Effects of land-use change on benthic macroinvertebrates in the upper reaches of the Apies-Pienaar catchment

by

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“Hard work is worthless for those that don’t believe in themselves.” – Uzumaki Naruto

DECLARATION

I, _____, declare that the mini-dissertation entitled: **Effects of land-use change on benthic macroinvertebrates in the upper reaches of the Apies-Pienaar catchment**, which I hereby submit for the degree **Master of Science (Environmental Management)** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

_____ _____

SIGNATURE **DATE**

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Abstract

Urbanisation of catchment areas is a major cause of freshwater ecosystem degradation worldwide. As catchments become more developed and river ecosystems become increasingly engulfed in various land use activities, there is a growing need to understand these impacts on freshwater ecosystems. Benthic macroinvertebrates are extensively used as indicators of ecosystem health and have been an instrumental tool in ecosystem monitoring and management. The effects of changing land use on macroinvertebrates at a fine scale however, have not been extensively investigated.

Therefore an investigation was conducted to compare chemical, physical and biological surface water quality parameters and aquatic macroinvertebrate community composition along the first 8 km of the Hartbeesspruit, which contains multiple land use types, in the upper Apies-Pienaar catchment in Gauteng, South Africa.

Five sampling sites corresponding to changes in land use were sampled four times at six-week intervals from September 2013 to February 2014. Influential variables that were recorded included in-stream habitat, riparian cover, flow regime and surface water quality parameters. Physical surface water parameters that were tested *in situ* included pH, salinity, total dissolved solutes, temperature, clarity and conductivity. *Ex situ* surface water parameters that were tested included physical parameters (alkalinity and turbidity), chemical parameters (major ions, metal ions and nutrients), and biological parameters (bacteria, coliforms and *Escherichia coli*). Macroinvertebrates were sampled using Hester-Dendy artificial samplers, which, following a 6 week

exposure period, were sampled three times from November 2013 to February 2014. Macroinvertebrates were identified to family level and counted.

Macroinvertebrate community composition across sites was assessed through macroinvertebrate abundance, family richness, SASS score, ASPT, Shannon-Wiener index, Pielou's evenness, non-metric multidimensional scaling and Indval analyses. Nineteen families were collected, of which only three made up 80% of macroinvertebrates sampled. These families were Hirudinea, Chironomidae and Oligochaeta.

Indices of macroinvertebrate community composition indicated a general increase in value from upstream to downstream which showed similar comparative variation between sites to physical water quality parameters (except temperature and clarity), major ions (except arsenic), the metal ion magnesium and nutrient sulphate. Surface water parameters showed patterns indicative of effects due to evaporation, dilution and connectivity of water flow along the stream due to the presence of dams and wetlands. Temperature was an important influence on macroinvertebrate abundance and family richness at a temporal scale.

On a spatial scale the most influential parameters on macroinvertebrate composition were seen to be depth, turbidity and conductivity, and temperature to a lesser extent. The land use types that showed the greatest association with various assemblages were the urban, recreational and least transformed wetland land uses. Although major influential factors, this pattern was not seen to be strictly due to the input of contaminants arising from associated activities, nor the variation in physical characteristics, but rather the discontinuity in flow regime.

It was concluded that at a fine scale, the strongest factors that influenced macroinvertebrate community composition along the Hartbeesspruit, was not land use type but rather the hydrological pathways of connectivity and stream flow that exist within the system. The hydrological pathways influenced values and concentrations of chemical and physical surface water parameters which in turn further influenced macroinvertebrate assemblages present.

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List of Accronyms

ANOVA	Analysis of Variance
ASPT	Average Score Per Taxon
CCA	Canonical Corrspondence Analysis
DO	Dissolved Oxygen
IndVal	Indicator Value Analysis
nMDS	Non-metric Multidimensional Scaling
TDS	Total Dissolved Solids
TEEB	The Economics of Ecosystems and Biodiversity
SASS	South African Scoring System

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Glossary of Terms

Assemblage:

A collection or grouping of taxa that are considered in a study of an ecological community.

Benthic Macroinvertebrates:

Invertebrate fauna that cannot pass through a 50 µm sieve mesh and which inhabit the bottom substrates of their aquatic habitat.

Biological Indicator:

Organisms which, by their own presence or absence, indicate the existence or abundance of a particular critical factor (Phillips and Rainbow 1993).

Catchment Area:

The area from which rainfall flows into a river or reservoir.

Contaminant:

A constituent put into water that alters the natural physical or chemical properties of surface water.

Ecosystem:

A dynamic complex of plant, animal and micro-organism communities and their abiotic environment interacting as a functional unit (TEEB Manual for CITIES 2011).

Ecosystem Health:

A description of the dynamic properties of an ecosystem. An ecosystem is considered stable or healthy if it returns to its original state after a disturbance, exhibits low temporal variability with time, or does not change dramatically in the face of disturbance (TEEB Manual for CITIES 2011).

Ecosystem Services:

The direct and indirect contributions of ecosystems to human well-being. The concept 'ecosystem goods and services' is synonymous with ecosystem services (TEEB Manual for CITIES 2011).

Urbanisation:

The alteration of landscape from natural to residential, commercial and industrial uses, through construction (Wheeler *et al* 2005).

Water Quality:

The physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems (DWAF Vol 1 1996).

Wetland:

"Land which is transitional between terrestrial and aquatic systems, where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil (NWA 36 1998)."

1. Introduction

1.1. Ecosystem Health and Monitoring in Urban Catchments

Ecosystems provide the biosphere with valuable services, which maintain natural processes and resources crucial to human well-being. These include provisional, regulatory, supporting and cultural services that provide for primary productivity, nutrient cycling, waste assimilation, water and air purification, disease and pest control, pollination, climate regulation, habitats for species, recreation and aesthetic appreciation (TEEB Manual for Cities 2011). As ecosystems are degraded, the efficiency of these services are reduced or even lost (Reiger and Baskerville 1986).

Water quality and community structure of a stream or river are dictated by the natural processes which occur in the catchment area. Some of these processes include geology, vegetation, topography, climate and hydrological pathways. An alteration of these processes by human activities will inadvertently affect the ecological integrity of rivers (Allan and Johnson 1997). Unfortunately, due to human activities, freshwater ecosystems are one of the most threatened ecosystems at a global scale (Abell 2001). Land use change and population growth are closely related to ecosystem degradation, loss in freshwater quality and decreasing groundwater supplies (Burcher *et al.* 2007; Smith *et al.* 1987). In South Africa, there has been an increase in human impacts on natural water resources due to changes in the socio-economic climate and high levels of development and urbanisation (Du Preez *et al.* 1999). This has led to a situation where freshwater resources, not only for ecological function but also human use, are becoming scarce (Davies *et al.* 1993), which is further exacerbated by seasonal rainfall and predicted drying due to climate change (Basson *et al.* 1997).

The urbanisation of catchment areas is a major cause of freshwater ecosystem degradation worldwide (Paul and Meyer 2001). Land use change and population growth are closely related to ecosystem damage (Burcher *et al.* 2007; Smith *et al.* 1987). With increased urbanisation comes an increase in population density, land transformation, land use activities and impervious surfaces. These factors all come with various effects on the associated catchment. Increased population density creates an increase in human waste and pollution density. Land transformation can include the alteration of waterways or removal of natural vegetation, which interfere with natural processes. Land use activities such as industrial, commercial or agricultural activities have associated chemical and physical impacts on catchments. With an increase in impervious land cover, comes an increase in the accumulation of effluents and surface runoff, which ultimately carries associated contaminants into freshwater river ecosystems. Reduced permeability of land cover is due to an increase in impermeable surface cover. An impervious surface is any surface which does not allow

water to infiltrate or be stored by the surface. Impervious surface cover is closely related to land transformation specifically in urban, commercial and industrial land use types. This cover typically includes roads, parking lots, roofs and any other surface which is impervious to water. Increasing impervious cover results in an increase in runoff into associated freshwater systems (Espy *et al.* 1966). This brings with it an increase of pollution levels, water velocity and physical alterations such as erosion and sedimentation. Finally, reduced infiltration increases the quantity of storm water which increases water load and subsequent intensity, increasing physical pressure on the ecosystem. Pollution collects on various impervious surfaces, the source of the pollution is from both the activities associated with the surface as well as atmospheric pollution settling on the surfaces (Horner *et al.* 2007). Runoff from storm water moving over impervious surfaces has a reduction in quality as it carries a higher pollutant load (Bannerman *et al. 2005*). Associated pollutants are solids (litter/waste), sediment, nitrogen and phosphorous, petroleum hydrocarbons, microbial traces (bacteria), organic chemicals (pesticides or herbicides) and trace metals (Horner *et al.* 2007).

To better understand the effects of urbanisation on these freshwater systems, urban catchments have been investigated to identify the cause and effect relationship between economic growth and environmental degradation. The literature is extensive when looking at these effects and there are a multitude of approaches that can be taken (Brown *et al.* 2005). A popular approach looks at a specific land use type (mining, agriculture, waste water works, roads and highways, turf management, residential) and all associated land transformations. The inputs (chemical, physical or biological) and subsequent impacts (loss of biodiversity, loss in ecosystem services) of such an activity on the associated ecosystem are then determined (Azrina *et al.* 2006; Bis *et al.* 2000; Hall *et al.* 2000; Lenat and Crawford 1994; Maldono 2010; Suren 2000; Qu *et al.* 2010; Winter and Duthie 1998).

Investigations which take this approach seem to typically test the effects of urbanisation in one of two ways. One approach is to investigate a single freshwater system and determine impacts and effects, between upstream (near pristine conditions) and downstream of a single land use type (Azrina *et al.* 2006; Baa-Poku *et al.* 2013; Cooper *et al.* 2006; Qu *et al.* 2010; Winter and Duthie 2013). The second approach is to investigate multiple independent tributaries at a large scale to determine impacts and effects within various land use types (Hall *et al.* 2001; Helms *et al.* 2009; Lenat and Crawford 1994; Maldono 2010). These investigations take a comparative approach, and because of the isolated nature of the impacts, provide a clear cause and effect relationship between land use types and impacts on natural systems. This being said, it is also important, due to the increasing level of development of catchments, to identify the impacts and effects of varied levels of urbanisation along an associated ecosystem at a small scale. The cause and effect relationship may

not be as clear in this case, as interactive effects between the various land use types may play a role. However, this will provide valuable insight, due to the relevant nature of such freshwater systems, into the effects that both; multiple land use changes as well as local processes and the specific physical nature of a specific system have on the abiotic and biotic characteristics of these systems.

The measure used to identify effects on an ecosystem must provide an accurate portrayal of what is happening in the system. Traditional measures of ecosystem health fall into the categories of structure, function and resilience investigations (Costanza and Mageau 1999). A deviation from natural conditions, after an impact, in any of these categories will indicate a deviation from a healthy ecosystem, thus allowing for valuable indices of change within a system when doing such a study. The structural measure is the most straightforward measure, and can be investigated through the use of indicator components, namely abiotic (chemical and physical) and biotic factors. The use of biotic indices is termed "bio-monitoring" whereby biological data is collected from a system and inferences are made about that system based on the findings (Hohls 1996). Both abiotic and biotic indicators have been used to measure ecosystem health. However, the use of biotic indicators is becoming an increasingly popular method to detrmine the health of an ecosystem (Uys 1994). This is not unusual as many biotic indicators represent ecosystem health on both a spatial and temporal scale, as opposed to traditional abiotic indicators, such as chemical and physical water quality parameters, which only allow for a "snap-shot" in time of the conditions in the ecosystem.

1.2. Benthic Macroinvertebrates as Indicators of Ecosystem Health

According to McGeoch (1998), an efficient bio-indicator for freshwater systems should be cheap in terms of resources (financial, time and effort) needed to investigate them and biologically efficient in indicating environmental change, represent biological diversity, and give an accurate representation of the ecological conditions in which they are found. Benthic macroinvertebrates are one such surrogate and have been extensively used as indicators of water quality and ecosystem health (Arman *et al.* 2012; Azrina *et al.* 2006; Baa-Poku *et al.* 2013; Cooper *et al.* 2006; Hall *et al.* 2001; Helms *et al.* 2009; Kemp *et al.* 2012; Lenat and Crawford 1994; Maldono 2010; Mason and Parr 2003; Morse *et al.* 2007; Qu *et al.* 2010; Winter and Duthie 2013;). Benthic macroinvertebrates are efficient indicators as they are readily found in all aquatic environments and are easily sampled. They have limited mobility and thus cannot easily move away from disturbances, have long enough lifespans so that their presence can indicate past events, and have a range of sensitivities to pollutants which is important in any bio-indicator (Barbour *et al.* 1999). This makes them an efficient tool to use in water quality and ultimately ecosystem health investigations.

Freshwater macroinvertebrates are a highly diverse faunal group which inhabit a multitude of niches and habitats throughout freshwater systems (Allan 1995). The distribution of macroinvertebrate taxa across a river system is strongly determined by the microhabitat that they occupy depending on their resource and habitat requirements (Evans and Norris 1997). The assemblages have adapted to specific conditions ideal for fecundity, growth and survival and are characteristic of specific flow regimes, physical habitat structure, water quality, and inputs from the catchment (Milhous and Bartholow 2004). An alteration of these conditions will cause subsequent changes to macroinvertebrate community structure (Thirion 2007).

1.2.1. Flow regime

The flow of water plays a major role in supporting river ecosystems (Allan 1995). Flow regimes influence the velocity, depth, wetted area, sedimentation and physical, chemical and biological characteristics of the system, which in turn influence the availability and suitability of habitats for aquatic organisms (Dewson *et al.* 2007; Donnely 1993; Hart and Finelli 1999).

In a natural system, wetlands moderate water flow as well as regulate water quality (Reddy and DeLaune 2008). Water flow is regulated by acting as a buffer for runoff in the wet season and allow for the slow release of water in the dry season. This prevents associated impacts of both high volumes of water entering the system (increased turbulence and water velocity) and reduced volumes of water entering the system (loss of water support in the system) (Reddy and DeLaune 2008). The regulation of flow allows for further water quality regulation within the wetland through various processes which in turn regulate suspended particles (Strecker *et al* 1992), nutrients (Reddy and DeLaune 2008), metals (Sheoran and Sheoran 2006), organic pollutants (Hemond and Benoit 1988) and bacteria (Rogers 1983).

In developed catchments, flow regime is altered through the presence of dams, impoundments or other river management activities which influence the connectivity of the system (Kingsford 2000). Variations in flow rate between running water and water within impoundments influence changes in physical, chemical and biological characteristics of the water (Baxter 1977). Temperature is altered within a dam due to the reduced surface exposure of the water to the surrounding atmosphere (Sinokrot *et al.* 1995) meaning dam water temperatures will be warmer in winter and cooler in summer compared to associated river water temperature. Variations in stream capacity cause sediment carried into a dam from an upstream to be deposited into the dam (which has a chance to settle), which causes a reduction in sediment load and subsequent turbidity downstream (Baxter 1977, Donnely 1993). Depending on the depth of the dam, deeper parts may become more anoxic due to the decomposition of vegetation which can cause the reduction of

various chemical constituents (Baxter 1977). Bacterial load also tends to decrease within dams (Stearns 1916).

1.2.2. Physical habitat

Physical habitat structure such as substrate varies between stream and river systems, and microhabitats within these systems also vary. River or stream substrate type is an important determinant of the distribution of benthic macroinvertebrate assemblages (Buss *et al.* 2004; Maldonado 2010). Allan (2005) indicated that with increasing particle size of sediments, comes an increase in diversity and abundance of invertebrates. Diversity and abundance also increase with increasing substrate diversity (Resh *et al.* 1995). This is due to an increase in microhabitats available for a variety of benthic macroinvertebrates to occupy.

Hawkins *et al.* (1982) showed that canopy cover played a major role in abundance of taxa, showing that streams with shading had a lower abundance of benthic macroinvertebrates than streams with no shading. An increase in sunlight causes an increase in primary productivity and a subsequent increase in baseline resources (Behmer and Hawkins 1986). Variation in riparian vegetative cover is the primary influence on shading of freshwater systems.

The depth of habitats in a freshwater system is an important determinant of benthic macroinvertebrate assemblages. Baumgartner *et al.* (2008) observed that depth played a major role in the community patterns of benthic macroinvertebrates. Brooks *et al.* (2005) showed that benthic macroinvertebrate abundance decreased as depth increased. Quinn and Hickey (1994) suggested that this could be explained by changes in algal biomass. Although, the relatively shallow depths commonly found in riffles of many streams will have negligent effects on benthic macroinvertebrate assemblages (Stark 1993).

1.2.3. Water quality

There is natural variation in pH in natural systems and ecosystems have adapted to these conditions. Season variation can cause changes in pH due to rainfall. This occurs through the introduction of organic acids due to leaching of the surrounding soil (Dallas *et al.* 1998). Variations in pH due to anthropogenic influences largely arise from land uses. An example is the release of sulphur and nitrogen into the atmosphere, through the burning of fossil fuels, poor management of animal manure, industrial processes and fertiliser, which are transformed into sulfuric acid and nitric acid when they react with rain water (Galloway *et al.* 1976). Changes in pH have an effect on other constituents present in water. Metals dissolve in water easier at a low pH. Metals including aluminium, cadmium, copper, silver, mercury, lead and zinc, become toxic as they are dissolved

(Sweeting 1994). The toxicity of these constituents will have an influence on the wellbeing of macroinvertebrates present. Changes in pH also cause changes in ionic and subsequently osmotic balance between the water and organisms (Dallas and Day 1993).

The temperature of water in river systems varies over space and time. In natural systems, these fluctuations are dependent on hydrological, climatic and structural features of the catchment area (Dallas and Day 1993). Hydrological variations are due to changes in the source and the flow rate of the water. Climatic variation is due to changes in the cloud cover, wind speed, air temperature, latitude and altitude of the river. Structural variations are due to the depth, turbidity and vegetation cover of the river (Ward 1985).

Benthic macroinvertebrates are ectothermic, meaning that their body temperatures are determined by the temperature of the water. Changes in water temperature will influence macro-invertebrates to varying degrees depending on their tolerance to temperature fluctuations. This will occur at a species level by influencing behavioural traits (such as activity, searching for resources, and migration) and physiological processes (such as nutrient assimilation, metabolic rate, growth, development and reproduction) (Allan 1995; Chown and Nicholson 2004). Fluctuations in temperatures may also affect interspecific relationships as optimal temperatures may differ between taxa, which influence competition and predator-prey interactions (Reid and Wood 1976). Ultimately, species more tolerant of changes in water temperature may flourish and replace the less tolerant dominant species. As well as influencing individual species and species interactions directly, temperature also influences the chemical, physical and biotic characteristics of water. Dissolved oxygen decreases with increased water temperature which has major influences on macroinvertebrates (Hynes 1970).

Dissolved oxygen (DO) is one of the most crucial factors effecting aquatic macroinvertebrate community structure (Hynes 1970). DO varies in natural systems due to variations in physical, chemical and biological factors. Turbulence increases the surface area of water with the atmosphere and allows the uptake of atmospheric oxygen into the water. Depth and velocity play important roles in this uptake as the slower the velocity and deeper the water body the less turbulence in the water thus less oxygen aeration. Atmospheric pressure also plays a role, with higher pressure permitting higher levels of dissolved oxygen in water (Dallas and Day 1993). Respiration by aquatic organisms and aquatic plant photosynthesis also affect DO to varying degrees depending on the level of activity. Conolly *et al.* (2004) showed that even though most macroinvertebrate taxa tolerated a range of DO saturations, different taxa of benthic macroinvertebrates have varying degrees of sensitivities to reduced DO. Various species within the Ephemeroptera (mayflies) are

highly sensitive (<20% saturation) whereas various species in Chironomidae (non-biting midges) were least sensitive (<8% saturation).

Turbidity in a natural system can be affected by the hydrology and geomorphology of the region, and natural processes such as seasonal variations resulting in wind and water erosion into river systems (Harrison and Elsworth 1958). In developed areas, turbidity can be caused by urban runoff, waste discharge, algal and phytoplankton growth, sediments and re-suspended bed sediments. Urban runoff can pick up sediments and waste with storm water from any impervious or pervious layer such as construction sites, lawns and landscaped areas. The introduction of sediments is closely related to an increase in storm water quantity that results from an increase in the percentage cover of impervious substrates in urban areas (Shi *et al.* 2007). This causes increased erosion, especially in areas where vegetation is reduced or absent due to overgrazing, agriculture or infrastructure development, which brings a subsequent increase in sediment runoff into associated river systems. An increase in algal or phytoplankton growth may be associated with an increase in nutrients introduced this way.

The initial effect of increased turbidity is a decrease in light penetration, which has indirect effects on aquatic ecosystems (Kirk 1985). Decreased light penetration leads to a decrease in photosynthesis and thus in primary production (Grobler *et al.* 1987). This in turn influences the availability of food and habitat resources for benthic macroinvertebrates. A reduction in light penetration into aquatic ecosystems also decreases heat absorption, causing a drop in temperature (DWAF Vol 7 1996) that may then affect biological and chemical processes. Suspended solids may also absorb nutrients or toxic components, thus either reducing nutrient availability or toxic concentrations in an aquatic system (Dallas and Day 1993). High levels of turbidity have been seen to increase the movement of macro-invertebrates downstream, thus a decrease in density of macro-invertebrates in the upper reaches of the affected system (Chessman *et al.* 1987). Azrina *et al.* (2006) illustrated how turbidity associated with land use intensity changes significantly decreased macroinvertebrate species richness and diversity. Gray and Ward (1982) observed that with increasing suspended solids, chironomid populations decreased initially and populations of mayflies and earthworms increased. They concluded that changes in macroinvertebrate assemblages for specific taxa were highly correlated to changes in turbidity.

Nutrients are elements that are required for primary production in a natural system. Natural sources of nutrients result from climatic conditions and the land form of an area i.e. rainfall, erosion and runoff (Dallas and Day 1993). The most important nutrients for freshwater ecosystem health are nitrogen in the form of nitrates (NO_3^-), nitrites (NO_2^-) and ammonium (NH_4^+), and phosphorus in the form of phosphate (PO_4^{3-}) (Davies and Day 1986). The major sources of nutrients such as

phosphorous and nitrogen in storm water runoff are chemical fertilisers used on golf courses, lawns and sports fields (Bannerman *et al.* 1993). Other contributors include atmospheric discharge from industrial activities and nutrient release from soil erosion (Horner *et al.* 2007), as well as the improper management of human and animal waste.

The freshwater ecosystem response to an increase in these nutrients is called eutrophication, which causes an increase in primary productivity. This causes an imbalance in the ecosystem where plant or animal communities increase rapidly, resulting in an increased oxygen demand and a subsequent depletion of dissolved oxygen, which brings with it its own repercussions. Phosphates exist in freshwater as ortho-phosphates or poly-phosphates and in high concentrations can be toxic (Horner *et al.* 2007). Rader and Richardson (1992) showed that macroinvertebrate density and diversity increased with phosphorus enrichment, but toxic effects were not investigated. Berenzen *et al.* (2001) showed that with an introduction of ammonium into a freshwater system, and subsequent ammonia and nitrate contamination, that macroinvertebrate taxon exhibited a range of sensitivities to the pollutants. Schofield *et al.* (1990) showed that high levels of ammonia in freshwater streams had negative effects on macroinvertebrate communities, with an increased proportion of pollution tolerant species. This illustrates the importance of investigating nutrient concentrations in a freshwater system in order to better understand macroinvertebrate assemblages.

The main sources of trace metals in freshwater systems are from geological weathering, industrial effluents, domestic effluents, agricultural runoff and acid mine drainage (Forstner and Wittmann 1983). Trace metals in urban environments are transported to stream systems by storm water runoff from sources such as parking lots, roads, industrial activities, and vehicle maintenance areas (Wilber and Hunter 1977; Steuer *et al.* 1997). Legret and Pagotto (1999) showed that the major metals associated with storm water runoff from highways were lead, copper, cadmium and zinc which are closely associated with automobiles.

Once trace metals enter a freshwater system they either stay in solution or they are absorbed by particles that accumulate in aquatic organisms. The effects of trace metals on aquatic organisms will depend on various factors such as temperature, pH, DO, alkalinity and salinity, amongst others, which affect the toxicity of the metal. Qu *et al.* (2010) saw that with an increased concentration of heavy metals came a distinct decrease in total abundance and species richness of benthic macroinvertebrates. Variation in sensitivities to changes in concentrations across taxa and functional feeding groups was also observed. Smolders *et al.* (2003) showed that high levels of zinc, copper, lead and cadmium from upstream mining activities greatly reduced macroinvertebrate species richness and allowed tolerant species to dominate. Roline (1988) found similar results with

the same metals as well as iron and manganese. Wren and Stephenson (1990) showed that mercury, lead and cadmium were all toxic to macroinvertebrates however, sensitivities to the toxicity varies with species and pH.

Conductivity is a measure of ionised substances present in water, which includes many dissolved solids. Conductivity is thus often reported instead of total dissolved solids (Dallas and Day 1993). Total dissolved solids (TDS) are the amount of soluble organic and inorganic materials dissolved in the water. The major ions found in natural freshwater systems are sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), chloride (Cl^{-}), carbonate (HCO_3^{-}) and sulfate (SO_4^{-2}) (Dallas and Day 1993). Natural sources of these solutes are from atmospheric and geological parameters such as air chemical composition (e.g. air carrying salts from the ocean) as well as that of associated soils and rock. Major sources of TDS include industrial waste, sewerage and agricultural and urban runoff.

Macroinvertebrate community composition reacts to changes in salinity (Horrigan *et al.* 2005). Nielson *et al.* (2003) showed that with increasing salinity, biota in freshwater ecosystems decreased. Azrina *et al.* (2006) showed that elevated conductivity, between upstream and downstream sites of intense land use, decreased macroinvertebrate species richness and diversity. Kefford *et al.* (2003) showed that freshwater macroinvertebrates demonstrated a range of sensitivities to changing salinity making them a suitable indicator for this parameter.

Microbial pollution is the most natural pollution occurring in water and is made up of bacteria, protozoans and viruses. Bacteria form a major part of freshwater ecosystems with various macroinvertebrate feeding groups, namely filterers, shredders and collectors, feeding directly on bacteria (Edwards and Meyer 1987; 1990). This provides an ecosystem service of regulating bacteria within freshwater systems. Unnatural sources of these are waste water treatment discharges, septic tank failures, livestock and human faecal matter and manure runoff (Horner *et al.* 2007). Mallin *et al*. (2000), showed that the most important human induced factor affecting faecal coliform abundance in nearby water systems is the percentage impervious cover of the associated watershed. With an increase in runoff comes an increase in introduced contaminants. *Escherichia coli* is a coliform bacteria which is extensively used as an indicator of faecal contamination in freshwater systems. The direct effects of heightened microbial pollution on macroinvertebrates are not clear.

Petroleum hydrocarbons come from anything made with crude oil. This includes fuels such as petrol and diesel and mineral oils. Primary sources of petroleum hydrocarbons are areas that are associated with vehicles such as parking lots, fuel stations and heavily used roads (Stein *et al.* 2006). These are typically introduced into natural systems via storm water runoff.

Organic chemicals such as pesticides and herbicides are used in agricultural areas and residential gardens and lawns. Hazardous organic compounds associated with industrial activities are polycyclic aromatic hydrocarbons, trihalomethanes, polychlorinated biphenyls, tetra chloromethane, trichloromethane and vinyl chloride. Insecticides generally have a negative effect on macroinvertebrate communities (Schulz and Liess 1999; Relyea 2005). Relyea (2005), however showed that herbicides and insecticides had varied negative and positive effects on zooplankton, amphibians and macroinvertebrate species found in freshwater ecosystems. Due to synergistic and antagonistic effects of these organic chemicals, their effects are not easily predicted. The effects of all of these constituents on benthic macroinvertebrate assemblages will depend on the concentrations, mixtures and the total load of pollutants introduced.

1.3. Bio-Monitoring in Practice

1.3.1. Current ecosystem integrity investigations

Various methods are used to investigate ecological integrity and water quality with benthic macroinvertebrates as surrogates. Some of these include the Rapid Field Screening Method (Moog 2005), the Australian River Assessment System (AusRivAS) (Smith *et al.* 1999), and the River Invertebrate Prediction Classification System (RIVPACS) in the UK (Wright *et al.* 2000). The South African Scoring System (SASS) is the most commonly utilised assessment method in South Africa. SASS was developed by Chutter (1998) to evaluate the health of an ecosystem using benthic macroinvertebrate presence and abundance. This method identifies seven biotopes, which when pooled together, contain a range of macroinvertebrates needed to make an effective assessment. These biotopes include a range of substrate particle sizes and aquatic vegetation in and out of the current, which ensures a wide range of benthic macroinvertebrate taxa are sampled. The samples are then scored based on the presence or absence of taxa. In this way, a site is given a standardised score (SASS Score) and an average score per taxon (ASPT), based on the tolerance values of various macroinvertebrate families to changes in preferred conditions. An abundance of taxa with higher tolerances for changing conditions will give a lower SASS Score indicating that a system displays high variations in conditions, which may be less healthy. The ASPT is valuable as it takes into account the evenness of the sample taken by dividing the final score by the number of taxon observed.

1.3.2. Sampling techniques

Various techniques can be used to sample benthic macroinvertebrates, which may often be prescribed for biomonitoring of aquatic health. Some of these techniques include hand held dip nets, artificial samplers, and drift net samplers. When sampling for benthic macroinvertebrate

assemblages using any of these methods it is important to take into account that the organisms sampled relate to substrate type (Allan 1995), microhabitat (Evans and Norris 1997), depth (Baumgartner *et al.* 2008), stream velocity, and exposure to sunlight (Hawkins *et al.* 1982). Consequently, various substrates, microhabitats, depths, velocities and exposures to sunlight are typically sampled with hand held dip nets or drift nets, to ensure the inclusion of a wide range of indicator taxon. This method of investigation however, is not always possible in smaller streams due to the streambed having few of the microhabitats that need to be sampled when using standardised biomonitoring regimes. In addition, high variation of streambed substrate type between sites does not always allow for a comparable investigation of the benthic macroinvertebrate assemblages.

The use of artificial samplers provides one way in which the variation between samples introduced by a heterogeneous stream bed may be removed (Rosenberg and Resh 1982). Artificial samplers standardise the microhabitat investigated across the entire study area, which reduces the influence of differences between natural substrates and allows the investigation to focus on spatial changes in disturbance rather than changes in microhabitat. This standardisation also allows for the reproducibility of investigations. For example, Modd and Drewes (1990), found that artificial substrates produced a more consistent and accurate representation of macroinvertebrate assemblages than natural substrate sampling methods in small streams. Even though both sampling techniques came to the same conclusions, sampling error between natural substrate sampling techniques was higher. As seen by Roby *et al.* (1978), when sampling in homogenous streambeds the advantages of using artificial substrates will not be significant enough to justify their use.

The use of artificial substrate samplers relies on the assumption that the composition of aquatic invertebrates on the artificial substrate represents the composition of the natural assemblage (Rosenberg and Resh 1982). Fullner (1971) showed that even though artificial substrate methods may fail to sample all aquatic invertebrates in a system, they do collect a wide variety of organisms with a range of sensitivities to disturbances, which is important in water quality investigations. Letovsky *et al.* (2012) showed that artificial substrate sampling methods produced a higher pooled Ephemeroptera, Plecoptera and Trichoptera (EPT) species richness than natural substrate methods although total species richness was lower. This seems like a major limitation, but these are orders that have a large range of sensitivities to impacts. The results obtained, however, can also be criticised due to the short time the artificial substrate was allowed to become inhabited by benthic macroinvertebrates (10 days). Samplers may also be lost, tampered with, become clogged with detritus or buried, may be used by predators as cover, and are relatively costly when compared to natural substrate sampling techniques (Roby *et al.* 1978). Samplers are constructed from a specific substrate, thus the material used to make the sampler will determine which

organisms settle on them. Samples obtained cannot be used to make inferences about conditions before the samplers were placed, thus giving an indication of conditions during the sample time (Environmental Protection Agency 1973, *cited in* Cover and Harrel 1978).

Two types of artificial substrate samplers are typically used. Firstly, a basket sampler which is made up of stone or porcelain balls inside a wire basket (Mason *et al.* 1967). Secondly, a Hester-Dendy Artificial Substrate Sampler (multi-plate sampler) comprising of multiple plates ordered parallel to each other at varied spaces appart (Hester and Dendy 1962). Both of these artificial substrate samplers work similarly in representing habitats for a range of taxonomic groups of benthic macroinvertebrates (Fullner 1971). Mason *et al.* (1973) showed that when both of these samplers had similar inhabitable surface area, results obtained from both could be comparable. Mason *et al.* (1973) also found that there was no difference in the number of taxa sampled between multi-plates constructed from hardboard and porcelain of the same surface area but the hardboard multi-plates collect a larger number of individuals than porcelain multi-plates. Based on these and other studies that have shown the reliability of Hester-Dendy samplers (Grubbs and Taylor 2004; Medeiros *et al.* 1983; Nedeau *et al.* 2003; Richards and Host 1993; Stewart *et al.* 2000), this means of benthic macroinvertebrate sampling has been approved by the US Environmental Protection Authority and the US Geological Survey. Macroinvertebrate assemblages collected using Hester-Dendy samplers, however, need to be interpreted in relation to conditions that may affect their performance such as the time allowed for sampling, stream depth and velocity (Roby *et al.* 1978), and exposure to the sun (Behmer and Hawkins 1986).

1.4. Rationale and Objectives

Human impacts on river ecosystems are magnified when looking at highly developed catchment areas. Various types of human activities affect water quality which invariably affects ecosystem integrity. For example, chemical concentrations in surface water may be correlated to percentage catchment transformed by land use types (Rhodes *et al*. 2001). In agricultural and recreational areas the natural landscape is altered, increasing the level of sediment runoff, erosion, flooding and chemical introduction (Skinner *et al.* 1997). Urbanisation is often associated with changes in chemical composition and the physical structure of associated freshwater systems, which ultimately impacts river ecosystems (Rhodes *et al* 2001; Suren 2001). Likewise, reduced soil permeability in urban areas increases storm water runoff, which carries with it associated pollutants such as trace metals, nutrients, petroleum hydrocarbons and other chemicals into nearby streams and rivers (Horner 2007). The level and type of impact that a land use has on a system will depend

on the activity upstream of a system (Hall *et al.* 2001, Lenat and Crawford 1994). As the intensity of land use changes, so will impacts to the system.

As landscapes become more developed and catchment areas and river ecosystems become increasingly engulfed in various land use activities, there is a need for monitoring of aquatic systems in impacted catchment areas. This will allow an understanding of the effects and ultimate changes that take place in these freshwater ecosystems. Knowing the state of an ecosystem allows for negative impacts or deteriorating conditions to be mitigated or responded to efficiently. Traditionally, chemical and physical water quality parameters have been used to determine the state of natural water resources. However, these investigations are not able to determine the health of an ecosystem, which takes into account the biotic wellbeing of the system (Cairns and Der Schalie 1980). To determine the health of our ecosystems, biotic indicators within a system must be identified to be used as a surrogate for the health of that system.

Benthic macroinvertebrates are one such surrogate, which provide evidence for changes in chemical and physical water quality parameters (Azrina *et al.* 2006; Bis *et al.* 2000; Hall *et al.* 2001*;* Lenat and Crawford 1994; Maldono 2010; Suren 2000; Winter and Duthie 1998). The general trend is that with increasing levels of land use intensity, comes a change in chemical and physical water quality parameters that in turn influence macroinvertebrate assemblages by causing a reduction in species diversity and an increase in dominant, pollution tolerant species. This trend allows for the efficient use of aquatic benthic macroinvertebrates as a tool for the assessment of the impacts of land uses on water quality and freshwater ecosystems.

This trend has mostly been determined through two types of investigation that have been instrumental in helping understand anthropogenic influences on macroinvertebrate assemblages.. Firstly, by investigating a single stream and determining changes in macroinvertebrate assemblages, between upstream (near pristine conditions) and downstream of a single land use type (Azrina *et al.* 2006; Baa-Poku *et al.* 2013; Cooper *et al.* 2006; Qu *et al.* 2010; Winter and Duthie 2013). Secondly, by investigating multiple independent tributaries at a large scale and determining macroinvertebrate assemblages within various land use types (Hall *et al.* 2001; Helms *et al.* 2009; Lenat and Crawford 1994; Maldono 2010). However, highly developed catchments have streams and rivers flowing through multiple land use types over relatively short distances. The results of the previous investigations may not apply to ecosystems of this nature, due to a lack of upstream land use interaction influences or a high variation in water quality conditions due to geographic isolation. Thus it would be advantageous to understanding of systems of this nature, to investigate influences of changing land use on riverine ecosystems at a fine scale.

There are an ever growing number of streams and rivers which flow through highly developed catchment areas. It is important to understand the dynamics behind management tools such as macroinvertebrates to be able to efficiently use them to maintain and ensure longevity of these highly vulnerable ecosystems. The project reported in this dissertation aimed to compare chemical, physical and biological surface water quality parameters and aquatic macroinvertebrate species composition at a fine scale along a single stream, the upper reaches of the Apies-Pienaar catchment area, which contains multiple land use types. Specifically, the project was designed to determine the:

1. Chemical and physical surface water quality parameters before and after each land use type along the first 8km of the upper reaches of the Apies-Pienaar catchment area, at various time intervals.
2. Composition of aquatic macroinvertebrate assemblages before and after each land use type along the first 8km of the upper reaches of the Apies-Pienaar catchment area, at various time intervals.
3. Relationship between specific changes in the chemical and physical surface water quality parameters and aquatic macroinvertebrate assemblages.
4. Major influences on chemical and physical surface water quality parameters and macroinvertebrate assemblages.

The nature of this study does not permit direct correlation of measured parameters with specific land use type. Land use types in the upper Apies-Pienaar catchment are adjacent to each other, meaning that interactions and dispersal of contaminants are likely. Consequently, the approach taken in this study is to not concentrate on the point sources of measured chemical, physical and biological parameters, but rather to look at changes in parameters that occur across various land use types. These patterns of change are then compared to macroinvertebrate assemblage patterns to propose likely drivers of these patterns.

It was anticipated that surface water chemistry would vary across space as a result of changing land use type due to associated activities. When repeatedly sampling over a period of several months, it was also predicted that measured parameters would change due to more frequent rainfall events during the transition from the cold, dry winter to hot, warm summer that is characteristic of the region where the Apies-Pienaar catchment is located. Where there is variation in surface water chemistry at a spatial level, it was also thought to be likely that there would be associated variation in the composition of the macroinvertebrate assemblage.

2. Materials and Method

2.1. Study Area

The upper reaches of the Apies-Pienaars catchment (Hartbeesspruit A23A-01072) were selected for this study (Figure 1). The catchment has an area of 682.38 km^2. It is an urban catchment with a large proportion being occupied by suburbs of the City of Tshwane (Pretoria). The upper Hartbeesspruit falls within the Apies/Pienaars sub-management area, and is part of the Crocodile (West) Marico water management area. The Hartbeesspruit flows to the northeast and merges with the Moreletaspruit then flows into Roodeplaat Dam, which is situated in the northeast of Pretoria. The study area began close to the source near the Frank Struben Bird Sanctuary and incorporated the first 8.06 km of the stream system. This stretch flows through the LC De Villiers sports grounds and Experimental Farm of the University of Pretoria, Colbyn Valley Wetland, the residential suburbs of Kilner Park and Waverly, the industrial and commercial node of East Lynne, and finally terminates just before the Ferro Holcim Quarry in Derdepoort.

The site is characterised by a moderate dry sub-tropical climate and is situated within the Grassland biome of southern Africa. It is a summer rainfall region receiving a mean of 537 mm of rainfall per year. Mean annual temperatures range from 16-20°C. The altitude is relatively constant at 1350 m

The study area is located on the shale and andesite of the Pretoria group on the Transvaal supergroup (Johnson *et al.* 2006). Red, iron-rich soils are widespread and are typically dystrophic and mesotrophic. The catchment is characteristic of a catena like sequence of soils, having originated from similar parent material but having varying characteristics due to variation in topography and drainage conditions.

2.2. Sampling Sites

The stream was separated into stretches based on land use type (Figure 1). Boundaries were determined by physical perimeters separating areas of land use. Sampling sites were selected at the beginning and end of each stretch of the stream located within the different land use types of the study area. This ensured that samples obtained gave an indication of results before and after each land use type. Five sample sites were chosen along the study area (Figure 1).

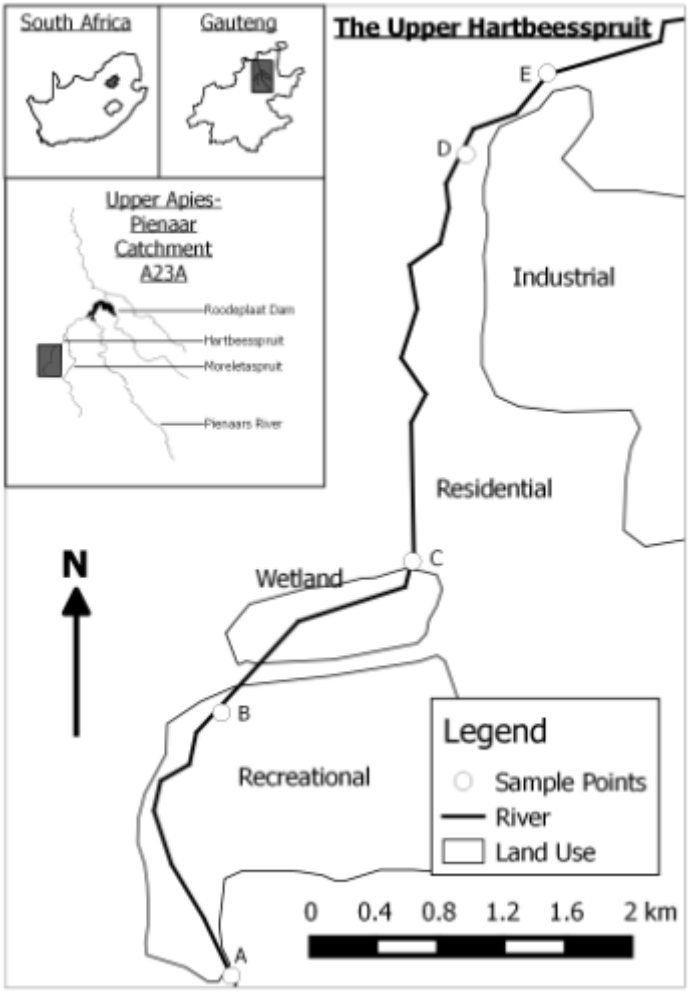

Figure 1: Map of the upper Hartbeesspruit (A23A-01072) study area, Gauteng, South Africa, indicating land use types and sampling sites A, B, C, D and E.

2.2.1. Site A

The source of the Hartbeesspruit has been totally transformed into a concrete canal that is devoid of all natural habitats. The first site (25°45'38.0" S, 28°15'03.5" E) was located approximately 50 m from the source, which was the closest point downstream with natural bed and banks. This site falls within the Frank Struben Bird Sanctuary, and had very little direct human impacts as it is fenced off. Upstream, at the end of the concrete canal, the stream bank was inhabited by squatters. There was a high level of development associated with the source but it flowed directly into the less intense recreational land use type. Immediately prior to the stream entering the recreational land use was a garbage collection point for nearby commerce, which was an obvious point source of refuse into the system.

2.2.2. Site B

Bordering the recreational and wetland land use types, this site (25˚44'38.7" S, 28˚15'01.3" E) was located against a concrete barrier separating the two land use types. The site fell within the recreational land use type of the University of Pretoria LC De Villiers sports fields. The site was highly accessible to patrons but there was no clear evidence of direct disturbance. There was a high level of upkeep associated with the land use type (i.e., lawns were mowed frequently in the riparian zone). There were two small dams approximately 50 m and 100 m upstream of the site. Fertilisers and irrigation were two obvious possible sources of contaminants.

2.2.3. Site C

Bordering the untransformed (wetland) and urban land use types, this site (25˚44’4.6” S, 28˚15’44.2” E) was immediately after a gabion wall bordering the Colbyn Valley Wetland and the suburb of Kilner Park. This site fell within the urban land use type and was characterised by high levels of riparian vegetation and a railway 20 m away. Direct inputs were not obvious but the site was relatively accessible to the public. On first inspection, this site seemed to be the most pristine.

2.2.4. Site D

Bordering the urban and industrial/commercial land use types, this site (25˚42'32,9" S, 28˚15'56,3" E) was located 50 m away from the N1 highway separating the residential suburbs of Waverley and the industrial/commercial node of East Lynn. The site was adjacent to a taxi pick up point on the N1 and thus had a high level of direct disturbance form the public. High levels of refuse and excrement characterised the site. The riparian zone was comprised of scattered vegetation and dry pedestrian paths on transported soils. Storm water drains and squatting were obvious potential sources of contaminants.

2.2.5. Site E

The final site (25˚42’14.6” S, 28˚16’14.6” E) was found within the commercial/industrial land use type and was located in the industrial node of East Lynn bordering (but still maintaining a constant geology to all sites) the change in geology of the area. The site was adjacent to the N1 highway and was highly accessible. It had a large amount of rubble associated with informal opportunistic scavenging by squatters in the area. The riparian zone was comprised of scattered vegetation and footpaths. Storm water drains and refuse were obvious sources of contaminants.

2.3. Site Land Use and Microhabitats

At each site, major influences to the stream system and physical characteristics of the site were determined. A site description was recorded which included visual observations, and assessment of riparian zone cover and in-stream habitat composition. Visual observations noted included point sources of pollution within 100m, storm water drains/tributaries, bank conditions, signs of direct human impact and level of accessibility to humans. Physical characteristics included the mean depth and width along each sampling site.

Conditions in the riparian zone were recorded using methods based on those described by Heiring *et al. 2003* and Suren *et al.* 2005a. The riparian zone was defined as the area 5 m perpendicular to the stream along the site. An estimation of cover in the riparian zone, larger than 5% within this area, was made to the nearest 5%. Cover smaller than 5% was not included in the total riparian cover, but was recorded as being present. This was done by observing the cover % at 5 m intervals from upstream to downstream of the sampling site. These observations were done on both sides of the stream along the sampling site. In areas where visual observation was not possible, aerial maps (Google Earth) were used to observe the characteristics of the riparian cover. Riparian cover classifications included trees, reeds (>2 m), sedges or grass (>2 m), grass (>30 cm), leaf litter, bare earth, tar or concrete and gravel.

In-stream habitat conditions were recorded using methods based on those described by Heiring *et al.* 2003. An estimate of microhabitat cover, larger than 5% within the length of the stream site, was made to the nearest 5%. Cover smaller than 5% was not included in the total cover, but was recorded as being present. This was done by observing in-stream microhabitat % at 5m intervals from upstream to downstream of the sampling site. Care was taken to not disturb in-stream invertebrate assemblages while ensuring accurate estimates of in-stream microhabitat. Microhabitat classifications were based on microhabitats used in the South African Scoring System (SASS) (Dickens and Graham 2002) and included both stones (2-25 cm diameter) and marginal vegetation in and out of the current, aquatic vegetation, gravel (<2 cm diameter), sand (<2 mm diameter) and mud/clay (<0.06 mm diameter).

2.4. Surface Water Chemistry

Precipitation data was obtained from the South African Weather Service for station (0513435A4) Pretoria University Proefplaas (-25,7510; 28,2850). Cumulative monthly precipitation was obtained in order to understand the total imputs of water into the system between sampling events. Water quality sampling was done once at each site on 4 occasions at 6-week intervals (13-17

September 2013, 1-5 November 2013, 17-21 December 2013 and 1-5 February 2014) to monitor potential seasonal changes. This coincides with the 18-week period that invertebrate samplers were deployed (see below). Surface water was collected in accordance with sampling protocol for inorganic chemical analysis (DWAF 2004). Water samples were collected using 500 ml sterile plastic bottles for later *ex situ* quality determination. Two were collected at each of the five sites. This made a total of 40 bottled samples. Bottles were first rinsed in the stream then submerged at 5 cm and sealed, ensuring no visible air bubbles were present inside the sealed bottles. Bottled water samples were immediately refrigerated at 2°C and taken to UIS Analytical Services (Pty) Ltd, within 3 days, where a full spectrum SANS 241 analysis was performed. The parameters that were tested are presented in Table 1.

Table 1: Surface water parameters tested *in situ** and *ex situ* along the upper Hartbeesspruit.

Physical	Chemical			Microbiolo-gical
	Trace elements	Major ions	Nutrients	
Temperature*	Aluminium (Al)	Arsenic (As)	Nitrites (NO_2^-)	Bacteria
pH*	Antimony (Sb)	Calcium (Ca)	Nitrates (NO_3^-) Total Nitrogen	Coliforms
Salinity*	Cadmium (Cd)	Chlorine (Cl)	Phosphate (PO_4^{3-})	*E. coli*
Conductivity*	Copper (Cu)	Fluorine (F)	Ammonia (NH_3)	
Total dissolved solutes*	Iron (Fe)	Manganese (Mn)	Sulphate ($S0_4$)	
Clarity*	Lead (Pb)	Potassium (K)		
Alkalinity	Magnesium (Mg)	Sodium (Na)		
Turbidity	Mercury (Hg)	Uranium (U)		
	Nickel (Ni)			
	Selenium (Se)			
	Vanadium (V)			
	Zink (Zn)			

In situ determination of temperature, pH, conductivity, salinity, TDS and clarity was performed at each of the five sites. It was the intention to also determine dissolved oxygen, but this could not be done due to malfunctioning equipment. Readings at each site were taken once at each of the four invertebrate samplers. Water was sampled in a 500 mL beaker and tested using a multi-parameter water quality tester (PCSTestr 35, Eutech Instruments, Oakton®, Singapore), or a clarity tube (Ground Truth, KwaZulu-Natal, South Africa; Kilroy and Biggs 2002) filled with water from the main flow of the stream. A clarity tube measures the depth in centimetres that light penetrates into the water. The tube was held perpendicular to the sun and, by moving a black disc in it with a magnet, the clarity of the water was measured at the point at which the disk was no longer visible.

Sampling was done from 10:00 to 14:00 to reduce diurnal variation in water quality parameters such as dissolved oxygen and temperature. Water quality was sampled prior to removal/replacement of macroinvertebrate samplers because sampler installation was relatively disruptive and may have affected surface water quality parameters.

2.5. Macroinvertebrates

2.5.1. Hester-Dendy artificial samplers

Hester-Dendy artificial samplers were constructed using the specifications detailed by Fullner (1971). This was different to the original Hester-Dendy sampler as it had more plates and varying spaces between each plate, which increased the surface area and increased comparability with basket samplers (Fullner, 1971). A similar modification was used by Mason *et al.* (1973), who found similar results. Hester-Dendy samplers were constructed using 0.3 cm tempered hardboard. The hardboard was cut into fourteen 7.6 cm x 7.6 cm square plates. Twenty three 2.5 cm x 2.5 cm square spacers were also cut. A 0.6 cm diameter hole was cut through all the plates and spacers which were fitted onto a 0.6 cm diameter eyebolt. The top 7 plates were fitted using one spacer, the next 4 plates were fitted using 2 spacers and the last 3 plates were fitted using 3 spacers to separate the plates. Plates were fitted with the rough side of the hardboard facing up and then down and plates were aligned corner to corner. A nut was added after the last plate to hold all plates in place. Due to the thickness of the spacers (0.3 cm), the spaces between plates were 0.3 cm, 0.6 cm and 0.9 cm. The distance from the top plate to the bottom plate was 11.1 cm and provided a total surface area of approximately 0.15m^2 for invertebrates to inhabit. The completed sampler was then attached to a perforated brick by placing the remainder of the eyebolt through the middle perforation and attached it using a size-appropriate washer and bolt. Nylon rope (length: 0.5 m) was then attached to the top of the eyebolt and a 5 cm diameter buoy attached to the end of the nylon rope. A total of 40 samplers were made to permit repeated sampling and the replacement of broken samplers.

The first 40 m of each study site was separated into four 10 m zones. One sampler was placed within each zone (Figure 2). Samplers were placed in areas where stream velocity, depth and solar exposure between zones and sites appeared largely similar. Samplers were placed at equal depths of 5 cm. Where possible, samplers were placed in areas with minimum canopy cover to ensure exposure to sunlight but in areas hidden from the public eye to prevent vandalism or removal. Placing samplers in the middle of the stream was avoided to prevent the clogging of samplers by passing detritus. Samplers were mounted in the substrate by digging a hole in the

substrate and inserting the perforated brick into the hole to ensure that the sampler was upright. The bottom plate was positioned above the natural substrate.

Based on the results of Mason *et al.* (1973) using similar hardboard multi-plate artificial samplers, the samplers were exposed for 6 weeks. Retrieval of samplers was done by placing a 30 cm x 30 cm net (1 mm mesh size) immediately downstream of the sampler before removing it from the stream and holding it over the net whilst removing it from the perforated brick and untying the nylon string, ensuring any samples dislodged from the sampler were caught in the net. The sampler was then placed in a clear sealable plastic bag. The net was rinsed out into the plastic bag using fresh water. Sample bags were then labelled accordingly and taken to the lab for processing and macroinvertebrate identification. Sample bags were processed on the same day to ensure preservation of the sample with 70% ethanol (See section below). Clean samplers were then bound to the perforated brick and nylon string with buoy attached, and reinserted into the substrate for the next period of sampling. A total of three sequential sampling periods (beginning 1-5 November 2013, 17-21 December 2013 and 1-5 February 2014) were performed to track potential changes in the sampled macroinvertebrate assemblage across changing wet and dry seasons.

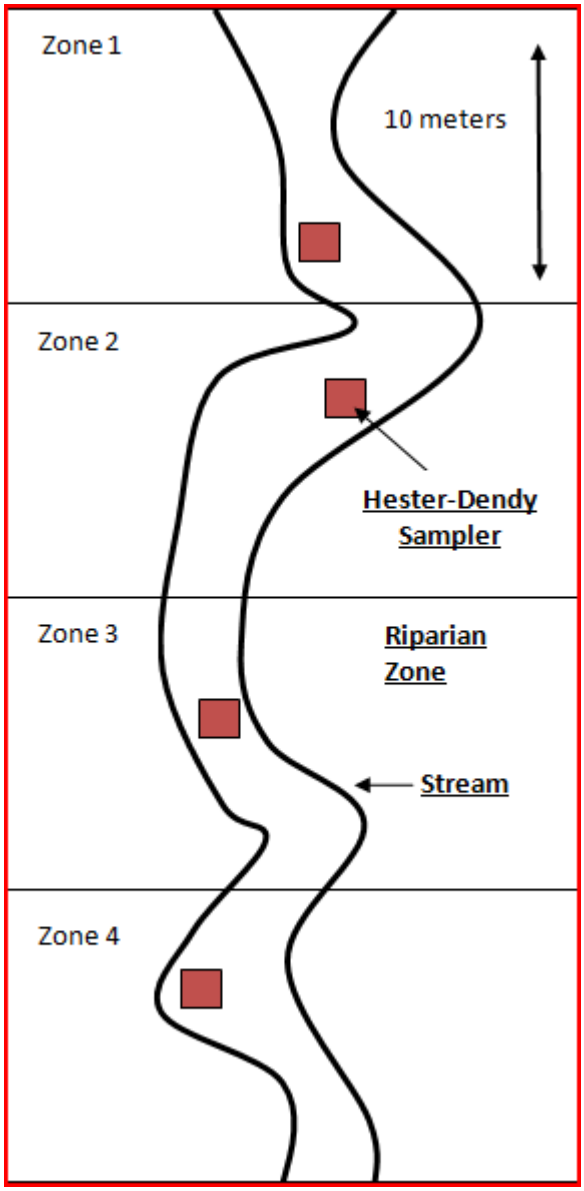

Figure 2: Zoning of sampling site and an example of placement of artificial samplers within each zone.

2.5.2. Identification

Samplers obtained were taken to the laboratory (on the same day as retrieval) to remove all benthic macroinvertebrates from the hardboard surfaces for thorough identification. Samplers were deconstructed and gently scrubbed with a soft brush to transfer all macroinvertebrates into a large dish. The sample was then rinsed through a 425 µm universal test sieve (Anemaet *et al.* 2005), to remove all excess soil and water. Samples were placed into jars to which 70% ethanol was added. A dissection microscope and light source were used to separate all macroinvertebrate samples from any detritus. Macroinvertebrate samples were pooled by site and repetition. Samples were identified to family level. Identification of macroinvertebrates to family level was sufficient for the

purposes of the study. Dickens and Graham (2002) and Mandaville (2002) compiled tolerance scores based on macroinvertebrate families for the purposes of comparing macroinvertebrate assemblages across a range of conditions. For the identification, "Aquatic Invertebrates of South African Rivers Field Manual (Gerber & Gabriel, 2002)" was used.

2.6. Statistical Analysis

50% of the limit of detection was used for results which were found to be below the limit of detection. Missing values were taken as gaps in the dataset. Parameter values that were below the minimum measurable level and remained so across both space and time were not analysed. These parameters were cadmium, copper, nickel, antimony, selenium, vanadium, nitrogen dioxide and phosphates (See appendix).

Macroinvertebrate abundance, family richness (number of taxa), the Shannon-Wiener diversity index and Pielou's eveness measure were calculated for each sampling period and site. The presence of macroinvertebrate families was also used to calculate the SASS score and average score per taxon (ASPT) for each sampling period and site following Dickens and Graham (2002). It must be noted that the use of the SASS score and ASPT were used merely as indicators for the purpose of comparing sites within this study, as done by Thirion (2000) to illustrate changes in macroinvertebrate assemblages collected from artificial samplers. They should not be considered equivalent to scores derived from the application of the complete SASS 5 methodology. The Shannon-Wiener diversity index and Pielou's evenness give an indication of macroinvertebrate diversity in the system. SASS score and ASPT gives insight into the state of the system based on the presence of macroinvertebrate families and their associated tolerance scores.

Statistical analysis was only performed on *in* situ measured parameters and summary statistics for macroinvertebrate assemblage composition. Data analysis was not performed on *ex situ* lab assays as only two samples were taken from each site due to the high cost of lab analysis. The distribution of data was investigated for normality and outliers using boxplots. Extreme values appeared to reflect real conditions. Normality was determined for each variable using a combination of Q-Q plots and the Shapiro-Wilk (sample size <40) or Kolmogorov-Smirnov (sample size >40) tests for normality. Homogeneity of variance and residuals were investigated using Levene's test and residual plots respectively. Variables that met these assumptions were family richness, Shannon-Wiener diversity, SASS score and ASPT. For variables which did not meet these assumptions, an appropriate transformation (Log10, reciprocal or square root) was used. If, following transformation, the variables still deviated from the assumptions of parametric tests, they were analysed using a

non-parametric test (see below). Variables which fell into this category included all *in situ* parameters, macroinvertebrate abundance and Pielou's evenness. Independence of data was not strictly met due to all sites being located along the same stream. However, due to the nature of the study, this was considered as acceptable.

A two-way analysis of variance (ANOVA) was used to determine if there were statistically significant effects of site and time, and their interaction, for family richness, Shannon-Wiener diversity index, SASS score and ASPT. Fisher's least significant difference (LSD) post-hoc tests were used to determine, where the differences between sites and times existed. The Scheirer-Ray-Hare test, a non-parametric equivalent of two-way ANOVA, was used to test the effects of site and time on all *in situ* parameters, namely temperature, conductivity, salinity, TDS, pH and clarity, and on the indices of macroinvertebrate abundance and Pielou's evenness. Multiple line charts depicting estimated marginal means against both time and sites, were used to further investigate trends in these parameters. These tests were performed using IBM SPSS Statistics v. 21 (IBM Corporation).

Non-metric multi-dimensional scaling (nMDS) was used to assess the effects of temporal and spatial variation on macroinvertebrate assemblages. Taxon by site abundance data was used to perform the ordination using the vegan library in R version 3.0.2. (R Development Core Team 2012). The Bray-Curtis index of dissimilarity was determined to be the most appropriate index to use on the data as it had the highest rank-order similarity with gradient separation (r=0.1792797). A dissimilarity matrix was generated and was reduced to two dimensions using the metaMDS procedure. Using randomized tests (999 permutations), an analysis of similarity (ANOSIM) was performed to determine whether there was a significant effect of time or site on macroinvertebrate assemblages. Ellipses representing the 95% confidence interval of the centroid were plotted on a two-dimensional ordination of sites. Indicator value analysis (IndVal) with 999 permutations was used to identify the families that were characteristic of each site.

A canonical correspondence analysis (CCA) was used to investigate macroinvertebrate composition against tested surface water parameters. Taxon by environmental variable data was used to perform the ordination using the vegan library in R version 3.0.2. (R Development Core Team 2012). A CCA tri-plot was constructed using only physical, chemical and biological surface water parameters with values that fell outside that of desired targets of water for domestic use in South Africa (DWAF 1996). These parameters (alkalinity, aluminium, ammonia, arsenic, conductivity, fluorine, iron, lead, mercury and turbidity) were seen as the most likely to have effects on macroinvertebrate assemblages.

3. Results

Full results are attached in the digital appendix.

3.1. Site Land Use and Microhabitats

Sites A, C and E had the most variability of widths compared to the other sites. Sites A and D had the lowest water level (Figure 3). Sites had relatively variable riparian zones, with the most abundant riparian cover being short grass (<30 cm) (Figure 4). Microhabitat composition was not consistent between sites (Figure 5). Sites A and B were dominated by marginal vegetation in the current and muddy substrates. Site C was characterised by a muddy substrate and stones out of current. Muddy substrates, marginal vegetation in the current, aquatic vegetation and sandy substrates characterised site D. Site E was characterised by a muddy substrate and stones in and out of the current.

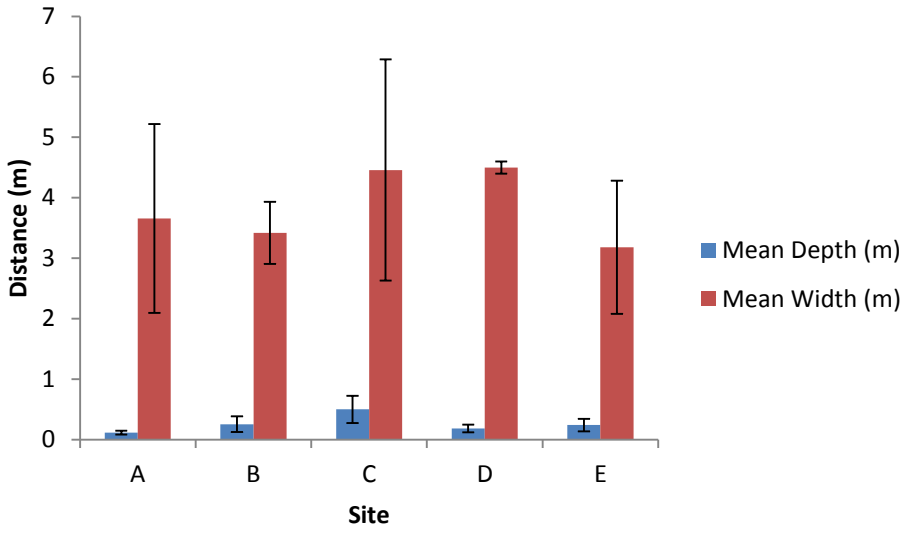

Figure 3: The mean depth and width along the first 40 metres of various sites along the upper Hartbeesspruit.

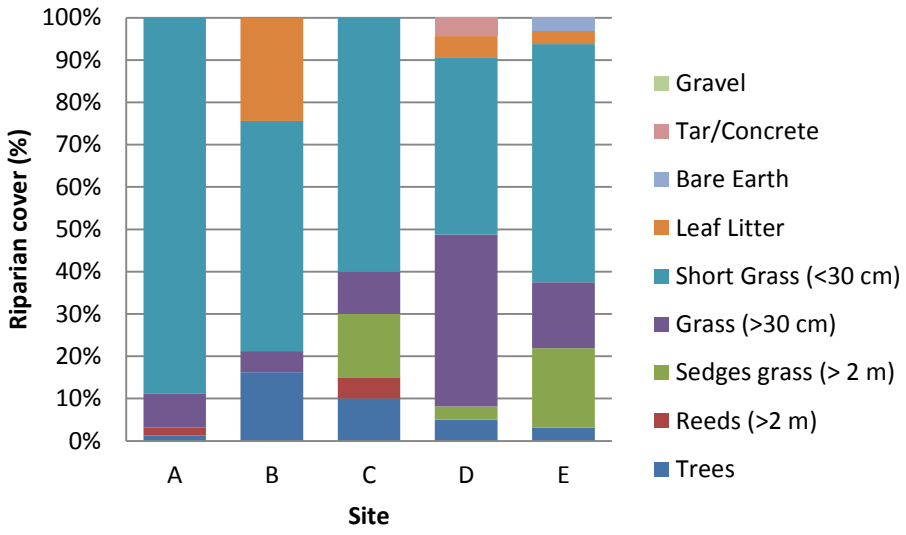

Figure 4: Riparian cover type along the first 40 metres of sampling sites along the upper Hartbeesspruit.

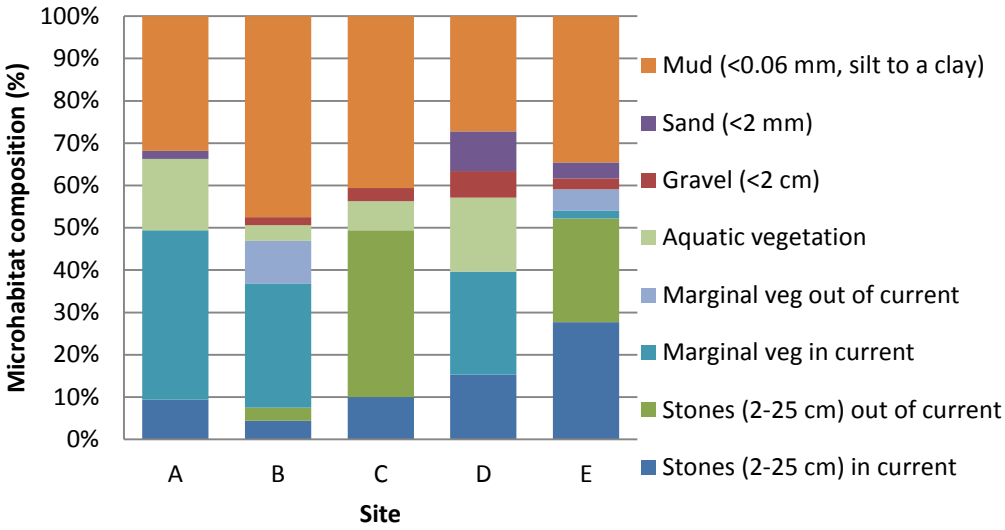

Figure 5: Microhabitat composition type within the aquatic zone along the first 40 metres of sampling sites along the upper Hartbeesspruit.

3.2. Surface Water Chemistry

3.2.1. Precipitation

Precipitation prior to sampling in September was the lowest with the second lowest being prior to the February sampling (Figure 6). The most precipitation occurred prior to December sampling with the second highest prior to November sampling. September water samples were

representative of 6 months of low rainfall prior to sampling. This differentiated the September samples from the subsequent months as this was an extreme case of low rainfall.

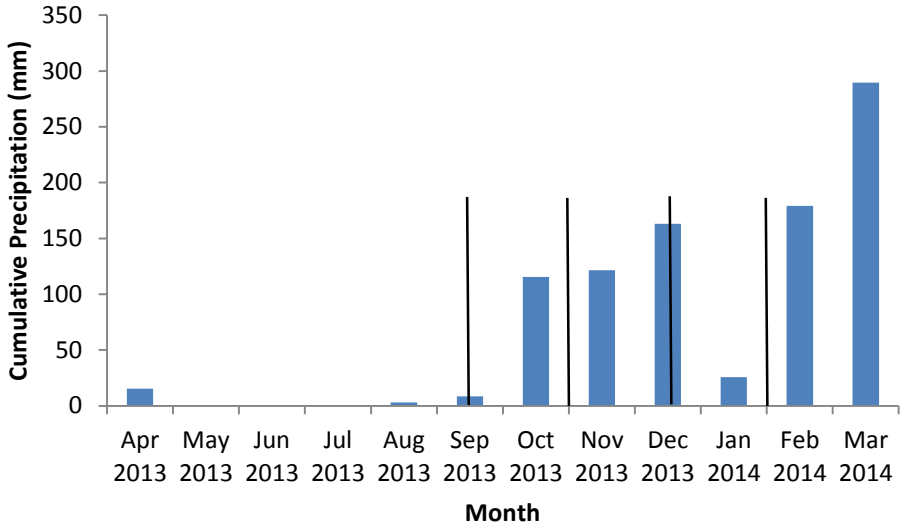

Figure 6: Cumulative monthly precipitation (mm) from April 2013 to March 2014 within the A23A quaternary catchment. Lines indicate sampling dates on the 13th September, 1st November, 18th December and 1st February.

3.2.2. *Ex-situ parameters*

Alkalinity values were generally stable from upstream to downstream other than variation seen at site B (Figure 7); with drops in value in November and December and peaks in values in September and February. Turbidity showed a similar trend except that site B in November and December remained relatively stable. All parameters remained relatively stable through sites C, D and E regardless of time of sampling.

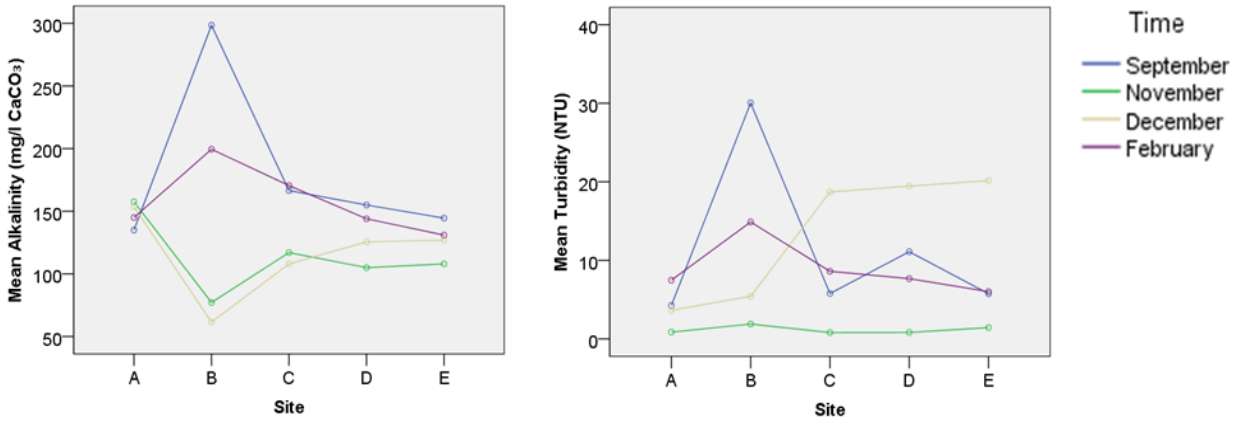

Figure 7: Mean values of surface water physical parameters measured *ex situ* at sampling sites along the upper Hartbeesspruit

Metal ions showed a range of results for concentrations across site and time (Figure 8). Aluminim, iron and zinc concentrations were close to a minimum throughout sites in September and November but increased, having variable concentrations, between sites in December and February. Across all sampling periods, zinc concentrations at site C were consistently the lowest. Lead had highly variable concentrations across site and time other than at site C where the concentration was the same in all samples. Magnesium concentrations were generally stable from upstream to downstream other than variation seen at site B; with lower values in November and December and peaks in September and February. Mercury concentrations declined from upstream to downstream, but a high concentration was detected at site A in November.

Values for major ions at site B were consistently different from generally stable or declining concentrations from upstream to downstream (Figure 9). Site B had the greatest variation of values across time periods. Calcium tended to decline from upstream to downstream, but there was a sharp peak in this variable at Site B in September and concentration was low at site B in November and December. September concentrations were the highest followed by February concentrations. Sodium, chlorine and potassium declined in concentration from upstream to downstream, having relatively high initial concentrations at site A. Concentrations of sodium and chlorine were the highest in September, followed by February. Uranium remained relatively stable across all sites and times other than September when a high concentration was detected at site B. Manganese tended to decline from upstream to downstream with a peak in concentration in February at site B. Concentrations of manganese in September were at a minimum at all sites. Fluorine was relatively stable across sites in September, November and February, but concentrations in December fluctuated between sites. Arsenic concentrations in November and February declined from upstream to downstream. Concentrations of arsenic in September and December were minimal across all sites. All parameters, except for arsenic, remained relatively stable through sites C, D and E regardless of time of sampling.

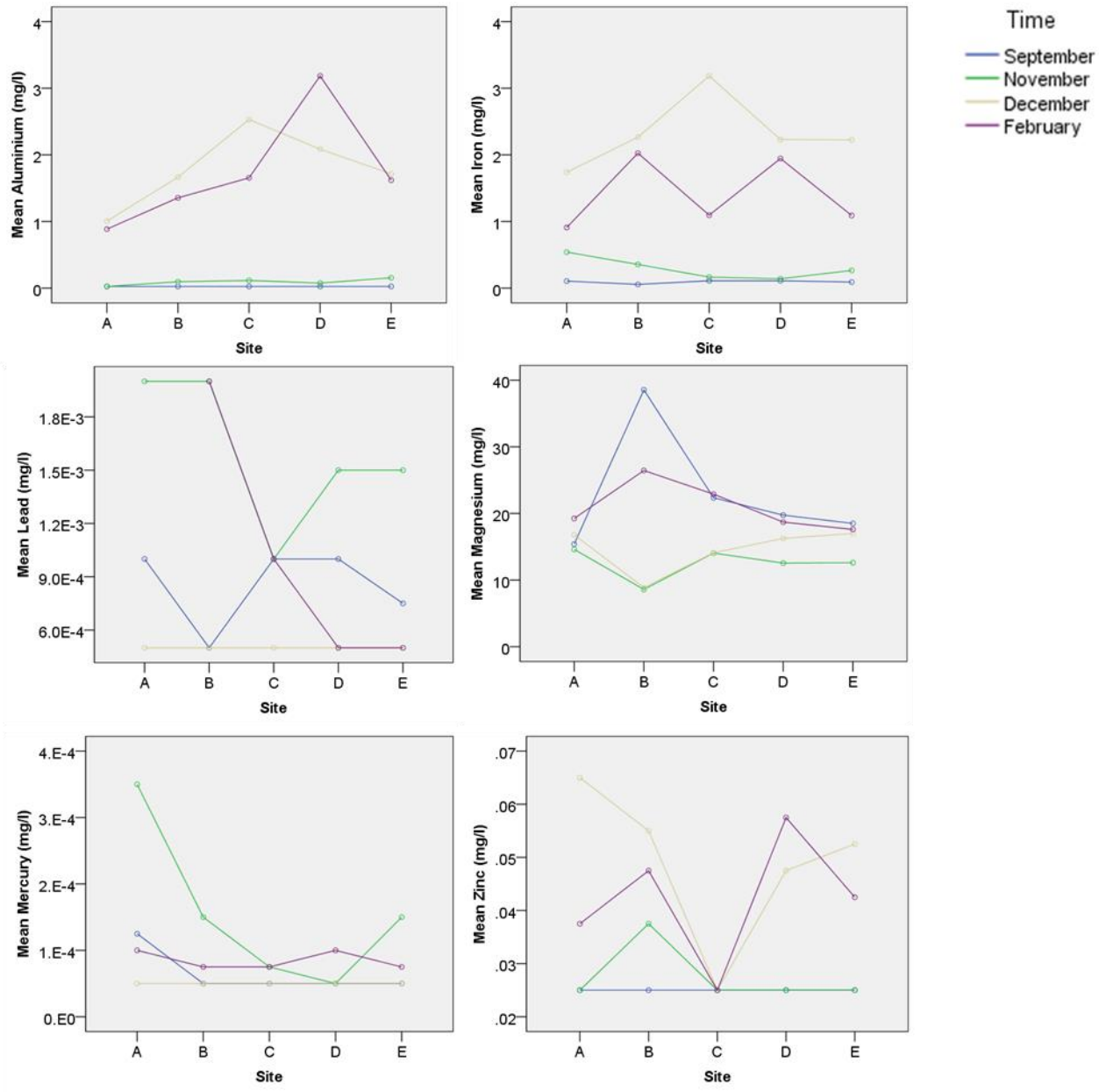

Figure 8: Mean concentrations of surface water metal ions measured *ex situ* at sampling sites along the upper Hartbeesspruit

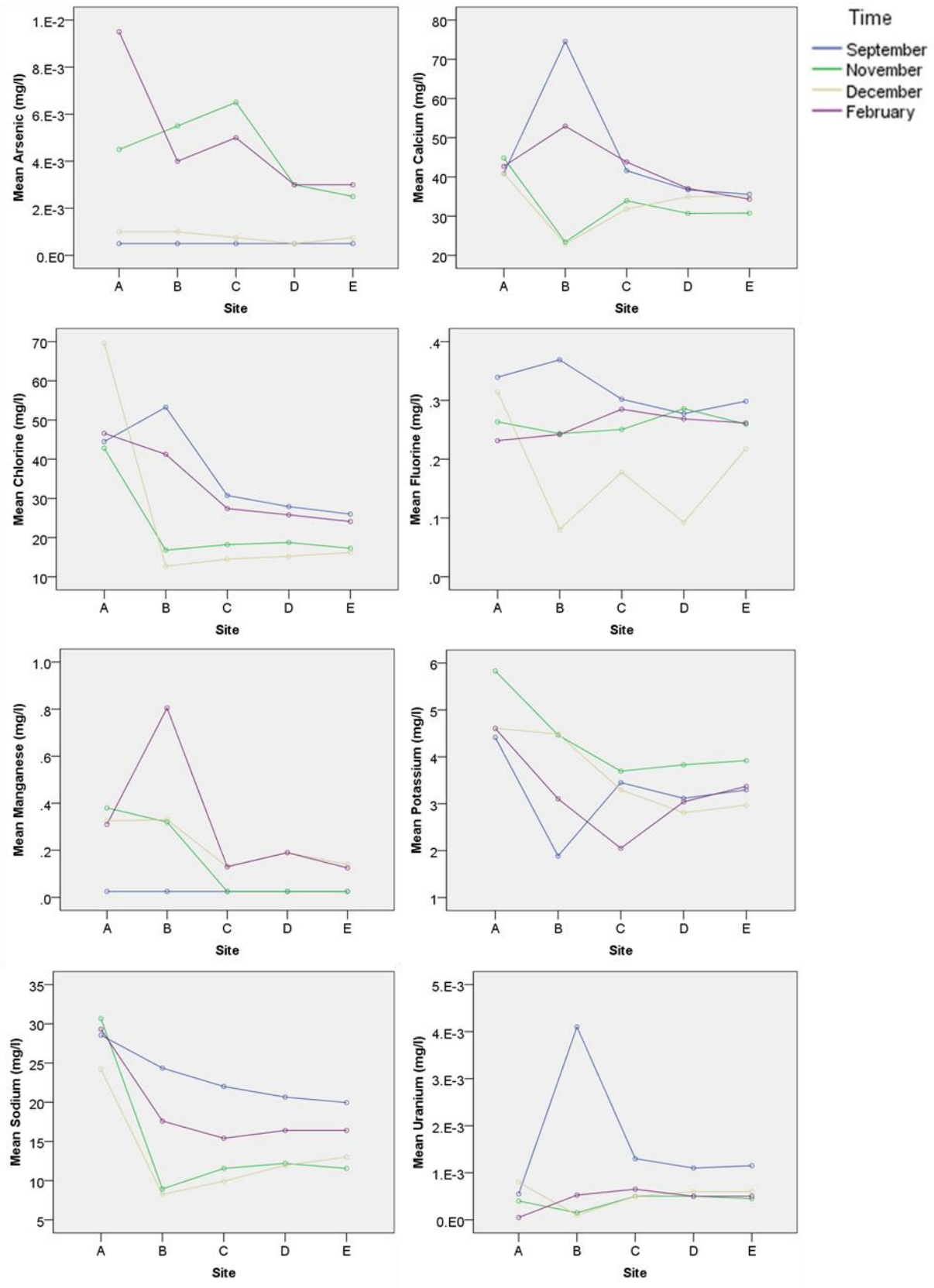

Figure 9: Mean concentrations of surface water major ions measured *ex situ* at sampling sites along the upper Hartbeesspruit

Sulphate was relatively high at site A, declining dramatically at site B and remaining relatively stable throughout sites downstream (Figure 10). Mean Nitrate and ammonia also had peaks at site A in December and November, respectively, but remained relatively stable downstream.

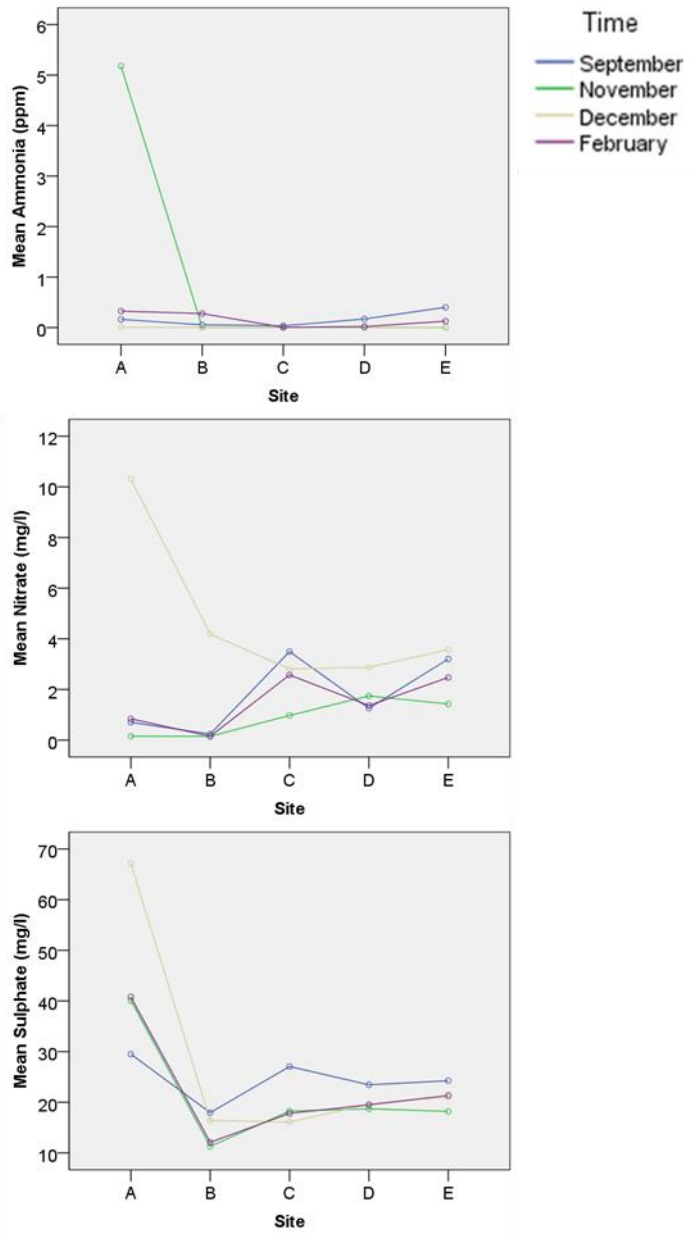

Figure 10: Mean concentrations of surface water nutrients measured *ex situ* at sampling sites along the upper Hartbeesspruit

Abundance of bacteria, coliforms and *E. coli* were highest at site A and D at various time periods (Figure 11). Overall, abundance of bacteria was high at site A and D in November and September respectively. Coliform bacteria were high in November at site A and B in November and at site D in September and February. *E coli.* was highest at site A and D in February.

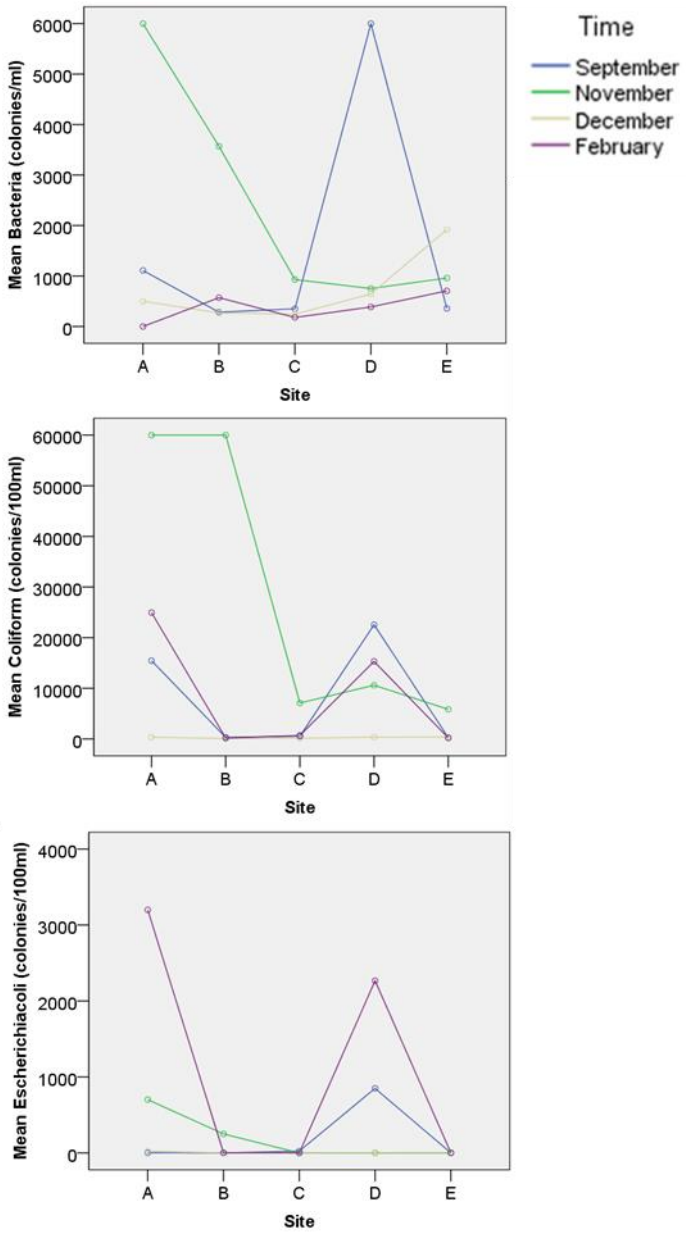

Figure 11: Mean abundance of surface water biological parameters measured *ex situ* at sampling sites along the upper Hartbeesspruit.

3.2.3. In-situ parameters

The time of sampling consistently affected water quality parameters that were measured *in situ* (Table 2). Water temperature was significantly affected only by the time when samples were taken; with the lowest temperature during the study being recorded in September at the end of the cold, dry season (Figure 12). The main effects of site and time affected water pH (Table 2); water pH was highest in September, and regardless of the time of sampling, was consistently the lowest at Site B (Figure 12). Salinity, conductivity, TDS and clarity were all affected by the interaction of site and

time (Table 2). Salinity, conductivity and TDS tended to decline from upstream to downstream, but there was a sharp peak in value at Site B in September and a drop in these variables at site B in November (Figure 12). Clarity was affected by the interaction of site and time (Table 2). Clarity tended to decline from upstream to downstream but there was a sharp drop in clarity at site B in September and February (Figure 12). All parameters, except for clarity remained relatively stable through sites C, D and E regardless of time of sampling.

Table 2: Summary of the Scheirer-Ray-Hare Test that describes the effects of time and site on temperature, pH, salinity, conductivity, total dissoved solids (TDS) and clarity. p<0,05 is underlined.

Parameter	F	df	*p*	Parameter	F	df	*p*
Temperature				Conductivity			
Intercept	11733,532	1	0,000	Intercept	2920,326	1	0,000
Time	1037,803	3	0,000	Time	77,395	3	0,000
Site	33,473	4	0,597	Site	67,588	4	0,000
Time*Site	42,275	12	0,572	Time*Site	32,239	12	0,001
pH				Tds			
Intercept	1836,743	1	0,000	Intercept	1234,334	1	0,000
Time	46,457	3	0,000	Time	31,262	3	0,000
Site	70,842	4	0,000	Site	22,79	4	0,001
Time*Site	9,533	12	0,234	Time*Site	13,028	12	0,002
Salinity				Clarity			
Intercept	4931,538	1	0,000	Intercept	1727,147	1	0,000
Time	94,686	3	0,003	Time	63,191	3	0,000
Site	155,878	4	0,000	Site	31,061	4	0,002
Time*Site	52,974	12	0,002	Time*Site	15,64	12	0,009

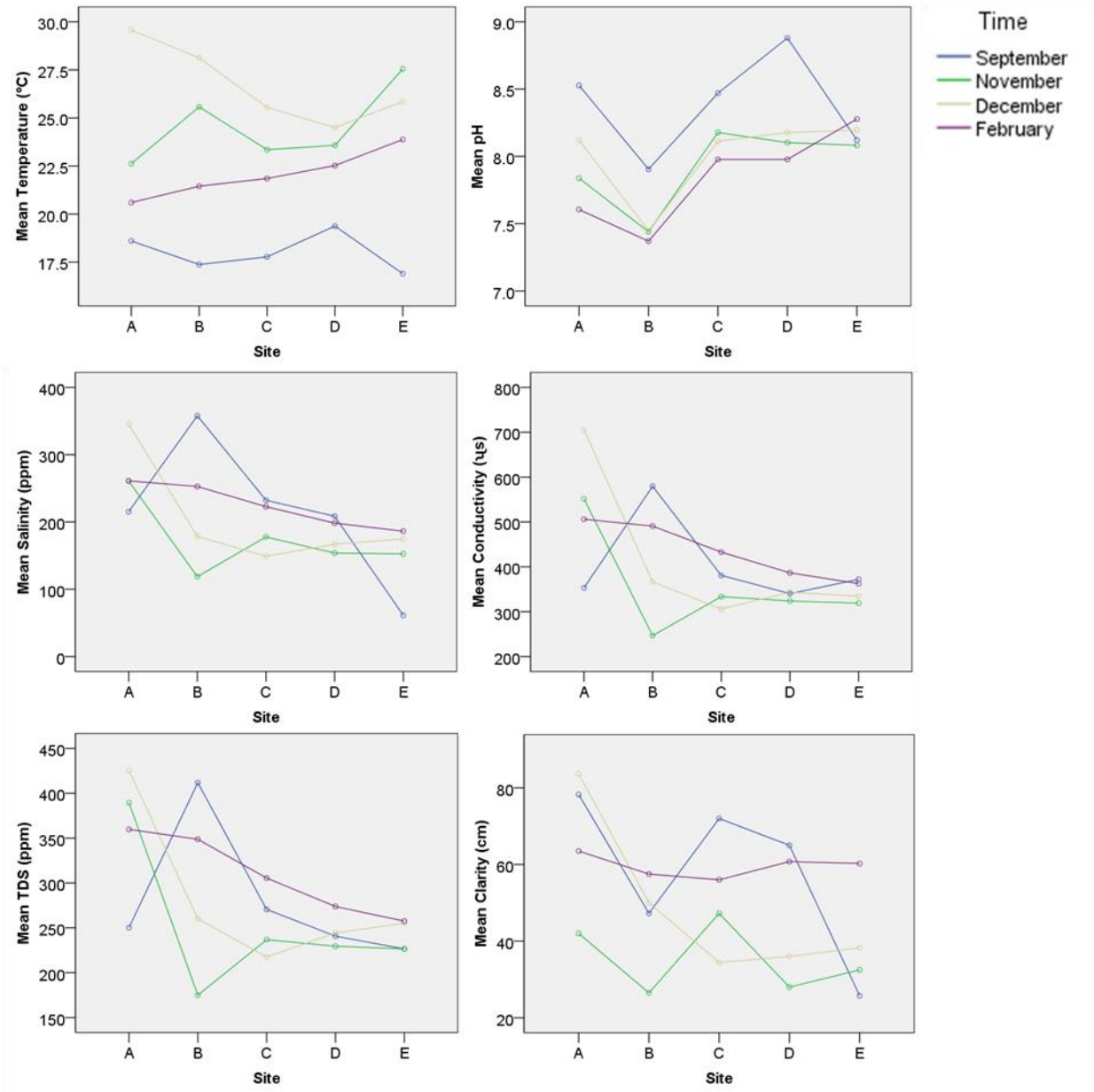

Figure 12: Mean values of surface water physical parameters measured *in situ* at sampling sites along the upper Hartbeesspruit

3.3. Macroinvertebrates

Nineteen macroinvertebrate families comprised of 2647 individuals were collected from 41 samplers. Nineteen samplers were lost, tampered with or deemed unreliable due to exposure to air. The most abundant families collected were Chironomidae, Oligochaeta and Hirudinea (Table 3).

Table 3: The phylum, class, order and family and the percentage of total abundance of macroinvertebrates sampled using Hester-Dendy artificial samplers in the upper reaches of the Hartbeesspruit. Families with total abundance >1% are underlined.

Phylum	Class	Order	Family	Total Abundance (%)
Annelida	Clitellata	Hirudinea		7.78
Annelida	Clitellata	Oligochaeta		15.72
Arthropoda	Malacostraca	Decapoda	Potamonautidae	3.25
Arthropoda	Insecta	Diptera	Chironomidae	56.52
Arthropoda	Insecta	Diptera	Psychodidae	0.79
Arthropoda	Insecta	Ephemeroptera	Baetidae	5.21
Arthropoda	Insecta	Ephemeroptera	Caenidae	0.08
Arthropoda	Insecta	Ephemeroptera	Leptophlebidae	0.04
Arthropoda	Insecta	Hemiptera	Belostomatidae	0.08
Arthropoda	Insecta	Hemiptera	Corixidae	1.81
Arthropoda	Insecta	Hemiptera	Naucoridae	0.15
Arthropoda	Insecta	Odonata	Coenagrionidae	0.19
Arthropoda	Insecta	Trichoptera	Ecnomidae	0.04
Arthropoda	Insecta	Trichoptera	Hydropsychidae	0.08
Mollusca	Gastropoda		Ancylidae	3.18
Mollusca	Gastropoda		Lymnaeidae	0.15
Mollusca	Gastropoda		Physidae	4.23
Mollusca	Gastropoda		Planorbidae	0.68
Mollusca	Gastropoda		Thiaridae	0.04

3.3.1. Indices of community composition

Family richness was affected by the interaction of site and time (Table 4). Least significant difference (LSD) post-hoc tests of variation over time and site, revealed an increase in December compared to the other months and a gradual increase in family richness from upstream to downstream with drops in value at site C and E (Figure 13). Shannon-Wiener diversity index, SASS score and ASPT were all affected by site (Table 4); the LSD test over site revealed a relatively low value at site A and increasing gradually to site D and finally dropping drastically at site E (Figure 13). Macroinvertebrate abundance was affected by time (Table 4); with there being an increase in abundance in December (Figure 13). Pielou's evenness was not affected by the major effects of time or site (Table 4).

Table 4: Summary of the two-way ANOVA and Shreirer-Ray-Hare test* that describes the effects of time and site on family richness, Shannon Wiener diversity, SASS score, ASPT, macroinvertebrate abundance and Pielou's eveness. p<0.05 is underlined.

Parameter	F	df	*p*	Parameter	F	df	*p*
Family richness				Abundance*			
Intercept	472,308	1	0,000	Intercept	249,434	1	0,000
Time	8,763	2	0,001	Time	8,74	2	0,017
Site	3,475	3	0,021	Site	1,398	3	0,625
Time*Site	1,283	4	0.295	Time*Site	2,425	4	0,338
Shannon Wiener diversity				Pielou's evenness*			
Intercept	536,774	1	0,000	Intercept	92,098	1	0,000
Time	2,148	2	0,137	Time	2,056	2	0,155
Site	7,848	3	0,000	Site	0,766	3	0,595
Time*Site	1,755	4	0,133	Time*Site	1,13	4	0,413
SASS score				ASPT			
Intercept	246,522	1	0,000	Intercept	571,475	1	0,000
Time	6,309	2	0,060	Time	0,331	2	0,721
Site	5,01	3	0,004	Site	3,351	3	0,024
Time*Site	1,473	4	0,215	Time*Site	0,971	4	0,479

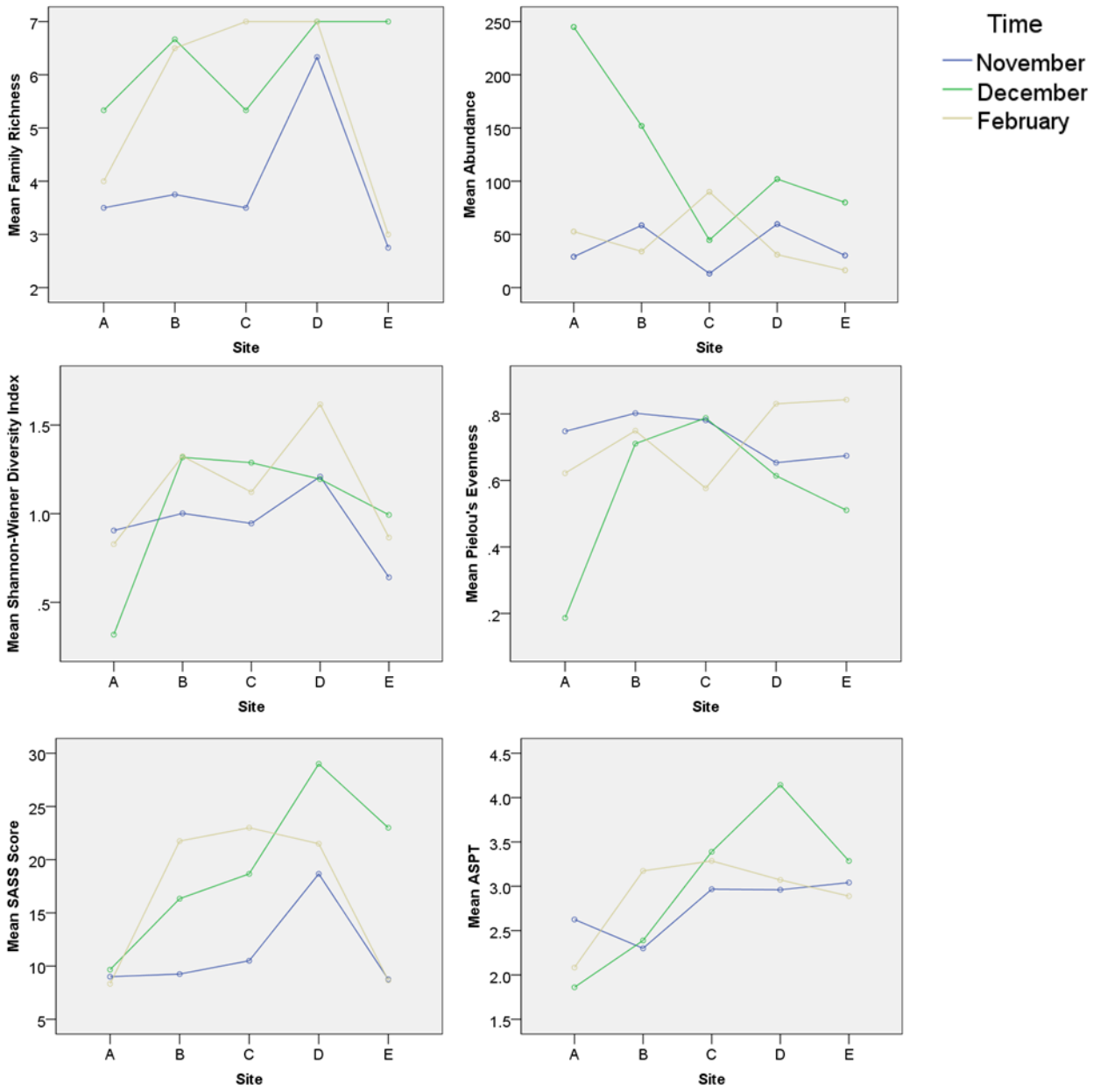

Figure 13: Mean macroinvertebrate index values of community composition at sampling sites along the upper Hartbeesspruit

3.3.2. Non-metric multidimensional scaling

Results of the ANOSIM showed that along the upper Hartbeespruit macroinvertebrate assemblages were structured by site (R=0.3641, p=0.001) but not time (R=0.0307, p=0.687). The stress value of the nMDS was 0.218, which showed a high degree of correspondence between data points (Kingston and Rosel 2004). The two dimensional plot of the nMDS, showed that sites C and D were very similar. Site E was the next closely related site to sites C and D but was unrelated to sites A and B. Sites A and B were closely related, both being the most unrelated to sites C, D and E (Figure 14).

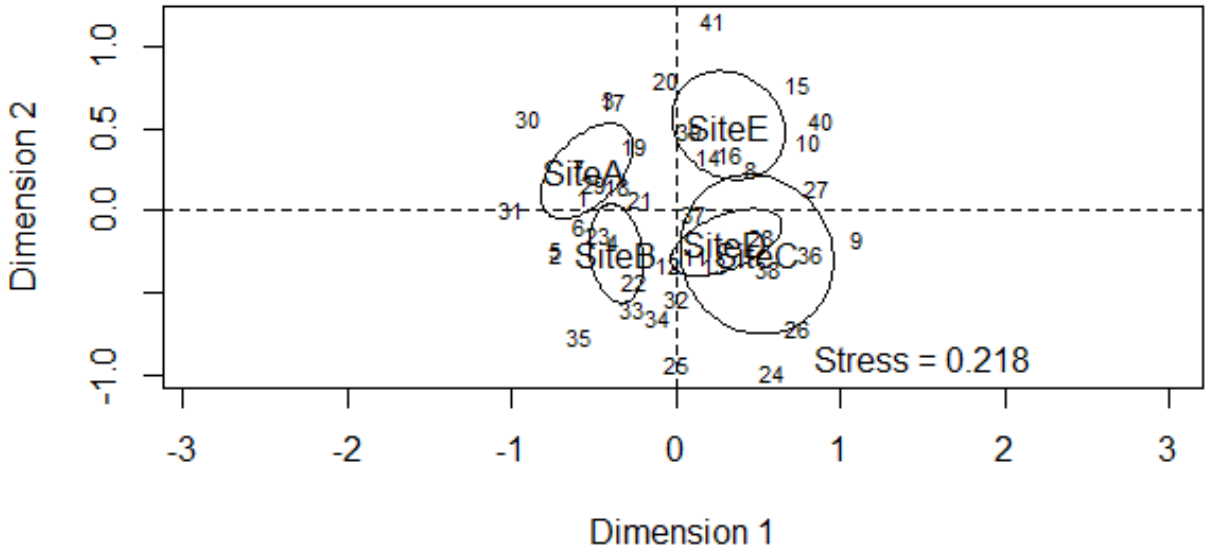

Figure 14: Non-metric multidimensional scaling ordination of aquatic invertebrate community assemblages from the upper Hartbeesspruit for sampling sites A, B, C, D and E. Elipses represent the 95% confidence interval of the centroid for each site.

3.3.3. Indicator Value Analysis

Indicator value (IndVal) analysis showed that five species were significant indicators of site (Table 5). Macroinvertebrates characteristic of each site were Psychodidae at site A (0.765, p=0.002), Planorbidae at site B (0.814, p=0.001), Physidae at sites A and B (0.847, p=0.001), Hirudinea at sites A, B and D (0.938, p=0.001), and Ancylidae at sites C, D and E (0.837, p=0.002). Tolerance values of the indicator families were low relative to possible maximum values of 15 (Dickens and Graham 2002).

Table 5: Macroinvertebrate families indicative of conditions at various sites as well as tolerance values according to Dickens and Graham (2002)

Site	Indicator Family	Tolerance value
		Dickens and Graham (2002)
A	Psychodidae	1
A, B	Physidae	3
A, B, D	Hirudinea	3
B	Planorbidae	3
C, D, E	Ancylidae	6

3.3.3 Cannonical Correspondence Analysis

In the CCA tri-plot, the total variation in macroinvertebrate families explained by environmental parameters was 69% (Figure 15). There was a strong association between the industrial land-use (Site E) and lead as well as macroinvertebrate families Caenidae and Chironomidae. The urban land use (Site A) showed a strong association with temperature, turbidity and conductivity (EC) as well as families Thiaridae and Psychodidae. The recreational (Site B) and residential (Site D) sites had a strong association with in stream habitat diversity, coliforms, arsenic and mercury, as well as families Physidae, Hirudinea, Oligochaeta and Planorbidae. The least transformed wetland (Site C) was generally associated with stream depth, width and aluminium, and the families Naucoridae, Baetidae and Hydropsychidae. The land uses which had the strongest influence on macroinverebrate distribution were the urban (Site A), recreational (Site B) and the least transformed wetland (Site C).

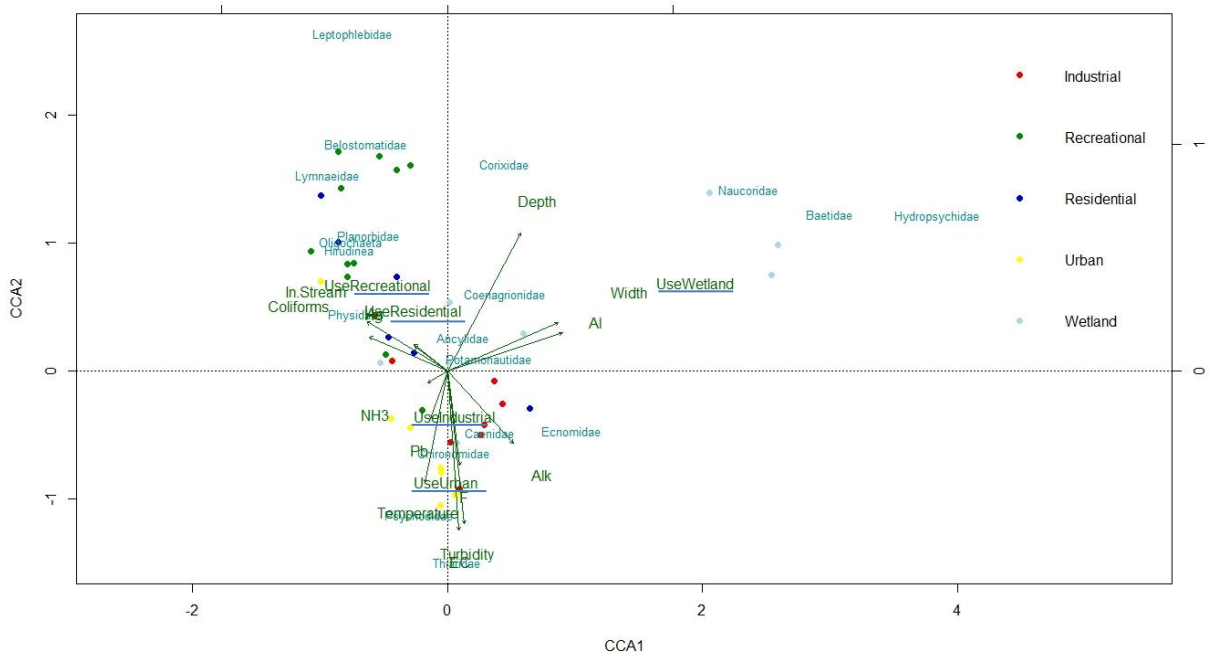

Figure 15: CCA tri-plot illustrating associations between sample sites (coloured circles), environmental factors (arrows) and land-use (underlined) recorded along the upper Hartbeesspruit, South Africa

4. Discussion

Of all the macroinvertebrates sampled, the ones with the highest abundance were those typical of polluted waters. Out of the 19 families collected, only 3 of these families made up 80% of macroinvertebrates sampled. These families were Hirudinea, Chironomidae and Oligochaeta, which have high tolerance for changes in water chemical and physical parameters (Dickens and Graham 2002). Chironomidae comprised 56.5% of total macroinvertebrates sampled which made them the dominant family within this stream. This is the most abundant of benthic macroinvertebrate families within river systems, and it is commonplace to find it consisting of half the richness of sampled species (Bouchard 2004).

4.1. Spatial Variation

Most measures of macroinvertebrate community composition varied significantly across sites along the Hartbeesspruit. Family richness, Shannon-Wiener diversity, SASS score and ASPT were all generally lowest at sites A and E, and highest at sites C and D. Across sites this was not found for macroinvertebrate abundance and Pielou's evenness which may have been due to high variability of samples obtained within sites for these parameters. Where changes between sites were found, this may be due to variations in the flow regime, width and depth, in-stream habitats and land use along the stream. In particular, patterns seen in macroinvertebrate assemblages may not necessarily vary in relation to decreased water quality but a low diversity of suitable habitats (Dias-Silva *et al.* 2010). In this study, physical parameters (except temperature and clarity), major ions (except arsenic), the metal ion magnesium, and nutrient sulphate did not directly correlate with measures of macroinvertebrate community composition. Rather, they showed a general decline in value from upstream to downstream with high variation at site B over time before remaining relatively stable through the downstream sites. Tolerance values of indicator families (Dickens and Graham 2002), also showed downstream sites to be more characteristic of less tolerant families. It is important to note that the highest quality score of 6 for the indicator family Ancylidae (Dickens and Graham 2002), as well as the highest ASPT values of approximately 4 for sites C, D and E, while being higher than from other sites, still indicate that the sampled stretch of the Hartbeesspruit is strongly impacted. This is because reference sites in the Highveld region of South Africa have a median ASPT of 5.7, but can be as high as 7.7 (Dallas 2007).

In comparison to downstream sites, indicators of community composition were lowest at site A. This may be due to a number of factors. Firstly, the low substrate diversity present and predominantly muddy substrate type may have played a role in the assemblages that were

recorded. Resh *et al.* (1995) and Allan (1995) showed that with reduced substrate diversity and particle size comes a decrease in macroinvertebrate diversity and abundance. Dewson *et al.* (2007) showed that with decreasing habitat diversity, macroinvertebrate richness decreased. The habitat diversity seen at site D, however, was similar to that seen at site A, but variation in species, particularly tolerant species was seen, indicating habitat diversity may not be the crucial driving factor. Secondly, the relatively shallow depths may have had an impact on the velocity of water. Stream water velocity influences substrate type and water quality parameters (Allan 1995), which ultimately influence habitat availability and suitability (Dewson *et al.* 2007, Hart and Finelli 1999). Lastly, the influence that the commercial/urban land use has on surface water quality was likely a major influence. Psychodidae, which was dominant at site A, has the highest tolerance for changes in conditions (Dickens and Graham 2002). This indicates that site A likely had the worst water quality of the five sites. This is further confirmed by the results of the indicators of macroinvertebrate community composition. Consistently over time there was a relatively higher value compared to the other sites in salinity, conductivity, TDS, clarity, calcium, chlorine, manganese, potassium, sodium and sulphate. The increased parameter values seen are most likely due to the remobilisation of contaminants after water discharge either due to rainfall events or through an outlet into the water at site A or directly upstream along the concreted canal.

Indicators of macroinvertebrate community composition showed site B to be intermediate in value between site A and C. The increase in macroinvertebrate index values at site B could be explained by deeper water and visually higher flow rate compared to site A. Site A and B, however, had similar substrate compositions, indicating that this was likely not the chief driver of patterns seen. The second and third most tolerant families identified to be characteristic of sites were Hirudinea and Physidae, which were characteristic of sites A, B and D, and sites A and B, respectively. This indicates that conditions at these sites allowed families which are indicative of poor water quality to flourish. The inclusion of site D was unexpected as the grouping of site A, B and D was not identified beforehand by other measures of community composition. Hirudinea are tolerant of a broad range of salinities, oxygen concentrations and temperature (Lenat 1993), which may explain their presence at the slower flowing sites A and B but not at site D. The influence of highly variable water quality parameters likely played a vital role in influencing macroinvertebrates observed at site B. Parameters which were especially variable over time at site B with the general trend of peaks in September, drops in November and December and stable to rising in February were salinity, conductivity, TDS, alkalinity, turbidity, calcium, chlorine, fluorine, manganese, sodium and magnesium.

These patterns were due to downstream dynamics after a dam, with a comparative reduction in suspended and dissolved solids at site B in November and December being due to flow rate variations. Suspended and dissolved load is high in flowing water, as flow rate decreases due to the increased size of a dam, the suspended and dissolved load reduces in the dam. Variations in flow rate between water flowing into a dam and the water already in the dam cause these changes to total suspended and dissolved solids according to Baxter (1977). Donnely (1993) showed that, due to these dynamics, dams act as storage for various inorganic compounds.

According to Brainwood *et al.* (2004) the two main factors which influence the ion concentration of water already in dams are groundwater recharge and evaporation. The influence of evaporation and reduced dilution were evident after drier periods but most notably September following 6 months of low to no rainfall. The decreased mobility of water and larger surface area in the dams allowed for increased evaporation together with reduced precipitation and thus reduced dilution caused a subsequent increase in concentrations of various parameters within the dams. Kalff (2002) showed a subsequent increase in salinity due to reduced dilution in dams in the dry season. Hamilton *et al.* (2004) showed that concentrations of chlorine and sodium ions increased due to water loss in waterholes. Dilution also seemed to play a major role as post wetter periods values decrease drastically due to increased rainfall which diluted concentrations at these time periods, notably in November and December. In February, the combination of reduced rainfall, dilution effects and flow variation resulted in a relatively stable or slight increase in concentrations. The patterns seen over the time period in parameters tested were likely due to the combination of effects from variations in flow rate, evaporation and dilution in the dam.

The pH varies temporally but retains the same general trend spatially. All measured pH-values were alkaline with Site B being distinctly closer to neutral values than any of the other sites. Reasons for the sudden reduction of pH at the recreation site may be due to anaerobic decomposition occurring in the dams upstream. However, this needs further investigation.

Indices of macroinvertebrate community composition had the highest values at sites C and D. This was not surprising as site C and D had relatively diverse in-stream habitats and riparian cover, relatively faster flow compared to upstream sites, and no restrictions in connectivity between these two sites. Ancylidae was characteristic of sites C, D and E, and had the lowest tolerance out of species identified as indicators of the various sites. This suggests that sites C, D and E were perhaps the least impacted by disturbance. There was also an elevated number of Baetidae observed at site C in December and February. This is a major indication of a comparatively stable system which has not been impacted heavily. The family Baetidae is comparatively less tolerant of pollution, and their presence indicates a relatively healthy system (Dickens and Graham 2002).

The major influences leading to the increase in index values, however, were likely due to site C being situated directly after the Colbyn Valley wetland, and the influence it had on surface water parameters. The influence of the wetland was evident when looking at water parameters tested across time periods and against upstream sites. Regardless of values at site B, parameter values returned to similar values across time at site C. Parameters which remained relatively stable over time and sites C, D and E were the physical parameters (except temperature and clarity), major ions (except arsenic), the metal ion magnesium and nutrient sulphate. Wetlands have regulatory effects on suspended particles (Strecker *et al.* 1992), nutrients (Reddy and DeLaune 2008), metals (Sheoran and Sheoran 2006), organic pollutants (Hemond and Benoit 1988), and bacteria (Rogers 1983).

Sites C, D and E were directly connected and had no natural or man-made obstructions to water flow. This indicates that at this fine scale, such as between sites C, D and E, and with unobstructed stream flow, changes in land use type, specifically due to residential and commercial/industrial use were not sufficient to cause significant variation in surface water parameter measured. This result should be treated with caution because the sampling design of this study may not have identified crucial changes in these parameters before they dispersed downstream between these three sites. However, the macroinvertebrates at site E showed less tolerant species and a drop in indices of community composition. This was not anticipated as flow regime, habitat diversity and most of the surface water parameters measured at site E were relatively similar to site C with no notable discontinuities in flow regime between the two. The non-metric multidimensional scaling indicated that sites A and B were not closely related to site E, indicating a different influence on the communities present at these sites. The land use characteristic of site E was industrial which likely had an influence on water quality and subsequently influenced conditions for macroinvertebrate survival. There is also no substantial change in surface water quality parameters at site E to explain the sudden change in grouping and drop in indicated favourable condition. This divergence may be attributed to one of two reasons: either the periodic nature of the water chemistry investigation was not sufficient in observing major influences, or hazardous contaminants which were not tested for, which are characteristic of an industrial land use type.

The patterns seen in indicator families at the various sites could further be explained due to substrate characteristics and sampling methods used. Psychodidae, which were indicative of site A, are often found within sediments (Bouchard 2004). Site A was characterised by a muddy substrate and Hester-Dendy samplers often contained settled muddy sediments after sampling. This is a possible limitation of the Hester-Dendy samplers where substrate type influences the samples regardless of standardisation due to artificial substrate use. However, the likely bias caused by this is

seen as negligible due to the high similarity of groupings of sites based on various measures of community composition. Three of the indicator families, Planorbidae, Physidae and Ancylidae, belong to the scraper feeding group (Mandaville 2002). These gastropods feed on algae, macrophytes and diatoms that settle on surfaces such as the Hester-Dendy samplers. This allows for a surface for feeding, perhaps introducing a sampling bias towards the scraper feeding group.

Based on the results of the CCA tri-plot it was evident that depth, turbidity and conductivity were the most influential factors when looking at the dispersal of macroinvertebrate families. This is not surprising as there is evidence of this occurring throughout the literature. The increased depth of a system has been shown to increase habitat type and thus be highly influential on macroinvertebrate composition (Baumgartner *et al.*2008). Azrina *et al.* (2006) showed that turbidity greatly decreased species richness and diversity of freshwater macroinvertebrates. The reason for this is likely the decrease in light penetration and a subsequent decrease in primary productivity (Grobler *et al.* 1987). Kefford *et al.* (2003) showed that macroinvertebrates could tolerate a range of salinities, but it has also been seen to decrease species richness and diversity with increasing conductivity (Azrina *et al.* 2006). Horrigan *et al.* (2005) also showed conductivity to be a major influence on macroinvertebrate composition.

The land uses which had the greatest influence on macroinvertebrate assemblage were the urban, recreational and least transformed wetland land uses. This was not surprising as these were the land uses which displayed the greatest variation between sites in tested chemical and physical water parameters. Again this can be attributed to the staggered connectivity between the sites due to the presence of dams and the wetland, as well as the quality regulating service of the wetland. The urban land use was greatly associated with increased temperature, turbidity and conductivity. This was likely due to the shallow depths observed at the site and turbidity and conductivity due to the high density of muddy substrate. The recreational land use was greatly associated with increased stream habitat diversity and coliforms, which may have influenced the presence of Physidae, Planorbidae, Hirudinea and Oligochaeta. The urban land use associated parameters seemed to lead to an increased abundance of Psychodidae and Thiaridae. The urban and recreational land uses had conditions that were associated with macroinvertebrates with high tolerances for polluted water (Dickens and Graham 2002), indicating a possible reduced health of those ecosystems. The environmental parameters associated with the urban and recreational sites vary to the extent that conditions allow for a variation in macroinvertebrate assemblages recorded, albeit with families tolerant of polluted water.

The least transformed, wetland land use was highly associated with increased depths, width and aluminium. These were associated with the presence of Naucoridae, Baetidae and Hydropsychidae, which have low tolerance scores for polluted water (Dickens and Graham 2002). The results confirm that the least transformed wetland land use has the most favourable conditions for less tolerant families, indicating higher ecosystem health.

4.2. Temporal Variation, Pulse Events and Point Sources

Measures of macroinvertebrate community composition which varied significantly over time periods included macroinvertebrate abundance and family richness. The combination of the two indices indicates an increase in favourable conditions for macroinvertebrate reproduction and survival in December compared to that of November and February. The variation seen in abundance was largely due to the large numbers of chironomids at sites A and B. Apart from this peak in December, abundance was relatively similar in other seasons (Table A1). This variation over the time periods for macroinvertebrate abundance fits well with the literature (Beche *et al.* 2006; Mesa 2012; Reece and Richardson 1998) but the observations for family richness do not. Mesa (2012) showed that community composition and abundance are highly influenced by seasonality, but presence and absence of species was not influenced as strongly. In Fourie *et al.* (2014) it was shown that SASS5 metrics, which are measured based on abundances and presence and absence of families, did not vary by season along the Skeerpoort River in the North West province in South Africa. The inconsistency here is likely due to the short time interval within which communities were observed. The temperature patterns seen in this study likely influenced the temporal patterns seen in macroinvertebrate assemblages. Temperature is a key factor influencing abundance and diversity of macroinvertebrates (Burgmer *et al.* 2007*;* Jacobsen and Marin 2008; Wallace and Anderson 1996). Kemp *et al.* (2014) showed that among the physical and chemical parameters (common to this study) measured along the Olifantsriver catchment, temperature variations were the major factor influencing family dispersion along the river.

The mean increase in these indices in December was likely due to the overall decrease in mean value of parameters in the periods when macroinvertebrates where sampled. It must be noted that the peaks in parameter values in September at site B may have had an influence on macroinvertebrate assemblages sampled in November. Seasonal changes in rainfall and subsequent water flow in the Hartbeesspruit probably led to improved mean conditions for macroinvertebrate survival at site B.

Biological water parameters showed an increase at sites A and D which were characterised by informal squatters and scattered faeces. The increase in indices of macroinvertebrate community

composition at site D, correlate with increased abundances of bacteria, coliforms and *E. coli*. These could have a positive influence on macroinvertebrate communities. However, these peaks typically occurred in November and February, correlating negatively with species abundance and family richness. Biological parameters also peaked at site A, which showed a decrease in macroinvertebrate communities and indicating a possible negative effect on the macroinvertebrate assemblage. In the literature there is no clear effect of these parameters on macroinvertebrate community composition, and further studies would need to be done to sensibly include them as a likely influence.

Metals ions (except magnesium), arsenic and nutrients (except sulphur) did not show a pattern over space and temporal effects were negligible. This was either due to (i) them not being influenced by the temporal dynamics of the dam and the regulation of the wetland, (ii) their presence ascribed to point sources along the upper Hartbeespruit arising from land use activities, or (iii) the variations in concentrations being insignificant with relation to the low levels of these metals detected. The source of arsenic is likely from geological weathering events and not a point source (as found by, for instance, Smedley and Kinniburgh 2001).

5. Conclusion

Indices of community composition indicated a general increase in value from upstream to downstream. The likely driver for assemblages was the flow regime along the river. The combination of the relative low flow upstream, dams and the wetland contributed influenced habitat diversity and water quality at the various sites.

Physical water quality parameters (except temperature and clarity), major ions (except arsenic), the metal ion magnesium and nutrient sulphate showed variations in value that were consistent with the influences of evaporation, dilution and connectivity of water flow along the river. However, variation between the last three sites was virtually non-existent regardless of changing land use types.

These water quality parameters showed similar groupings of sites compared to the measures of community composition. This suggests that these parameters had a cumulative influence on the favourability of conditions for macroinvertebrates across space. Although major variations in habitat diversity were not seen, the low flow regime upstream was seen to influence habitat availability and suitability for macroinvertebrates. Temperature was seen as an important influence on macroinvertebrate abundance and family richness at a temporal scale. The drop in macroinvertebrate measures at site E could not be explained by tested variables, with the only explanation being an unrecorded influence from the industrial land use type.

On a spatial scale the most influential parameters on macroinvertebrate composition were depth, turbidity and conductivity, and temperature to a lesser extent. The land use types that showed the greatest association with the sampled assemblages were the urban, recreational and least transformed wetland land uses. Although major influential factors, this pattern was not seen to be strictly due to the input of contaminants arising from associated activities, nor variation in physical characeristics, but rather the discontinuity in flow regime.

It can be concluded that at a fine scale, the strongest factors that influence macroinvertebrate community composition, are not land use type but rather the hydrological pathways of connectivity and stream flow that exist within a given system. These hydrological pathways influence values and concentrations of chemical and physical surface water parameters which in turn further influence macroinvertebrate assemblages.

6. References

Abell, R. 2001. Conservation Biology for the Biodiversity Crisis: A Freshwater Follow-Up. Conservation Biology. 16: 1435-1437

Allan, J.D. 1995. Stream Ecology Structure and Function of Running Waters. Chapman & Hall

Allan, J.D., Johnson, L.B. 1997. Catchment-Scale Analysis of Aquatic Ecosystems. Freshwater Biology. 1: 107–111

Anemaet, E.R., Taylor, J., Mitsch, W.J. 2005. Macroinvertebrate Diversity and Abundance in Two Created Wetlands in Ohio. Schiermeier Olentangy River Wetland Research Park, Schoolof Environment and Natural Resources, The Ohio State University

Arman, N.Z., Salmiati, Said, M.I., Azman, S., Azuri, S., Safeai, M. 2012. Benthic Macroinvertebrates as an Alternative Tool for Biological Monitoring in Assessing River Water Quality. International Proceedings of Chemical, Biological and Environmental Engineering. 35: 52-56

Azrina, M.Z., Yap, C.K., Rahim Ismail, A., Ismail, A., Tan, S.G. 2006. Anthropogenic Impacts on the Distribution and Biodiversity of Benthic Macroinvertebrates and Water Quality of the Langat River, Peninsular Malaysia. Ecotoxicology and Environmental Safety. 64: 337-347

Baa-Poku, J., Asante, F., Amakye, S. 2013. Impact of Urban Effluents on the Macroinvertebrates of a Creek in Accra, Ghana. West African Journal of Applied Ecology. 21: 97-109

Bannerman, R., Clark, S., Pitt, R., Williamson, D. 2005. Sources of Pollution in Urban Areas (Part 1): Older monitoring projects, 465-484 and 507-530 In: James, W., Irvine, K.N., McBean, E.A., Pitt, R. E. (Eds) 2005. Effective Modeling of Urban Water Systems, Monograph 13. Guelph, Ontario

Bannerman, R., Owens, D.W., Dodds, R.B., Hornewer, N.J. 1993. Sources of Pollutants in Wisconsin Storm Water. Water Science and Technology. 28: 241-259

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B. 1999. Rapid Bio-assessment Protocols for use in streams and rivers. U. S. E. P. Agency

Basson, M.S., Van Niekerk, P.A., Van Rooyen, J.A. 1997. Overview of Water Resources Availability and Utilization. Department of Water Affairs and Forestry, Pretoria.

Baumgartner, D., Mortl, M., Rothhaupt, K. 2008. Effects of Water Depth and Water Level Fluctuations on the Macroinvertebrate Community Structure in the Littoral Zone of Lake Constance. Hydrobiologia. 613: 97-107

Baxter, R.M. 1977. Environmental Effects of Dams and Impoundments. Review of Ecology and Systematics. 8: 255-283

Beche, L. A., Mcelravy, E. P., Resh, V. H. 2006. Long-Term Seasonal Variation in the Biological Traits of Benthic Macroinvertebrates in Two Medaterranean-Climate Streams in California U.S.A. Freshwater Biology. 51: 56-75

Behmer, D.J., Hawkins, C.P. 1986. Effects of Overhead Canopy on Macroinvertebrate Production in a Utah Stream. Freshwater Biology. 16: 287-300

Berenzen, N., Schulz, R., Liess, M. 2001. Effects of Chronic Ammonium and Nitrate Contamination on the Macroinvertebrate Community in Running Water Microcosms. Water Research. 35: 3478-3482

Bis, B., Zdanowicz, A., Zalewski, M. 2000. Effects of Catchment Properties on Hydrochemistry, Habitat Complexity and Invertebrate Community Structure in Lowland Rivers. Hydrobiologia. 423: 369-387

Bouchard, R.W., Jr. 2004. Guide to Aquatic Macroinvertebrates of the Upper Midwest. Water Resources Center, University of Minnesota, St. Paul, MN. Pp208

Brainwood, M.A., Burgin, S., Maheshwari, B. 2004. Temporal Variations in Water Quality of Farm Dams: Impacts of Land Use and Water Sources. Agricultural Water Management. 70: 151-175

Brooks, A.J., Haeusler, T., Reinfelds, I., Williams, S. 2005. Hydraulic Microhabitats and the Distribution of Macroinvertebrate Assemblages in Riffles. Freshwater Biology. 50: 331-344

Brown, L. R. Gray, R, H. Hughes, R, M. Meador, M. R. 2005. Introduction to effects of urbanization on stream ecosystems. American Fisheries Society Symposium. 47: 1-8

Burcher, C.L., Valett, H.M., Benfield, E.F. 2007. The Land-Cover Cascade: Relationships Coupling Land and Water. Ecology. 88: 228–242

Burgmer, T., Hillebrand, H., Pfenninger, M. 2007. Effects of Climate Driven Temperature Chnges on the Diversity of Freshwater Macroinvertebrates. Global Change and Conservation Ecology. 151: 93-103

Buss, D.F., Baptista, D.F., Nessimian, J.L., Egler, M. 2004. Substrate Specificity, Environmental Degradation and Disturbance Structuring Macroinvertebrate Assemblages in Neotropical Streams. Hydrobiologia. 518: 179-188

Cairns, J., Der Schalie, W.H.V.D. 1980. Review paper: Biological Monitoring. Part I- Early Warning Systems. Water Research. 14: 1179–1196

Chessman, B.C., Robinson D.P., Hortle K.G. 1987. Changes in the Rifle Macroinvertebrate Fauna of the Tanjil River, South-eastern Australia, During Construction of Blue Rock Dam. Regulated Rivers Research and Management. 1: 317-329

Chown, S.L., Nicolson, S.W. 2004. Insect Physiological Ecology: Mechanisms and patterns. Oxford University Press, New York.

Chutter, FM. 1998 Research on the Rapid Biological Assessment of Water Quality Impacts in Streams and Rivers. Report to the Water Research Commission, Pretoria. WRC Reports No. 422/1/98

Clarke, W.C., Richards, J. Flint, E. 1986. Human Transformations of the Earth's Vegetation Cover: Past and Future Impacts of Agricultural Development and Climatic Change. 54-59

Costanza, R., Mageau, M. 1999. What is a healthy ecosystem?. Aquatic Ecology. 33:105-115

Conolly, N.M., Crossland, M.R., Pearson, R.G. 2003. Effect of Low Dissolved Oxygen on Survival, Emergence, and Drift of Tropical Stream Macroinvertebrates. Journal of the North American Benthological Society. 23: 251-270

Cover, E.C., Harrel, R.C. 1978. Sequences of Colonization, Diversity, Biomass and Productivity of Benthic macroinvertebrates on Artificial Substrates in a Freshwater Canal. Hydrobiologia. 59: 81-95

Dallas H.F., Day J.A. 1993. The Effects of Water Quality Variables on Riverine Ecosystems: A Review. Water Research Commission. Report No. 351

Dallas, H.F., Day, J.A., Musibono, D.E. Day, E.G. 1998. Water Quality for Aquatic Ecosystems: Tools for Evaluating Regional Guidelines. WRC Report No. 626/1/98

Dallas, H.F. 2007. River Health Programme: South African Scoring System (SASS) Data Interpretation Guidelines. Department of Water Affairs and Forestry, Pretoria.

Davies, B.R., Day, J.A. 1986. The Biology and Conservation of South Africa's Vanishing Waters. Centre for Extra-Mural Studies, University of Cape Town. Pp 186

Davies, B.R., O'Keeffe, J.H., and Snaddon, C.D. 1993. A Synthesis of the Ecological Functioning, Conservation and Management of South African River Ecosystems. WRC Report No. TT 62/93

Dewson, Z.S., James, A.B.W., Death, R.G. 2007. A Review of the Consequences of Decreased flow for Instream Habitat and Macroinvertebrates. Joyrnal of the North American Benthological Society. 26: 401-415

Dias-Silva, K., Cabette, H.S., Juen, L., De Marco Jr P. 2010. The Influence of Habitat Integrity and Physical Chemical Water Variables on the Structure of Aquatic and Semi-aquatic Heteroptera. Zoologia. 27: 918–930.

Dickens, C.W.S., Graham, P.M. 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science. 27: 1-10

Donnely, T.W. 1993. Impoundment of Rivers: Sediment Regime and its Effect on Benthos. Aquatic Conservation: Marine and Freshwater Ecosystems. 3: 331–342

Du Preez, H.H., Kempster, P.L., Kleynhans, C.J., Roux, D.J., Van Vliet, H.R. 1999. Integrating Stressor and Response Monitoring into a Resource-Based Water Quality Assessment Framework. Environmental Management. 23: 15-30

DWAF. 1996. South African Water Quality Guidelines: Volume 1: Domestic Use. Department of Water Affairs and Forestry, Pretoria

DWAF. 1996. South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems. Department of Water Affairs and Forestry, Pretoria

DWAF. 2004. Water Resource Quality Monitoring: Volume 1-Sampling Protocol for Inorganic Chemical Analysis. Resource Quality Services, Department of Water Affairs.

Edwards, R.T., Meyer, J. L. 1987. Bacteria as a food source for black fly larvae in a blackwater river. Journal of the North American Benthological Society. 6: 241-250.

Edwards, R.T., Meyer, J. L. 1990. Bacterivory by deposit-feeding mayfly larvae (*Stenonema* sp.). Freshwater Biology. 24: 453-462.

Environmental Protection Agency. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. Environmental Protection Agency, Cincinnati, Ohio. EPA-67014-73-001-38

Espy, W.H., Morgan, C.W., Masch, F.D. 1966. A Study of Some Effects of Urbanization on Storm Runoff from a Small Watershed. Report 23. Texas Water Development Board

Evans, L.J., Norris, R.H. 1997. Prediction of Benthic Macroinvertebrate Composition Using Microhabitat Characteristics Derived from Photography. Freshwater Biology. 37: 621-633

Forstner, U., Wittmann, G.T.W. 1983. Metal pollution in the aquatic environment. Springer-Verlag. 18: 194

Fourie, H.E., Thirion, C., Weldon, C.W. 2014. Do SASS5 Scores vary with Season in the South African Highveld? A Case Study on the Skeerpoort River, North West Province, South Africa. African Journal of Aquatic Science. 39: 369-376

Fullner, R.W. 1971. A Comparison of Benthic macroinvertebrates Collected by Basket and Modified Multiple-Plate Samplers. Water Pollution Control Federation. 43: 494-499

Galloway, J.N., Likens, G.E., Edgerton, E.S. 1976. Acid Precipitation in the North Eastern United States: pH and Acidity. Science. 194: 722-724

Gerber, A., Gabriel, M.J.M. 2002. Aquatic Invertebrates of South African Rivers, Field Guide. Institute for Water Quality studies, Department of Water Affairs and Forestry, Pretoria

Gray, L.J., Ward, J.V. 1982. Effects of Sediment Releases from a Reservoir on Stream Macroinvertebrates. Hydrobiologia. 96: 177-184

Grobler, D.C., Toerien, D.F., Rossouw, J.N. 1987. A Review of Sediment Water Quality Interaction with Particular Reference to the Vaal River System. Water SA. 13:15-22

Grubbs, S.A., Taylor, J.M. 2004. The Influence of Flow Impoundment and River Regulation on the Distribution of Riverine Macroinvertebrates at Mammoth Cave National Park, Kentucky, U.S.A. Hydrobiologia. 520: 19-28

Hall, M.J., Gerhard, P.C., Ralph, H.R. 2001. Relationship between Land Use and Stream Invertebrate Community Structure in a South Island, New Zealand, Coastal Stream Catchment. New Zealand Journal of Marine and Freshwater Research. 35: 591-603

Hamilton, S.K., Bunn, S.E., Thoms, M.C., Marshall, J.C. 2004. Persistence of Aquatic Refugia between Flow Pulses in a Dryland River System (Cooper Creek, Australia). Limnology and Oceanography. 50: 743-754

Harrison A.R., Eldsworth J.F. 1958. Hydrobilogical Studies on the Great Berg River, Western Cape Province. Part 1. General Description, Chemical Studies and Main Features of the Flora and Fauna. Transactions of the Royal Society of South Africa. 35: 125-226

Hart, D.D., Finelli, C.M. 1999. Physical-Biological Coupling in Streams: The Pervasive Effects of Flow on Benthic Organisms. Annual Review of Ecology and Systematics. 30: 363–395

Hawkins, C.P., Murphy, M.L., Anderson, N.H. 1982. Effects of Canopy, Substrate Composition, and Gradient on the Structure of Macroinvertebrate Communities in Cascade Range Streams of Oregon. Ecology. 63: 1840-1856

Helms, B.S., Schoonover, J.E., Feminella, J.W. 2009. Seasonal Variation of Land use Impacts on Macroinvertebrate Assemblages in Streams of Western Georgia, USA. Journal of the North American Benthological Society. 28: 991-1006

Heiring, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., Feld, C., Johnson, R.K., Pinto, P., Skoulikidis, N., Verdonschot, P.F.M., Zahrádková, S. 2003. The Development of a System to Assess the Ecological Quality of Streams based on Benthic Macroinvertebrates – Design of the Sampling Programme within the AQEM Project. International Review of Htdrobiology. 88: 345–361

Hemond, H.F., Benoit, J. 1988. Cumulative Impacts on Water Quality Functions of Wetlands. Environmental Management. 12: 639-653.

Hester, F.E., Dendy, J.S. 1962. A Multiple-Plate Sampler for Aquatic Benthic macroinvertebrates. Transactions of the American Fisheries Society. 91: 4

Hohls, D.R. 1996. National Biomonitoring Programme for Riverine Ecosystems: Framework Document for the Programme. RHP Report Series No. 1. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria

Horrigan, N., Choy, S., Marshall, J., Recknagel, F., 2005. Response of Stream Macroinvertebrates to Changes in Salinity and the Development of a Salinity Index. Marine and Freshwater Research. 56: 825-833

Horner, R., May, C., Ridley, G., Shaver, E., Skupien, J. 2007. Fundamentals of Urban Runoff Management: Technical and Institutional Issues. U.S. Environmental Protection Agency. North American Lake Management Society

Hynes, H.B.N. 1970. The Ecology of Running Waters. Liverpool University Press, Liverpool, UK

Jacobsen, D., Marin, R. 2008. Bolivian Altiplano Streams with Low Richness of Macroinvertebrates and Large Diel Fluctuations in Temperature and Dissolved Oxygen. Aquatic Ecology. 42: 643-656

Johnson, M.R., Anhaeusser, C.R. and Thomas, R.J. 2006. The geology of South Africa, Geological Society of South Africa and Council for Geoscience: Johannessburg

Kalff, J. 2002. Limnology. Prentice Hall, Upper Saddle River.

Kefford, B.J., Papas, P.J., Nugegoda, D., 2003. Relative Salinity Tolerance of Macroinvertebrates from the Barwon River, Victoria, Australia. Marine and Freshwater Research. 54: 755-765.

Kemp, M., de Kock, K.N., Wepner, V., Roets, W., Quinn, L., Wolmarans, C.T. 2014. Influence of Selected Abiotic Factors on Aquatic Macroinvertebrate Assemblages in the Olifants River Catchment, Mpumalanga, South Africa. African Journal of Aquatic Science. 39: 141-149

Kingsford, R.T. 2000. Ecological Impacts of Dams, Water Diversions and River Management on Floodplain Wetlands in Australie. Austral Ecology. 25: 109-127

Kingston, S., Rosel, P. 2004. Genetic Differentiation among Recently Diverged Delphinid Taxa Determined using AFLP Markers. Journal of Heredity. 95: 1–10

Kilroy, C., Biggs, B.J.F. 2002. Use of the SHMAK Clarity Tube for Measuring Water Clarity: Comparison with the Black Disk Method. New Zealand Journal of Marine and Freshwater Research. 36: 519-527

Kirk, J.T.O. 1985. Effects of Suspensoids (Turbidity) on Penetration of Solar Radiation in Aquatic Ecosystems. Hydrobiol. 125: 195-208

Legret, M., Pagotto, C. 1999. Evaluation of Pollutant Loadings in the Runoff Waters from a Major Rural Highway. The Science of Total Environment. 235: 143-150

Lenat, D.R. 1993. A Biotic Index for the Southeastern United States: Derivation and List of Tolerance Values, with Criteria for Assigning Water-Quality Ratings – Journal of North American Benthologic Society. 12: 279–290.

Lenat, D.R., Crawford, K. 1994. Effects of Land Use on Water Quality and Aquatic Biota of Three North Carolina Piedmont Streams. Hydrobiologia. 294: 185-199

Letovsky, E., Myers, I. E., Canepa, A., McCabe, D.J. 2012. Differences between Kick Sampling Techniques and Short-Term Hester-Dendy Sampling for Stream Benthic macroinvertebrates. Biological Society. 83: 47-55

Maldonado, V.G.M. 2010. Land Use Influence on Benthic Macroinvertebrate Communities of streams in Nyangores and Amala Tributaries of Mara River, Kenya. MSc Thesis, UNESCO-IHE Institute for Water Research, Delft, Netherlands

Mallin, M., Williams, K., Esham, E., Lowe, R. 2000. Effect of Human Development on Bacteriological Water Quality in Coastal Watersheds. Ecological Applications. 10: 1047-1056

Mandaville, S.M. 2002. Benthic Macroinvertebrates in Freshwaters-Taxa Tolerance Values, Metrics and Protocols. Soil and Water Conservation Society of Metro Halifax

Mason, W.T., Anderson, J.B., Morrison, G.E. 1967. A Limestone Filled, Artificial Substrate Sampler-Float Unit for Collecting Benthic macroinvertebrates in Large Streams. The Progressive Fish-Culturist. 29: 74

Mason, W.T., Weber, C.I., Lewis, P.A., Julian, E.C. 1973. Factors Effecting the Performance of Basket and Multi-plate Macroinvertebrate samplers. Freshwater Biology. 3: 409-436

Mason, C.F., Parr, B.L. 2003. Long-term Trends in Water Quality and their Impact on Macroinvertebrate Assemblages in Eutrophic Lowland Rivers. Water Research. 37: 2969–2979

McGeoch, M.A. 1998. The Selection, Testing and Application of Terrestrial Insects as Bioindicators. Biological Reviews. 73: 181–201

Medeiros, C., LeBlanc, R., Coler, R.A. 1983. An In Situ Assessment of the Acute Toxicity of Urban Runoff to Benthic Macroinvertebrates. Environmental Toxicology and Chemistry. 2: 119-126

Mesa, L.M. 2012. Interannual and Seasonal Variability of Macroinvertebrates in Monsoonal Climate Streams. Brazilian Archives of Biology and Technology. 55: 403-410

Milhous, R.T., Bartholow, J.M. 2004. Physical Habitat as a Limit to Aquatic Ecosystems. IAHR Congress Proceedings. Fifth International Symposium onEcohydraulics. Aquatic Habitats: Analysis and Restoration. September 12-17, 2004, Madrid, Spain. *In:* Thirion, C. 2007. Module E: Macroinvertebrate Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (version 2). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No.

Moog, O. 2005. Manual on Pro-Rata Multi-Habitat-Sampling of Benthic Invertebrates from Wadeable Rivers in the HKH region. Kathmandu, Deliverable No. 8 - Part 1

Modd, T., Drewes, H.G. 1990. Comparison of Biotic Index Values for Invertebrate Collections from Natural and Artificial Substrates. Freshwater Biology. 23: 171-180

Morse, J.C., Bae, Y.J., Munkhjargal, G., Sangpradub, N., Tanida, K., Vsh ivkova, T.S., Wang, B., Yang, L., Yule, C.M. 2007. Freshwater Biomonitoring with Macroinvertebrates in East Asia. Frontiers in Ecology and the Environment. 5: 33-42

Nielson, D.L., Brock, M.A., Rees, G.N., Baldwin, D.S. 2003. Effects of Increasing Salinity on Freshwater Ecosystems in Australia. Australian Journal of Botany. 51: 655-665

Nedeau, E.J., Merritt, R.W., Kaufman, M.G. 2003. The Effects of an Industrial Effluent on an Urban Stream Benthic Community: Water Quality vs Habitat Quality. Environmental Pollution. 123: 1-13

NWA [National Water Act] 36:1998. Government Printer. Pretoria.

Paul, M. J., Meyer, J. L. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics. 32: 333–365.

Phillips, D.J.H., Rainbow, P.S. 1993. Biomonitoring of Trace Aquatic Contaminants, Environmental Management Series. Chapman and Hall, London, UK

Qu, X., Wu, N., Tang, T., Cai, Q., Park, Y. 2010. Effects of Heavy Metals on Benthic Macroinvertebrate Communities in High Mountain Streams. International Journal of Limnology. 46: 291-302

Quinn, J.M., Hickey, C.W. 1994. Hydraulic Parameters and Benthic Invertebrate Distributions in Two Gravelbed New Zealand Rivers. Freshwater Biology. 32: 489–500

Rader, R.B., Richardson, C.J. 1992. The Effects of Nutrient Enrichment on Algae and Macroinvertebrates in the Everglades: A Review. Wetlands. 12: 121-135

Reddy, K.R., DeLaune, R.D. 2008. Biochemistry of Wetlands: Science and Applications. CRC Press, Taylor and Francis Group, Boca Raton, Florida.

Reece, P. F., Richardson, J. S. 1998. Seasonal Changes of Benthic Macroinvertebrate Communities in Southwestern British Columbia. Aquatic and Atmospheric Sciences Division. Report Number: DOE-FRAP1998-33

Reid, G.K., Wood R.D. 1976. Ecology of Inland Water and Estuaries. D. Van Nostrand Company, New York.

Reigier, H.A., Baskerville, G.L. 1986. Sustainable Redevelopment of Regional Ecosystems Degraded by Exploitive Development, Pp. 75–103. In: Clark, W.C., and Munn, R.E. (Eds). 1986. Sustainable Development of the Biosphere. Cambridge University Press, Cambridge

Relyea, R. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecological Applications. 15: 618-627

Resh, V.H., Norris, R.H., Harbour, M.T. 1995. Design and Implementation of Rapid Assessment Approaches for Water Resource Monitoring using Benthic Macroinvertebrates. Australian Journal of Ecology. 20: 108-121

Rhodes, A.L., Newton, R.M., Pufall, A. 2001. Influences of Land Use on Water Quality of a Diverse New England Watershed. Environmental Science and Technology. 35: 3640-3645

Richards, C., Host, G.E. 1993. Identification of Predominant Environmental Factors Structuring Stream Macroinvertebrate Communities within a Large Agricultural Catchment. Freshwater Biology. 29: 285-294

Roby, K.B., Newbold, J.D., Erman, D.C. 1978. Effectiveness of an Artificial Substrate for Sampling Benthic macroinvertebrates in small streams. Freshwater Biology. 8: 1-8

Roline, R.A. 1988. The Effects of Heavy Metal Pollution of the Upper Arkansas River on the Distribution of Aquatic Macroinvertebrates. Hydrobiology. 160: 3-8

Rogers, F.E.J. 1983. Wetlands as Accreting Systems: Wastewater Treatment. Journal of the Limnological Society of South Africa. 9: 110-116.

Rosenberg, D.M. and V.H. Resh. 1982. The Use of Artificial Substrates in the Study of Freshwater Benthic macroinvertebrates. 175-235. In: Cairns, J.Jr. (editor) Artificial substrates. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Schofield, K., Seager, J., Merriman, R.P. 1990. The Impact of Intensive Dairy Farming Activities on River Quality: The Eastern Cleddau Catchment Study. Journal of the Institution of Waterand Environmental Management. 4: 176-186

Schulz, R., Liess, M. 1999. A field study of the effects of agriculturally derived insecticide input on stream macroinvertebrate dynamics. Aquatic Toxicology. 46: 155-176

Sheoran, A.S., Sheoran, V. 2006. Heavy Metal Removal Mechanisms of Acid Mine Drainage in Wetlands: A Critical Review. Minerals Engineering. 19: 105-116

Shi, P., Yuan, Y., Zheng, J., Wang, J., Ge, Y., Qui, G. 2007. The Effects of Land Use/Cover Change on Surface Runoff in Shenzen Region China. Catena. 69: 31-35

Sinokrot, B.A., Stefan, H.G. McCormick, J.H., Eaton, J.G. 1995. Modelling of Climate Change Effects on Stream Temperatures and Fish Habitats below Dams and Near Groundwater Inputs. Climatic Change. 30: 181-200

Skinner, J.A., Lewis, K.A. Bardon, K.S. Tucker, P. Catt, J.A. Chambers, B.J. 1997. An Overview of the Environmental lmpact of Agriculture in the U.K. Journal of Environmental Management. 50: 111-128

Smedley, P.L., Kinniburgh, D.G. 2001. Synthesis Report on Arsenic in Drinking Water: Chapter 1. Source and Behaviour of Arsenic in Natural Waters. British Geological Survey, Wallingford, U.K.

Smith, R.A., Alexander, R.B., Wolman, M.G. 1987. Water-quality trends in the Nation's rivers. Science. 235: 1607-1615

Smith, M.J., Kay, W.R., Edward, D.H.D., Papas, P.J., Richardson, K.S.J., Simpson, J.C., Pinder, A.M., Cale, D.J., Horwitz, P.H.J., Davis, J.A., Yung, F.H., Norris, R.H., Halse, S.A. 1999. AusRivAS: Using Macroinvertebrates to Assess Ecological Condition of Rivers in Western Australia. Freshwater Biology. 41: 269-282

Smolders, A.J.P., Lock, R.A.C, Van der Velde, G., Medina Hoyos, R.I., Roelofs, J.G.M. 2003. Effects of Mining Activities on Heavy Metal Concentrations in Water, Sediment, and Macroinvertebrates in Different Reaches of the Pilcomayo River, South America. Archives of Environmental Contamination and Toxicology. 44: 314-323

Suren, A. M., Riis, T., Biggs, B. J. F., McMurtrie, S., Barker, R. 2005a. Assessing the Effectiveness of Enhancement Activities in Urban Streams: 1. Habitat Responses. River Research and Applications. 21: 381-401

Suren, A.M. 2001. Effects of Urbanisation. In: Hall, M.J., Gerhard, P.C., Ralph, H.R. 2001. Relationship between Land Use and Stream Invertebrate Community Structure in a South Island, New Zealand, Coastal Stream Catchment. New Zealand Journal of Marine and Freshwater Research. 35: 591-603

Stark, J.D. 1993. Performance of the Macroinvertebrate Community Index: Effects of Sampling Method, Sample replication, Water Depth, Current Velocity, and Substratum on Index Values. New Zealand Journal of Marine and Freshwater Research. 27: 463-478

Stearns, R.H. 1916. Decolonization of Water by Storage. Journal of the New England Water Works Association 30:20-34 In*:* Baxter, R.M. 1977. Environmental Effects of Dams and Impoundments. Review of Ecology and Systematics. 8: 255-283

Stein, E.D., Tiefenthaler, L.L., Schiff, K. 2006. Watershed-Based Sources of Polycyclic Aromatic 3-72 Fundamentals of Urban Runoff Management Hydrocarbons in Urban Storm water. Environmental Toxicology and Chemistry. 25: 373-385.

Steuer, J., Selbig, W., Hornewer, N., Prey, J. 1997. Sources of Contamination in an Urban Basin in Marquette, Michigan and an Analysis of Concentrations, Loads, and Data Quality. US Geological Survey (USGS) Water Resources Investigation Report 97-4242

Stewart, P.M., Butcher, J.T., Swinford, T.O. 2000. Land Use, Habitat, and Water Quality Effects on Macroinvertebrate Communities in Three Watersheds of a Lake Michigan Associated Marsh System. Aquatic Ecosystem Health and Management. 3: 179-189

Strecker, E.W., Kersnar, J.M., Driscoll, E.D., Horner, R.R., Davcenport, T.E. 1992. The Use of Wetlands for Controlling Stormwater Pollution. The Terrene Institute, Washington, DC.

Sweeting, R.A. 1994. River Pollution. 23-32 In: Calow, P., Petts, G.E. (Eds.) The Rivers Handbook, Vol2. Blackwell Scientific Publications, Oxford

TEEB. 2011. The TEEB Manual for Cities: Ecosystem Services in Urban Management.

Thirion, C. 2000. A New Biomonitoring Protocol to Determine the Ecological Health of Impoundments using Artificial Substrates. African Journal of Aquatic Science. 25:1

Thirion, C. 2007. Module E: Macroinvertebrate Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (version 2). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No.

Uys, M.C. 1994. Classification of Rivers and Environmental Health Indicators. Proceedings of a joint South African /Australian workshop. February 7-14 1994, Cape Town, South Africa. Water Research Commission Report No. TT 63/94

Wallace, J.B., Anderson, N.H. 1996. Habitat, Life History, and Behavioural Adaptations of Aquatic Insects. *In:* Kemp, M., de Kock, K.N., Wepner, V., Roets, W., Quinn, L., Wolmarans, C.T. 2014. Influence of Selected Abiotic Factors on Aquatic Macroinvertebrate Assemblages in the Olifants River Catchment, Mpumalanga, South Africa. African Journal of Aquatic Science. 39(2): 141-149

Ward, J.V. 1985. Thermal Characteristics of Running Water. Hydrobiology. 125: 31-46

Wheeler, A. P., Angermeier, P. L. Rosenberger, A. E. (2005). Impacts of New Highways and Subsequent Urbanization on Stream Habitat and Biota. Reviews in Fisheries Science. 13: 141-164

Wilber, W.G., Hunter, J.V. 1977. Aquatic transport of heavy metals in the urban environment. Water Resources Bulletin 13. 4: 721-734

Winter, J.G., Duthie, H.C. 1998. Effects of Urbanisation on Water Quality Periphyton and Invertebrtate Communities in a Southern Ontario Stream. Canadian Water Resource Journal. 23: 245-257

Wren, C.D., Stephenson, G.L. 1990. The Effects of Acidification on the Accumulation and Toxicity of Metals to Freshwater Invertebrates. Environmental Pollution. 71: 205-241

Wright, J.F., Sutcliffe, D.W., Furse, M.T. 2000. Assessing the Biological Quality of Fresh Waters: RIVPACS and Other Techniques. Freshwater Biological Association. Ambleside, UK

Appendix

Time	Site	Repetition	Hydropsychidae	Ecnomidae	Baetidae	Caenidae	Leptophlebidae	Coenagrionidae	Corixidae	Belostomatidae	Naucoridae	Chironomidae	Psychodidae	Oligochaeta	Potamonautidae	Physidae	Lymnaeidae	Ancylidae	Planorbidae	Thiaridae	Hirudinea
November	A	1	0	0	0	0	0	0	0	0	0	34	0	2	0	12	0	0	0	0	1
November	A	2	0	0	0	0	0	0	0	0	0	1	0	1	0	6	0	0	0	0	1
November	A	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
November	A	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
November	B	1	0	0	0	0	0	0	0	0	0	21	0	51	0	0	0	0	0	0	0
November	B	2	0	0	0	0	0	0	0	0	1	29	0	13	0	18	0	0	1	0	0
November	B	3	0	0	0	0	0	0	0	0	0	1	0	6	0	11	0	0	0	0	1
November	B	4	0	0	0	0	0	0	0	0	0	24	0	19	0	5	0	0	0	0	33
November	C	1	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0
November	C	2	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	5	0	0	0
November	C	3	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	8	1	0	0
November	C	4	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	7	1	0	0
November	D	1	0	0	0	0	0	0	0	0	0	33	0	13	0	0	1	3	7	0	10
November	D	2	0	0	0	0	0	0	0	0	0	7	1	16	0	0	1	2	1	0	13
November	D	3	0	0	0	0	0	1	0	0	0	5	0	57	0	0	0	2	2	0	4
November	D	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
November	E	1	0	0	0	0	0	0	0	0	0	12	0	15	0	0	0	1	0	0	0
November	E	2	0	0	0	1	0	0	0	0	0	43	0	0	0	0	0	3	0	0	0
November	E	3	0	0	0	0	0	0	0	0	0	11	0	1	0	0	0	3	0	0	0
November	E	4	0	0	0	0	0	0	0	0	0	18	0	13	0	0	0	0	0	0	0
December	A	1	0	0	0	0	0	0	0	0	0	94	1	5	1	4	0	0	0	0	3
December	A	2	0	0	0	0	0	0	0	0	0	442	2	3	9	2	0	0	0	0	2
December	A	3	0	0	0	0	0	0	0	0	0	162	0	3	1	0	0	0	0	1	0
December	A	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	B	1	0	0	0	0	0	0	0	0	0	155	0	9	25	25	0	1	0	0	31
December	B	2	0	0	0	0	0	0	16	0	0	8	0	66	4	12	1	1	1	0	39
December	B	3	0	0	1	0	0	0	0	0	0	16	0	29	0	5	0	0	0	0	11
December	B	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	C	1	0	0	1	0	0	1	1	0	1	7	0	2	0	0	0	0	0	0	0
December	C	2	0	0	8	0	0	0	4	0	0	4	0	2	0	0	0	0	0	0	0
December	C	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	C	4	0	0	55	0	0	0	8	0	2	32	0	0	3	0	0	3	0	0	0
December	D	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	D	2	0	1	12	1	0	0	0	0	0	62	0	0	6	0	0	18	0	0	2
December	D	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	D	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	E	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	E	2	0	0	5	0	0	1	1	0	0	59	0	7	3	0	0	4	0	0	0
December	E	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
December	E	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	A	1	0	0	0	0	0	0	0	0	0	70	2	5	0	1	0	0	0	0	2
February	A	2	0	0	0	0	0	0	0	0	0	32	8	0	0	0	0	0	0	0	2
February	A	3	0	0	0	0	0	0	0	0	0	16	7	0	0	5	0	0	0	0	8
February	A	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	B	1	0	0	4	0	0	0	0	0	0	0	0	21	1	0	1	1	0	0	6
February	B	2	0	0	1	0	1	0	12	0	0	2	0	25	1	2	0	1	0	0	9
February	B	3	0	0	3	0	0	0	3	1	0	0	0	13	1	2	0	1	0	0	6
February	B	4	0	0	0	0	0	0	3	0	0	0	0	4	0	0	0	0	0	0	11
February	C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	C	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	C	4	2	0	47	0	0	0	0	0	0	32	0	1	6	0	0	1	1	0	0
February	D	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	D	2	0	0	0	0	0	0	0	0	0	7	0	3	3	1	0	1	1	0	1
February	D	3	0	0	0	0	0	1	0	1	0	7	0	7	0	0	0	17	2	0	10
February	D	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	E	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	E	2	0	0	1	0	0	0	0	0	0	8	0	1	5	0	0	0	0	0	0
February	E	3	0	0	0	0	0	0	0	0	0	2	0	0	6	0	0	1	0	0	0
February	E	4	0	0	0	0	0	0	0	0	0	14	0	0	11	0	0	0	0	0	0

Table A1: Abundance of macroinvertebrate families sampled at various times, sites and repetitions along the upper Hartbeesspruit.

Digital Appendices

The following are included in the companion CD.

Appendix A2: Analysed data used in this study.

Table A2.1: Abundance of macroinvertebrate families sampled at various times, sites and repetitions along the upper Hartbeesspruit.

Table A2.2: Values of chemical parameter field results sampled at various times, sites and repetitions along the upper Hartbeesspruit.

Table A2.3: Values of chemical parameter lab results sampled at various times, sites and repetitions along the upper Hartbeesspruit.

Table A2.4: Rainfall (mm) per day for one year, ranging from 1st April 2013- 31st March 2014, along the upper Hartbeesspruit.

Table A2.5: The percentage riparian cover type along the first 40 metres of various sites along the upper Hartbeesspruit.

Table A2.6: The percentage microhabitat type within the aquatic zone along the first 40 metres of various sites along the upper Hartbeesspruit.

Appendix A3: Raw analytical results received from the laboratories.

Table A3.6: Water quality laboratory results for September 2013 samples along the upper Hartbeestspruit.

Certificate A3.1: Final certificate of analysis for September 2013 samples along the upper Hartbeestspruit.

Table A3.2: Water quality laboratory results for November 2013 samples along the upper Hartbeestspruit.

Certificate A3.2: Final certificate of analysis for November 2013 samples along the upper Hartbeestspruit.

Table A3.3: Water quality laboratory results for Decemberr 2013 samples along the upper Hartbeestspruit.

Certificate A3.3: Final certificate of analysis for December 2013 samples along the upper Hartbeestspruit.

Table A3.4: Water quality laboratory results for February 2013 samples along the upper Hartbeestspruit.

Certificate A3.4: Final certificate of analysis for February 2013 samples along the upper Hartbeestspruit.