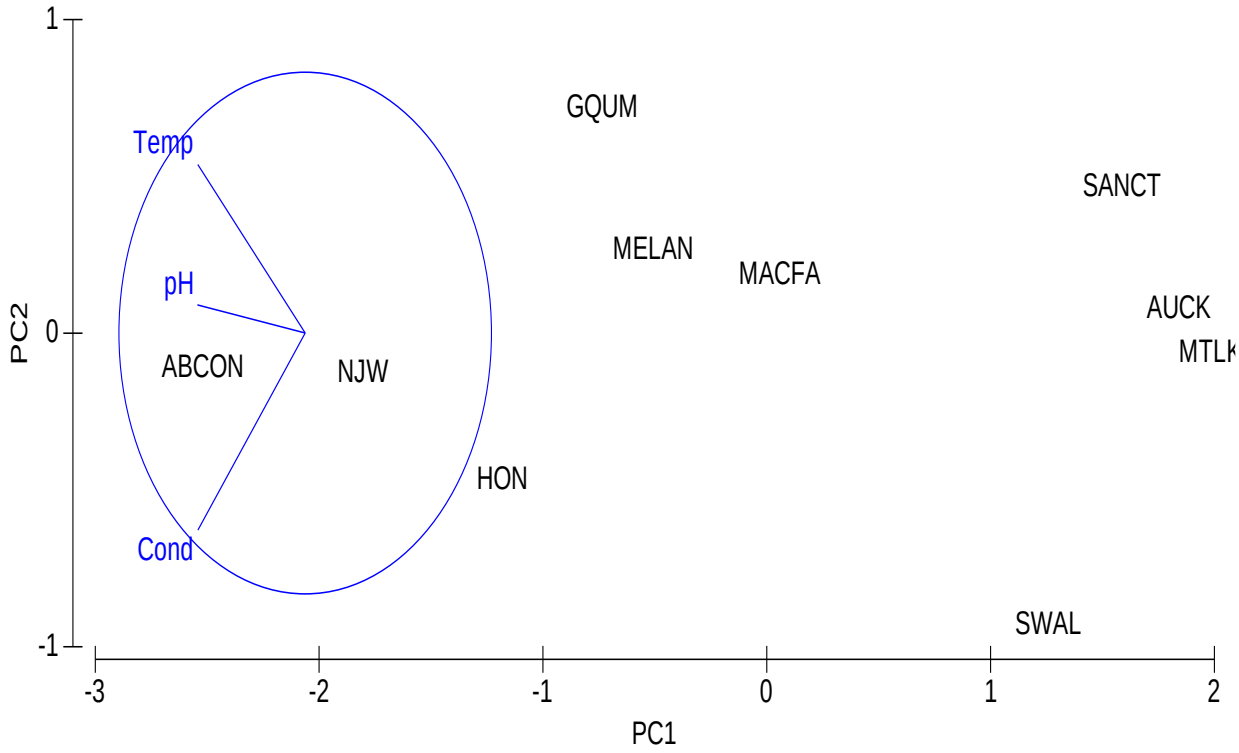

Fig. 3.8 Principal Component Analysis (PCA) Ordination of sampling sites regarding physico-chemical parameters along the Tyhume River

Figure 3.9 showed MDS of site classification with respect to physico-chemical parameters with very low stress value (0.01), implying that physico-chemical parameters influenced species distribution within each sampling site. Sites close together on this map-like demonstration have similar resemblance in community structure as opposed to sites further apart. Sites (ABCON, NJW and HON) formed the first group; Sites (GQUM, MELAN and MACFA) formed the 2nd group; and Sites (SANCT, AUCK, MTLK and SWAL) formed the 3rd last group (Fig. 3.9).

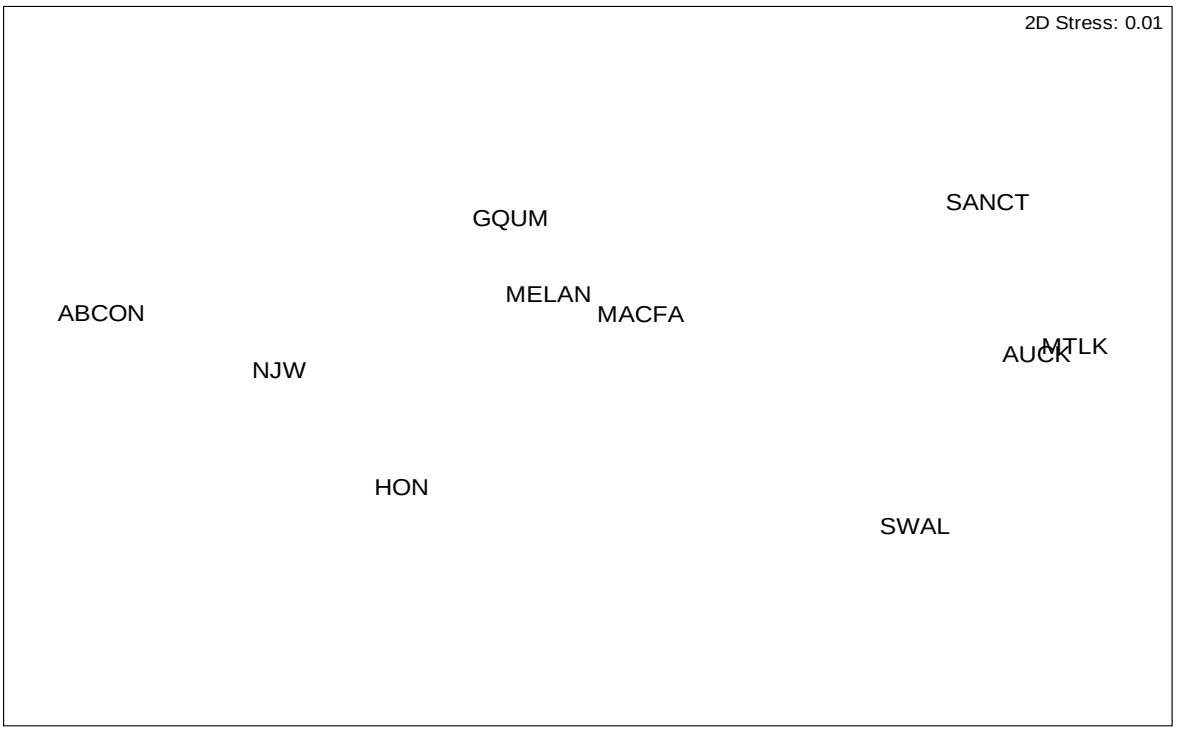

Fig. 3.9 MDS Ordination showing the classification of sites with respect to influence of physico-chemical parameters

CHAPTER 4: DISCUSSION

4.1 Macroinvertebrates inhabiting Tyhume River.

Four families that are very low tolerant (highly sensitive) to pollution (Baetidae >2sp; Heptaginiidae; Perlidae and Notonemouridae) were collected in the majority (at least six) of the 10 study sites. According to Gerber & Gabriel (2002), healthy streams are mostly dominated by the Heptageniidae and Oligoneuridae families (both in the Order Ephemeroptera). The same was reported by Abong'o *et al.*, 2015) who reported families under Order Ephemeroptera high in abundance in the upper and mid-stream areas. Maseti (2005) also found Perlidae and Notonemouridae in river sites that were regarded healthy. These families are all regarded as highly sensitive to environmental stresses of fresh water systems. In this study, sixteen moderately pollution tolerant families such as in Order Odonata (Chlorolestidae, Lestidae, Aeshnidae, Corduliidae, Gomphidae), Order Trichoptera (Ecnomidae, Hydropsychidae 2sp, Psychomyiidae, Leptoceriidae), Order Coleoptera (Elmidae, Psephenidae), Order Hemiptera (Naucoridae), Order Diptera (Athericidae), and Order Ephemeroptera (Baetidae 2sp, Caenidae, Tricorythidae), were collected. These families were in high densities on the upstream, middle stream, and downstream. Abong'o *et al.* (2015) reported similar results; families under Orders Hemiptera and Plecoptera were highly abundant in the upper and mid-stream areas. A river site which has little impairment is often indicated by the presence of Tricorythidea and Ecnomidae families which tolerate little range of pollution; and they are an indication of the beginning of pollution on water quality of a river (Ollis *et al*., 2010). Twenty-five highly tolerant families such as in the Order Diptera (Chironomidae, Culicidae, Muscidae, Simuliidae, Syrphidae, Tabanidae, Tipuliidae), Order Hemiptera (Belostomatidae, Corixidae, Gerridae, Nepidae, Veliidae), Order Odonata (Coenagrionidae, Libellulidae), Order Amphipoda (Potamonautidae), Order Annelida

(Leeches, Oligochaetae), Order Pelecypoda (Corbiculidae, Sphaeriidae), Order Gastropoda (Planorbidae, Physidae, Thiaridae), Order Coleoptera (Dytiscidae, Gyrinidae), and Order Trchoptera (Hydropsychidae 1sp), were also collected. Oligochaeta and Chironomidea families are an indication of pollution, due to their ability to tolerate high pollutants loads that occur on fresh water systems (Ollis *et al*., 2010). A site with no pollution intolerant species carries a red flag of pollution indication, and their return to the site will be an indication of improvement in water quality on fresh water streams (Jackson & Fureder, 2006).

Study Site 5 had the highest number of individual macroinvertebrate families than Site 8. According to Ollis *et al.* (2010), healthy waters support high densities of macroinvertebrate families which are very low tolerant to pollution. There was good presence of stoneflies within the upper stretch of the river (including the Site 5), which suggested that conditions were consistently suitable for the pollution-sensitive organisms and were relatively unaffected by pollution. The disappearance of stoneflies at Site 8 indicated that health conditions steadily declined within the lower stretch of the river (including the Site 8). Stoneflies are well known for requiring highly aerated microhabitats which are easily found in riffle areas (Gerber & Gabriel, 2002b). Downstream from Site 5, the macroinvertebrate diversity and community were increasingly dominated by highly tolerant macroinvertebrates families, especially dipterans, particularly the Chironomidae and Simuliidae. The decline in river health conditions was evident and became more serious as the river approached and flowed through Site 8. Storm water canals from Alice Town and effluents form University of Fort Hare farm were observed entering into the river above Site 8. The storm- and effluent-water was green coloured with a pungent odour and there was dominance of low-scoring and tolerant dipterans which were signs of possible water quality impairment. Abong'o

et al. (2015) stated that lower abundance of macroinvertebrates downstream due to water quality deterioration resulting from various reasons (e.g. local land use; sewage effluent discharge; annual floods).

4.2 Biotic Integrity of sites based on their ecological classification

Sixty percent of study sites along Tyhume River (Sites 1 (SWALL), 2 (MTLOK), 3 (SANCT), 4 (AUCKL), 7 (GQUMA), and 10 (ABCON)), were in "NATURAL" state. Several authors have reported some of their sampling sites to be in Natural state (Maseti, 2005 for the Buffalo River; Mbikwana *et al*., 2010 for Keiskamma River; Odume *et al.*, 2012 for Swartkops River).

In this study, "NATURAL" meant either absence of human interference or negligible modification of in-stream and riparian habitats and biota. Diversity of macroinvertebrate families in these six sites suggested that river health conditions were Natural and consistently suitable for pollution-sensitive organisms (e.g. Perlidae and Notonemouridae) to thrive (Al-shami *et al.*, 2011).

Twenty percent of study sites along Tyhume River (Sites 5 (MACFA) and 7 (GQUMA)), were in "GOOD" state. Several authors have reported some of their sampling sites to be in Good state (Maseti, 2005 for the Buffalo River; Mbikwana *et al*., 2010 for Keiskamma River; Odume *et al.*, 2012 for Swartkops River). In this study, "GOOD" meant characterised by the ecosystem where biodiversity is largely intact. Previous River Health Programme (RHP) studies reported sites with "GOOD" condition as having macroinvertebrate biodiversity largely intact (RHP, 2004). Diversity of macroinvertebrate families in these two sites suggested that river health conditions were good and

consistently suitable for pollution-sensitive organisms (e.g. Heptaginiidae and Baetidae >2sp) to thrive.

Twenty percent of study sites along Tyhume River (Sites 8 (HONEY) and 9 (NJWAX)), were in “FAIR” state. Several authors have reported some of their sampling sites to be in Fair state (Maseti, 2005 for the Buffalo River; Mbikwana *et al*., 2010 for Keiskamma River; Odume *et al.*, 2012 for Swartkops River). In this study, “FAIR” meant characterised by multiple disturbances associated with socio-economic development. Multiple disturbances associated with socio-economic development result in river water quality degradation (RHP, 2004). Various researches (Bredenhand, 2008; Abong’o *et al.*, 2015; Maseti, 2005; Mbikwana *et al*., 2010; Odume *et al.*, 2012; Rajele 2004) also confirmed water quality impairments, habitat destruction and species diversity reduction where “FAIR” conditions were reported. In such situationsI species may be lost, with tolerant or opportunistic species dominating (Mbikwana *et al*., 2010; RHP, 2004). The macroinvertebrates collected from the two sites of this study, lacked any pollution-intolerant organism, but dominated by moderately pollution-tolerant (e.g. Elmidae and Pisuliidae) and pollution-tolerant (e.g. Chironomidae and Planorbinae) families.

4.3 The effect of habitat preference on macroinvertebrate distribution

Stone biotope contained higher macroinvertebrate diversity and abundances, followed by vegetation biotope, and then the Gravel-Sand-Mud biotope. Many researchers of South African rivers (e.g. Dallas, 2005, 2007; Maseti, 2005; Mbikwana *et al*., 2010; Odume *et al*., 2012; Rajele, 2004), have also reported similar results. These may be associated with the fact that stone biotopes are available in most of the river sites in South Africa (Dallas, 2005; Maseti, 2005). The macroinvertebrate representatives

collected from stone biotope, in these previous and the current studies were dominated by moderately to highly pollution intolerant families (e.g. Tricorythidae, Perlidae, Notonemouridae, Psephenidae and Syrphidae).

The vegetation biotope contained the second higher macroinvertebrate diversity and abundances of all sampled biotopes. Dallas (2007), Maseti (2005) and Rajele (2014) have also reported similar results. In this study, the macroinvertebrate representatives collected from vegetation biotope were dominated by moderate and pollution-tolerant families (e.g. Psychodidae, Gyrinidae, Veliidae, Notonectidae, Coenagrionidae, Leptoceriidae and Belostomatidae). Dallas (2007), Maseti (2005) and Rajele (2014) attest to similar results.

The Grave-Sand-Mud biotope contained the lowest diversity and abundances of macroinvertebrates than all sampled biotopes. Many studies (e.g. Dallas, 2007; Odume *et al*., 2012; Parsons *et al*., 2003), also attest to Gravel-Sand-Mud supporting few macroinvertebrates; sand has been seen as the poorest habitat. In this study, the macroinvertebrates collected from Grave-Sand-Mud biotope were dominated by pollution-tolerant representatives (e.g. baetid 1sp, chironomids, oligochaetes and simulids).

The mean temperature at the 10 study sites ranged between 12.3 ˚C and 18.1 ˚C; the mean temperature ranges of between 15 ˚C and 30 ˚C, which is preferred for the survival of majority of macroinvertebrates. Temperature is one of the most influential abiotic devises of aquatic ecosystem processes affecting macroinvertebrate distribution (Dallas and Ross-Gillespie, 2015; Ramulifho *et al.*, 2018). For instance, Ross-Gillespie *et al.* (2018), reported that temperature influenced the successful development and hatching rates in eggs of certain macroinvertebrates. Temperature can be modified by various factors such as weather, removal of riparian vegetation,

large wood debris accumulation, turbidity, (Dallas & Rivers-Moore, 2014; Farrell, 2014; Odhiambo & Mwangi, 2014; Walters *et al*., 2009).

CHAPTER 5: CONCLUSION & RECOMMENDATIONS

The number of macroinvertebrate taxa found in this study, along with the number of pollution-intolerant taxa, indicated that Tyhume River is relatively healthy. As expected, macroinvertebrates were widespread, and provided a spectrum of responses to disturbances, and acted as monitors of the river health. Diversity was a strong sign of health, especially the orders with diverse families that are pollution intolerant. Thus, the presence or absence of specific types of macroinvertebrates was one way in which information was obtained about the river health status.

The study observed different types of macroinvertebrate taxa, some kinds of which only survive in waters that are virtually free of pollution, or very low tolerant to pollution, and which disappear when water become contaminated. The study also observed moderately pollution-tolerant macroinvertebrates, and which occur more typically in waters that are somewhat contaminated. Yet other macroinvertebrates observed were very tolerant to polluted conditions, and which dominates areas that are highly contaminated. These differences in pollution-tolerance made macroinvertebrates excellent indicators of the health of where they live along Tyhume River. The majority of Tyhume River is healthy, especially above Binfield Dam. The deterioration in health conditions was evident with the downstream continuum, especially below UFH and Alice Town.

The Tyhume River appeared to contain ideal SASS sampling site. The majority of sites along the river possessed three (at least 2) biotopes types required for SASS5 sampling. The results from this study compare with other studies regarding the

response within ranges of SASS5 weightings. This study, therefore, provided useful data for assessing riverine macroinvertebrate diversity, the present ecological health and the habitat preference of the selected catchments using the SASS5 index. Studies conducted in Tyhume River provide valuable contribution towards the macroinvertebrate database in South Africa. This can provide valuable benchmarks for future biomonitoring of the same and other river catchments.

The following tips are recommended for the design of Tyhume River Programmes which would promote the restoration and protection of the good health of the river:

- concrete effort should be taken by the government and civil society to engage continued monitoring of the river health (e.g. biological and physico-chemical conditions).
- increasing education and awareness on water pollution and involvement of the public in river stewardship could minimize the negative consequences due to urbanization (e.g. effluent and stormwater seapage directly into the river; sewage treatment plants should be built before discharging wastes into the river).
- promote acts of community ownership of resources: engaging watershed residents (e.g. river services; agreed-to charges or penalties; downstream health preservation; controlled gracing, overgrasing, etc.
- strict polluter-pay penalty provisions on the culprit.

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Appendix I: SASS5 Score sheet

SASS Version 5 Score Sheet

Date: / /200__

Collector:

Grid Reference: WGS-84 Cape datum

S: ° ' " E: ° ' "

Site code:

River:

Site description:

Weather Condition:

Temp: °C pH:

DO: mg/l Cond: mS/m

Biotopes sampled:

SIC Time: minutes

SOOC Time: minutes

Average size of stones: cm

Bedrock

Aquatic veg'n. Dom. sp

MvegIC Dom. sp

MvegOOC Dom. sp

Gravel Sand

Mud

Hand picking/Visual observation

Flow: Low/Medium/High/Flood

Turbidity: Low/Medium/High

Riparian land use:

Disturbance in the river: eg. sandwinning, cattle drinking point, floods etc.

Observations: eg. smell and colour of water, petroleum, dead fish, etc.

Taxon	S	Veg	GSM	TOT
PORIFERA	5			
COELENTERATA	1			
TURBELLARIA	3			
ANNELIDA				
Oligochaeta	1			
Leeches	3			
CRUSTACEA				
Amphipoda	13			
Potamonautidae*	3			
Atyidae	8			
Palaemonidae	10			
HYDRACARINA	8			
PLECOPTERA				
Notonemouridae	14			
Perlidae	12			
EPHEMEROPTERA				
Baetidae 1sp	4			
Baetidae 2 sp	6			
Baetidae > 2 sp	12			
Caenidae	6			
Ephemeridae	15			
Heptageniidae	13			
Leptophlebiidae	9			
Oligoneuriidae	15			
Polymitarcyidae	10			
Prosopistomatidae	15			
Teloganodidae SWC	12			
Tricorythidae	9			
ODONATA				
Calopterygidae ST,T	10			
Chlorocyphidae	10			
Chlorolestidae	8			
Coenagrionidae	4			
Lestidae	8			
Platycnemidae	10			
Protoneuridae	8			
Aeshnidae	8			
Corduliidae	8			
Gomphidae	6			
Libellulidae	4			
LEPIDOPTERA				
Pyralidae	12			

Taxon	S	Veg	GSM	TOT
HEMIPTERA				
Belostomatidae*	3			
Corixidae*	3			
Gerridae*	5			
Hydrometridae*	6			
Naucoridae*	7			
Nepidae*	3			
Notonectidae*	3			
Pleidae*	4			
Veliidae/Mesoveliidae*	5			
MEGALOPTERA				
Corydalidae	8			
Sialidae	6			
TRICHOPTERA				
Dipseudopsidae	10			
Ecnomidae	8			
Hydropsychidae 1 sp	4			
Hydropsychidae 2 sp	6			
Hydropsychidae > 2 sp	12			
Philopotamidae	10			
Polycentropodidae	12			
Psychomyiidae/Xiphocentronidae	8			
Cased caddis:				
Barbarochthonidae SWC	13			
Calamoceratidae ST	11			
Glossosomatidae SWC	11			
Hydroptilidae	6			
Hydrosalpingidae SWC	15			
Lepidostomatidae	10			
Leptoceridae	6			
Petrothrincidae SWC	11			
Pisulidae	10			
Sericostomatidae SWC	13			
COLEOPTERA				
Dytiscidae*	5			
Elmidae/Dryopidae*	8			
Gyrinidae*	5			
Haliplidae*	5			
Helodidae	12			
Hydraenidae*	8			
Hydrophilidae*	5			
Limnichidae	10			
Psephenidae	10			

Taxon	S	Veg	GSM	TOT
DIPTERA				
Athericidae	10			
Blepharoceridae	15			
Ceratopogonidae	5			
Chironomidae	2			
Culicidae*	1			
Dixidae*	10			
Empididae	6			
Ephydridae	3			
Muscidae	1			
Psychodidae	1			
Simulidae	5			
Syrphidae*	1			
Tabanidae	5			
Tipulidae	5			
GASTROPODA				
Ancylidae	6			
Bulininae*	3			
Hydrobiidae*	3			
Lymnaeidae*	3			
Physidae*	3			
Planorbinae*	3			
Thiaridae*	3			
Viviparidae* ST	5			
PELECYPODA				
Corbiculidae	5			
Sphaeriidae	3			
Unionidae	6			
SASS Score				
No. of Taxa				
ASPT				

Sample collection effort exceeds method? .....

Other biota including juveniles:

Comments:

Appendix II: List of taxa and collection sites

Sites	**1**			**2**		**3**			**4**			**5**			**6**			**7**		**8**		**9**			**10**	
Families	**S**	**V**	**G**	**S**	**V**	**S**	**V**	**G**	**S**	**V**	**G**	**S**	**V**	**G**	**S**	**V**	**G**	**S**	**V**	**S**	**V**	**S**	**V**	**G**	**S**	**V**
TURBELLARIA	P	P		P					P						P	P		P				P				P
Oligochaeta	P	P	P		P						P	P			P		P	P	P		P			P	P	P
Leeches				P																	P	P			P	P
AMPHIPODA																										
Potamonautidae	P	P		P		P	P		P	P	P	P		P	P		P	P		P	P		P		P	
PLECOPTERA																										
Notonemouridae	P								P			P			P			P							P	
Perlidae	P			P	P	P	P		P			P	P	P	P		P	P	P						P	P
EPHEMEROPTERA																										
‘Baetidae 1 Sp							P		P		P		P						P					P		
Baetidae 2 Sp	P	P	P	P	P	P	P	P	P	P		P	P			P		P	P	P	P	P	P	P	P	P
Baetidae > 2 Sp	P	P			P	P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P		P	P
Caenidae	P			P	P	P												P							P	
Heptageniidae	P			P		P	P	P	P	P	P	P			P	P	P	P	P	P	P		P		P	P

Leptophlebiidae				P																					
Tricorythidae	P	P		P		P			P	P		P			P	P	P	P	P					P	
ODONATA																									
Chlorolestidae			P				P									P					P				
Coenagrionidae	P	P					P			P	P		P			P			P		P	P	P			P
Lestidae		P																							
Zygoptera Juvs										P		P						P	P		P		P			P
Aeshnidae	P	P		P		P		P	P		P	P			P	P	P	P			P		P	P	P	P
Cordulidae				P																					
Gomphidae							P	P				P		P	P	P	P	P							P	P
Libellulidae								P			P															P
HEMIPTERA																									
Belostomatidae								P								P	P		P		P		P			P
Corixidae							P	P		P	P						P		P		P		P	P		P
Gerridae										P			P			P		P	P	P		P				P
Naucoridae																P	P				P					
Nepidae		P			P		P										P		P	P	P		P	P		P
Notonectidae																P			P							

Pleidae		P					P						P			P			P		P		P			P
Veliidae		P	P	P	P	P										P										
TRICHOPTERA																										
Ecnomidae	P	P		P					P			P				P									P	
Hydropsychidae 1 Sp																							P			
Hydropsychidae 2 Sp												P												P	P	
Psychomyiidae	P	P			P		P			P			P			P	P	P	P		P		P		P	P
Leptoceridae	P									P						P				P						P
Pisuliidae							P			P			P		P	P					P	P	P	P		P
COLEOPTERA																										
Dytiscidae															P	P		P			P		P		P	P
Elmidae/ Dryopidae										P		P	P			P		P	P		P	P	P			P
Gyrinidae		P			P					P		P	P				P				P	P				
Helodidae													P						P							
Hydraenidae	P						P												P		P		P			P
Hydrophilidae	P								P	P					P			P					P		P	P
Psephenidae	P	P		P	P	P		P	P	P		P			P			P	P							
DIPTERA																										

Athericidae	P																							P	
Chironomidae	P	P		P	P	P	P	P			P	P	P		P	P	P	P	P		P	P	P	P	P	P
Culicidae																	P									
Muscidae	P	P		P	P	P	P		P	P	P	P	P		P	P	P	P	P		P		P	P	P	P
Psychodidae	P				P		P	P		P											P		P			
Simuliidae	P											P	P		P			P					P		P	P
Syrphidae									P		P	P														
Tabanidae	P	P			P		P			P											P		P			
Tipulidae		P																			P		P			P
GASTROPODA																										
Physidae	P																	P	P							
Planorbinae							P								P						P	P	P		P	P
Thiaridae	P						P	P														P			P	
PELECYPODA																										
Corbiculidae	P																									
Sphaeriidae												P	P	P	P	P										

Appendix III: Ecological classes based on ASPT scores at different sites for all the biomonitoring months

SITE No	March	April	May	June	July	Aug	Sep	Result
1	G	G	N	N	N	G	N	N
2	N	F	G	F	N	N	N	N
3	F	N	P	N	N	N	N	N
4	G	N	N	N	N	G	N	N
5	F	G	N	N	N	N	G	G
6	N	G	G	N	N	G	N	G
7	N	G	F	N	N	N	G	N
8	G	F	F	N	F	P	G	F
9	P	F	G	F	P	F	N	F
10	G	F	N	N	N	G	G	N

Appendix IV: Principal Component Analysis (PCA) of sampling sites

Eigenvalues

PC	Eigenvalues	%Variation	Cum.% Variation
1	2.580	85.9	85.9
2	0.217	7.2	93.2
3	0.205	6.8	100.0

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3
pH	-0.579	0.108	-0.808
Cond	-0.576	-0.755	0.312
Temp	-0.577	0.646	0.499