THE ECOLOGICAL IMPACTS OF POLLUTION ON A RIVER ECOSYSTEM: A COMMUNITY INDEX AND STABLE ISOTOPE APPROACH

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ABSTRACT

For decades, urbanized rivers have been modified to meet the needs of constantly expanding human populations in many countries around the world. The Bloukrans River in Grahamstown is one of the polluted and structurally modified urban rivers in South Africa, and there is no published information regarding its water quality and ecological status. Water quality is threatened by human activities including the disposal of treated and raw sewage, livestock farming, and agriculture. This study was conducted to determine the ecological status of the river by assessing its biological, chemical, and physical components in relation to man-mediated activities. Biological responses of macroinvertebrates were used to assess changes in water quality through space and time based on the South African Scoring System version 5 and the Average Score per Taxon water quality indices. The results showed poor water quality due to high nitrate and ammonium concentrations derived from sewage, and channel modifications by agricultural activities and dumping of solid waste. Channel width, water depth, dissolved oxygen, nitrate, and ammonium concentrations were the main drivers of macroinvertebrate distribution patterns and had the most influence on the variability in macroinvertebrates taxa richness, diversity and abundance. Diatoms were also used to assess water quality, specifically to indicate the trophic status of the river based on changes in the Trophic Diatom Index. The results suggested that the Bloukrans River was eutrophic during the course of this study. However, the trophic status varied with freshwater input, resulting in mesotrophic conditions during flooding and eutrophication in dry seasons. Changes in pH, phosphate concentration, water velocity (current speed), and temperature influenced the distribution of diatoms in the Bloukrans River. However, only pH was important at the community level and significantly influenced diatom abundances. Stable nitrogen isotope ratios ($\delta^{15}N$) of autotrophs and primary and secondary consumers revealed noticeable differences between tissues of organisms exposed to treated sewage and those without any exposure. The $\delta^{15}N$ values in biota occurring above the sewage treatment discharge point were low, and those collected below the sewage point were higher. Although fertilizer derived nitrogen is generally depleted in ^{15}N, agriculture-derived nitrogen could not be excluded as a possible source since animals at the sample site that was most affected by agricultural activities had the highest $\delta^{15}N$ values. This study provided valuable information on the ecological status of the Bloukrans River and identified the major activities associated with reduced biodiversity and water quality.

Keywords: water quality, biomonitoring, eutrophication, stable isotope tracers, diatoms, macroinvertebrates, community indices

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ABBREVIATIONS AND ACRONYMS

% PTV	Percentage of Pollution Tolerant Valves
ASPT	Average Score per Taxon
BMWP	Biological Monitoring Working Party
CSIR	Council for Scientific and Industrial Research
DEAT	Department of Environmental Affairs and Tourism
DO	Dissolved Oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
FAII	Fish Assemblage Integrity Index
GSM	Gravel Sand and Mud
IDP	Integrated Development Plan
IHI	Index of Habitat Integrity
NAEHMP	National Aquatic Ecosystem Health Monitoring Programme
NWA	National Water Act
NPS	Non-Point source
NWRS	National Water Resources Strategy
RHP	River Health Programme
RVI	Riparian Vegetation Index
SASS	South African Scoring System
TDI	Trophic Diatom Index
TDS	Total Dissolved Solids
WMS	Weighted Means index
WRC	Water Research Commission

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DECLARATION

I declare that this thesis submitted for a degree at Rhodes University (Grahamstown, South Africa) has never been submitted to any other university. The work presented here is that of the author unless otherwise stated.

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1 GENERAL INTRODUCTION

Water scarcity has become a global concern affecting billions of people worldwide (Hassan *et al.* 2005). This is no surprise since the freshwater available for aquatic life and human consumption represents 0.26% of the world's resources (Shiklomanov 1998). Freshwater availability outweighs the current water demands as human population growth expands more rapidly each year (Shiklomanov 1998), and it serves as a natural resource for domestic use, agriculture, power generation, waste disposal, navigation routes, and recreational activities (Malmqvist & Rundle 2002). Freshwater is a particularly scarce natural resource in most parts of South Africa (DWAF 2004). The country is located in the semi-arid part of the world, where climatic conditions vary from desert and semi-desert to sub-humid, with an average annual rainfall of about 450 mm (DWAF 2004). There is a high annual evaporation rate (1100 to 3000 mm) relative to the average annual rainfall (Dallas 2000a; DWAF 2004). Although the supply of water is limited, a majority of South Africans depend primarily on running water rather than harvested rainwater (DWAF 2004). The importance of water to man has driven a surge of human settlement closer to rivers and streams (Paul & Meyer 2001; Malmqvist & Rundle 2002), resulting in increased urbanization and industrialization in catchment areas (Malmqvist & Rundle 2002; Vörösmarty *et al.* 2013). Most river channels have been modified by the construction of dams and reservoirs for water storage (Davies & Day 1998). Water pollution becomes an important issue following urbanization and expanding human populations, especially in developing countries (Dudgeon 1992) including South Africa (Thiere & Schulz 2004; Oberholster *et al.* 2008; Simaika & Samways 2010), simply because most developing countries lack sufficient and adequate water treatment facilities and have limited knowledge regarding natural water resource management and conservation (Dudgeon 1992; Malmqvist & Rundle 2002).

1.1 WATER POLLUTION IN URBAN RIVERS

Although water quality should ideally be controlled by natural processes such as the rates of precipitation and weathering, anthropogenic influences from land use could significantly contribute to variations in water chemistry and quality (Carpenter *et al.* 1998; Jarvie *et al.* 1998; Singh *et al.* 2005). Large amounts of pollutants from diffuse and point sources easily find their way into urban rivers (Singh *et al.* 2005) through run off from agriculture, storm water drains, and road ways (Ongley *et al.* 2010). Diffuse source nutrients such as nitrogen (N) and phosphorus (P) are responsible for eutrophication, hypoxia, and water quality degradation in many rivers (Nikolaidis *et al.* 1998). Researchers have modelled the movements of nutrients from agricultural landscapes into some Australian rivers (Hunter & Walton 2008; Thorburn *et al.* 2011; Biggs *et al.* 2013), but quantifying diffuse pollutants is time consuming and expensive (Kemp *et al.* 2014). Point source discharges such as sewage effluents also contribute to nutrient influx in lowland rivers (Neal *et al.* 2000). In South Africa, the Water Act (Act 54 of 1956) requires that water extracted from rivers for industrial and municipal use is returned to rivers as treated effluent based on acceptable standards (Morrison *et al.* 2001). Returning treated effluent to rivers seems reasonable from a natural resource management standpoint, but the amounts of phosphorus and nitrogen entering the system could easily increase above the recovery capabilities of a river (Yount & Niemi 1990), especially when effluent treatment standards are not met due to lack of adequate infrastructure (Schoeman & Steyn 2003; Akcil & Koldas 2006). Maintaining river health in the presence of expanding human development has received much attention over the years (e.g. Allan & Flecker 1993; Harding *et al.* 1998; Sponseller *et al.* 2001).

The Bloukrans River in Grahamstown is a small polluted urban river in South Africa without documented information regarding its water quality status. Water quality is threatened by human activities including sewage effluent disposal, livestock farming and agriculture. The river provides habitat for a variety of aquatic plants and animals, and is used by humans for recreational and religious purposes. This study investigates the ecological integrity of the Bloukrans River by assessing the influence of anthropogenic pollution on diatoms and macroinvertebrates based on South African aquatic ecosystem guidelines.

1.1.1 The National River Health Programme (RHP)

In 1994, the country's first national biomonitoring programme for river ecosystems (i.e. the RHP) was initiated by the Department of Water Affairs and Forestry (DWAF 2006). The River Health Programme (RHP) was a successful achievement designed in collaboration with entities including the Department of Environmental Affairs and Tourism (DEAT) and the Water Research Commission (WRC), and aided by the Council for Scientific and Industrial Research (CSIR). The RHP was designed to provide necessary information about the ecological state of rivers, which would help in the development of a long-term and sustainable management system (DWAF 2006). The RHP uses the responses of fish, macroinvertebrates and riparian vegetation to changes in abiotic factors such as habitat, geomorphology, hydrology, and water chemistry to characterize a site and determine its water quality status (DWAF 2000). River health indices were developed to suit different biological indicators. For example, the Fish Assemblage Integrity Index (FAII) for fish, the South African Scoring System (SASS) for macroinvertebrates, and the Riparian Vegetation Index (RVI) for vegetation (DWAF 2006). To aid the interpretation of the biotic indices, the Index of Habitat Integrity (IHI) that assesses the impact of human disturbance on the riparian and in-stream habitats was designed (DWAF 2006). Such disturbances could be in the form of bed and channel modifications, removal of indigenous riparian vegetation, water abstraction and flow regulation (DWAF 2006). Increased anthropogenic disturbances not only affect water quality, but they also alter the natural occurrence of important ecological processes such as nutrient cycling, reproduction, and the feeding habits of aquatic animals (Hassan *et al.* 2005). When the natural state of a river is changed, aquatic residents detect the change and respond in ways that scientists can identify through rapid biomonitoring (e.g. Li *et al.* 2010; Culp *et al.* 2011). In South Africa, as in other countries such as the United States, Chile and Australia, the method of biomonitoring has gained much attention (Dallas 2012).

1.2 MANAGEMENT OF WATER RESOURCES IN SOUTH AFRICA

The national Department of Water and Sanitation (DWS), formerly DWAF, is the lead agent authorized by the government to ensure the best management and rapid monitoring of water resources (DWAF 1997). This department performs its duties under a number of policies and legislations, chief of which is the National Water Act of 1998 (Act 36 of 1998) which seeks to provide long-term protection and equal distribution of water resources to all citizens (NWA 1998). One of the core responsibilities of DWS is to develop, implement, and sustain necessary monitoring programmes that will continuously provide information about the water quality status of different systems through a national water resource strategy framework (DWAF 2004). To achieve that goal, a National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) was initiated to develop a national database of aquatic ecosystems that would document all water quality reports countrywide (DWAF 2006). Within the NAEHMP are sub-monitoring programs including those that monitor rivers (e.g. the River Health Programme), wetlands, estuaries, and groundwater dependent ecosystems (DWAF 2004).

1.2.1 Biological Assessment and Monitoring

Biological monitoring, often referred to as 'biomonitoring', can be defined as "an evaluation of the condition of a water body using biological surveys and other direct measurements of the resident biota in surface waters" (Engel & Voshell 2002). Members of aquatic communities spend part or all of their life cycles in rivers, exposing themselves to various physical and chemical influences that occur over time (Roux *et al.* 1999), so they are considered good indicators of ecological health (Dallas 2012). The ultimate goal of biological monitoring is to evaluate the effect of human activities on biological resources (Fore *et al.* 1996). The evaluation of anthropogenic activities in rivers has been in practice even before the establishment of the River Health Programme in South Africa (Roux *et al.* 1996). In the past several decades, information provided to assist the management of ecological resources was mostly non-ecological (Roux *et al.* 1996), as river monitoring was focused on measuring chemical and physical variables (Oberholster *et al.* 2008). However, the challenge with measuring only chemical and physical variables is that no information is provided on the biota (Roux *et al.* 1996). River ecosystems are complex, and factors such as water chemistry, flow regime, habitat geomorphology, biological interactions, and energy sources contribute to the ecological integrity of river ecosystems (Karr 1993; Ollis *et al.* 2006). However, it is logistically difficult to measure all of these contributing factors in one setting (Ollis *et al.* 2006). Difficulties in measuring the complex nature of river systems have led to the emphasis of biological indicators as tools to determine the ecological status of a river (Karr 1999). According to Markert *et al.* (1999), a bioindicator is "an organism or a part of an organism or a community of organisms that contains information on the quality of the environment or a part of the environment". A good or ideal biological indicator should satisfy some of the following characteristics: (1) easy to identify using taxonomical methods, (2) present in local habitats and having low mobility, (3) high sensitivity to environmental disturbances, (4) well-known ecological characteristics such as position in food the chain, (5) wide distribution and abundance for continuous sampling and comparisons, (6) life span long enough for comparisons between age groups, and (7) high ability for quantification and standardization (Hilty & Merenlender 2000; Zhou *et al.* 2008). Biological indicators can be applied at different levels of organization (Adams

& Greeley 2000). For the purpose of this study, macroinvertebrate and diatoms were used at species and community levels to assess abundance/dominance, diversity, richness, tolerance and sensitivity levels, and the biological integrity of the river.

1.3 THE USE OF AQUATIC MACROINVERTEBRATES IN BIOMONITORING

Lotic ecosystems are characterized by macroinvertebrate species whose presence or absence potentially indicates unfavorable conditions in the habitat (Walker 1992). Macroinvertebrates provide a rage of essential services that are critical in maintaining a river's functional integrity (Angermeier & Karr 1996). The most critical role of these organisms is their ability to acquire energy from the primary producers and serve as links to the upper levels of the food web (Wallace & Webster 1996), and many species occupy wide ranges of trophic levels and pollution tolerances (Li *et al.* 2010; Pelletier *et al.* 2010). Benthic macroinvertebrates have been commonly used in the biological assessment of the health and water quality of lotic ecosystems (e.g. Delong & Brusven 1998; Rempel *et al.* 2000; Bonada *et al.* 2007; Johnson *et al.* 2013), mainly because of their unique characteristics. First, they are sedentary and abundant in most habitats (Ollis *et al.* 2006) and are therefore representative of site-specific ecological conditions (Li *et al.* 2010). Second, their life cycles are long enough to detect temporal changes caused by disturbances, while having life spans short enough to enable the observation of recolonization patterns following a disturbance (Ollis *et al.* 2006). Finally, macroinvertebrates respond relatively quickly to pollution, and responses to different types of pollution have been established for many species (Ollis *et al.* 2006; Pelletier *et al.* 2010). Macroinvertebrate community structure often changes in response to environmental disturbances through a reduction in taxa diversity and an increase in the dominance of species with rapid reproduction rates and short life cycles (Gray 1989). Such responses were reported for rivers that were impacted by heavy metals (Clements 1994; Hickey & Clements 1998) and organic pollution (Whitehurst & Lindsey 1990; Bacey & Spurlock 2007). Pollutats tend to reduce species diversity and richness, resulting in the dominance of tolerant species in highly polluted areas and sensitive species in areas of fewer disturbances (Whitehurst & Lindsey 1990; Bacey & Spurlock 2007). In many countries, macroinvertebrates are used in the biological monitoring of lotic systems that are impacted by stressors such as nutrient enrichment (Hering *et al.* 2006; Justus *et al.* 2010), organic pollution (Gray 2010), and hydromorphological degradation (Lorenz *et al.* 2004; Friberg *et al.* 2009). In South Africa, early work on the application of macroinvertebrates as monitoring tools dates back to the 1950's and 1960's (Ollis *et al.* 2006), when numerous surveys were conducted across several rivers (e.g. Chutter 1972; King 1981; Scott 1988). The use of macroinvertebrates gained popularity over time, and this is currently the most applied and reliably tested method for the assessment of water quality in the country (e.g. Dickens & Graham 2002).

1.4 THE USE OF FRESHWATER DIATOMS IN BIOMONITORING

Diatoms are microscopic algal cells that represent a primary producer level of riverine food webs (Mayer & Likens 1987). There are two main groups of diatoms, distinguished according to their habitats: (1) the river plankton is commonly made of floating communities that include species from the genera *Stephanodiscus*, *Cyclotella* and *Cyclostephanos* (Round 1991), and (2) the river benthos is characterized by communities that form attached colonies on the surfaces of embedded substrates (Round 1991). The benthic diatoms are found in four major habitats: epiphyton occurs on the surfaces of aquatic plants, epilithon colonizes stones, epipsammon occurs within the sand, and epipelon colonizes silted surfaces (Round 1991). Diatoms have rapid reproduction rates and very short life cycles, strategies that allow them to successfully inhabit most water bodies (Sabater & Roca 1992; Passy 2001). Because they are found everywhere in streams, diatom communities can indicate sudden or gradual changes in water quality (McCormick & Stevenson 1998). These organisms are sensitive to changes in water velocity, temperature, grazing by consumers, and nutrient levels (Pan *et al.* 1996). For example, Salomoni *et al.* (2006) found that species abundance and composition of epilithic diatoms were highly controlled by pollution gradients of phosphate, ammonium, total organic nitrogen, and faecal coliforms in the Gravataı´ River, Brazil. Jüttner *et al.* (2003) reported that diatom richness and diversity were high in streams that drained agricultural fields and urban settlements. Their study specifically identified species that belonged to the genera *Navucula* and *Planotidium* as those most abundant in sites that were characterized by high levels of conductivity and concentrations of NO_3^-, PO_4^{3-}, N, and Cl^- (Jüttner *et al.* 2003). The high abundances of *Navucula* and *Planotidium* were not surprising since these taxa are very common in electrolyte rich waters and can tolerate extremely polluted waters, especially organically polluted areas (Taylor *et al.* 2007). Diatoms have diverse tolerances to water pollution, especially the concentrations of nutrients (Rey *et al.* 2004). Early ecologists who conducted studies in polluted streams reported two different responses from diatom communities:

either diatoms are enhanced (Bahls 1973), or negatively impacted (Berthon *et al.* 2011) by increased concentrations of nutrients. Habitat availability and structure also influence the composition and diversity of diatoms (Kutka & Richards 1996). The amount of light penetrating the water column affects diatom succession, especially during long photoperiods in summer when photosynthesis rates are high (Ajithamol *et al.* 2014). Moreover, at very high water velocities most diatoms (especially pelagic forms) tend to be swept away (Ghosh & Gaur 1998). Diatom assemblages also differ with habitat heterogeneity, and are often affected by bank conditions, substrate embeddedness, and the slope of microhabitats (Kutka & Richards 1996).

1.5 ECOLOGICAL ASSESSMENT OF RIVERS USING BIOTIC INDICES

A biotic index measures the quality of an environment by the types of organisms that reside in it (Lenat 1993). Biological community data can be summarized and presented as simple numeric or categorized indices (Ollis *et al.* 2006). Biotic indices allow for species to be allocated scores based on their relative abundance in conjunction with their sensitivity or tolerance to pollution (Ollis *et al.* 2006). Scores allocated per taxon are summed and/or averaged to provide a value by which site specific water integrity can be gauged (Ollis *et al.* 2006). Amongst the most widely used biotic indices is the Biological Monitoring Working Party (BMWP) scoring index that was initially developed by Armitage et al. (1983) and Hawkes (1979) to biologically classify water quality in British rivers. Since its development, the BMWP has been extensively employed in Thailand (Mustow 2002), the United Kingdom (Metcalfe 1989), Europe and Spain (Rodriguez & Wright 1991; Zamora-Muñoz & Alba-Tercedor 1996). Later on, the BMWP was modified to suit a range of different areas including the Iberian Peninsula (Zamora-Muñoz *et al.* 1995) and South Africa (Dickens & Graham 2002). After several validity tests were completed in South African rivers, the BMWP was formally adopted and modified into the South African Scoring System (SASS) through the work of Chutter in the 1990's (Dickens & Graham 2002).

1.5.1 The South African Scoring System Version 5 (SASS5)

The South African Scoring System is a macroinvertebrate based biotic index that assesses the ecological condition of rivers that are potentially subject to anthropogenic influences (Dickens & Graham 2002). The SASS method was specifically developed for quick and cost effective assessment of water quality in South African rivers (Chutter 1972). Macroinvertebrate families are given sensitivity scores ranging from 1 to 15 in increasing order of their sensitivity to water quality changes, and the results are expressed as an index score (SASS Score) and an average score per recorded taxon (ASPT) value (e.g. Dickens & Graham 2002; Bere & Nyamupingidza 2013). Early versions of the SASS have been vigorously tested and modified to suit a range of habitats and account for catchment structure (Dickens & Graham 2002). The current version (SASS5) has received preference from environmental practitioners and is currently used as the backbone of the River Health Programme (Uys *et al.* 1996; Dickens & Graham 2002). The application of SASS has not only yielded great success in South Africa (Dallas 2004; Vos *et al.* 2004), but also in several Southern African countries including Zimbabwe, Zambia and Mozambique (Bere & Nyamupingidza 2013). In Namibia, SASS has been modified and standardized into the Namibian Scoring System (NASS) to account for additional tropical invertebrate taxa that occur specifically in northern Namibia (Palmer & Taylor 2004). Although SASS5 can detect past and present ecological changes in rivers, it could easily be affected by factors such floods, seasonal variation in habitat conditions, and the reproduction cycles of priority taxa (DWAF 2000; Dickens & Graham 2002; Dallas 2004). For these reasons, most studies have suggested that one group of organisms may not be entirely reliable to reflect the overall ecological integrity of an environment (Triest *et al.* 2001; Belore *et al.* 2002; Hering *et al.* 2006; Beyene *et al.* 2009). The current study was designed to assess the responses of both macroinvertebrates and diatoms to anthropogenic disturbances.

1.5.2 The Trophic Diatom Index (TDI)

The TDI is amongst the most evaluated indices for its potential use as a tool to determine the effects of pollution, especially eutrophication, in aquatic ecosystems (Potapova *et al.* 2004; Atazadeh *et al.* 2007). This index was developed in the 1990's with the aim to using diatom responses as indicators of eutrophication in European rivers (Kelly & Whitton 1995). The TDI was later adopted in South Africa and was standardized using the BMWP (macroinvertebrate index) as a benchmark for allocating diatom sensitivity values (Kelly 1998). TDI scores range between 0 and 100, with the largest values indicating high levels of eutrophication (Kelly 1998; Kelly *et al.* 2001). This biotic index has been successfully used in different geographical regions including China (Yang *et al.* 2015), Poland (Rakowska & Szczepocka 2011), Kenya (Ndiritu *et al.* 2006), and South Africa (Taylor *et al.* 2007), as the specific climatic conditions and environmental pressures of the region were taken into account. Diatom based indices have been frequently implemented in water quality analyses (Kelly & Whitton 1995; Rott *et al.* 1998; Belore *et al.* 2002). The dominance of nutrient sensitive species like *Epithemia adnata*, *Eunotia pectinalis* and *Rhopalodia* sp. coupled with low abundances of species that prefer nutrient rich environments is indicative of brackish, alkaline and electrolyte-rich environments (Kelly & Whitton 1995; Kelly 1998). A study conducted by Atazadeh *et al.* (2007) to evaluate the use of the TDI in Iran reported that high values were significantly associated with high phosphate and nitrate concentrations in sites impacted by human disturbances. Such findings led the researchers to conclude that the application of the TDI for monitoring ecological conditions of rivers in Iran was suitable to diagnose causes of river impairment (Atazadeh *et al.* 2007). There is still more to be done in South African rivers regarding the usage of the TDI and its validity for local rivers.

1.5.3 The Index of Habitat Integrity (IHI)

The IHI assesses the presence, if any, and severity of anthropogenic disturbances and the damage they cause in a river (Dallas 2005). Disturbances including water abstraction, weirs, dams, pollution, and dumping of rubble that modify the habitat are assessed on a field-based site assessment approach (Dallas 2005). Habitat assessment includes identifying in-stream and riparian zone integrity by compiling information about the presence of floods, water clarity, nutrients, bed modification, algal growth, and other in-stream physico-chemical factors (Kleynhans *et al.* 2008). Determining the state of the riparian zone habitat is achieved by evaluating riverbank modifications, erosions, substrate type and exposure, and the presence of invasive vegetation (Kleynhans *et al.* 2008). Both the in-stream and riparian zones are eventually rated as individual metrics according to the severity and the extent of modifications by allocating impact scores (Kleynhans *et al.* 2008). Habitat integrity is determined by classifying IHI scores into percentage classes A to F, where class A indicates unmodified habitat at 90% to 100% (Dallas 2005). The use of IHI in South African Rivers has provided important information about the overall ecological status of aquatic ecosystems (Kleynhans 1996; Amis *et al.* 2007). However, more work is still to be done, especially in the rivers that do not form part of the national river network (i.e. the main stem rivers; Nel *et al.* 2004). A national assessment of the status of the main stem rivers reported that 44% of the rivers were critically endangered by the year 2004 (Nel *et al.* 2004). This assessment did not include the tributaries of the main stem rivers (see Nel *et al.* 2004), even though most of these tributaries were structurally modified by anthropogenic factors (Amis *et al.* 2007). Researchers have emphasized the importance of habitat assessment and argued that changes in the physical structure of a river channel are related to changes in flow regime and loss of habitat heterogeneity, and in turn, changes in the composition of the biotic community (Munn & Brusven 1991; Cobb *et al.* 1992; Bunn & Arthington 2002).

1.5.4 Limitations of biotic indices

Although biotic indices integrate the characteristics of a community into a single ecological category, the generation of a single category could result in the loss of single species' spatiotemporal interactions within a community (Van den Brink *et al.* 2003). Several authors have argued that responses of biotic indices to environmental conditions are not always predictable (e.g. Jüttner *et al.* 1996; Hill *et al.* 2001; Stevenson *et al.* 2010), simply because species diversity can decrease with pollution (Rott & Pfister 1988) or fluctuate based on the type of pollution present (Jüttner *et al.* 1996; Hillebrand & Sommer 2000). Studying the effects of high nutrient concentrations on biota is important, although community responses may not provide a conclusive picture of the influences of nutrient enrichment simply because biota can develop adaptation strategies to survive in stressful conditions (Hillebrand & Sommer 2000; Kay *et al.* 2001; Artigas *et al.* 2008). For this reason, conducting tissue analysis of biota becomes important in determining the influence of nutrient enrichment (Bunn *et al.* 1995; Costanzo *et al.* 2005; Pruell *et al.* 2006)

1.6 THE APPLICATION OF STABLE ISOTOPES IN WATER QUALITY STUDIES

Stable isotope analysis has gained much attention in hydrology and food web studies over the past several decades (Fry 1991; Hansson *et al.* 1997; Anderson & Cabana 2007). In this thesis, stable isotope techniques were incorporated to assess the influence of nutrients from point sources on the benthos of a small river. Nitrogen (N) is one of the well-studied elements in ecology since it forms part of the essential nutrients needed for organisms to survive (Marsh & Tenore 1990). Nitrogen has stable isotopes that differ in the number of neutrons, and the ratio of these isotopes (i.e. $^{15}N/^{14}N$) is calculated based on how the sample deviates from a standardized value and expressed in delta notation as $\delta^{15}N$ with units of parts per thousand (‰; Peterson & Fry 1987; Fry

1991). The isotope ^{14}N is lighter and abundantly available in nature, whereas the ^{15}N isotope is heavier and more rare, and the ratio of these forms changes through the occurrence of chemical reactions and biological processes such as photosynthesis and metabolism (Kendall *et al.* 2007). Isotopic fractionation, which is the enrichment of the heavier relative to the lighter isotope (Fry 2007), occurs during chemical and biological processes because the chemical bonds between lighter isotopes are weaker than those of heavier isotopes, causing the lighter isotopes to react faster (Boon & Bunn 1994). Isotopic fractionation occurs as heavier isotopes accumulate in the tissues of consumers through metabolism (Kendall & McDonnell 2012), and stable isotope ratios in animals can indicate their trophic level relative to primary producers (Peterson & Fry 1987).

In nutrient rich waters, benthic organisms can assimilate polluted organic material derived from sewage (Vander Zanden *et al.* 2005), and stable isotopes of macroinvertebrates (di Lascio *et al.* 2013; Morrissey *et al.* 2013) and autotrophs (Costanzo *et al.* 2005) can be used to assess water quality and eutrophication (Voβ & Struck 1997). Nitrogen isotopes have been successfully used to identify nutrient subsidies from urban watersheds, especially in receiving waters affected by wastewater effluent and agriculture (Heaton 1986; Cole *et al.* 2004; Camargo & Alonso 2006; Diebel & Vander Zanden 2009). Effluent from treated sewage has high values of $\delta^{15}N$ because of the denitrification activity of wastewater bacteria that preferentially use the lighter ^{14}N isotope (Morrissey *et al.* 2013). High $\delta^{15}N$ values in freshwater systems typically indicate the influence of sewage and wastewater effluent discharges (e.g. Wayland & Hobson 2001; Costanzo *et al.* 2005; Kaushal *et al.* 2011) rather than inorganic fertilizers and untreated raw sewage (Kreitler 1979; Heaton 1986; Anderson & Cabana 2007). Relative to atmospheric N_2, the typical $\delta^{15}N$ values of nitrate from commercial fertilizer range from -2.5 to +2.0‰; soil nitrate ranges from -2 to +9‰, and human and animal waste range from +10 to +20‰ (Heaton 1986; Kendall *et al.* 1995). Although fractionation through nitrification, volatilization and denitrification may result in overlapping values of $\delta^{15}N$ as nitrogen moves from one medium to another (Kendall *et al.* 2010), $\delta^{15}N$ values can reflect the external sources of nitrogen into surface water (Anderson & Cabana 2005; Diebel & Vander Zanden 2009; Kaown *et al.* 2009; Connor 2012). In water quality studies, the interpretation of the stable isotope data from biota can be strengthened by simultaneously assessing the chemical and hydrogeological status of the area under study (Kaown *et al.* 2009).

1.7 RATIONALE AND SIGNIFICANCE OF THE STUDY

Although the South African government has established water quality standards and regulations, the continued deterioration of rivers calls for additional rapid water assessment programs using different approaches (Karr 1991). Extensive work has been done in many impacted rivers in the country (Dallas 1997; Taylor *et al.* 2007; Bollmohr & Schulz 2009; Kemp *et al.* 2014). However, little is known regarding water pollution and its effects on the biological communities of macroinvertebrates and diatoms in the Bloukrans River in Grahamstown. This river is economically important as it supplies water to the Belmont Valley agricultural farms that feed the residents of Grahamstown, Rhini, Port Alfred and other neighboring towns. Water quality is a social and ecological concern owing to the multiple anthropogenic influences that could reduce the river's ability to provide ecosystem functions that are essential for aquatic life. According to the Makana Municipality Report (2008), there are no water quality data available from the Bloukrans River, and data generation and input for this system by the DWA was an ongoing process. Additionally, there are no data available for this river in the National Rivers Water Quality Database of South Africa. There is an urgent need for the publication of water quality data from this river system to address concerns for public health and safety, and to inform authorities and managers on the associated ecological issues. The purpose of this study was to assess the water quality and the effects of pollution on the biological communities in the Bloukrans River. To accomplish these aims, the possible sources of pollution were identified, the habitat characteristics of the river, including chemical and hydrological data at different locations, were recorded, water quality and habitat integrity classifications were done using the biological responses of diatoms and macroinvertebrates, and the nitrogen isotope signatures of macroinvertebrates were determined in relation to the nutrient concentrations and isotopic signatures of the surrounding waters.

1.8 APPROACH TO THE STUDY

This research was designed to employ multivariate and multimetric methods to determine relationships between environmental characteristics and biological communities. I used descriptive methods to analyse habitat structure and man-made modifications by taking measurements and recording observations on standardized field-data sheets. The primary goal was to test whether pollutants derived from activities that occur in catchment areas influence the aquatic biota of the river. Diatoms and macroinvertebrates were collected at different locations in the Bloukrans River, with each location having a unique source of pollution. The main predictions were that there would be different levels of pollutants in the form of nutrients (ammonia, nitrate, and phosphates) at each location, and such changes would result in variations in diatom and macroinvertebrate species composition and community structure. Regression methods were used to further test the predictions.

1.9 RESEARCH QUESTIONS AND HYPOTHESES

The main objectives of this study were to answer the following questions:

I. what are the most important environmental variables influencing the structures of the macroinvertebrate and diatom communities,
II. do the water quality indices SASS5 and TDI provide consistent results about the water quality status of the Bloukrans River, and
III. can stable nitrogen isotope ($\delta^{15}N$) values differentiate the sources of pollution from urban, sewage, agriculture, and recreational activities in the river?

Based on published results from human impacted rivers, the following hypotheses were tested:

1. The concentrations of inorganic nitrate, ammonium, and phosphate increase with an increase in human disturbance, therefore significantly higher nutrient concentrations occur upstream, with a decrease in the downstream direction away from human influences.
2. As a result of increased nutrient loads, macroinvertebrate total abundance, diversity, and taxa richness tend to decrease, hence a significant decrease in diversity, total abundance, and taxa richness occur at the sites with the highest ammonium, nitrate, and phosphate concentrations.
3. Since macroinvertebrates have diverse strategies that allow them to thrive under variable nutrient conditions, their dominance shifts from pollution-tolerant taxa upstream to pollution-sensitive taxa downstream following a land use gradient.

4. A longitudinal gradient in SASS5 and ASPT scores (with significantly lower scores upstream and higher scores downstream) indicates water quality deterioration in the upper reaches of the river where human influences are greater.
5. The responses of flora to increased nutrient concentrations differ from those of fauna, resulting in contradictory responses. Nutrient loading causes eutrophication and stimulates the growth of aquatic flora, so diatom total abundance, diversity, and taxa richness is greater at the sites where nutrient concentrations are higher.
6. Trophic Diatom Index values are higher at the sites where phosphate concentrations are high, indicating some degree of eutrophication.
7. Variation in $\delta^{15}N$ values occurs in water subjected to sewage, agriculture and domestic uses, so $\delta^{15}N$ values are higher where concentrations of ammonia and nitrate are highest, and are lower at sites with decreased concentrations of nutrients.

1.10 STRUCTURE OF THE THESIS

Chapter 1 is a general introduction and literature review on the global and national water resource situations. The management and monitoring strategies of water resources in South Africa are introduced and explained, and the approaches applied for the ecological assessment of the Bloukrans River are described. The rationale, objectives, and hypotheses of this study are presented, and the structure of the thesis is outlined. **Chapter 2** describes the study area and the major anthropogenic activities that influence the ecological integrity of the Bloukrans River. Included are descriptions of the physico-chemical parameters measured, organism sampling, identification protocols for diatom and macroinvertebrates, and the preparation and analysis protocols for deriving stable isotope data. **Chapter 3** contains the results on the effects of pollution on the macroinvertebrate community structure and water quality. Relationships between physico-chemical parameters and the macroinvertebrate communities are described. **Chapter 4** includes the descriptions of the diatom communities in relation to environmental parameters and the trophic state of the Bloukrans River. **Chapter 5** contains the results on spatio-temporal variations in the isotopic $\delta^{15}N$ values of biota, and the relationships between these values and nutrient concentrations in the water. **Chapter 6** is a conclusive summary of the results obtained from all the approaches applied in this study, and general recommendations are made according to my findings.

2 MATERIALS & METHODS

2.1 STUDY AREA

The Bloukrans River (Figure 2.1) is a small heavily polluted perennial river of approximately 59 km length in the Eastern Cape province of South Africa. This river is a tributary of the Kowie River located at the heart of Makana Municipality in the Belmont Valley 6.5 Km east of Grahamstown (Rivers-Moore *et al.* 2006). The river source lies in the low lands approximately 4 km from Grahamstown, in a subtropical north coast climatic zone (Sinchembe & Ellery 2010). Seasonal mean water temperatures are 20.3°C and 12.3°C in summer and winter, respectively. Water supply to the river is through groundwater influx and direct rainfall, with an annual rainfall of approximately 680 mm (Sinchembe & Ellery 2010). For many years, the Bloukrans River has been subjected to various sources of anthropogenic disturbance as it drains residential, business, and agricultural areas. The middle reach occasionally receives partially treated sewage wastewater that frequently increases the water velocity and potentially introduces microbial infestations, faecal coliforms, nutrient loading, turbidity and bad odor (Wutor *et al.* 2009). The lower reach drains a protected area (Bloukrans Nature Reserve) that is privately owned by the community. As the river passes through the nature reserve it forms a pool that is preserved for recreational and religious ceremonies by local communities. Approximately 20 km from the nature reserve the river flows into the Kowie River, which eventually enters the Indian Ocean at the town of Port Alfred.

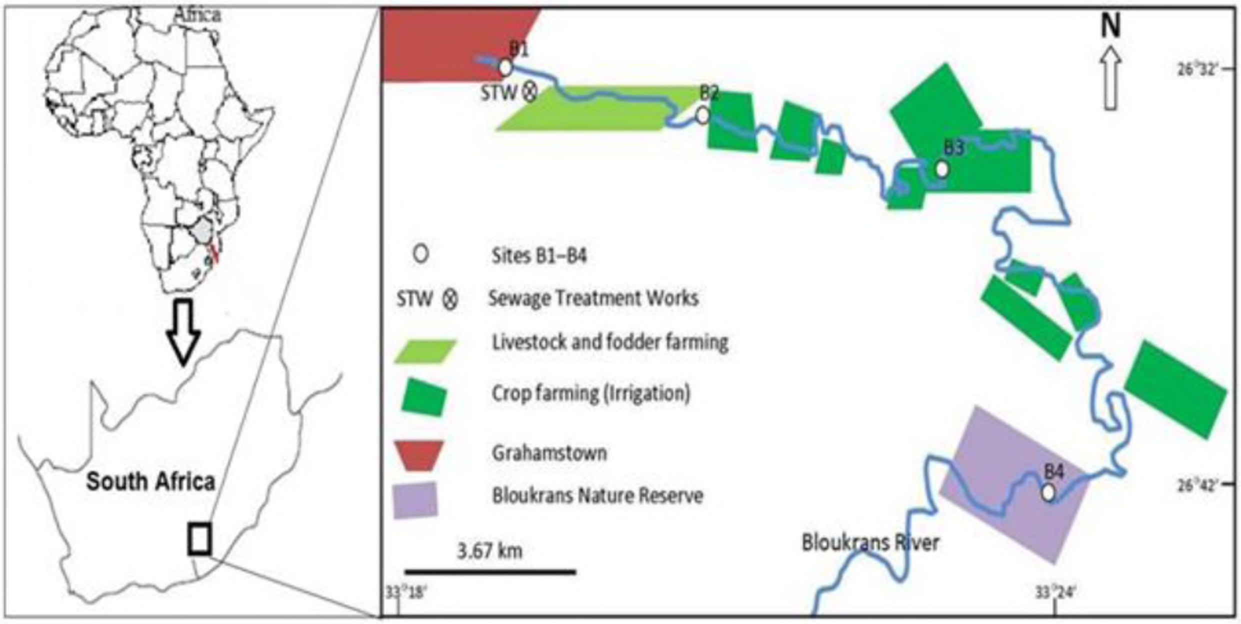

Figure 2.1: Map showing the separation of study sites along a land use gradient in the Bloukrans River. The abbreviations B1-B4 (Bloukrans 1-4) denote the locations of sites from upstream to downstream, where B1 is Urban, B2 Sewage, B3 Agriculture, and B4 Recreational.

2.2 STUDY SITES

Four study sites were identified and named based on the associated type of land use: Urban, Sewage, Agriculture, and Recreational (Figure 2.1). Each of the sites covered an area of approximately 50m^2. Data collection was conducted four times at each site (i.e. once in April, July and October 2013, and January 2014) to account for seasonality. Bridges that cut through the river channel and gravel roads constructed for supplying water to farms were both prevalent in the entire area. During high rainfall periods, large amounts of litter and solid waste would flood the river banks and clog the water passage holes underneath the bridges, preventing easy flow of water.

2.2.1 Site Urban

This site was located at the coordinates 33°18'51.9"S, 26°33'07.01"E, and was considered the sampling starting point at 0 km. The site was situated in an area of informal settlements and urban construction works at about 4.23 km below the river spring. The channel was narrow (3.4 ± 1.1 m) and shallow (35.3 ± 13.9 cm), with the presence of roads, litter, debris and overgrazing by cattle (Figure 2.2). Instream conditions included high levels of turbidity and odor and a lack of incurrent vegetation. Channel morphology was characterized by sand, mud/clay, and cobble of about 100-256 mm in size. Grass was the most dominant riparian vegetation. Sampled biotopes included submerged stones, marginal riparian vegetation and sediments.

Figure 2.2: The most upstream site of the Bloukrans River (site Urban). Evidence of previous flooding events, domestic garbage and livestock was apparent. Water chemistry was impaired and the riparian habitat had undergone anthropogenic modifications.

2.2.2 Site Sewage

This site was 7 km downstream of site Urban (33°19'25.1"S, 26°36'01.81"E), and it occasionally experienced high water velocity due to sewage effluent discharge from a neighbouring treatment plant. The site had a typically wide (5.9 ± 2.3 m) channel (Figure 2.3). Fodder and livestock farming were in practice a few meters away from the site. Flow was generally slow, and there was evidence of channel modification and deforestation. Instream morphology was characterized by mixed bedrock, sand, and cobble. The site had very little canopy and water depth was shallow.

Figure 2.3: The second site (Sewage) showing the foamy water produced from the discharge point located upstream. Exposure of the riparian root network indicated bank erosion had occurred.

2.2.3 Site Agriculture

The site was situated 14.7 km from site Urban, downstream of site Sewage at 33°19'47.08"S, 26°38'47.41"E co-ordinates (Figure 2.4). It drained crop and fodder production farmed land, with a ± 54.5 m wide channel. A bridge, litter/debris, in-channel fences and an adjacent road were all present. Instream morphology was characterized by active flow except for impounded areas with flow modifications, and bank modifications and a partially open canopy were noted.

Figure 2.4: Site Agriculture of the Bloukrans River, which experienced extensive disturbances from bank erosion caused by transportation of agricultural machinery and water abstraction for irrigation.

2.2.4 The Recreational site

The most downstream site at coordinates 33°23'29.04"S, 26°42'32.60"E was located 46.4 km from site Urban (Figure 2.5). The site was situated within a privately owned nature reserve upstream of a natural pool that was preserved for cultural and religious cleansing ceremonies by local people. Observed uses of the catchment area included man-made impoundments and defined fire areas that were associated with the recreational activities occurring at the pool. Instream morphology was dominated by mixed bedrock and gravel, and side bars indicated the potential of channel width increase during flooding periods. Instream vegetation was observed onsite, mostly *Cyperus eragrostis*. Water temperatures were generally low due to moderate canopy cover.

Figure 2.5: The Recreational site showing sampled biotopes and the associated cultural land uses at about 16 m upstream of the Bloukrans natural pool.

2.3 SAMPLE COLLECTION

2.3.1 Physico-chemical parameters

Physico-chemical parameters were measured at each site during April (autumn), July (winter), October (spring) 2013, and January (summer) 2014. Water quality parameters were recorded prior to disturbing the water while sampling the biological components (i.e. macroinvertebrates, diatoms and plants). Three local habitat areas were selected for the collection of all instream environmental data at each site (i.e. the left littoral zone, the middle pelagic zone, and the right littoral zone), providing three replicates per site in one sampling occasion. Electrical conductivity, total dissolved solids, sodium chloride, pH and temperature were measured on site using a CyberScan Series 600 water proof multi-probe meter (Singapore). A YSI incorporated-550A (U.S) handheld meter was used to measure dissolved oxygen. Water velocity was obtained using a Marsh McBirney 2000 portable flow meter. Channel depth and width were measured using a tape measure. For nutrient analysis, three 500mL water samples were collected in separate plastic containers directly from the river (i.e. each 500mL representing each of the microhabitats selected). Samples were stored on ice and nutrient analyses were performed using an HI 83203 multiparameter bench photometer (Hanna Instruments Inc., Rhode Island). Ammonium ($NH4^{+}$) concentrations were obtained by mixing 10 ml of water with 4 and 6 drops of reagents HI 93715A-0 and HI 93715B-0, respectively. Nitrate (NO_3^-) concentrations were obtained by mixing 6 ml of water with the HI 93728-0 reagent, and the concentrations of phosphate (PO_4^{3-}) were obtained by mixing 10 ml of water with 10 drops of HI 93717A-0 Moly date reagent and one packet of HI 93717B-0 Phosphate HR Reagent B (HI 83203 multiparameter bench photometer instruction manual).

2.3.2 Determination of habitat characteristics

Site characterization was completed according to Dallas (2005). The condition of the local catchment was assessed by identifying the occurrences of activities such as deforestation, agriculture (crops, livestock and irrigation), impoundments through formation of man-made dams and diversion weirs, disturbance by wildlife (wildlife watering), and the presence of areas with limited anthropogenic modification. The condition of the channel at each site was assessed by evaluating the presence or absence of any channel and bank modifications. Channel morphology was assessed by critically identifying the type of river bed at each site. Features such as substrate type and flood alleviations were recorded with specific interest in the presence of sand, gravel, cobble, and/or boulder. Substrate size was recorded for silt/clay/mud, sand, gravel, pebble, cobble, boulder, and bedrock. The abundance and dominance of each identified substrate instream and on the river bank was estimated based on the scale: 0 – absent; 1 – rare; 2 – sparse; 3 – common; 4 - abundant; 5 – entire (Dallas 2005).

2.3.2.1 Analysis of Habitat Integrity

Habitat integrity at each site was analyzed by determining IHI scores according to a method that critically assesses modifications of instream and riparian habitat (Dallas 2005), as highlighted in Chapter One. In this thesis, instream habitat refers to the area of water within the demarcated area of 50 m^2 per site, and riparian habitat was the land area covering a 10 m buffer zone away from the water. The analysis of IHI was conducted in three steps. Firstly, the presence of instream or riparian zone disturbances was recorded. Impact classes were chosen based on the extent and severity of the impact, ranging from 'no impact' to 'critically impacted' and scored between 0 to 25 (Table 2.1). Secondly, the impacts were described according to specific criteria on water quality, water abstraction, and solid waste disposal. Each criterion was weighed and allocated a standardized rating value (Dallas 2005; Table 2.2). The final impact value of each site was obtained by dividing the values of impact classes by 25 (maximum impact score) and multiplying by the criterion weight. For example, if the water quality score at the Urban site was 10, with a weight of 14, then the final impact score was: 10/25 * 14 = 5.6. Lastly, the IHI scores for both instream and riparian zones were calculated for each site using the equation:

$$\mathrm{IHI} = 100 - [\left(\frac{\Sigma\left(\frac{\mathrm{Mg}}{\mathrm{MV}}\right) \times \mathrm{Wt}}{\mathrm{MV}}\right) \times 100]$$

where: IHI = index of habitat integrity (%), Mg = criterion rating value, Wt = criterion weight, and MV = the maximum value per criterion based on criterion weights (Dallas 2005; Kleynhans *et al.* 2008). The resulting instream and riparian IHI values were interpreted as habitat integrity classes raging from A to F (Table 2.3), where class A represented unmodified habitat (Dallas 2005).

Table 2.1: The scoring guidelines (extracted from Dallas 2005) used to determine the degree of impact for the instream and riparian zones at each site in the Bloukrans River.

Impact Class	Description	Score
None	Any modifications are not located in such a way that they have an impact on habitat quality, diversity, size and variability.	0
Small	The modification is limited to very few localities and the impact on habitat quality, diversity, size, and variability is limited.	1-5
Moderate	The modifications occur at a small number of localities and the impact on habitat quality, diversity, size and variability are fairly limited.	6-10
Large	The modification is generally present with a clearly detrimental impact on habitat quality, diversity, size, and variability. However, large areas are not affected.	11-15
Serious	The modification is frequently present and the habitat quality, diversity, size, and variability in almost the whole of the defined area are affected. Only small areas are not influenced.	16-20
Critical	The modification is present overall with a high intensity. The habitat quality, diversity, size, and variability in almost the whole of the defined section are influenced detrimentally.	21-25

Table 2.2: Weightings (Wgt) for instream and riparian zone criteria (extracted from Dallas 2005) used to develop the Index of Habitat Integrity for the Bloukrans River.

Instream Criteria	**Wgt**	**Riparian Zone Criteria**	**Wgt**
Water abstraction	14	Water abstraction	13
Extent of inundation	10	Extent of inundation	11
Water quality	14	Water quality	13
Flow modification	7	Flow modification	7
Bed modification	13		
Channel modification	13	Channel modification	12
Presence of exotic macrophytes	9		
Presence of exotic fauna	8		
Solid waste disposal	6		
		Decrease of indigenous vegetation from the riparian zone	13
		Exotic vegetation encroachment	12
		Bank erosion	14

Table 2.3: Habitat Integrity classes (extracted from Dallas 2005) used to characterize instream and riparian zones for each study site in the Bloukrans River.

Class	Description	Score (%)
A	Unmodified, natural	90 - 100
B	Largely natural with few modifications. A small change in natural habitats and biota may have taken place, but the assumption is that ecosystem functioning is essentially unchanged.	80 - 89
C	Moderately modified. A loss or change in natural habitat and biota has occurred, but basic ecosystem functioning appears predominantly unchanged.	60 - 79
D	Largely modified. A loss of natural habitat and biota and a reduction in basic ecosystem functioning are assumed to have occurred.	40 - 59
E	Seriously modified. The loss of natural habitat, biota and ecosystem functioning are extensive.	20 - 39
F	Modifications have reached a critical level and there has been an almost complete loss of natural habitat and biota. In the worst cases, the basic ecosystem functioning has been destroyed.	0 - 19

2.3.3 Collection and treatment of biological data

2.3.3.1 Macroinvertebrate communities

Macroinvertebrates were collected using a 35 cm x 35 cm square shaped kick sampler SASS net of 0.08 mm mesh size. Vigorous kicking and sweeping was conducted for a duration of 3 to 5 minutes in three distinct biotopes including stone, vegetation, sand (Dickens & Graham 2002). An area of approximately 1 m^2 of marginal vegetation was sampled at each site. The SASS net was vigorously pulled through the vegetation and rocks in the direction opposite the water flow, moving back and forth through the same area with the net kept below the water surface. Hand picking was done where animals were seen attached on rocks. Three invertebrate samples were collected per site on each occasion (i.e. from gravel sand and mud, marginal vegetation, and sand biotopes). Samples were transferred into separate sorting trays for identification on site. For further laboratory and statistical analyses, each sample unit was placed into a 250 ml plastic jar with 70% ethanol, and were analyzed separately for each respective site. For the taxa abundances, the animals were counted and identified at family level using Gerber & Gabriel (2002). The animals were preserved in 100% alcohol in 2.0 ml mono-polymer microtubes.

To determine the SASS scores, macroinvertebrates were allocated sensitivity scores between 1 and 15, with the most sensitive taxa scoring the highest (15) and the most tolerant taxa the lowest scores (Dickens & Graham 2002; Simaika & Samways 2012). Three principal indices were determined: (1) the SASS Score – which was generated by adding all the sensitivity scores for each taxon present, (2) the Number of Taxa – which was the total number of taxa present in the sample, and (3) the Average Score Per Taxon (ASPT) was calculated by dividing the SASS Scores with the Number of Taxa in the sample (Dickens & Graham 2002). The SASS Score and ASPT values were used for the analysis and interpretation of SASS data (Chutter 1994). In general, higher SASS and ASPT values indicate less impact on water quality (Dickens & Graham 2002). However, Chutter (1998) emphasized that the true quality of water is most accurately reflected by the ASPT rather than the SASS score, as the former accounts for taxa richness. Chutter's view was that factors such as substrate type and physiological changes in habitat have the potential to cause variations in SASS scores, but high abundances of pollution sensitive taxa increase the ASPT values and allow for accurate interpretation of variations in SASS scores (Chutter 1998).

2.3.3.2 Collection of diatoms

Diatom assemblages were collected from four different microhabitats within a 50 m^2 sampling area following Taylor *et al.* (2005). Epilithon samples were collected by haphazardly selecting five submersed pebbles of approximately 60-200 mm in size. The stones were carefully rinsed in river water to remove attached sand particles and transferred into a bowl filled with 500 mL river water, where they were brushed using a tooth brush. Care was taken to brush only the top areas of stones that had been exposed to flowing water and light (to minimize inputs of excess sand into the samples). Epipsammon and epipelon were suctioned from the top undisturbed layer of sediment using a 15 mL syringe. Epiphyton was collected by cutting off 10 stalks of instream *Cyperus eragrostis* using a field knife. The stalks were brushed in a similar manner as the epilithon. Fine suspended particulates were collected with a 20 µm mesh net towed against the direction of water flow and horizontally in standing water. The sample was transferred into a 500 mL sample jar and preserved in Lugol's iodine and 70% ethanol. Three units of samples were prepared for statistical and site comparison purposes (i.e. an epilithon sample, an epipsammon / epipelon sample, and an epiphyton sample). All samples were stored on ice and transferred to the laboratory for identification. In the laboratory, the samples were allowed to defrost and settle in a refrigerator for 24 hours. Organic material was removed from the diatom valves using hot potassium permanganate and hydrochloric acid (Taylor *et al.* 2005), and diatoms were counted under a phase-contrast inverted light microscope at 1000x and identified to species (when possible) using an illustrated guide (Taylor *et al.* 2007).

2.3.3.3 Stable isotope preparation

Samples of autotrophs and consumers intended for stable isotope analyses were cleaned and sorted according to genus upon arrival in the laboratory. Autotrophs were represented by water samples containing diatoms, filamentous algae, and coarse particulate organic matter (decomposing leaves and twigs). Water samples were filtered through ashed 47 mm glass fibre filters (GF/F) to isolate the diatom material. Live macroinvertebrates were separated according to family. All samples were frozen at -20 °C for 24 hours and later freeze-dried. The dried samples were homogenized into fine powder, and approximately 1 mg per sample was loaded into separate tin capsules and sent to IsoEnvironmental Laboratory in Grahamstown, South Africa, for isotope ratio analysis. The nitrogen ratios were obtained using a mass spectrometer with a Europa Scientific 20-20 IRMS linked to an ANCA SL prep unit. Isotopic composition was expressed in parts per mil relative to an international standard (atmospheric nitrogen) as per the equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{15}N and R is the $^{15}N/^{14}N$ ratio.

2.4 STATISTICAL ANALYSIS

2.4.1 Analyzing environmental data

Data for habitat assessment were collected onsite. Site characterization scores were preserved as the original scores per site and occasion to reveal the spatial and temporal changes in habitat structure, and then averaged to make generalizations. Physico-chemical data were handled in two separate ways based on the hypotheses tested. Environmental variables intended for multivariate analyses were normalized in PRIMER V6 statistical package to improve normal distribution of data (Clarke & Ainsworth 1993). Resemblance matrices were calculated for each variable using Euclidean distance similarity measures (Clarke 1993). Spatiotemporal changes in environmental variables were explored using canonical analysis of principal coordinates (CAP), an ordination procedure that uses a resemblance matrix to analyze (dis)similarities between environmental variables in PRIMER V6 (Clarke & Gorley 2005). A permutational analysis of variance (PERMANOVA) was performed to test for significant differences in environmental variables amongst sites and periods at $p \leq 0.05$ level of significance (Clarke & Gorley 2005). For simple linear regressions, environmental data were natural log transformed (LN (x+1)) to minimize the effects of different measurement scales, while avoiding the possibility of negative values that are not suitable for regression models (Smith 1993).

2.4.2 Analyzing community data

Macroinvertebrate and diatom abundance data were log (x+1) transformed to decrease the dominance of highly abundant species over the least abundant (Clarke & Ainsworth 1993). In PRIMER V6, the Bray-Curtis similarity coefficient was used to calculate a resemblance matrix (Bray & Curtis 1957; Clarke 1993). A non-metric multidimensional scaling (nMDS) method was used to visualize spatio-temporal patterns in both the diatom and macroinvertebrate communities based on the Bray-Curtis similarity coefficient (Clarke & Gorley 2005). Biotic community indices were calculated for each site to analyze community structure. The calculated indices were total abundance, taxa richness, Shannon diversity index (*H'*) for both diatom and invertebrates, SASS5 with ASPT for invertebrates and TDI for diatoms. Values obtained from the biotic indices were used as independent variables in the simple regression models to assess the effects of ammonia, nitrate, and phosphate on biotic communities. Water quality was assessed based on the SASS5, ASPT and the TDI indices (Kelly 1998; Dickens & Graham 2002), and the dominance of indicator taxa. Indicator invertebrate taxa were pooled into three groups according to their pollution tolerances. Group one was named 'Tolerant' and consisted of all tolerant taxa with scores between 1 and 5. Group two was named 'Intermediate' and was made of taxa with scores from 6 to 10. The third group 'Sensitive' included all the sensitive taxa with scores ranging from 11 to 15. The dominance of each group was reported as a relative percentage contribution to the entire community across sites and periods. Diatom indicator taxa were pooled into one of five groups based on their tolerance to phosphorus (Kelly & Whitton 1995). Group one was made of all sensitive taxa that can only tolerate <0.01 mg/L of phosphorus. In group two were taxa that tolerated low to moderate phosphorus levels (>0.01, <0.035 mg/L). Group three were intermediate taxa, tolerant to >0.035, < 0.1 mg/L phosphorus concentrations. The fourth group had taxa that were tolerant to high phosphorus concentrations (>0.1, <0.3 mg/L). The last group was made up of very tolerant taxa that preferred and survived in environments with high phosphate concentrations (>0.3 mg/L).

2.4.3 Multivariate species-environment analysis

Relationships between environmental variables and communities of macroinvertebrates and diatoms were evaluated using PRIMER V6 (Clarke & Ainsworth 1993). Distance-based linear models (distLM) and distance-based redundancy analysis (dbRDA) (Clarke & Gorley 2005) were used to analyze spatial and temporal patterns in community distribution. The dstLM routine is a multiple linear regression procedure for analyzing and modelling patterns in biological data using predictor or environmental data (Clarke & Gorley 2005). To find out if there were any similarities between biological and environmental data, a RELATE routine was applied. The RELATE procedure performs non-parametric correlations between the resemblance matrix of the environmental data and that of the biological data using the Spearman rank correlation coefficient (Rho), where a correlation value closest to +1 indicates strong similarities in the two groups

(Clarke & Gorley 2001). After relating the biological data with the environmental parameters, a stepwise search was performed to select the environmental variables that were best correlated with patterns in biological data using a BVSTEP function within the BEST routine in PRIMER V6 (Clarke & Gorley 2001). The BVSTEP method performs forward selections and backward eliminations of variables by systematically adding them in the order of maximum coefficient match, while eliminating variables with the lowest contributions to the model (Clarke & Gorley 2001). The selected environmental variables were used in the distLM model to explain species-environment relationships.

2.4.4 Analyzing stable isotope data

Biota prepared for stable nitrogen analysis were divided into three trophic niche groups (i.e. autotrophs, primary consumers, and secondary consumers). The autotroph group consisted of epipsammon, epilithon, phytoplankton, epiphyton, filamentous algae, and course particulate organic matter. Primary consumers included macroinvertebrate families Baetidae, Chironomidae, Corixidae, Simuliidae, Caenidae, and Amphipoda. Secondary consumers were represented by the families Potamonautidae, Aeshnidae, Lestidae, Gomphidae, and Tabanidae. Nitrogen values obtained from tissue analysis of the biota were log (X+1) transformed and used as independent variables in a regression model to assess if nitrogen signatures in water were related to those of biota.

2.4.5 Analysis of spatial and temporal changes

Each data set was arranged according to sampling site and period to reveal spatial and temporal variability. Data collected at each site were used as replicates to compare sites based on environmental variables, biotic indices, and $\delta^{15}N$ values. Data collected during each period were used as replicates to compare differences across periods. One-way ANOVA were performed to analyze variations across sites and periods at $p \leq 0.05$ level of significance. A two-tailed post-hoc student t-test was used to identify the significant changes between any two groups at $\alpha \leq 0.05$ significance level.

3 RESULTS: EFFECTS OF POLLUTION ON WATER QUALITY AND MACROINVERTEBRATE COMMUNITIES

3.1 HABITAT CHARACTERIZATION

In this thesis, the concept of habitat modification refers to man-made activities or natural events that have altered the state of the habitat (Pollock *et al.* 2014). An overall assessment indicated that habitat modification did not vary extensively within the sampling year. During all sampling occasions, instream habitat was in category D, while riparian habitat was in category C, indicating large and moderate habitat modifications, respectively (Table 3.1). Across-site assessments indicated that the upstream Urban site was the most altered compared with all the other sites. Instream and riparian habitats were in categories E and D, indicating serious and large habitat alterations, respectively. At the Sewage site, instream and riparian habitat conditions were in category C, indicating moderate modifications of the natural habitat. At the Agriculture site, the riparian habitat was moderately modified (category C), but the condition of the instream habitat changed from moderate to large modifications during the last two sampling occasions. Habitat conditions varied with sampling time downstream at the Recreational site. For example, instream and riparian habitats were in their natural state (category B) during the first period, riparian habitat remained natural in the second period, but instream habitat was moderately altered (category C). By the third sampling period, habitat alterations had increased such that instream habitat was seriously modified (category E), while riparian zones were largely modified (category D). During the last sampling period, both instream and riparian habitats were largely modified (category D) with the loss of natural habitat and disturbances of biological processes at the site.

Table 3.1: The integrity of instream and riparian habitat based on the Index of Habitat Integrity (IHI) scores calculated for each site between April 2013 and January 2014 at the Bloukrans River. The scores suggested whether the habitat was seriously modified (E), largely modified (D), moderately modified (C), or largely natural with few modifications (B) (Kleynhans *et al.* 2008).

Period	**Sites**	**Urban (0 Km)**	**Sewage (7 Km)**	**Agriculture (14.7 Km)**	**Recreational (46.7 Km)**
	Habitat	**IHI Score : Category**	**IHI Score : Category**	**IHI Score : Category**	**IHI Score : Category**
April	Instream	21.9 : E	69.6 : C	60 : C	79.84 : B
	Riparian	48 : D	72.2 : C	79 : C	81.28 : B
July	Instream	22.8 : E	70.2 : C	79 : C	77.6 : C
	Riparian	48 : D	74.2 : C	79.2 : C	81.28 : B
October	Instream	21.9 : E	70.7 : C	55.2 : D	31.52 : E
	Riparian	48 : D	64 : C	79.2 : C	52 : D
January	Instream	20.9 : E	62.6 : C	44.8 : D	42.4 : D
	Riparian	48 : D	74.2 : C	79.2 : C	47.84 : D

3.2 SPATIOTEMPORAL PATTERNS IN ENVIRONMENTAL VARIABLES

Twelve environmental variables were measured at each site during the course of the study (see Appendix A). A CAP analysis revealed that environmental variables varied between sites and sampling periods (Figure 3.1 A & B). PERMANOVA confirmed significant differences in environmental variables by period ($F_3 = 223.5$, $p = 0.001$) and by site ($F_3 = 217.9$, $p = 0.001$).

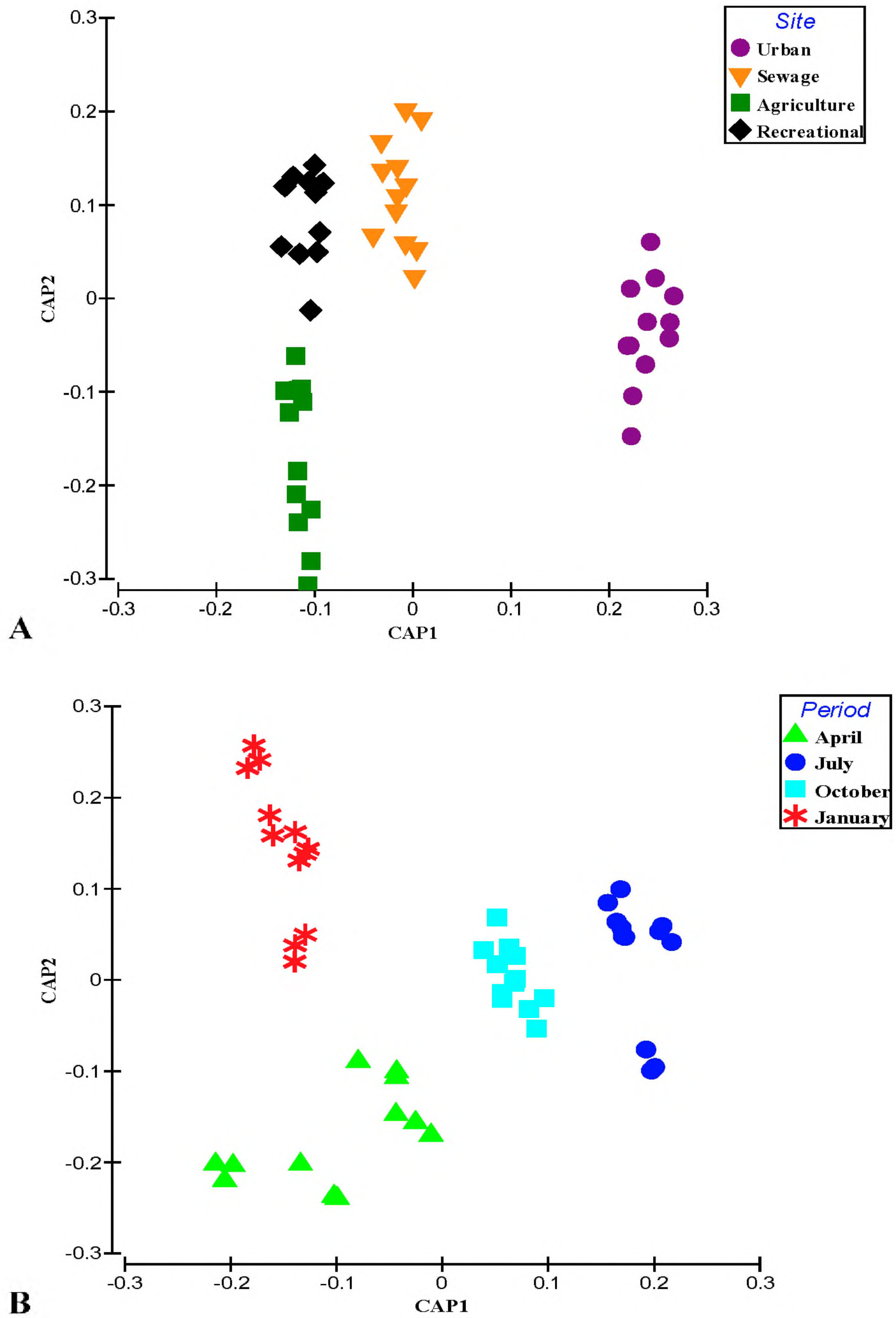

Figure 3.1: The first and second axes of a canonical analysis of principal coordinates showing the separations of sites based on measured environmental variables per site (A) and sampling period (B). Similar sites or periods were grouped closer together based on Euclidean distances.

3.2.1 Spatial changes in physico-chemical variables

The environmental variables changed across sites (Figure 3.2). One-way ANOVA indicated significant spatial variations in nitrate concentrations, ammonium concentrations, DO, and channel width. Nitrate concentrations were significantly higher at the sewage impacted site when compared to the Urban, Agriculture and Recreational sites ($t_{22} = -3.01$, $p = 0.007$; $t_{22} = 4.6$, $p = 0.0001$; $t_{22} = 2.8$, $p = 0.01$). There was a longitudinal gradient of higher ammonium concentrations upstream (Urban) and lowest downstream (Recreational). Ammonium concentration differed significantly when sites were compared to each other (i.e. Urban- Sewage at $t_{22}= 12.1$, $p< 0.0001$); Urban- Agriculture at $t_{22} = 17.3$, $p< 0.0001$; Urban- Recreational at $t_{22} = 19.5$, $p< 0.0001$; Sewage- Agriculture at $t_{22} = 4.3$, $p = 0.0003$; Sewage- Recreational at $t_{22} = 6.2$, $p< 0.0001$; Agriculture- Recreational at $t_{22} = 2.7$, $p = 0.01$). DO concentrations were significantly higher downstream ($t_{22} = -3.1$, $p = 0.01$) at the recreational site when compared to the upstream Urban site, but not significant when compared to other sites. The upstream Urban site had a significantly narrow channel when compared to the other three sites (Urban-Sewage at $t_{22} = -8.4$, $p< 0.0001$; Urban- Agriculture at $t_{22} = -2.9$, $p = 0.008$; Urban- Recreational at $t_{22} = -4.4$, $p< 0.0002$), and the Sewage impacted site was wider than the Agriculture ($t_{22} = 5.9$, $p< 0.0001$) and similar to the Recreational site.

3.2.2 Temporal variations in environmental parameters

Environmental variables varied differently during this study (Figure 3.2). Results from a one-way ANOVA indicated that the only variables that changed significantly over time were nitrate, dissolved oxygen, and depth ($F_3 = 7.29$, $p = 0.0004$; $F_3 = 6.59$, $p = 0.0008$; and $F_3 = 8.03$, $p = 0.0002$, respectively). A post-hoc test showed that nitrate concentrations were higher in July compared to other periods (July vs April at $t_{22} = -3.4$, $p = 0.002$; July vs October at $t_{22} = 3.04$, $p = 0.006$; July vs January at $t_{22} = 2.42$, $p = 0.01$). DO and conductivity were also high in July compared with other sampling periods. Dissolved oxygen was significantly high in July when compared to April and January ($t_{22} = -3.3$, $p = 0.003$ and $t_{22} = 4.1$, $p = 0.0004$, respectively). Water depth was higher in October (i.e. October vs April at $t_{22} = -3.7$, $p = 0.001$; October vs July at $t_{22} = -3.9$, $p = 0.0001$ and October vs January at $t_{22} = 4.1$, $p =0.0001$). A one-way ANOVA revealed no statistical variations ($F_3 = 2.27, p = 0.09$; $F_3 = 0.55, p = 0.65$; $F_3 = 1.80, p = 0.16$) in channel width, ammonium and phosphate across periods.

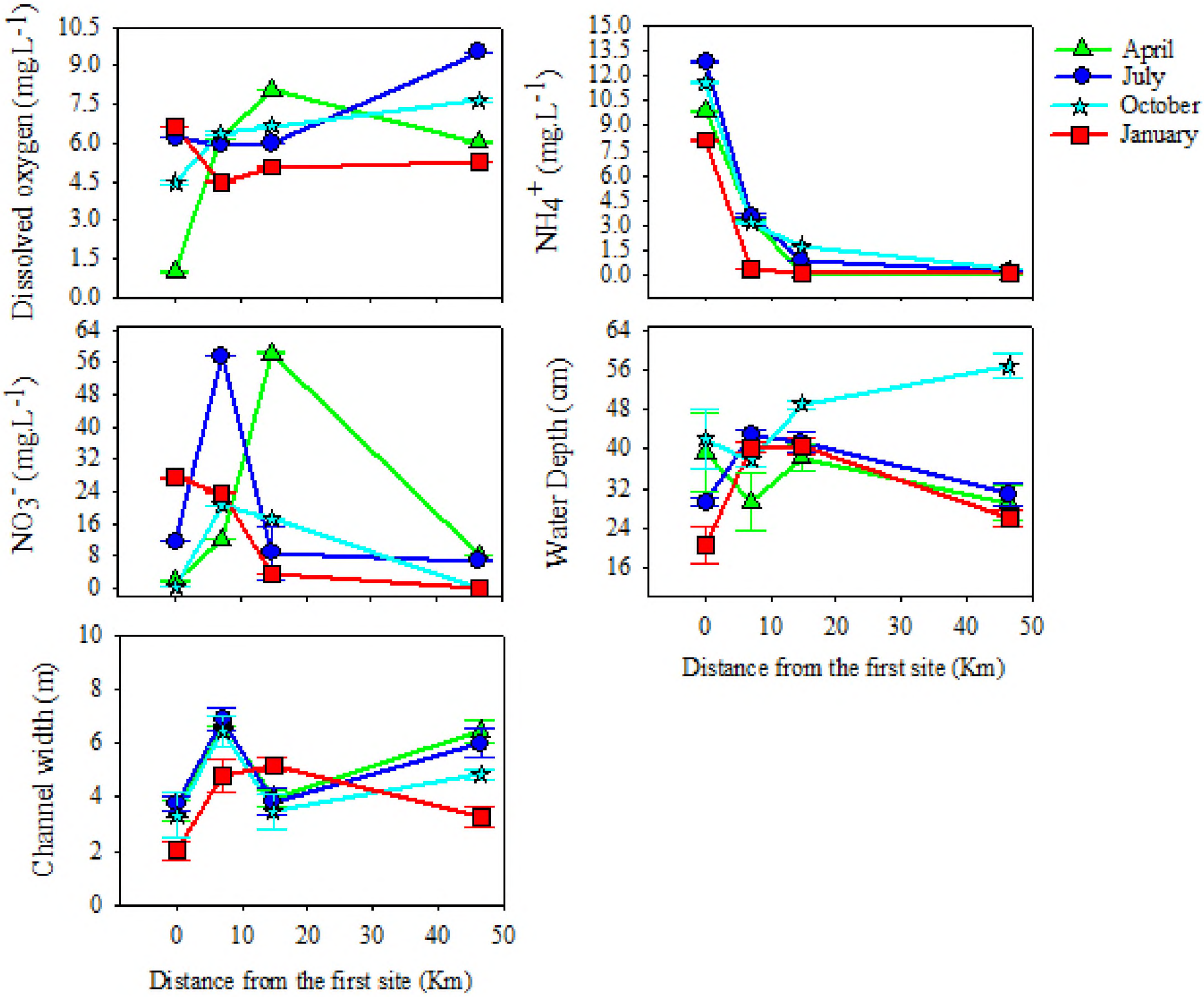

Figure 3.2: Spatial variation of environmental variables between April 2013 and January 2014 at the Bloukrans River. A maximum of 5 minutes was allocated for the collection of each data point. For each environmental variable, three readings were taken at each site, from which means and standard deviations (indicated as error bars) were calculated. Differences among sites were determined at $p < 0.05$ level of significance. Sites were reported in distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

3.3 SPATIOTEMPORAL PATTERNS IN MACROINVERTEBRATE DISTRIBUTION

The nMDS plots indicated clear differences in species composition between sites and sampling periods (Figure 3.3 A & B). The upstream Urban and downstream Recreational sites differed significantly in species composition when compared to the sites located adjacent to sewage and agriculture. Although the latter two sites appeared closely grouped together (Figure 3.3 A), a PERMANOVA suggested that all four sites were significantly different ($F_3 = 56.05$, $p = 0.001$), as were the sampling periods ($F_3 = 16.58$, $p = 0.001$).

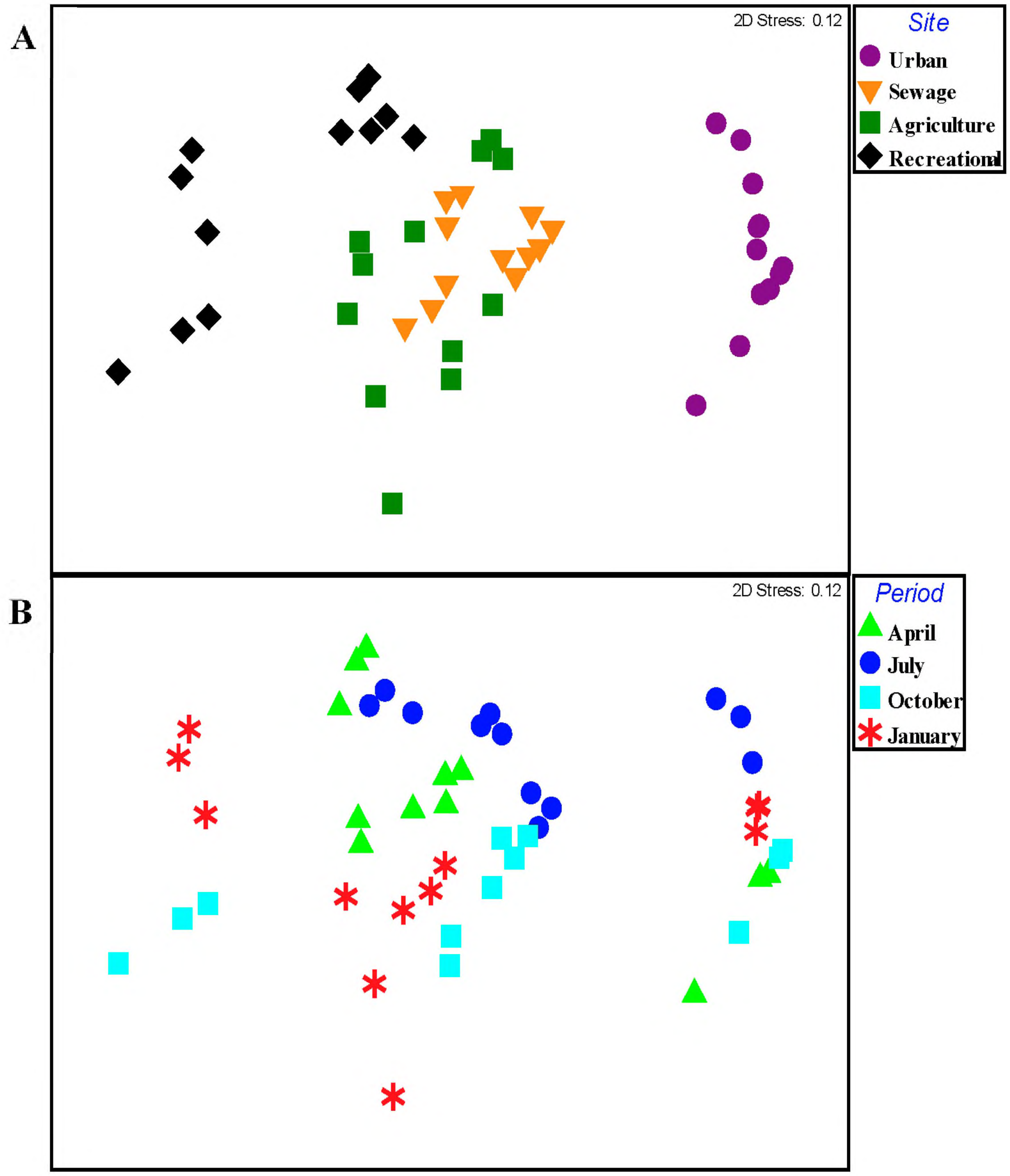

Figure 3.3: nMDS ordination in two dimensions (stress: 0.12) computed using a Bray-Curtis ecological distance measure to determine rank similarities in macroinvertebrate distribution across sites (A) and sampling periods (B). Sites and periods with more similar species compositions were located closest to each other.

3.3.1 Spatial and temporal changes in macroinvertebrate community structure

In total, 4076 individual macroinvertebrates belonging to 43 taxa were identified during the period of April 2013 to January 2014 (Appendix B). Macroinvertebrate community indices revealed some noticeable patterns in community structure (Figure 3.4). A one-way ANOVA indicated that there were no significant differences in total macroinvertebrate abundance across sites (F_3 = 2.91, p = 0.064). Taxa richness and diversity varied significantly across sites (richness F_3 = 14.33, p< 0.0001 and diversity F_3 = 30.77, p< 0.0001). A post-hoc test indicated that the Urban site had fewer taxa than the other three sites (Urban- Sewage at t_{22} = -5.13, p< 0.0001; Urban- Agriculture at t_{22} = -7.08, p< 0.0001; Urban- Recreational t_{22} = -4.35, p = 0.0003). Diversity was lowest at the Sewage impacted site when compared to all other sites (i.e. Sewage- Urban at t_{22} = 5.76, p< 0.0001; Sewage- Agriculture at t_{22} = -10.06, p< 0.0001; Sewage- Recreational at t_{22} = -6.85, p< 0.0001). Community structure changed over time (Figure 3.4). A one-way ANOVA indicated that only total abundances varied significantly across sampling periods (F_3 = 5.60, p = 0.02), and no statistical variations were detected for taxa richness (F_3 = 1.66, p = 0.19) or diversity (F_3 = 0.22, p = 0.88). Results from a post-hoc test revealed that macroinvertebrate abundances were significantly higher in April and July than they were in October and January (i.e. April vs October at t_{22} = 2.69, p = 0.01; April vs January at t_{22} = 2.70, p = 0.01; July vs October at t_{22} = 3.03, p = 0.01; July vs January at t_{22} = 3.08, p = 0.005). Both the April and July periods had similar invertebrate abundances, as did the October and January periods.

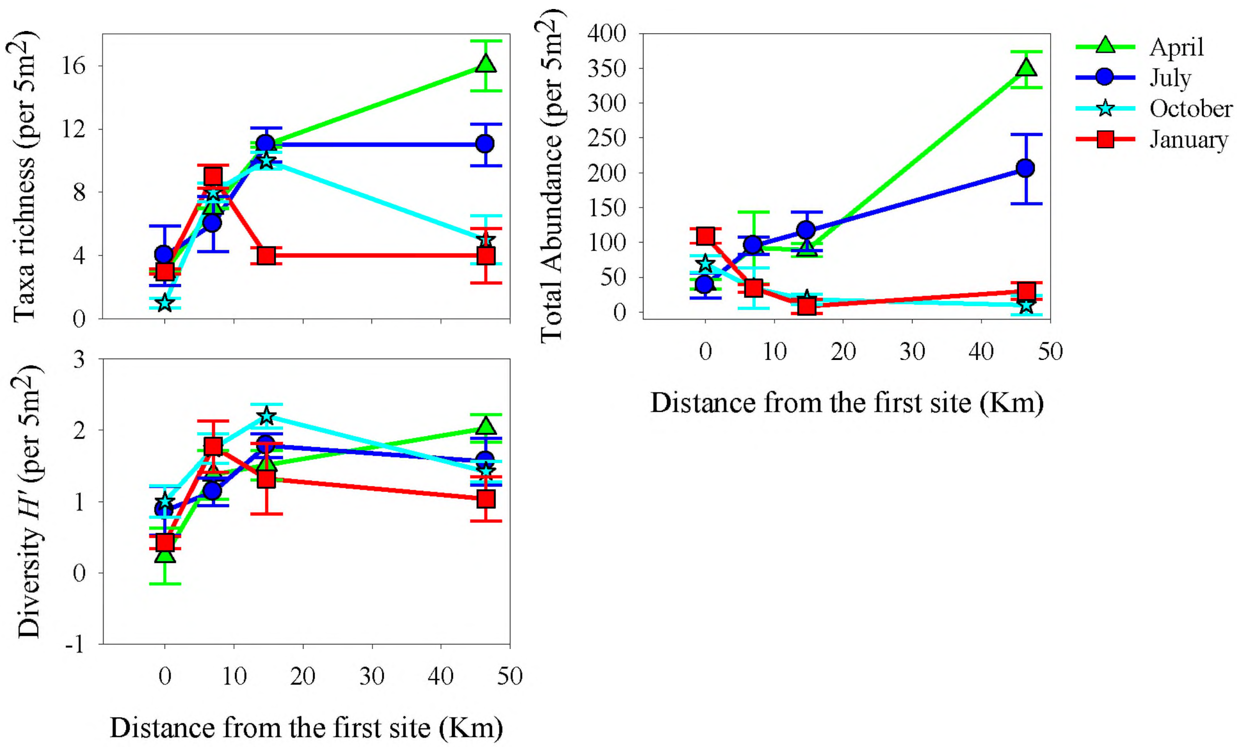

Figure 3.4: Spatiotemporal changes in macroinvertebrate community indices across sites, indicating variation of community structure between April 2013 and January 2014. At each site, invertebrates were collected from gravel sand and mud, marginal vegetation, and sand biotopes for a maximum of 10 minutes in a 5 m^2 area, providing three readings for each index that were averaged (± standard deviations indicated as error bars). Calculated community indices were transformed for easy comparison across sites. Sites were reported in distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km. ASPT- average score per taxon, SASS- South African Scoring System

3.4 SPECIES-ENVIRONMENT INTERACTIONS

3.5 Factors influencing macroinvertebrate distribution

The RELATE function within PRIMER V6 indicated that there was a significantly weak (Rho = 0.39, p = 0.01) relationship between the environmental variables and macroinvertebrate communities. A distance based linear model (Figure 3.5) revealed that DO, water depth, channel width, ammonia and nitrate were the five significant variables explaining macroinvertebrate community structure (i.e. df= 49: DO at F= 5.61, p= 0.002; water depth at F= 3.56, p= 0.006;

channel width at $F= 5.93$, $p= 0.001$, ammonium $F= 27.14$, $p= 0.001$; nitrate at $F= 3.99$, $p= 0.004$). Ammonium concentrations were important in explaining invertebrate distribution across all four sampling periods, but only at the Urban site (Figure 3.5). Nitrate, dissolved oxygen, channel width and water depth were all important at the Sewage site. At the more downstream Agriculture and Recreational sites, invertebrate distributions were associated with water depth, channel width and DO. Nitrate concentrations were important only in July. Channel width and the concentrations of ammonium, nitrate, and DO were important in April. The most important variables in October were water depth, channel width, and DO. In January, the only important variable was water depth.

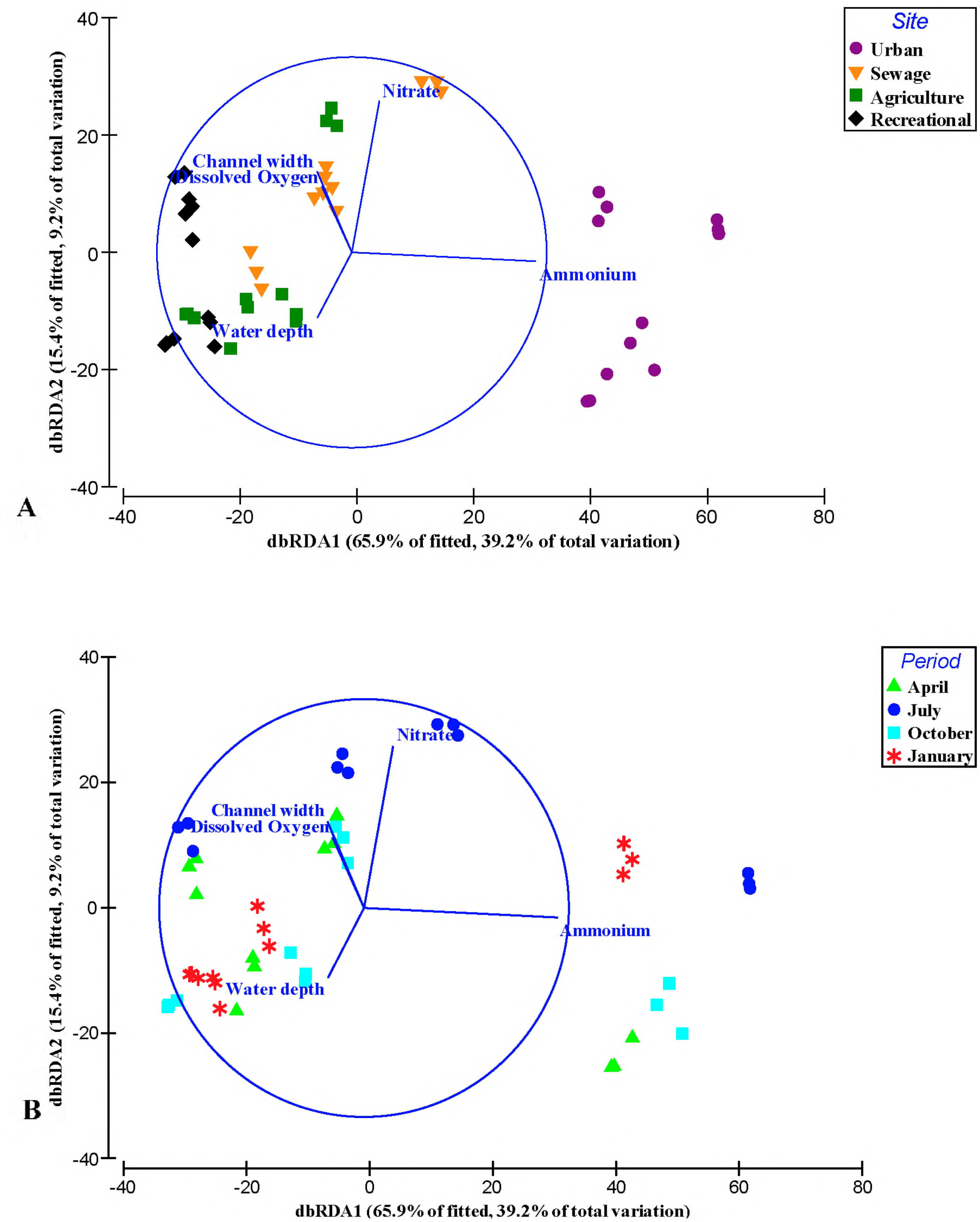

Figure 3.5: The first two dbRDA axes explaining 81.3% of variance in the distribution of macroinvertebrates relative to environmental variables in the Bloukrans River from April 2013 to January 2014. The model revealed the most influential environmental variables that structured

macroinvertebrate communities in (A) four sites that received pollution from different sources across (B) several sampling periods.

3.5.1 Spatiotemporal shifts in macroinvertebrate taxa dominance

An overall analysis showed that 86% of the macroinvertebrates collected during this study were tolerant to water pollution, 13% fell on the borderline between tolerant and sensitive, and only 1% were sensitive. The number of pollution tolerant invertebrates decreased in the downstream direction, creating a longitudinal gradient (Figure 3.6 A). Only one species from the Baetidae family (*Baetis* sp.) was identified during the entire course of this study. The lack of additional Baetidae species indicated that *Baetis* sp. had developed some tolerances to the disturbed environment (Dickens & Graham 2002). Ninety-eight percent of the invertebrates collected at the Urban site were tolerant to pollution, 88% of them belonged to family Chironomidae, 7% to the class Oligochaeta, and 3% to Physidae. At the Sewage site, 97% of the invertebrates were tolerant to pollution. The most dominant taxa included *Baetis* sp. (50%), Chironomidae (22%) and Simuliidae (13%). The Agriculture site was also dominated (80%) by tolerant taxa. The most dominant (40%) were *Baetis* sp., 16% Simuliidae and 14% Corixidae. At the downstream Recreational site, 78% were tolerant invertebrates belonging to families such as Baetidae (only *Baetis* sp. present at 43%), Hydropsychidae (19%), Corixidae (14%), and Simuliidae (11%). The dominance of Chironomidae species decreased in the downstream direction. The only highly sensitive taxon present during this study belonged to class Amphipoda, and specimens occurred only at the Recreational Site. Tolerant macroinvertebrates were highly dominant (86%, 85%, 87%, 91%) in April, July, October and January, respectively (Figure 3.6 B). *Baetis* sp. was most dominant in April (52%) and July (33%) relative to all other invertebrates. Chironomidae species were most abundant in October (66%) and January (47%).

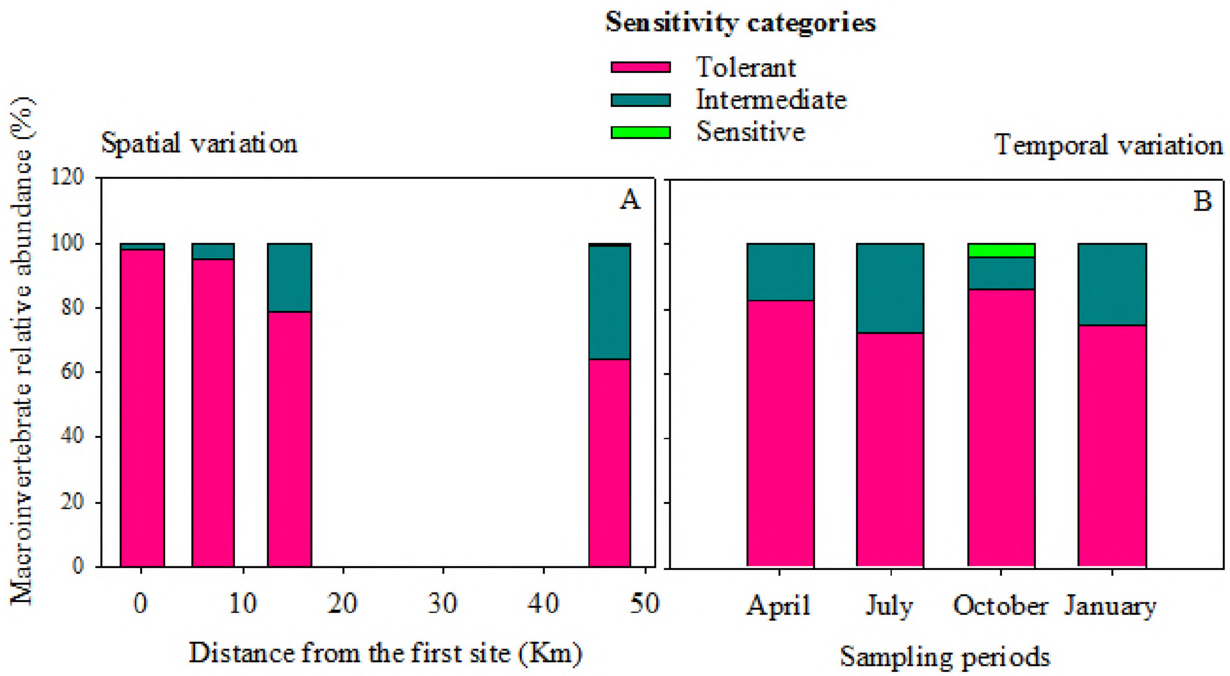

Figure 3.6: Macroinvertebrate dominance plots showing the relative abundances of tolerant, intermediate and sensitive species per site (A) and period (B) from April 2013 to January 2014. Sites were reported as distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

3.6 DIRECT RELATIONSHIPS BETWEEN ENVIRONMENTAL VARIABLES MACROINVERTEBRATE INDICES

3.6.1 Spatial and temporal effects

The second hypothesis of this study predicted that the macroinvertebrate community indices decrease with an increase in nutrient concentrations. Independent linear regression results indicated that nutrient concentrations were not the only significant predictors of changes in macroinvertebrate community structure, as DO concentration and physical parameters including water depth and channel width were also influential (Table 3.2). Although ammonium concentration was highest at the Urban site, it was not a significant predictor of changes in invertebrate community indices. Instead, DO concentration and channel width were significant in predicting changes in invertebrate diversity at this site. At the Sewage site, nitrate concentration was high, but was not statistically associated with changes in invertebrate community indices.

However, the variations in total invertebrate abundance were significantly predicted by changes in channel width, DO and ammonium concentrations. DO and ammonium concentrations were also significant in predicting changes in taxa richness at this site. At the Agriculture site, ammonium concentrations were lowest and significant in predicting increases in invertebrate diversity. DO concentrations were also significant in predicting an increase in invertebrate diversity and taxa richness. Water depth was a significant predictor of changes in total invertebrate abundance. Downstream at the Recreational site, nitrate concentration was lowest and significant as a predictor of increased invertebrate diversity, taxa richness and total invertebrate abundance. Additionally, channel width was significant at the Recreational site.

Ammonium and DO concentrations were significant predictors of changes in taxa richness, diversity and total invertebrate abundances across sampling periods (Table 3.3). Additionally, nitrate concentration was significant in predicting variations in total invertebrate abundance and taxa richness in April and October, respectively. The January period was particularly interesting because in addition to nutrient influences, water depth and channel width were influential on macroinvertebrate community structure.

Table 3.2: Simple linear regression results for spatial relationships between macroinvertebrate community matrices and environmental variables. The equation y = ax+ b was used to describe the relationships between environmental variables (x) and macroinvertebrate community indices (y), where a = y intercept and b = slope of the regression line. Relationships were explored per site and were considered statistically significant at $p \leq 0.05$, indicated in bold. DO = dissolved oxygen

Sites	Variables	Community Indices	R^2	F	*p* - value	a	b
Urban	DO ($mg.L^{-1}$)	Taxa richness	0.02	0.25	0.63	-0.06	0.72
		Total Abundance	0.22	2.89	0.12	0.15	1.50
		Diversity index (*H'*)	**0.49**	**9.73**	**0.01**	**0.10**	**0.23**
	Channel width (m)	Taxa richness	0.31	4.53	0.06	0.59	0.59
		Total Abundance	0.20	2.48	0.15	-0.37	2.26
		Diversity index (*H'*)	**0.41**	**6.93**	**0.03**	**-0.24**	**0.72**
Sewage	NH_4^+-N ($mg.L^{-1}$)	**Taxa richness**	**0.43**	**7.59**	**0.02**	**-0.10**	**1.07**
		Total Abundance	**0.41**	**7.04**	**0.02**	**0.29**	**1.49**
		Diversity (*H'*)	0.02	0.17	0.69	0.03	0.13
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.32**	**4.79**	**0.05**	**-0.38**	**1.68**
		Total Abundance	**0.33**	**5.03**	**0.05**	**1.14**	**-0.34**
		Diversity index (*H'*)	0.01	0.07	0.80	-0.08	0.30
	Channel width (m)	Taxa richness	0.23	2.95	0.12	-0.27	1.48
		Total Abundance	**0.56**	**12.83**	**0.01**	**1.24**	**-0.61**
		Diversity index (*H'*)	0.09	1.01	0.34	0.23	-0.30
Agriculture	NH_4^+-N ($mg.L^{-1}$)	Taxa richness	0.11	1.20	0.30	0.11	1.02
		Total Abundance	0.00	0.02	0.88	-0.04	1.66
		Diversity (*H'*)	**0.35**	**5.50**	**0.04**	**0.08**	**0.44**
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.40**	**6.65**	**0.03**	**0.78**	**-0.54**
		Total Abundance	0.25	3.38	0.10	1.74	-1.57
		Diversity index (*H'*)	**0.53**	**11.38**	**0.01**	**0.34**	**-0.27**

	Water depth	Taxa richness	0.32	4.61	0.06	-1.02	4.83
		Total Abundance	**0.57**	**13.12**	**<0.01**	**-3.83**	**16.33**
		Diversity index (*H'*)	0.01	0.14	0.72	-0.08	0.72
Recreational	NO_3^--N ($mg.L^{-1}$)	**Taxa richness**	**0.83**	**50.25**	**<0.01**	**0.15**	**0.85**
		Total Abundance	**0.84**	**50.59**	**<0.01**	**0.43**	**1.44**
		Diversity (*H'*)	**0.46**	**8.42**	**0.02**	**0.04**	**0.36**
	Channel width (m)	**Taxa richness**	**0.73**	**26.85**	**<0.01**	**0.68**	**-0.21**
		Total Abundance	**0.45**	**8.22**	**0.02**	**1.51**	**-0.80**
		Diversity index (*H'*)	**0.67**	**20.39**	**<0.01**	**0.25**	**-0.04**

Table 3.3: Simple linear regression results showing relationships between macroinvertebrate community matrices and influential environmental parameters over time. The equation y = ax+ b was used, where x = environmental variable, y= invertebrate community index, a = y intercept and b = slope of the regression line. Relationships were explored for each sampling period and were considered statistically significant when $p \leq 0.05$ (indicated in bold). DO = dissolved oxygen

Periods	Variables	Community Indices	R^2	*F*	*p* - value	a	b
April	NH_4^+-N ($mg.L^{-1}$)	**Taxa richness**	**0.87**	**66.99**	**<0.01**	**-0.22**	**1.23**
		Total abundance	**0.67**	**20.19**	**<0.01**	**-0.32**	**2.40**
		Diversity (*H*')	**0.81**	**43.75**	**<0.01**	**-0.14**	**0.49**
	NO_3^--N ($mg.L^{-1}$)	Taxa richness	0.23	2.92	0.12	0.14	0.70
		Total abundance	**0.34**	**5.15**	**0.05**	**0.29**	**1.48**
		Diversity (*H*')	0.26	3.48	0.09	0.10	0.15
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.57**	**13.10**	**<0.01**	**0.29**	**0.51**
		Total Abundance	**0.57**	**13.03**	**<0.01**	**0.48**	**1.25**
		Diversity index (*H*')	**0.57**	**13.42**	**<0.01**	**0.19**	**0.04**
	Channel width (m)	Taxa richness	0.19	2.31	0.16	0.36	0.34
		Total Abundance	**0.62**	**16.44**	**<0.01**	**1.08**	**0.11**
		Diversity index (*H*')	0.14	1.58	0.24	0.20	-0.01
July	NH_4^+-N ($mg.L^{-1}$)	**Taxa richness**	**0.70**	**23.64**	**<0.01**	**-0.14**	**1.14**
		Total abundance	**0.65**	**18.62**	**<0.01**	**-0.18**	**2.13**
		Diversity (*H*')	**0.74**	**28.43**	**<0.01**	**-0.07**	**0.47**
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.57**	**13.48**	**<0.01**	**0.75**	**-0.59**
		Total Abundance	**0.52**	**10.76**	**0.01**	**0.98**	**-0.16**
		Diversity index (*H*')	**0.45**	**8.18**	**0.02**	**0.33**	**-0.30**
October	NH_4^+-N ($mg.L^{-1}$)	**Taxa richness**	**0.39**	**6.41**	**0.03**	**-0.20**	**1.11**
		Total abundance	**0.63**	**16.74**	**<0.01**	**0.27**	**1.23**
		Diversity (*H*')	**0.64**	**17.78**	**<0.01**	**-0.18**	**0.58**
	NO_3^--N ($mg.L^{-1}$)	**Taxa richness**	**0.41**	**7.02**	**0.02**	**0.12**	**0.65**

		Total abundance	0.01	0.05	0.82	0.01	1.55
		Diversity (*H'*)	0.32	4.72	0.06	0.07	0.23
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.50**	**9.85**	**0.01**	**1.12**	**-1.36**
		Total Abundance	**0.55**	**12.12**	**0.01**	**-1.22**	**3.98**
		Diversity index (*H'*)	**0.75**	**29.43**	**<0.01**	**0.94**	**-1.50**
	Water depth (cm)	Taxa richness	0.10	1.08	0.32	0.51	-1.10
		Total Abundance	**0.53**	**11.07**	**0.01**	**-1.22**	**6.27**
		Diversity index (*H'*)	0.15	1.71	0.22	0.42	-1.28
January	NH_4^+-N ($mg.L^{-1}$)	**Taxa richness**	**0.57**	**13.36**	**<0.01**	**-0.20**	**0.96**
		Total abundance	**0.48**	**9.39**	**0.01**	**0.21**	**1.44**
		Diversity (*H'*)	**0.64**	**17.61**	**<0.01**	**-0.12**	**0.43**
	DO ($mg.L^{-1}$)	**Taxa richness**	**0.69**	**22.69**	**<0.01**	**-1.70**	**3.97**
		Total Abundance	**0.32**	**4.64**	**0.06**	**1.27**	**-0.76**
		Diversity index (*H'*)	**0.80**	**40.55**	**<0.01**	**-1.05**	**2.28**
	Water depth (cm)	**Taxa richness**	**0.43**	**7.60**	**0.02**	**0.54**	**-1.03**
		Total Abundance	**0.54**	**11.77**	**0.01**	**-0.66**	**3.87**
		Diversity index (*H'*)	**0.73**	**27.43**	**<0.01**	**0.40**	**-1.04**
	Channel width (m)	**Taxa richness**	**0.66**	**19.13**	**<0.01**	**0.66**	**-0.18**
		Total Abundance	**0.49**	**9.44**	**0.01**	**-0.63**	**2.54**
		Diversity index (*H'*)	**0.82**	**44.16**	**<0.01**	**0.42**	**-0.30**

3.7 EFFECTS OF ENVIRONMENTAL VARIABLES ON WATER QUALITY

3.7.1 Overall water quality status of the Bloukrans River

Water quality was poor in the Bloukrans River during the course of this study (Table 3.4). Overall, water quality shifted from critically impaired (upstream) to generally poor (downstream). There were consistent patterns of serious impairment of the ecological integrity coupled with the dominance of pollution tolerance taxa throughout all sampling periods.

Table 3.4: The ecological status of the Bloukrans River between April 2013 and January 2014. Classification categories were allocated according to Dallas (2007)

SITE	SASS Score	ASPT	IHI CLASS	DESCRIPTION
Urban	13	3.0	F (Critically modified)	Critically impaired. A few tolerant taxa present.
Sewage	29	3.5	F (Critically modified)	Critically impaired. A few tolerant taxa present.
Agriculture	48	4.4	E (Seriously modified)	Severely impaired. Only tolerant taxa present.
Recreational	54	5.7	D (Poor)	Considerably impaired. Mostly tolerant taxa present.
PERIOD				
April	41	3.8	E (Seriously modified)	Severely impaired. Only tolerant taxa present.
July	30	4.1	E (Seriously modified)	Severely impaired. Only tolerant taxa present.
October	33	4.5	E (Seriously modified)	Severely impaired. Only tolerant taxa present.
January	30	4.1	E (Seriously modified)	Severely impaired. Only tolerant taxa present.

3.7.2 Variability in water quality

Macroinvertebrate response to changes in water quality differed significantly across sites, as the SASS5 and ASPT matrices varied greatly (SASS5 $F_3 = 19.59$, $p < 0.0001$ and ASPT $F_3 = 15.98$, $p < 0.0001$). Both the SASS5 and ASPT scores increased in the downstream direction (Figure 3.7), supporting the fourth hypothesis. The Urban upstream site had significantly lower scores when compared to all other sites (SASS5: Urban- Sewage at $t_{22} = -3.27$, $p = 0.003$; Urban-Agriculture at $t_{22} = -6.55$, $p < 0.0001$; Urban- Recreational at $t_{22} = -6.20$, $p < 0.0001$ and ASPT: Urban- Sewage at $t_{22} = -1.92$, $p = 0.03$; Urban- Agriculture at $t_{22} = -3.21$, $p = 0.004$; Urban-

Recreational at $t_{22} = -5.56$, $p < 0.0001$). Low SASS5 and ASPT scores indicated water quality deterioration upstream at the Urban site. A one-way ANOVA indicated no statistical differences in SASS5 ($F_3 = 0.62$, $p = 0.60$) and ASPT ($F_3 = 0.56$, $p = 0.64$) across sampling periods.

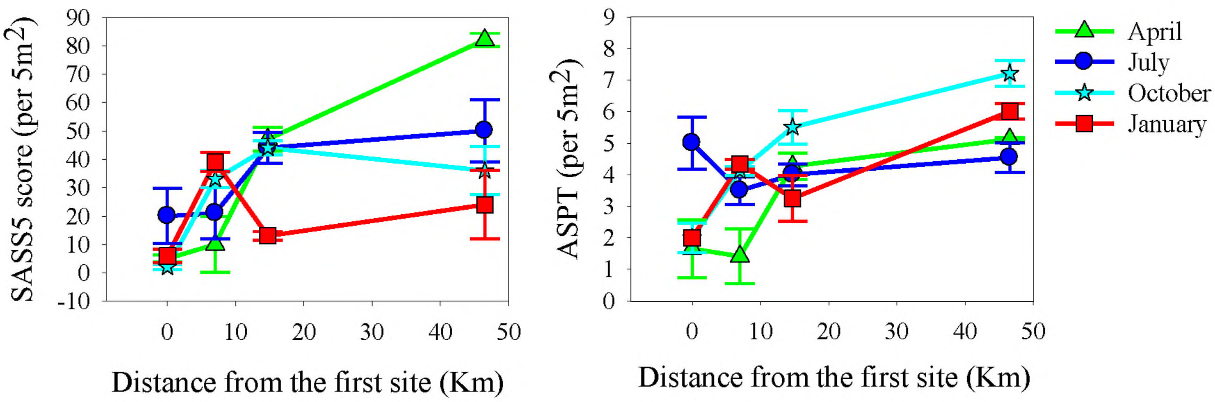

Figure 3.7: Spatio-temporal changes in SASS and ASPT values across sites, indicating variation of community structure between April 2013 and January 2014. At each site invertebrates were collected from gravel sand and mud, marginal vegetation, and sand biotopes for a maximum of 10 minutes in a 5 m^2 area, providing three readings for each index that were averaged (± standard deviation indicated as error bars). Calculated community indices were transformed for easy comparison across sites. Sites were reported in distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km. ASPT- average score per taxon, SASS- South African Scoring System.

3.7.3 Spatial effects of environmental variables on water quality

Since the SASS and ASPT scores are best interpreted as pairs, I first report the environmental factors that influenced them both, and later those that had an influence on one score without affecting the other. Based on the regression results, water depth was a significant predictor of changes in SASS and ASPT scores (Table 3.5) at the Sewage site. At the Recreational site, nitrate concentrations and channel width were significant predictors of variations in SASS and ASPT scores. Channel with was significant in predicting changes in SASS scores at the upstream Urban site. At the Agriculture site, dissolved oxygen concentration was significant in predicting changes in SASS score, while ammonium concentration was a significant predictor of changes in ASPT.

Table 3.5: Simple linear regression results showing how the variation in environmental variables affected the water quality scores across sites from April 2013 to January 2014. The equation y = ax+ b was used, where x = environmental variable, y = water quality index, a = y intercept and b = slope of the regression line. Relationships were explored for each site and considered statistically significant when $p \leq 0.05$ (indicated in bold). DO= dissolved oxygen, SASS Scores= South African Scoring System Scores, ASPT= Average Scores per Taxon.

Sites	Environmental variable	Index scores	R^2	F	*p*-value	a	b
Urban	NH_4^+-N ($mg.L^{-1}$)	SASS	0.15	1.80	0.21	0.94	-1.31
		ASPT	0.19	2.42	0.15	0.36	-0.30
	NO_3^--N ($mg.L^{-1}$)	SASS	0.00	0.04	0.84	0.02	0.95
		ASPT	0.00	0.02	0.89	-0.01	0.59
	DO ($mg.L^{-1}$)	SASS	0.05	0.47	0.51	-0.15	1.23
		ASPT	0.07	0.72	0.42	-0.06	0.68
	Water depth (cm)	SASS	0.02	0.21	0.66	0.18	0.35
		ASPT	0.03	0.34	0.58	0.08	0.31
	Channel width (m)	**SASS**	**0.36**	**5.51**	**0.04**	**1.10**	**-0.56**
		ASPT	0.28	3.86	0.08	0.33	0.12
Sewage	NH_4^+-N ($mg.L^{-1}$)	SASS	0.30	4.21	0.07	-0.22	1.69
		ASPT	0.19	2.29	0.16	-0.09	0.75
	Nitrate (NO_3^-)	SASS	0.10	1.17	0.30	0.06	1.33
		ASPT	0.10	1.08	0.32	0.03	0.58
	DO ($mg.L^{-1}$)	SASS	0.24	3.08	0.11	-0.86	3.06
		ASPT	0.16	1.86	0.20	-0.38	1.36
	Water depth (cm)	**SASS**	**0.52**	**10.80**	**0.01**	**0.87**	**-1.73**
		ASPT	**0.73**	**27.41**	**<0.01**	**0.56**	**-1.39**
	Channel width (m)	SASS	0.22	2.85	0.12	-0.70	2.80
		ASPT	0.17	2.05	0.18	-0.33	1.29
Agriculture	NH_4^+-N ($mg.L^{-1}$)	SASS	0.12	1.38	0.27	0.16	1.59
		ASPT	**0.45**	**8.10**	**0.02**	**0.09**	**0.69**
	NO_3^--N ($mg.L^{-1}$)	SASS	0.08	0.84	0.38	0.04	1.54
		ASPT	0.01	0.09	0.77	0.00	0.72
	DO ($mg.L^{-1}$)	**SASS**	**0.49**	**9.67**	**0.01**	**0.74**	**0.24**
		ASPT	0.05	0.48	0.51	-0.09	1.00
	Water depth (cm)	SASS	0.23	2.93	0.12	-0.74	4.51
		ASPT	0.26	3.53	0.09	0.32	-0.38

	Channel width (m)	SASS	0.02	0.25	0.63	-0.17	1.98
		ASPT	0.01	0.05	0.82	0.03	0.77
Recreational	NH_4^+-N (mg.L^{-1})	SASS	0.10	1.17	0.31	-0.51	1.78
		ASPT	0.04	0.39	0.54	0.12	0.80
	NO_3^--N (mg.L^{-1})	**SASS**	**0.56**	**12.86**	**0.01**	**0.11**	**1.60**
		ASPT	**0.77**	**34.06**	**<0.01**	**0.05**	**0.87**
	DO (mg.L^{-1})	SASS	0.01	0.07	0.80	0.06	1.58
		ASPT	0.22	2.80	0.13	-0.15	1.12
	Water depth (cm)	SASS	0.05	0.51	0.49	-0.11	2.11
		ASPT	0.23	2.97	0.12	0.10	0.47
	Channel width (m)	**SASS**	**0.67**	**20.21**	**<0.01**	**0.56**	**0.71**
		ASPT	**0.34**	**5.04**	**0.05**	**0.16**	**1.11**

3.8 DISCUSSION

The important environmental factors controlling macroinvertebrates in the Bloukrans River during the period of April 2013 to January 2014 were channel width, water depth, DO, ammonium, and nitrate concentrations (Figure 3.5). These factors were examined spatially and temporally to evaluate their effects on community structure using community indices.

3.8.1 Spatio-temporal variations of significant environmental parameters

3.8.1.1 Variations in channel width and water depth and their effects on macroinvertebrates

Bank to bank channel width and water depth varied across sites, with a narrow and shallow channel upstream at the Urban site. The river continuum concept (RCC) predicts that a river channel is naturally narrow and shallow upstream, and broader and deeper towards the mouth (Vannote *et al.* 1980). Such a prediction is normally true in un-impacted rivers. Anthropogenic and climatic conditions can, however, cause similar morphological changes in river channels as those predicted by the RCC (Montgomery 1999). For example, land use practices and flooding events played a noticeable role in channel alteration in the current study. Although the morphological makeup of the Bloukrans River resembled the RCC predictions, man-made alterations such as water abstraction and direct sewage effluent discharge also contributed significantly to the downstream widening of the channel. The upstream Urban site was the most narrow and shallow, and had the lowest habitat integrity scores, indicating major habitat degradation (Table 3.1). Serious alterations at this site were caused by the direct disposal of solid waste including bottles, sanitary towels, baby diapers, and bricks, collectively decreasing the naturally available instream habitat. During the wet season (September-November), runoff from human settlements, grazing, and brick construction activities deposited large amounts of sediment into the channel. Increased sedimentation resulted in low water velocity and reduced water depth during the dry season (May-July). The effects of sedimentation have been reported in several studies, particularly for rivers that drain urban settlements (e.g. Gregory & Chin 2002; Chin & Gregory 2005; Downs & Gregory 2014). When examining the effects of channel width and depth on macroinvertebrates at the Urban site of the Bloukrans River, it was apparent that only channel width was influential as there was a positive relationship between width and macroinvertebrate diversity upstream. The narrow bank to bank distance limited habitat heterogeneity and complexity, which consequently reduced macroinvertebrate diversity. Since macroinvertebrates have diverse habitat preferences (Jowett & Richardson 1990), the low species diversity was attributed to the lack of natural habitat at the Urban site. Species composition is highly controlled by the availability of multiple substrate types in microhabitats (Jowett & Richardson 1990; Johnson & Goedkoop 2002). In the Bloukrans River, however, natural habitat was limited, and the addition of motile artificial substrates such as bottles and solid litter instream probably prohibited macroinvertebrate larvae from colonizing.

The second site (Sewage) was the widest and deepest of all three sites, located just downstream of a wastewater discharge point, and just upstream of an impervious bridge that restricted water flow. Channel width and water depth increased as a result of wastewater discharge upstream and water flow regulation downstream. The increased bank to bank cross section caused by bank erosion may have occurred over time at the site, and was attributed to flooding and the growth of riparian vegetation. Although riparian vegetation may provide bank stability due to the holding ability of roots (Wenger 1999), excessive growth of littoral vegetation may also erode river banks under persistent anthropogenic influences (Montgomery 1997; Buckingham & Whitney 2007), especially during flooding seasons. Furthermore, Buckingham & Whitney (2007) provided evidence that wastewater discharge facilitates bank erosion and can increase channel width over time. In the current study, the widening of the channel facilitated habitat heterogeneity and complexity, resulting in increased macroinvertebrate abundance. Among the dominant microhabitats were riffles, pools, gravel (stones), and marginal vegetation that provided refuge to dominant invertebrate nymphs belonging to the families Baetidae, Chironomidae, and Simuliidae (450, 201, and 118 individuals per 5 m^2, respectively). The dominance of these invertebrates was facilitated by the availability of their desired habitat. For example, the family Baetidae prefers stony coarse rifles, Chironomidae dominates in pools, and Simuliidae attaches to any solid material present in the water column including stones, debris, and marginal vegetation (Gerber & Gabriel 2002).

The last two downstream sites (Agriculture and Recreational) were wider compared with the upstream Urban site, but less so compared with the Sewage site. The Agriculture and Recreational sites were closest to agricultural croplands and were structurally modified to store volumes of water for irrigation. Flow was regulated by the construction of small weirs, resulting in the formation of minor pools within the sampled area. Water extraction pipes occurred at the Agriculture site, and these were actively extracting water for the surrounding farms. In-channel and bank modifications resulted in a habitat characterization score between 1 and 4, an indication of extensive habitat alterations. Channel size was artificially increased due to the removal of in-channel boulders to facilitate the transportation of agricultural machinery and produce between farms at the Agriculture site. Turunen *et al.* (2016) documented similar artificial increases in channel width and water depth in agricultural river reaches in Finland streams as a result of the removal of boulders to create a path for the transportation of timber. At the Recreational site of the Bloukrans River, no weirs were observed during this study, but there was evidence of previous water diversion attempts that altered the natural state of the river banks and consequently increased channel size. The recreational activities that occurred at the Recreational site did not have significant impacts on channel size or water depth. For example, Figure 2.5 shows some evidence that minor fires were common on site, and were prepared along the riparian habitat at distances close enough to have had direct impacts on channel structure. Furthermore, while spiritual cleansing and body purifications occurred within the instream habitat at the pool, these activities did not result in enlargement of the channel or increased water depth. Instead, agriculture related activities were those identified as prominent contributors to increased water depth and channel width at the Recreational site.

A general assessment of temporal changes in channel morphology indicated that channel width did not differ significantly across sampling periods, but water depth did. The Bloukrans River had the highest volumes of water and the deepest channel reaches in October 2013 (Figure

3.2). Increases in water depth were coupled with an increase in water velocity, and were strongly attributed to high freshwater discharge during October. Massive rainfall affected the river, resulting in large volumes of water flowing at relatively high velocities. Maximum monthly rainfall levels between 54-63 mm occurred in spring between the 1st September and 30th November in Grahamstown (http://www.timeanddate.com/weather/southafrica/grahamstown/historic?month=1&year=2014).

The effects of increased water depth on macroinvertebrates were prominent at the Agriculture site. High water depth was positively associated with taxa richness but negatively associated with invertebrate abundance. The highest number of taxa (i.e. 24 families in total) with the lowest total abundances (i.e. 688 individual invertebrates) were observed at this site. These findings contradicted those of Hille *et al.* (2014), who found that increased water depth in a Danish lowland stream did not necessarily provide refuge for more taxa and did not affect taxa richness. Instead, the current results were supported by the findings of Collier *et al.* (2013), who reported high taxa richness in deeper rivers in New Zealand. The high habitat (substrate) heterogeneity at the Agriculture site of the Bloukrans River may have contributed to high taxa richness. The most abundant taxa were from the families Baetidae and Simuliidae, whose habitat preferences include riffles, stones, attachment biotopes and deeper water, all characteristics of this site.

Water depth had no significant effect on macroinvertebrates at the Recreational site; however, positive relationships were observed between channel width and all macroinvertebrate indices (i.e. total abundance, diversity, and taxa richness). The Recreational site was wider and supported high invertebrate abundances, family diversity, and richness mainly because it was forested and fairly pristine with the highest IHI scores (Table 3.1). Several studies have reported that forested sites are wider than urbanized ones (e.g. Allmendinger *et al.* 1999, 2005; Burchsted *et al.* 2010), and that the relative abundance, diversity, and richness of macroinvertebrates are usually high in forested river reaches (Sweeney *et al.* 2004). Wide forested reaches are heterogeneous and have high detrital loads from riparian vegetation, providing a major source of energy to sustain a variety of invertebrates (Johnson & Covich 1997). Other factors that strongly structured the longitudinal and temporal variation in macroinvertebrate distribution at the Recreational site of the Bloukrans River included oxygen availability, nutrient loading and water quality.

3.8.1.2 Variation of dissolved oxygen and its impacts on macroinvertebrates

For the most part, the Bloukrans River was not under hypoxic stress since the minimum DO concentrations were above 2 mg.L^{-1} (Yin *et al.* 2004), with the exception of site Urban during April when the concentrations were as low as ±0.9 mg.L^{-1}. A longitudinal gradient in DO was observed, with the lowest DO occurring at the upstream Urban site and highest at the downstream Recreational site (Figure 3.2). Changes in DO were attributed to variations in environmental factors such as temperature, water flow velocity, and biological consumption. High water temperature at the Urban site greatly reduced DO since there was no canopy cover and samples were collected in broad daylight under sunny weather conditions. Oxygen solubility decreases with increase in water temperature (e.g. Wilcock *et al.* 1998; Ilarri *et al.* 2010; Eliason *et al.* 2011).

In the current study, DO concentration improved as canopy cover increased in the downstream direction, and the maximum values were recorded at the Recreational site. At the Sewage site, low DO concentrations were strongly influenced by the reduction in flow velocity that hindered atmospheric reaeration, and increased biological oxygen demand caused by elevated bacterial activities associated with sewage decomposition (see Caraco *et al.* 2000; Cox 2003). DO depletion by respiration was most likely due to bacterial activity rather than macroinvertebrate metabolism, mainly because invertebrate abundances did not change significantly across sites but DO concentrations did. Furthermore, if invertebrate respiration caused low DO, then the site with the highest invertebrate abundance was expected to have the lowest DO level, which was not the case as both DO levels and invertebrate abundances were highest at the same site (Recreational site). The general findings suggested that macroinvertebrate taxa richness and diversity decreased with decreasing DO concentrations, and increased as DO increased. Although DO was abundantly available at the Recreational site, macroinvertebrate responses were not significantly associated with DO concentrations (Table 3.2). However, in the other three sites where DO was lower, macroinvertebrate community structure changed greatly, probably because the animals adjusted their respiration patterns or reduced their levels of activity when DO concentrations decreased (Cox 2003). Such biological responses to DO depletion may result in poor species composition, and a dominance of invertebrates that are capable of switching to atmospheric oxygen (Justus *et al.* 2014). The results indicated that low DO concentrations were associated with a reduced family diversity at the Urban site, while some improvements in DO levels at the Agriculture site were associated with increased family diversity and taxonomic richness. Lower DO concentrations at the Sewage site resulted in a reduction of taxa richness even though total invertebrate abundance was higher (Table 3.2 & Figure 3.2). Spatially, low DO concentration reduced macroinvertebrate taxonomic richness, diversity, and abundance. Ding *et al.* (2016) reported a similar pattern of reduced taxa richness and general invertebrate abundances in the DO depleted Ziya River Basin in China. A study conducted by Justus *et al.* (2014) also showed that diversity and taxa richness decreased with a decrease in DO minima, and most of the observed taxa were tolerant to low DO concentrations. Although Kaller & Kelso (2006) observed a contrasting pattern of high invertebrate abundance, diversity and taxa richness at low DO concentrations in selected south eastern USA streams, their observations supported the current findings in the sense that the highly abundant and diverse taxa were more tolerant to low DO concentrations. The two upstream sites were the most polluted and oxygen depleted, and were dominated by tolerant taxa including Chironomidae and Simuliidae that have permanent air storage respiratory structures to help them withstand low DO. Ephemeroptera was also dominant, and although this order is sensitive to pollution and oxygen declines (Arimoro & Muller 2009), the Baetidae family consists of pollution resistant species (Buss & Salles 2006; Menetrey *et al.* 2007).

DO concentration varied significantly with season in the Bloukrans River (Figure 3.2 and Table 3.3). Significantly lower concentrations were observed in April (autumn), while the highest were in July (winter), primarily because of temperature variations. The April period was relatively warmer than July, thereby facilitating DO depletion. Macroinvertebrate community matrices did not change significantly across periods except for total invertebrate abundance, which was highest in April. The relationships between DO concentration and macroinvertebrate abundance indicated that there were 57% and 52% probabilities that changes in macroinvertebrate abundances were due to variations in DO during April and July, respectively. High abundances of invertebrates regardless of low DO concentrations in April strongly indicated specialized respiratory adaptations and coping strategies for the invertebrates. Family Baetidae had the most abundant naiads in both April and July. Coping mechanisms employed by Baetidae include reducing their level of activity during the day and being more active at night (Elliott 1967) when DO saturation is relatively higher due to cooler temperatures. Maximum DO occurred during the coldest period (July), when more sensitive species began to emerge, which in turn increased diversity and taxa richness (Table 3.3).

3.8.1.3 Variations in nutrients and their effects on macroinvertebrates

Of the measured nutrients, only ammonium and nitrate varied significantly across sites, whereas phosphates did not. The first hypothesis that the concentrations of nitrate, ammonium, and phosphate decrease in the downstream direction following a human influence gradient was partially supported because only ammonium concentrations matched the human influence gradient. Considering that ammonium is an easily alterable form of nitrogen (Webster *et al.* 2003), its high concentration at the Urban site may have originated from mineralization and external inputs from land (Bu *et al.* 2011). According to the World Health Organization (WHO 1993) and the South African Water Quality Guidelines for Aquatic Ecosystems (DWA 1996), the amount of ammonium naturally available in surface waters typically does not exceed 0.2-3 $mg.L^{-1}$ in rivers. In the Bloukrans River, only the three more downstream sites complied with the WHO and DWA standards. At the Urban upstream site, however, ammonium concentrations were as high as 10 $mg.L^{-1}$. Such high NH_4^+ levels indicated direct anthropogenic inputs from residential and industrial runoff. At close proximity to the Urban site were human settlements with watersheds affected by poor sanitation practices located a few meters above the river banks, providing easy flow and deposition of nitrogenous material into the river during heavy rain.

Underdeveloped watersheds act as primary stores for nitrogenous waste (Hundecha & Bárdossy 2004; Burns *et al.* 2005; Bernhardt *et al.* 2008) that eventually enter rivers through run off. The greatest contributors to nitrogenous waste onsite were human faecal material, dumping landfills, and decomposing cow dung. Ngoye & Machiwa (2004) reported similar findings of high ammonium concentrations at a reach that drained human settlements at the Ruvu River in Tanzania. David *et al.* (2016) observed highest concentrations of ammonium at the section of the Pamba River, India, that drained the largest pilgrimage temple on earth (the Sabarimala Temple). During the worship season, the temple receives approximately 555,000 pilgrims per day, whose activities resulted in elevated ammonium concentrations derived from sanitary latrines and liquid waste disposal (David *et al.* 2016). In the current study, the highest ammonium concentrations were observed in October when rainfall was high and resulted in significant runoff. Randall & Mulla (2001), Jiao *et al.* (2010), and Bu et al. (2011) have also reported significantly high ammonium concentrations in rivers when precipitation rates were highest.

Although nitrate patterns in the Bloukrans River did not support the first hypothesis, the highest concentrations were observed at the Sewage site immediately downstream of the Urban site. Based on the South African Water Quality Guidelines for Aquatic Ecosystems, naturally occurring nitrates in surface water typically range from 0.1-10 $mg.L^{-1}$ (DWA 1996). At the Sewage site, however, nitrate concentrations averaged as high as 28.6 $mg.L^{-1}$, suggesting anthropogenic inputs. Additional nitrates were probably a result of the combined effects of nitrification of ammonium derived from upstream regions, nitrate deposition from fertilizers, and direct sewage disposal. Ammonium oxidation probably occurred due to the high DO concentrations observed at this site, as ammonium is rapidly oxidized to nitrate in well oxygenated waters (Sabalowsky 1999; Kurosu 2001). On the other hand, sewage effluent and agricultural runoff primarily contain inorganic nitrate, among other pollutants (Drury *et al.* 2013; Kamjunke *et al.* 2013). The impacts of sewage disposal in the Bloukrans River were most prominent during July, when the highest nitrate concentration of 58.3 $mg.L^{-1}$ occurred. The July period was generally dry with an average rainfall of 24 mm, so freshwater input was limited, thereby reducing the natural dilution of sewage discharge. Additionally, sewage discharge into the river from the Grahamstown sewage treatment plant had occurred two days prior to the sampling day, resulting in high nutrient concentrations.

The second hypothesis that sites with the highest nutrient concentrations have low invertebrate abundances, taxa richness and diversity was partially supported in a sense that not all the community indices decreased with increases in NH_4^+ or NO_3^-. Instead, only taxa richness was lowest at the highest NH_4^+ concentrations (at the Urban site), while only diversity was lowest at the highest NO_3^- concentrations (at the Sewage site). Although these patterns supported the second hypothesis, there was no evidence to suggest that ammonium and nitrate concentrations caused the reduction in taxa richness and diversity at the Urban and Sewage sites, respectively. However, a relationship between each of the three indices was observed when analyzed in relation to nitrate concentrations at the downstream Recreational site. Low nitrate concentrations were positively associated with high invertebrate taxa richness, total abundance and family diversity downstream. Overall spatial observations indicated that high ammonium and nitrate concentrations did not favor taxa richness and diversity, while a decrease in these nitrogenous nutrients improved the emergence of taxa and favored invertebrate diversity and abundance. Usseglio-Polatera & Beisel (2002) found a similar pattern when assessing the longitudinal variation of macroinvertebrates in the Meuse River in Europe. Their main findings indicated that macroinvertebrate taxa richness, abundance and diversity were higher at the river reaches that had low nitrogenous nutrient concentrations, and increased when concentrations decreased (Usseglio-Polatera & Beisel 2002). Similarly, macroinvertebrate community structure changed with variations in ammonium and nitrate concentrations at the Sidabra and Tatula Rivers in Lithuania, Northern Europe (Pliuraite & Mickėnienė 2009). The relationship between nitrogenous nutrients and macroinvertebrates was weak in the Bloukrans River when analyzed across sampling periods. Instead, factors including high flow velocity and increased water abstractions probably contributed greatly to the observed invertebrate distributions, and not just the nutrients. For instance, ammonium concentrations did not vary significantly across sampling periods, and even though the concentrations were slightly higher in October and lowest in January (Figure 3.2), both high and low concentrations were associated with low invertebrate taxa, diversity and total abundance during the two periods (Table 3.3). These findings suggested that although there was a relationship between low or high ammonium and macroinvertebrates, such a relationship was not ecologically conclusive because taxa richness, diversity and abundance remained low in October and January regardless of the slight changes in ammonium concentrations. As mentioned earlier, water velocity was greatest in October due to flooding, while extensive water abstractions were observed in January. Both these incidents probably contributed to low invertebrate diversity since both flooding and reduced flow negatively affect invertebrate diversity (Dickens & Graham 2002; Hillman & Quinn 2002; Wood & Armitage 2004). The nitrate-invertebrate relationship was also inconclusive. Although the concentrations varied significantly across periods (with the highest in July and lowest in October), no significant relationships were observed at high nitrate concentrations in July. Low concentrations were significantly associated with low invertebrate taxa richness, diversity and total abundance in October, suggesting that the indirect effects of flooding were strong enough to dilute nitrates while simultaneously sweeping away invertebrates.

3.8.2 Variability in water quality

Water quality was poor at the Bloukrans River between April 2013 and January 2014. Changes in water quality did not occur over time, but along the longitudinal gradient of the river, indicating the importance of spatial habitat conditions (Table 3.4). Habitat integrity was lost due to man-made modifications of the local channel to sustain different economical and recreational activities. Changes in habitat morphology had significant impacts on water quality and biological communities. For instance, more than 80% of the invertebrates collected during the study were resilient to habitat alterations and water pollution (Figure 3.6). There were no significant changes in taxa dominance across sampling periods, but there were across sites. The third hypothesis that macroinvertebrate dominance shifts from tolerant taxa upstream and to sensitive ones downstream following a land use gradient was supported because more than 95% of the taxa collected upstream were tolerant to pollution and habitat alterations. The relative dominance of tolerant taxa shifted from 98% at the Urban site to 64% at the downstream Recreational site. The general underrepresentation of sensitive macroinvertebrates was indicative of unfavorable conditions due to loss of natural habitat and impaired water quality (Dickens & Graham 2002), and therefore only tolerant taxa could survive. Tolerant taxa have low sensitivity scores (e.g. Chironomidae: 2, Corixidae: 3, Baetidae 1sp: 4, and Simuliidae: 5), and their dominance in the Bloukrans River explained the progressively low SASS and ASPT scores. Dallas (2007b) and Smith-Adao & Scheepers (2007) also reported that SASS and ASPT scores were low in rivers that were dominated by tolerant taxa in Mpumalanga and the Western Cape, respectively. In the Bloukrans River, SASS and ASPT varied significantly across sites, supporting the fourth hypothesis that there is a longitudinal gradient in SASS5 and ASPT scores, with increasing scores occurring in the downstream direction. Since SASS and ASPT values are directly linked to macroinvertebrate diversity and number of taxa (Dickens & Graham 2002), a reduction in diversity and taxa richness would significantly reduce SASS and ASPT scores (Chutter 1998). At the first three sites (Urban, Sewage, and Agriculture), SASS and ASPT scores were below 50 and 5, respectively, indications of major deterioration in water quality (see Dallas 2007a; Dickens & Graham 2002). Habitat variability had the strongest influence on the reduction of SASS and ASPT scores at the Urban and Sewage sites. The reduction in channel width coupled with loss of natural habitat restricted macroinvertebrate diversity and lowered SASS scores at the Urban site. Low SASS and ASPT scores at the Sewage site were due to the indirect effects of increased water depth, which restricted diversity since the deep waters were oxygen deficient. Water quality scores began to increase towards the Agriculture and Recreational sites (Table 3.4) as taxa richness and diversity increased. In both sites, variations in scores were attributed to changes in water chemistry and habitat structure. For instance, the combination of low ammonium with high DO concentrations favored macroinvertebrate diversity and taxa richness at the Agriculture site. However, water quality scores remained below the acceptable health standards of the RHP (Dallas 2007a). This finding was attributed to the October flooding events and the extensive instream modifications that occurred in January. Dicken & Graham (2002) reported that flooding and the loss of natural habitat could affect SASS and ASPT scores even in areas with low concentrations of nutrients and organic pollutants. The condition of the channel was generally better at the last site (Recreational), where the channel was large enough for the occurrence of diverse microhabitats. Nutrient enrichment was low and invertebrate diversity was high, and consequently the highest water quality scores (SASS 54 and ASPT 5.7) were recorded at this site. Nitrate concentration and channel width contributed significantly to higher values of water quality scores observed downstream. However, the values were still lower than those required by the RHP, indicating some deterioration in water quality downstream (Dallas 2007a; Dickens & Graham 2002). The low scores were also attributed to the significant reduction in macroinvertebrate taxa richness and diversity caused by flooding and water abstraction. Based on the IHI scores, the effects of flooding and water abstraction that occurred in October and January were more prominent at the downstream site since channel conditions changed from natural (before disturbances) to seriously modified (after disturbances).

3.9 SUMMARY AND CONCLUSIONS

The ecological impacts of pollution in the Bloukrans River were analyzed through habitat, biological, and water quality assessments. The general findings suggested that the natural state of the river was completely lost, most critically during the period of April 2013 to January 2014. Habitat integrity, water quality, and macroinvertebrate diversity were poor across sites and sampling periods. The poor conditions did not change significantly with time, but with site, suggesting that the ecological state of the river was strongly influenced by factors occurring at a local scale. Major water quality deteriorations and reductions in invertebrate taxa richness were observed at the upstream site where habitat was critically modified by anthropogenic activities. Although natural events such as flooding affected the river in October, anthropogenic effects such as water abstraction and nutrient enrichment remained important. The first two sites (Urban and Sewage) were critically affected by high ammonium and nitrate concentrations, and had the lowest observed macroinvertebrate taxa richness and diversity, respectively. The concentrations of these two nutrients were lower at the Agriculture and Recreational sites, and were associated with some increases in taxa richness and diversity. However, the reduction in nutrient concentrations was coupled with episodic increases in water abstraction and impoundments at these two sites, affecting invertebrate diversity and consequently the water quality index values downstream. Water quality in the Bloukrans River was not only affected by nutrient enrichment, but also by habitat degradation. Currently, the river is one of the many understudied aquatic ecosystems in South Africa, and this study represents data that can be included in the national state of rivers database that will assist managers and policy makers in making informed decisions regarding its ecological role in the society at large.

4 RESULTS: THE TROPHIC STATE OF THE BLOUKRANS RIVER

4.1 INTRODUCTION

The goal of this chapter was to determine the ecological status of the Bloukrans River using diatoms. To achieve this goal, the factors that influence diatom distributional patterns (**Question i**) were identified. Secondly, community indices were used to determine whether these factors were important at community level. The hypothesis was that community indices are influenced by nutrient concentrations such that the values of community indices are high when nutrient concentrations are high (**Hypothesis 5**). Lastly, the trophic state of the river was determined by applying the Trophic Diatom Index (TDI), with the expectation that high TDI values occur upstream where direct human disturbance and pollution levels are high (**Hypothesis 6**). Since TDI varies based on diatom tolerances, highly tolerant species were expected to dominate upstream following a pollution gradient (**Hypothesis** 7).

4.2 SPATIOTEMPORAL DISTRIBUTION OF DIATOMS

Forty-four diatom taxa (Appendix C) were observed in samples collected from rocks, vegetation, sediment, and the water column within a 50 m^2. A PERMANOVA revealed no significant differences (F_3= 0.85, p= 0.69) in diatom distribution across sites, but across sampling periods (F_3= 3.75, p= 0.001). The nMDS results (Figure 4.1) also indicated no clear separation in diatom distribution across sites (A), but communities collected in January were slightly separated from those collected in the other three periods (B).

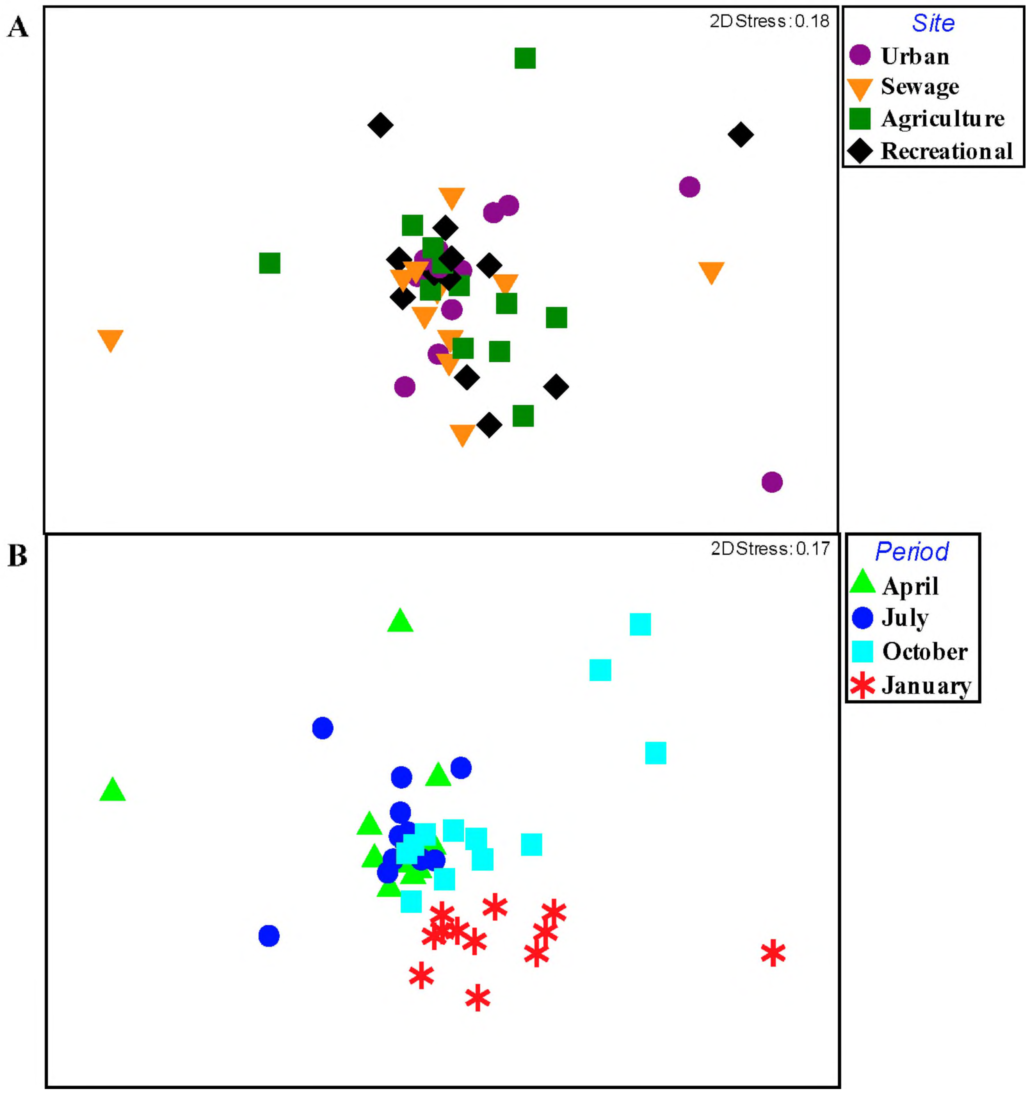

Figure 4.1: nMDS ordination in two dimensions, computed using a Bray-Curtis ecological distance measure to determine rank similarities in diatom distribution (A) across sites and (B) sampling periods. Sites and periods with similar species compositions are located closest to one another.

4.3 ENVIRONMENTAL FACTORS AFFECTING DIATOM DISTRIBUTION

4.3.1 Variation in influential environmental variables

Of the measured environmental variables, water velocity (F_{46}= 2.19, p= 0.02), pH (F_{46}= 2.51, p= 0.004), temperature (F_{46}= 4.75, p= 0.001), and phosphate concentration (F_{46}= 1.52, p= 0.01) were influential on the distributional patterns of diatoms (Figure 4.2). All four variables did not change significantly across sites (pH $F_3 = 0.69$, $p = 0.56$, temperature $F_3 = 2.64$, $p = 0.06$, flow velocity $F_3 = 1.73$, $p = 0.17$ and phosphate concentration $F_3 = 2.25$, $p = 0.1$).

The pH varied significantly with sampling period ($F_3 = 68.8$, $p < 0.01$), with the highest value (8.4) occurring in January and lowest in April (7.1). Water temperature also varied with time ($F_3 = 56.9$, $p < 0.01$), with the lowest occurring in July (mean 11.8 °C) and the highest in January (mean 23.3 °C). Water velocity was significantly higher in October compared with the other periods (October vs April $t_{22} = -4.7$, $p < 0.01$; October vs July $t_{22} = -5.8$, $p < 0.01$; October vs January at $t_{22} = 7.3$, $p < 0.01$). Phosphate concentrations did not change over time ($F_3 = 2.2$, $p = 0.1$) although high concentrations were observed at the urban site in January.

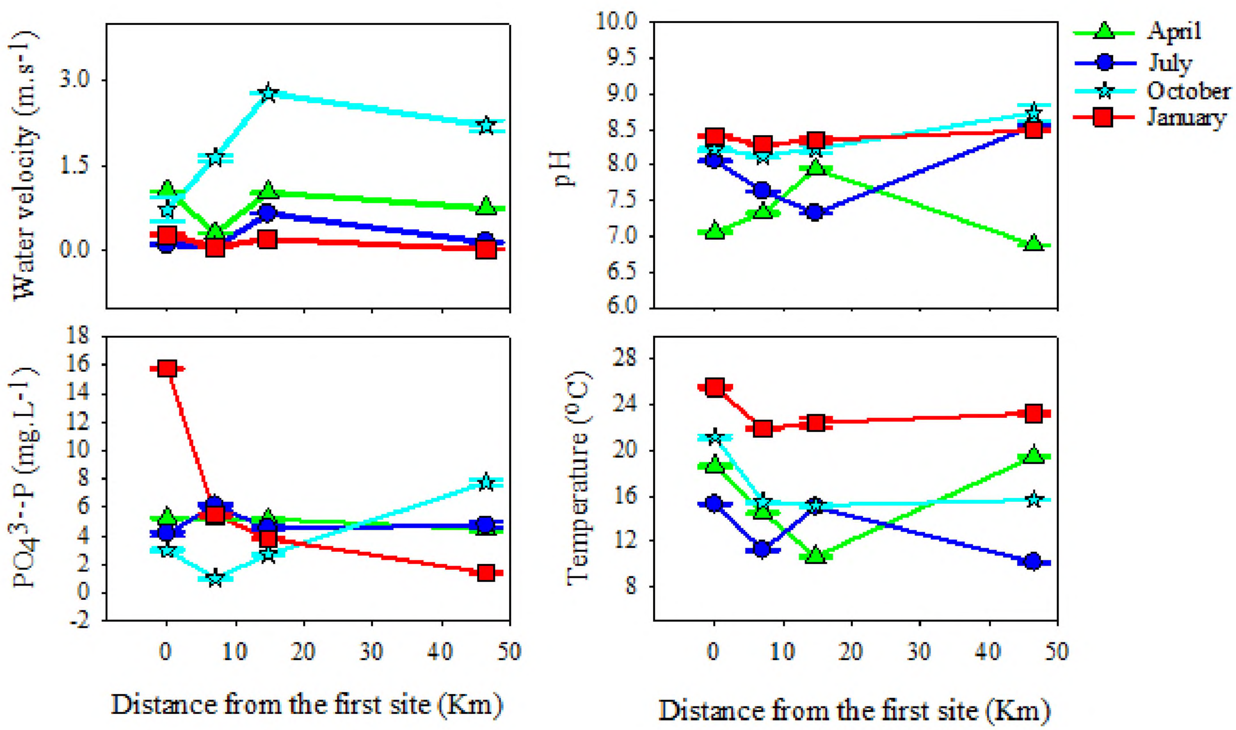

Figure 4.2: Spatio-temporal variations of key environmental factors influencing diatoms in the Bloukrans River between April 2013 and January 2014 (means ± standard deviations). Sites are reported in distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km. PO_4^{3-}-P= phosphate concentration.

Water temperature and water velocity explained most of the variation in diatom distribution in the distLM ordination analysis. In the first axis, temperature explained 86% of the variance while water velocity explained 91% variance in the second axis. Diatoms at the Urban site were mostly influenced by high temperatures and low pH (Figure 4.3 A). Low pH, water temperature, and phosphate concentrations were influential for diatoms at the Sewage site. High water velocity, low temperature and low phosphate concentrations were influential at the Agriculture site. At the Recreational site, pH, phosphate concentration and water temperature were all influential. Diatoms collected in October were influenced by high water velocity, pH and water temperature (Figure 4.3 B). In January the diatom community distribution was closely associated with high water temperatures. Diatoms collected during April and July were influenced by variations in phosphate concentrations.

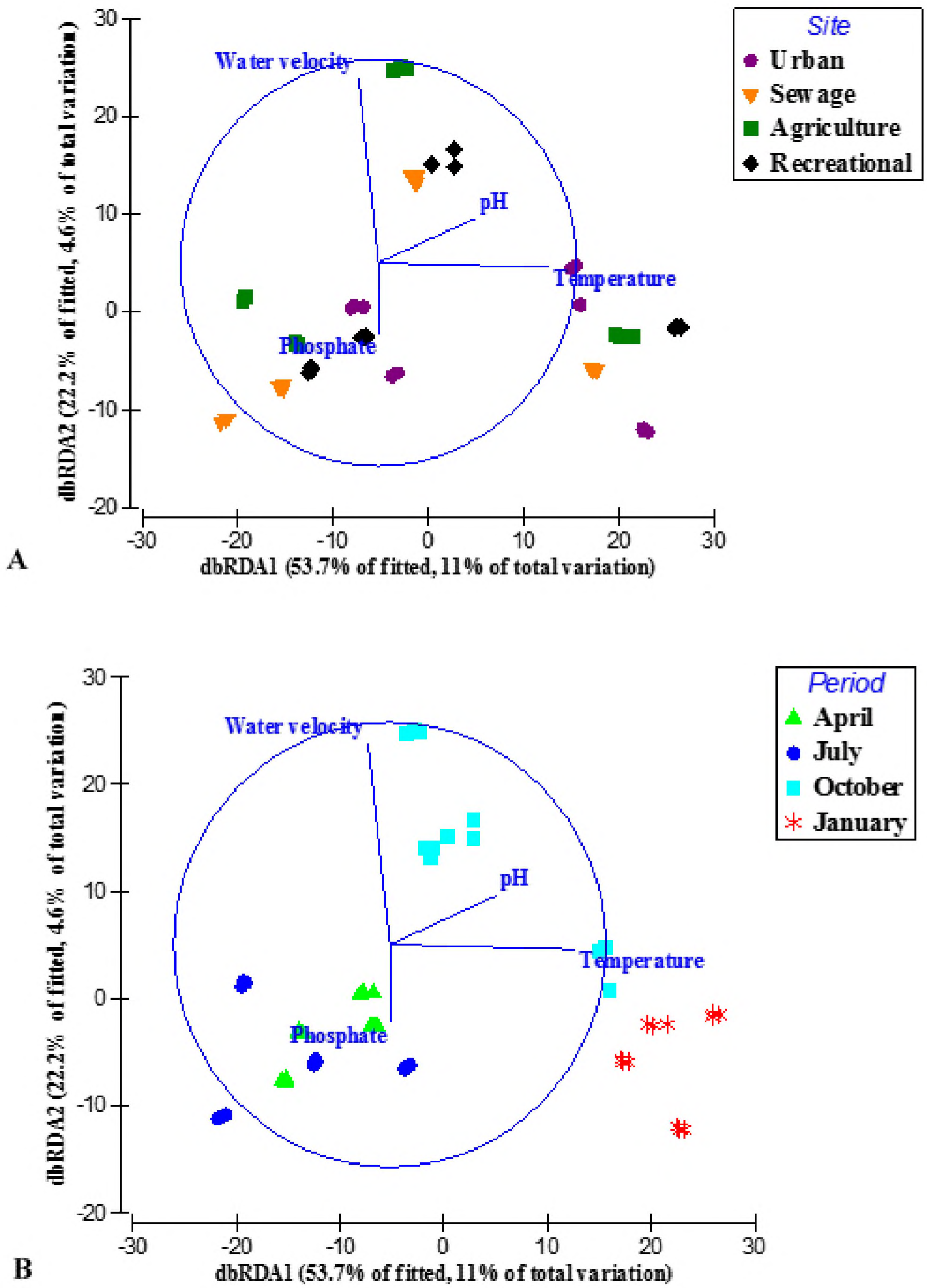

Figure 4.3: The first two dbRDA axes explaining 91.5% of variance in the distribution of diatoms in relation to environmental variables from April 2013 to January 2014. The model revealed the most influential environmental variables structuring diatom distribution at (A) four sites and (B) four sampling periods.

4.4 COMMUNITY LEVEL ANALYSES OF THE RELATIONSHIPS BETWEEN ENVIRONMENTAL PARAMETERS AND DIATOMS

4.4.1 Spatial and temporal variations in community structure

No spatial variations were observed in diatom indices across sites (Figure 4.4). One-way ANOVA revealed no statistical differences in taxa richness (F_3= 0.01, p= 0.99), total abundance (F_3= 0.04, p= 0.98), or diversity (F_3=0.03, p= 0.99) across sites. Diatom total abundance varied significantly with sampling period (F_3= 5.61, p= 0.002), but there were no significant changes in taxa richness (F_3= 2.13, p= 0.11) and diversity (F_3= 0.91, p= 0.44) over time. Diatom abundances were greatest in July and April, and lowest in October and January.

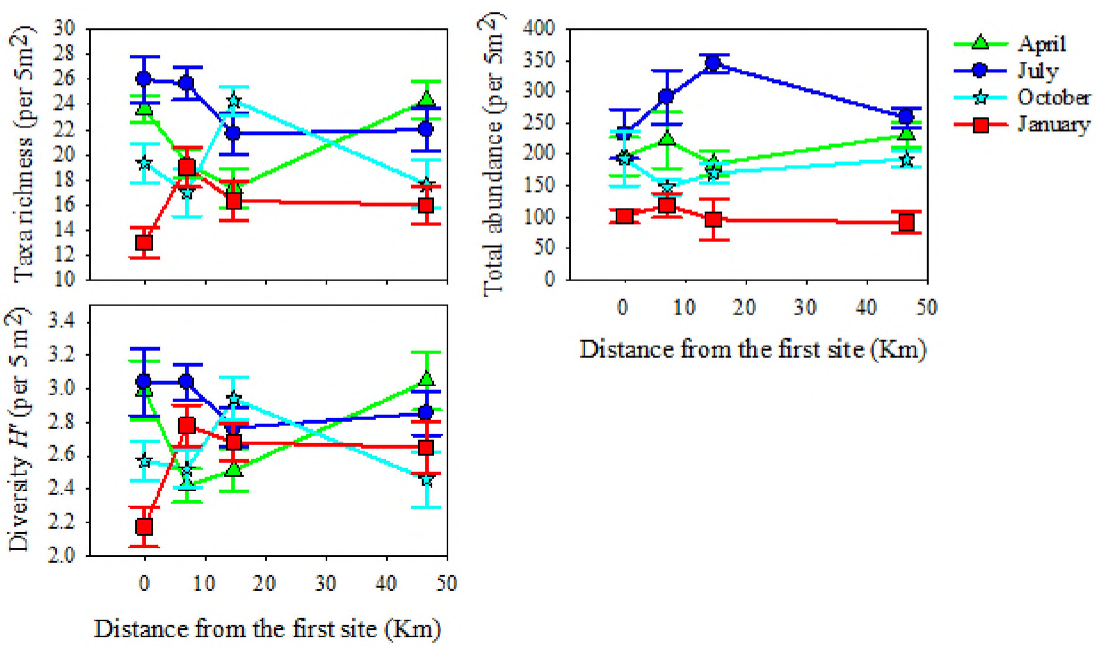

Figure 4.4: Diatom community index values (means ± standard deviations) across sites between April 2013 and January 2014. Sites were reported as distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

The regression results indicated that pH was the only significant environmental factor that caused variations in diatom abundance across sites (Table 4.1). No other significant relationships were observed between environmental variables and diatom community matrices across sites or sampling periods (Table 4.2).

Table 4.1: Spatial relationships between diatom community matrices and environmental variables. The regression equation y = ax+ b was used to model the relationships, where x = environmental variable, y = diatom community index, a = y intercept, and b = slope of the line. Relationships were considered statistically significant when $p \leq 0.05$ (indicated in bold). PO_4^{3-}–P= phosphate concentration.

Sites	Environmental variable	Community Indices	R^2	F	*p*-value	*a*	b
Urban	Water Velocity (ms^{-1})	Taxa richness	0	0.53	0.94	0	0.01
		Total Abundance	0	0.58	0.9	0	0.02
		Diversity index (*H'*)	0	0.46	0.85	0.03	0.04
	pH	Taxa richness	0.12	8.37	0.27	-0.02	1.36
		Total Abundance	0.04	8.13	0.52	0	0.45
		Diversity index (*H'*)	0.15	8.76	0.22	-0.3	1.7
	Temperature (°C)	Taxa richness	0.3	5.89	0.06	-1.26	4.38
		Total Abundance	0.19	3.07	0.16	-0.02	2.28
		Diversity index (*H'*)	0.25	7.89	0.1	-2.87	3.3
	PO_4^{3-}–P ($mg.L^{-1}$)	Taxa richness	0.22	12.76	0.12	-0.28	2.83
		Total Abundance	0.2	11.09	0.15	-0.02	2.45
		Diversity index (*H'*)	0.17	15.83	0.18	-3.27	2.12
Sewage	Water Velocity (ms^{-1})	Taxa richness	0.07	0.98	0.42	-0.02	0.72
		Total Abundance	0.04	0.75	0.51	0	0.46
		Diversity index (*H'*)	0.04	1.16	0.52	-0.24	0.45
	pH	Taxa richness	0.03	8.04	0.56	-0.01	0.36
		Total Abundance	0.2	8.13	0.15	0	2.47
		Diversity index (*H'*)	0	7.72	0.83	0.05	0.05
	Temperature (°C)	Taxa richness	0.07	18.45	0.42	-0.13	0.7
		Total Abundance	0.25	18.92	0.1	-0.02	3.3
		Diversity index (*H'*)	0	16.99	0.84	-0.45	0.04
	PO_4^{3-}–P ($mg.L^{-1}$)	Taxa richness	0.1	2.74	0.32	0.08	1.07

		Total Abundance	0.08	3.52	0.38	0	0.85
		Diversity index (*H'*)	0.06	2.21	0.46	0.83	0.6
Agriculture	Water Velocity (ms^{-1})	Taxa richness	0.11	0.38	0.3	0.04	1.19
		Total Abundance	0	1.21	0.92	0	0.01
		Diversity index (*H'*)	0.04	0.06	0.51	0.41	0.46
	pH	Taxa richness	0.01	8.08	0.72	-0.01	0.13
		Total Abundance	**0.35**	**8.27**	**0.04**	**0**	**5.44**
		Diversity index (*H'*)	0	7.94	0.97	0.01	0
	Temperature (°C)	Taxa richness	0.01	16.98	0.73	-0.06	0.12
		Total Abundance	0.07	17.33	0.39	-0.01	0.81
		Diversity index (*H'*)	0.01	14.14	0.82	0.62	0.05
	PO_4^{3-}–P ($mg.L^{-1}$)	Taxa richness	0.08	4.68	0.39	-0.03	0.82
		Total Abundance	0.03	3.83	0.56	0	0.36
		Diversity index (*H'*)	0.1	5.63	0.32	-0.58	1.1
Recreational	Water Velocity (ms^{-1})	Taxa richness	0	0.93	0.86	-0.01	0.03
		Total Abundance	0.01	0.64	0.77	0	0.09
		Diversity index (*H'*)	0.05	1.88	0.47	-0.4	0.57
	pH	Taxa richness	0.09	8.77	0.35	-0.03	0.97
		Total Abundance	0.02	8.35	0.67	0	0.2
		Diversity index (*H'*)	0.1	9.45	0.32	-0.47	1.08
	Temperature (°C)	Taxa richness	0.03	19.42	0.59	-0.11	0.31
		Total Abundance	0.22	21.1	0.13	-0.02	2.75
		Diversity index (*H'*)	0	17.7	0.95	-0.2	0
	PO_4^{3-}–P ($mg.L^{-1}$)	Taxa richness	0.01	3.99	0.76	0.03	0.1
		Total Abundance	0.12	3.19	0.26	0.01	1.41
		Diversity index (*H'*)	0.02	6.1	0.7	-0.55	0.15

Table 4.2: Temporal relationships between diatom community matrices and environmental variables. The linear regression equation y = ax+ b was used to model the relationships, where x = environmental variable, y = diatom community index, a = y intercept, and b = slope of the line. Relationships were statistically significant when $p \leq 0.05$. PO_4^{3-}–P= phosphate concentration.

Period	Environmental variable	Community Indices	R^2	F	*p*-value	a	b
April	Water Velocity (ms^{-1})	Taxa richness	0.05	0.54	0.48	0.01	0.55
		Total Abundance	0.0	0.73	0.84	0.0	0.04
		Diversity index (*H'*)	0.13	0.29	0.26	0.15	1.45
	pH	Taxa richness	0.11	7.31	0.29	-0.01	1.24
		Total Abundance	0.0	7.17	0.84	0.0	0.04
		Diversity index (*H'*)	0.17	7.48	0.19	-0.12	2.0
	Temperature (°C)	Taxa richness	0.12	14.97	0.27	0.09	1.34
		Total Abundance	0.0	16.68	0.86	0.0	0.03
		Diversity index (*H'*)	0.19	12.85	0.16	1.48	2.36
	PO43-_P ($mg.L^{-1}$)	Taxa richness	0.0	4.92	0.83	0.0	0.05
		Total Abundance	0.02	4.93	0.7	0.0	0.16
		Diversity index (*H'*)	0.02	5.06	0.68	-0.08	0.18
July	Water Velocity (ms^{-1})	Taxa richness	0.03	0.57	0.58	-0.01	0.32
		Total Abundance	0.08	0.08	0.36	0.0	0.91
		Diversity index (*H'*)	0.05	0.98	0.48	-0.22	0.53
	pH	Taxa richness	0.02	8.2	0.66	-0.01	0.21
		Total Abundance	0.02	8.15	0.68	0.0	0.18
		Diversity index (*H'*)	0.01	8.32	0.72	-0.09	0.13
	Temperature (°C)	Taxa richness	0.04	10.54	0.54	0.05	0.41
		Total Abundance	0.05	12.83	0.5	0.0	0.48
		Diversity index (*H'*)	0.03	9.16	0.56	0.91	0.36
	PO_4^{3-}_P ($mg.L^{-1}$)	Taxa richness	0.0	4.93	0.89	0.0	0.02

		Total Abundance	0.04	4.69	0.52	0.0	0.44
		Diversity index (*H'*)	0.0	4.83	0.9	0.07	0.02
October	Water Velocity (ms^{-1})	Taxa richness	0.03	1.54	0.6	0.02	0.3
		Total Abundance	0.0	1.9	0.87	0.0	0.03
		Diversity index (*H'*)	0.03	1.33	0.62	0.19	0.26
	pH	Taxa richness	0.0	8.34	0.97	0.0	0.0
		Total Abundance	0.02	8.28	0.65	0.0	0.22
		Diversity index (*H'*)	0.0	8.36	0.93	-0.01	0.01
	Temperature (°C)	Taxa richness	0.0	17.01	0.94	-0.01	0.01
		Total Abundance	0.01	16.48	0.75	0.0	0.11
		Diversity index (*H'*)	0.0	17.43	0.87	-0.21	0.03
	PO_4^{3-}_P ($mg.L^{-1}$)	Taxa richness	0.0	3.96	0.85	-0.02	0.04
		Total Abundance	0.02	3.14	0.7	0.0	0.15
		Diversity index (*H'*)	0.01	4.78	0.73	-0.45	0.12
January	Water Velocity (ms^{-1})	Taxa richness	0.1	0.26	0.31	-0.01	1.15
		Total Abundance	0.0	0.16	0.96	-3.58	0.0
		Diversity index (*H'*)	0.15	0.37	0.21	-0.09	1.8
	pH	Taxa richness	0.15	8.49	0.21	-0.01	1.77
		Total Abundance	0.09	8.43	0.33	0	1.04
		Diversity index (*H'*)	0.09	8.51	0.35	-0.05	0.95
	Temperature (°C)	Taxa richness	0.13	24.96	0.25	-0.1	1.47
		Total Abundance	0	23.33	0.97	0	0
		Diversity index (*H'*)	0.19	26.58	0.16	-1.28	2.29
	PO_4^{3-}_P ($mg.L^{-1}$)	Taxa richness	0.08	11.84	0.36	-0.33	0.91
		Total Abundance	0	6.01	0.86	0.01	0.03
		Diversity index (*H'*)	0.19	19.4	0.16	-4.97	2.3

4.5 WATER QUALITY AND TROPHIC STATUS

There were no statistical differences in TDI across sites (one-way ANOVA F_3= 0.09, *p*= 0.97) or sampling periods (F_3= 2.67, p= 0.06; Figure 4.5). There were also no relationships between TDI and any environmental variables across sites or periods (Tables 4.3 and 4.4).

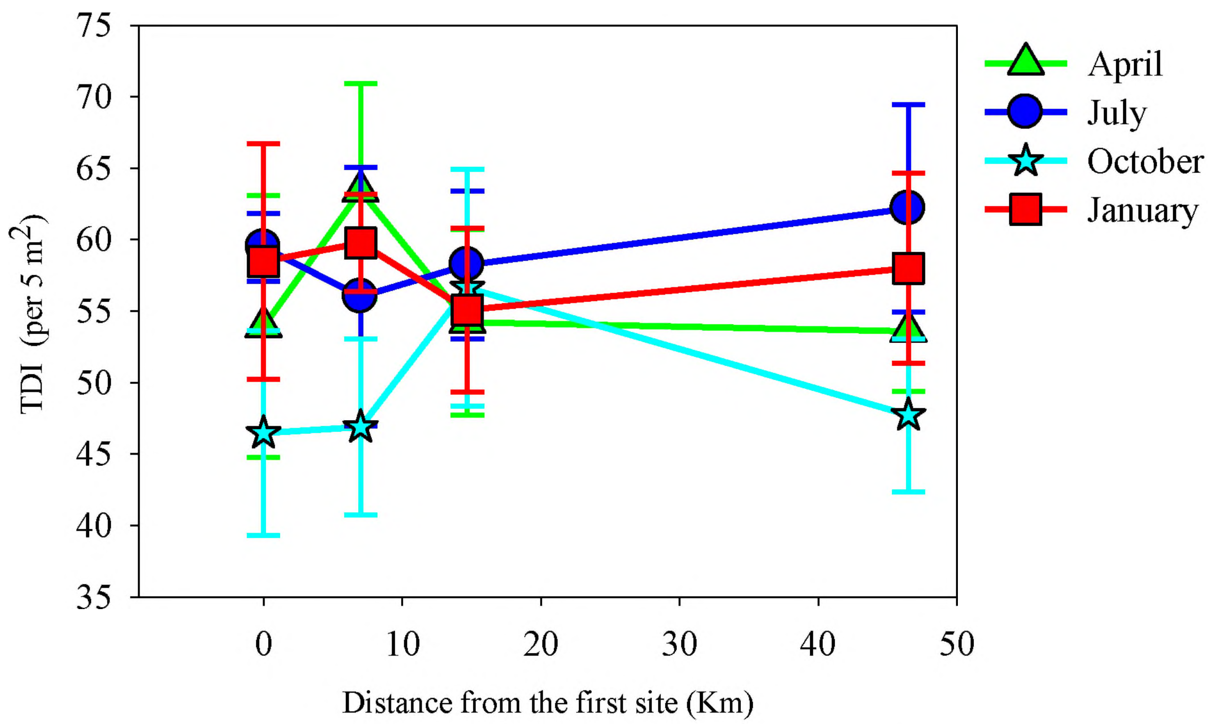

Figure 4.5: Trophic Diatom Index (TDI; ±SD) values in the Bloukrans River between April 2013 and January 2014. Sites were reported as distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

Table 4.3 Spatial relationships between the Trophic Diatom index (TDI) and environmental variables. The regression equation y = ax+ b was used to model the relationships, where x = environmental variable, y = diatom community index, a = y intercept, and b = slope of the line. Relationships were considered statistically significant when $p \leq 0.05$. PO_4^{3-}–P= phosphate concentration

Site	Environmental variable	R^2	F	p-value	a	b
Urban	Water Velocity (ms^{-1})	0.12	1.21	0.27	-0.01	1.39
	pH	0	7.88	0.95	0	0
	Temperature (°C)	0	0.96	0.9	-0.01	0.02
	PO_4^{3-}_P ($mg.L^{-1}$)	0.06	0.94	0.46	0.11	0.6
Sewage	Water Velocity (ms^{-1})	0.27	2.49	0.08	-0.03	3.67
	pH	0.11	8.58	0.3	-0.01	1.21
	Temperature (°C)	0.01	13.33	0.74	0.04	0.12
	PO_4^{3-}_P (mg.L-1)	0.27	-1.69	0.08	0.11	3.75
Agriculture	Water Velocity (ms^{-1})	0	0.91	0.91	0	0.01
	pH	0.02	8.37	0.68	-0.01	0.18
	Temperature (°C)	0	16.54	0.95	-0.01	0
	PO_4^{3-}_P ($mg.L^{-1}$)	0.01	4.62	0.8	-0.01	0.06
Recreational	Water Velocity (ms^{-1})	0.27	3.43	0.08	-0.05	3.71
	pH	0.01	7.75	0.77	0.01	0.09
	Temperature (°C)	0.02	20.99	0.67	-0.07	0.19
	PO_4^{3-}_P ($mg.L^{-1}$)	0.15	9.62	0.22	-0.09	1.71

Table 4.4: Temporal relationships between Trophic Diatom Index (TDI) and environmental variables. The regression equation y = ax+ b was used to model the relationships, where x = environmental variable, y = diatom community index, a = y intercept, and b = slope of the line. Relationships were considered statistically significant when $p \leq 0.05$. PO_4^{3-}–P= phosphate concentration

Period	Environmental variable	R^2	F	p-Value	a	b
April	Water Velocity (ms^{-1})	0.24	1.72	0.11	-0.02	3.09
	pH	0.11	6.65	0.3	0.01	1.21
	Temperature (°C)	0.14	23.36	0.23	-0.11	1.64
	PO_4^{3-}_P ($mg.L^{-1}$)	0.05	4.22	0.49	0.01	0.51
July	Water Velocity (ms^{-1})	0	0.48	0.9	0	0.02
	pH	0.11	7.11	0.29	0.02	1.23
	Temperature (°C)	0	12.32	0.93	-0.01	0.01
	PO_4^{3-}_P ($mg.L^{-1}$)	0.08	6.79	0.39	-0.03	0.82
October	Water Velocity (ms^{-1})	0.09	0.8	0.35	0.02	0.95
	pH	0	8.32	0.97	0	0
	Temperature (°C)	0	4.17	0.88	-0.01	0.02
	PO_4^{3-}_P ($mg.L^{-1}$)	0.01	0.21	0.79	0	0.07
January	Water Velocity (ms^{-1})	0.01	0.21	0.79	0	0.07
	pH	0	8.36	0.88	0	0.02
	Temperature (°C)	0	23.55	0.93	0	0.01
	PO_4^{3-}_P ($mg.L^{-1}$)	0	5.08	0.89	0.03	0.02

4.5.1 Diatom species dominance across sites and periods

Based on the five categories of tolerance by diatoms (Figure 4.6), 48% of the species obtained across sites were tolerant to very high phosphorus concentrations. This was followed by species that were favored by very high phosphorus concentrations, which accounted for up to 21% in relative abundance. Overall, pollution tolerant taxa constituted more than 60% of the identified diatoms in all sites and across sampling periods, while less than 40% were sensitive and intermediate taxa. The tolerant group was dominated by the genera *Nitzschia* and *Navicula*.

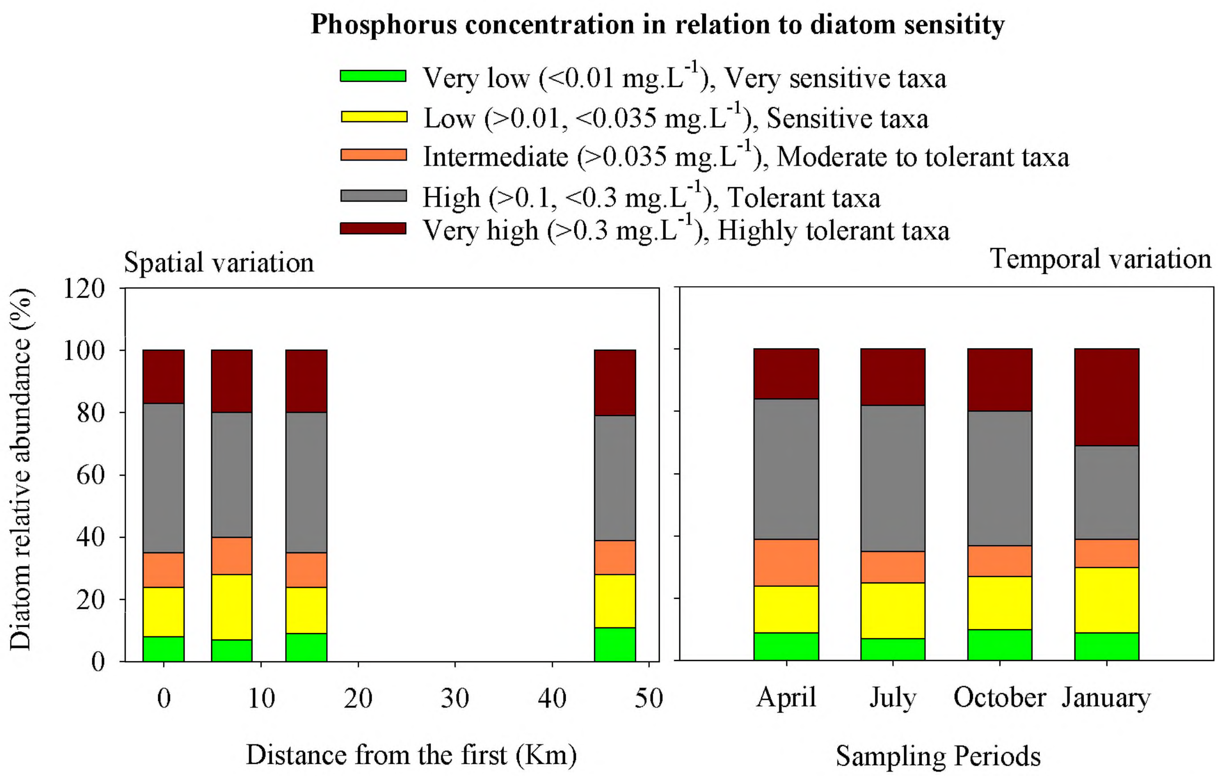

Figure 4.6: The relative abundance of diatoms at phosphorus concentrations ranging between <0.01 and >0.3 mg/L. Species dominance was assessed across (A) sites and (B) periods between April 2013 and January 2014. Sites were reported in distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

4.5.2 Water quality in relation to diatom distribution

Pollution tolerant diatoms varied in a similar pattern with the TDI (Figure 4.7). October was the only period in which TDI and pollution tolerant species were below 50 and 50%, respectively. The tolerant species were those favored by phosphorus concentrations greater than 0.1 mg.L^{-1}.

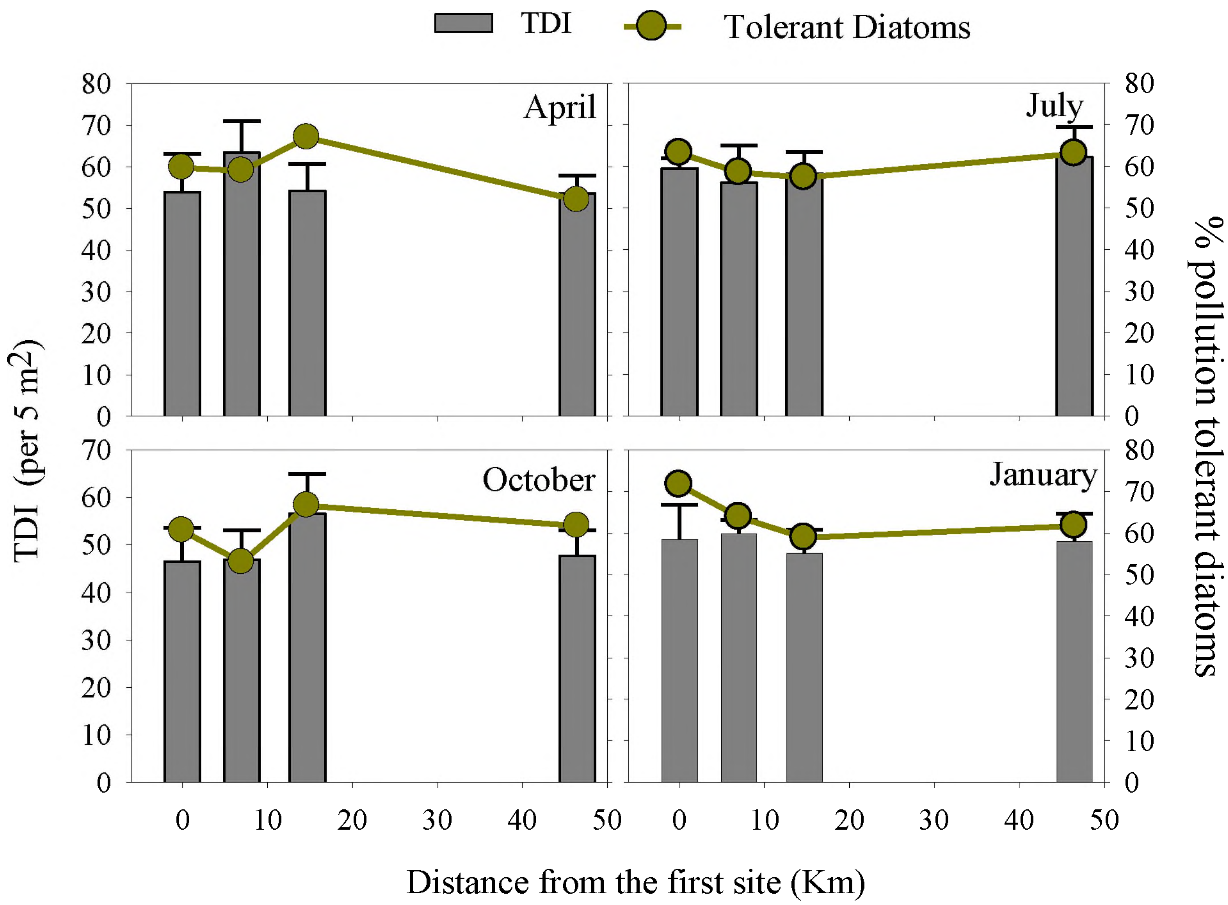

Figure 4.7: Changes in the Trophic Diatom Index (TDI) relative to the distribution of tolerant diatoms across sites between April 2013 and January 2014. Sites are reported as distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

4.6 DISCUSSION

4.6.1 Spatio-temporal changes in the environmental variables associated with diatom distribution

The first research question was addressed using the ordination model, which indicated that diatom distribution patterns in the Bloukrans River were influenced by changes in pH, temperature, water velocity, and phosphate concentrations. Variability in pH (Pan *et al.* 1996; Fabricius *et al.* 2003), temperature (Leira & Sabater 2005), water velocity (Tang *et al.* 2002), and phosphate/phosphorus concentrations (Tang *et al.* 2002; Urrea & Sabater 2009) have influenced diatom distributions in other studies. In the Bloukrans River, water velocity was highest in October due to flooding and lowest in January due to water abstraction. The Agriculture site was the most affected by flow variability. For example, water flow changed from moderate (ranging between 0.7-1.0 $m.s^{-1}$) in April and July to high in October (mean 2.7 $m.s^{-1}$), to low in January (mean 0.2 $m.s^{-1}$). The anthropogenic influences were most evident during the warm January period when precipitation was lowest and the demand for irrigation was greatest.

Changes in water temperature were attributed in part to changes in canopy cover (direct radiant energy into the water column), seasonal variations in weather conditions, and variability in the mixing process that is highly controlled by current fluctuations. As expected, highest water temperatures occurred in summer (January), and the lowest in winter (July). Radiant energy increased temperatures at the Urban site due to the lack of canopy. Water temperatures remained high at the Urban site during each sampling period when compared to the other sites. High water temperatures influenced pH levels and phosphorus concentrations in the warm January period, when both pH and phosphate values were highest (8.4 and 6.6 $mg.L^{-1}$, respectively). Increased temperature in a liquid medium causes dissociation of molecules and facilitates the movement of ions, resulting in an ion imbalance and eventually changing the pH of the medium (Kelter & Zumdahl 1993; Barron *et al.* 2006).

4.6.2 Diatom responses to environmental changes

The distance linear model (distLM) identified some distributional patterns of diatoms, and water velocity and temperature combined explained more than 88% of the variation. The effects of water flow at the Agriculture site in October indicated the importance of flooding. During the period of high flow, the site was dominated by smaller diatoms (valve length ranged between 20-70 µm) that migrated easily with the current and attached on the rocky benthic substrate. A shift to larger *Fragilaria* species (valve length range 27-600 µm) was observed at the same site during low flow periods. Similar results were reported by Fabricius *et al.* (2003) studying the distribution patterns of benthic diatoms in the Cuarto River in Argentina, where larger diatom species occurred during low flow and smaller species dominated during high flow occasions. The larger species typically form colonies that settle in the benthic zones during low flow (Taylor 2007), while the smaller ones are usually favored at high flow provided the benthic zone is rocky (Ács & Kiss

1993), as was the case was in the Bloukrans River. Furthermore, fast flowing waters create turbulent flux that enhances nutrient delivery to diatom cells, thus stimulating rapid growth and reproduction (Ács & Kiss 1993; Song 2007).

The effects of water temperature on diatoms were prominent at all sites during the warm periods (October and January). The upstream Urban and downstream Recreational sites were characterized by particularly high water temperatures in these warm periods. For example, the mean daytime temperatures at the Urban and Recreational sites in October were 21°C and 16°C, respectively, and 26°C and 23°C at the same sites in January. Diatom abundances were reduced in October and January at both the sites when water temperature was highest. High temperatures can enhance growth rates (Montagnes 1996; Montagnes *et al.* 2002; Cossins 2012), and therefore the abundance and density of diatoms (Ajithamol *et al.* 2014). However, the current findings were consistent with those of Schabhüttl *et al.* (2013), who ran laboratory experiments on the effects of temperature on phytoplankton communities and found that diatoms preferred cooler waters. There is a continued debate regarding the responses of diatoms to temperature variations, because there is much natural variability in climatic conditions and local habitats (Pajunen *et al.* 2016). Often the general assumption is that diatom communities do not respond consistently to temperature variations (Smol 1988), although Pienitz *et al.* (1995) developed a diatom-based model for quantitatively analyzing climatic temperature variations in Canada. My results from the Bloukrans River indicated that diatoms responded to short term changes in temperature and nutrient inputs (e.g. through agricultural runoff).

Overall, diatom community structure (as indicated by community indices) and the four environmental factors of interest did not vary significantly across sites, but they did show important changes across periods. The fifth hypothesis that diatom abundance, diversity, and taxa richness are significantly greater at the sites where nutrient levels are higher was rejected. There was no evidence that high concentrations of ammonium or phosphate caused an increase in taxa richness, so the community level analysis of diatoms did not indicate any effects of nutrient enrichment in the Bloukrans River. However, there were effects of pH on diatom abundance. The highest diatom abundances were observed at the Agriculture site, where pH ranged from 7.3 to 8.4 across sampling periods, in contrast with other studies that showed diatom abundance increases at pH ≤ 7 (Kröger *et al.* 1999; Vrieling *et al.* 1999; Sumper & Kröger 2004). The proposition is that the development of diatom cell walls is enzyme mediated, and the enzymes involved function optimally in acidic conditions (Vrieling *et al.* 1999). However, at the Agriculture site of the Bloukrans River, the diatoms *Fragilaria ulna*, *Nitzschia desertorum*, *Navicula heimansiodes*, and *N. vandamii* were dominant. These species are cosmopolitan and typically found in circumneutral to alkaline waters (Taylor 2007), hence their abundance under high pH conditions.

4.6.3 Water quality and the trophic status of the Bloukrans River

One goal was to determine whether the Trophic Diatom Index (TDI) would indicate eutrophication in the Bloukrans River. The hypothesis that TDI values are higher at the sites where phosphate concentrations are high, indicating some degree of eutrophication, was not supported as TDI values did not vary significantly with site or phosphate concentration. The lack of spatial variations in phosphate concentration indicated that the different anthropogenic sources of external phosphate were of similar magnitude and were influential from upstream to downstream. TDI values also did not vary across sites, mainly because of the lack of spatial variations in diatom distribution. Although statistically insignificant, slight increases in TDI values occurred at all sites in April, July and January, and a decrease in October when flooding occurred. Interestingly, the values obtained at the Agriculture site remained slightly higher even under the influence of flooding, mainly due to nutrient inputs from sewage works and agriculture since the site was located immediately downstream of the Sewage site. Other researchers reported high TDI values in sites located below sewage treatment works. For example, Kelly & Whitton (1995) reported that TDI values downstream of a sewage treatment remained high due to nutrient enrichment at the River Browney in England. Similarly, Soininen (2002) found that sewage and agriculture contributed to water quality deterioration when assessing the trophic status of Finland rivers using diatoms. Although there was no statistical evidence to suggest the importance of nutrient inputs from sewage discharge in the Bloukrans River, the slight increase in TDI values obtained at the two sites closest to the outlet point (the Sewage and Agriculture sites) were probably due to sewage inputs. Additionally, the index values were generally high in July, when partially treated sewage was discharged into the river. Nutrient dilution occurred during the flooding in October, resulting in low TDI values. Among other factors, high rainfall and nutrient variability can influence diatom composition and ultimately the TDI values of water bodies (e.g. Kelly & Whitton 1995; Iriarte & Purdie 2004; Maraslioglu *et al.* 2016). For this reason, Kelly & Whitton (1995) emphasized that TDI values should be supplemented with information on the proportions of pollution tolerant species in the studied areas. Although the Bloukrans River was eutrophic at all times (with average TDI values above 50, and orthophosphate concentrations above 0.03 $mg.L^{-1}$) except for the wet season (September-November) when mean TDI values ranged from 46.5 to 56.6 across sites, the proportions of tolerant taxa were determined to augment any final conclusions (Figure 4.7). Tolerant taxa were expected to dominate upstream where human influences are high, and decrease in the downstream direction. However, pollution sensitive taxa represented a small proportion (<40%) of diatoms across sites and periods (Figure 4.6), and more than 60% were pollution tolerant. There were no major variations in the dominance of tolerant taxa across sites, therefore the hypothesis was rejected. The combination of the TDI and % pollution tolerant taxa results indicated that the Bloukrans River was eutrophic in the dry seasons (May-July), and mesotrophic when freshwater inputs increased. The consistent dominance of tolerant taxa at all sites indicated that pollution was derived from multiple sources along the river. Among the most dominant tolerant taxa were species from the genera *Nitzschia* and *Navicula.* A dominance of *Nitzschia* species was also reported in the Buyuku Gure stream in Turkey, where TDI values revealed that the stream was eutrophic in that tolerant taxa represented 41-60% of the community (Maraslioglu *et al.* 2016). When assessing the applicability of the TDI, Kelly & Whitton (1995) found that the proportions of species from the genera *Nitzschia* and *Navicula* fluctuated with the external input of fairy clean mine discharge, where the highest proportions occurred when there was no discharge, and the lowest proportions occurred during and immediately after discharge periods. Their findings indicated that properly treated discharges could be used to help ecologically restore affected aquatic ecosystems (Kelly & Whitton 1995). In the Bloukrans River, partially treated sewage discharge had the opposite effect and was coupled with high proportions of *Nitzschia* and *Navicula*, particularly during the discharge period (July).

4.7 SUMMARY AND CONCLUSION

Although the community index did not indicate longitudinal effects of pollution on diatoms in the Bloukrans River, the ordination analysis indicated some effects of environmental factors on the diatom assemblages. Several authors have highlighted the difficulties in obtaining reliable water quality results using diatom community data (Sullivan 1986; Round 1991). Essentially, community indices may not vary significantly across space or time owing to persistent stressors of variable magnitudes (Round 1991), or the loss of vital data when grouping organisms based on shared numerical characteristics such as diversity and evenness (Sullivan 1986). However, diatom community structure can provide useful information about the trophic status of water bodies (Kelly & Whitton 1995). In the Bloukrans River, the TDI indicated that a eutrophic state occurred during the dry season, and a mesotrophic state occurred during the wet season. The lack of significant relationships between TDI and nutrient concentrations may be attributed to the lack of any calibration of the index specifically for South African rivers. Additional studies using diatom-based indices including the TDI in other South African locations have similarly reported no significant correlations between TDI and nutrient concentrations (e.g. Taylor *et al.* 2007a, b; Dalu *et al.* 2016). Such calibrations need to be completed to establish how researchers can most beneficially apply TDI data as a monitoring tool for South African rivers.

5 RESULTS: STABLE NITROGEN RATIOS ($\delta^{15}N$) AS INDICATORS OF ANTHROPOGENIC NITROGEN

5.1 INTRODUCTION

Stable nitrogen isotope ratios can be used to indicate anthropogenic nitrogen inputs into water bodies (Lake *et al.* 2001; Vander Zanden *et al.* 2005; Bannon & Roman 2008). This chapter investigates whether $\delta^{15}N$ values can distinguish nitrogen inputs from urban, sewage, agricultural, and recreational activities in the Bloukrans River. The hypothesis (**Hypothesis 8**) was that $\delta^{15}N$ values are higher where concentrations of ammonia and nitrate are highest, and lower in areas where nutrient concentrations are low. To test this hypothesis, two-way ANOVA were used to determine variability in $\delta^{15}N$ values of autotrophs and consumers across sites and periods, and within sites and periods. Linear regressions were used to identify any relationships between biota $\delta^{15}N$ values and nitrogenous nutrient concentrations in the water.

5.2 SPATIO-TEMPORAL VARIATIONS IN BIOTA $\delta^{15}N$ VALUES

5.2.1 Overall isotopic variation

Stable nitrogen isotope values varied in similar ways in autotrophs and primary and secondary consumers in the Bloukrans River (Figure 5.1). The lowest isotopic values were observed at the Urban site and the highest at the Agriculture site. Over time, $\delta^{15}N$ values of the autotrophs and primary consumers were lowest in April and showed a temporal gradient with the highest values occurring in January. The pattern was slightly different in secondary consumers, and the lowest values were observed in July instead of April. A two-way ANOVA indicated that $\delta^{15}N$ values of autotrophs varied significantly across sites ($F_3= 44.3$, $p< 0.01$), but not across periods ($F_3= 1.2$, $p= 0.32$). No significant site-period interactions were observed for autotrophs ($F_9= 0.9, p= 51$). Variations in $\delta^{15}N$ values of primary consumers were significant across sites ($F_3= 84.4$, $p< 0.01$), periods ($F_3= 5.2$, $p= 0.005$), and there were site-period interactions ($F_9= 3.2$, $p= 0.007$). Secondary consumer $\delta^{15}N$ values also changed significantly across sites ($F_3= 119, p< 0.01$), periods ($F_3= 17.6$, $p< 0.01$), with a significant interaction between site and period ($F_9= 9.7$, $p< 0.01$).

5.2.2 Variability of autotroph $\delta^{15}N$ values

On average, $\delta^{15}N$ values of autotrophs were lowest at the Urban site, ranging from 1.4-4.2‰, with no significant changes across periods (F_3= 1.5, p= 0.3). The values increased and ranged between 10.6-15.9‰ at the Sewage site, with no significant changes across periods (F_3=3.3, p= 0.1). Autotroph $\delta^{15}N$ values were highest at the Agriculture site, ranging between 14.4-17.7‰, with no significant variation across sampling periods (F_3=0.5, p= 0.7). At the downstream Recreational site, the values decreased slightly and ranged between 9.6-12.9‰, with no significant changes across periods (F_3= 0.4, p= 0.7).

5.2.3 Variability of primary consumer $\delta^{15}N$ values

Mean $\delta^{15}N$ values of primary consumers were higher than those of autotrophs in the Bloukrans River. Primary consumer $\delta^{15}N$ values were lowest at the Urban site, ranging from 0.3-6.2‰, with no significant variations across periods (F_3=1.6, p= 0.3). The values increased at the Sewage site and ranged between 12.1-27.2‰, with significantly higher values occurring in January and lowest values in July (t_4= -6.1, p< 0.01). The highest mean $\delta^{15}N$ values (ranging from 21.4-27.2‰) were obtained at the Agriculture site, with no significant differences across periods (F_3=3.5, p= 0.1). Primary consumer $\delta^{15}N$ values did not vary with sampling period at the Recreational site (F_3= 1.3, p= 0.3), and they ranged between 14.6-17.1‰.

5.2.4 Variability of secondary consumer $\delta^{15}N$ values

At the Urban site, $\delta^{15}N$ values of secondary consumers were the lowest among all the sites and ranged between 6.9-16.9‰ from April to January. These values varied significantly across sampling periods (F_2= 13.9, p< 0.01), with the highest occurring in October and lowest in April (T_4= -3.9, p= 0.02). No secondary consumer species were obtained at the Urban site in July (Figure 5.1). At the Sewage site, $\delta^{15}N$ values were higher in January (mean 27.94‰) when compared with the other sites. The values decreased in the downstream direction and ranged between 23.2-25.9‰ at the Agriculture site, with no significant variation across periods (F_3= 0.74, p= 0.56). The $\delta^{15}N$ values obtained at the Recreational site ranged between 16.8-19.4‰, with no variation across periods (F_3= 0.77, p= 0.54).

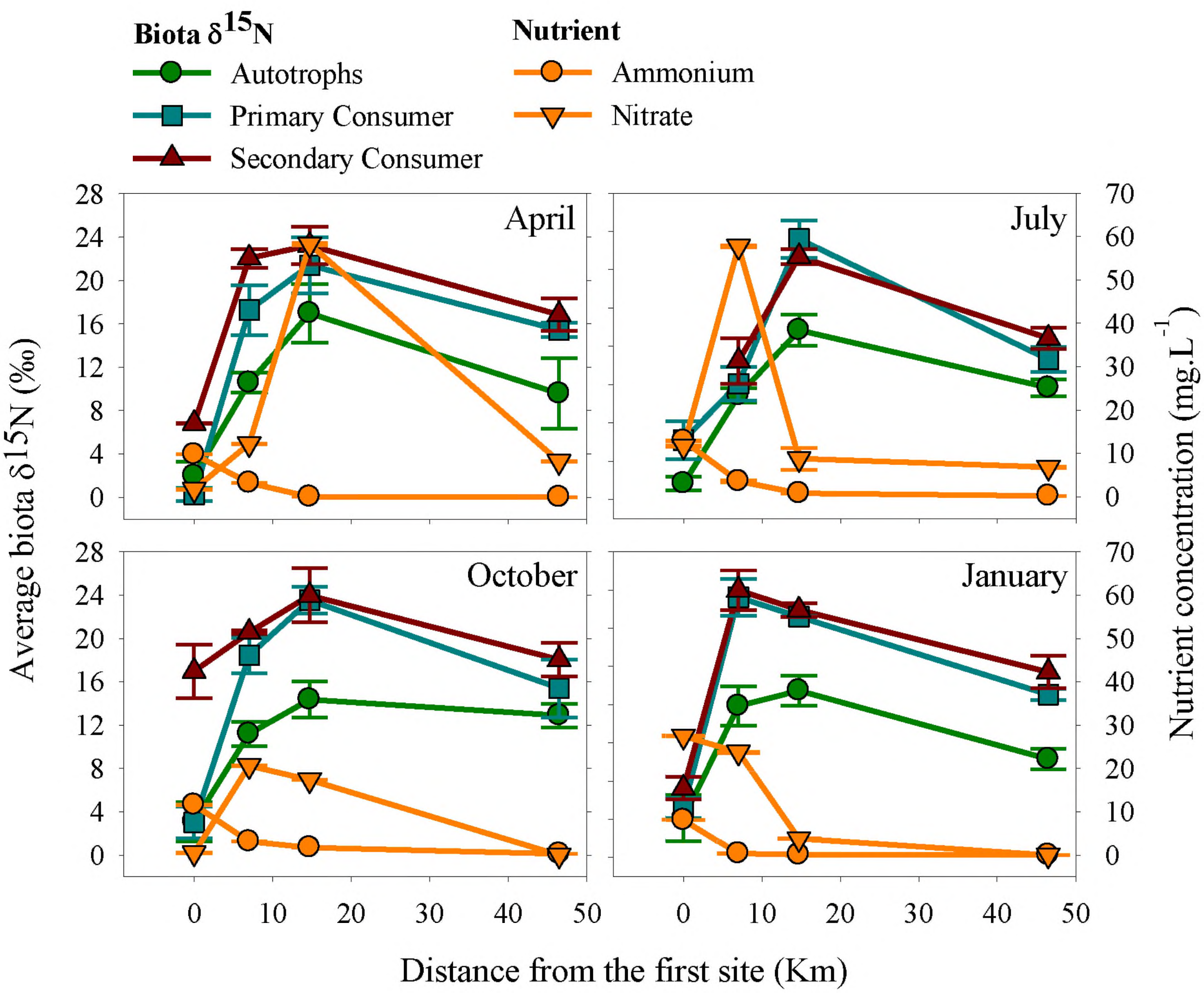

Figure 5.1: Spatio-temporal variations in $\delta^{15}N$ values in biota in relation to changes in nutrient concentrations in the water column between April 2013 and July 2014. Sites were reported as distance from the most upstream site: site Urban = 0 Km, site Sewage = 7 Km, site Agriculture = 14.7 Km, and site Recreational = 46.5 Km.

5.3 RELATIONSHIP BETWEEN $\delta^{15}N$ AND NUTRIENT CONCENTRATIONS

5.3.1 Biota $\delta^{15}N$ and ammonium ($NH4^+$)

Ammonium concentrations showed an opposite pattern when compared with biota $\delta^{15}N$ (Figure 5.1), and there were no significant relationships between these variables across sites in April and July (Table 5.1). In October, however, high ammonium concentrations were significantly associated with low $\delta^{15}N$ values in primary consumers, contradicting my hypothesis.

Low ammonium concentrations were also significantly related to the reduction in biota $\delta^{15}N$ values observed at the Recreational Site in January.

5.3.2 Biota $\delta^{15}N$ and nitrates (NO_3^-)

Although nitrate concentrations varied in a similar manner as biota $\delta^{15}N$ from April to October, these patterns were not significantly related (Table 5.2) except for the relationship at the Agriculture site in October. Nitrate concentrations were undetectable (less than 0.001 $mg.L^{-1}$) at the Recreational site in October and January, so models for these months were not possible.

Table 5.1: Temporal relationships between diatom community matrices and environmental variables. The regression equation y = ax+ b was used to model the relationships, where x = ammonium concentration, y = biota $\delta^{15}N$, a = y intercept, and b = slope of the regression line. Relationships were considered statistically significant when $p \leq 0.05$ (indicated in bold). P = primary consumer, S = secondary

		April			**July**			**October**			**January**		
Site	**Biota type**	**R^2**	**F**	***P***	**R^2**	**F**	***P***	**R^2**	**F**	***P***	**R^2**	**F**	***P***
Urban	Autotrophs	0.9	9.8	0.2	0.01	0.01	0.9	0.01	0.01	0.9	0.9	7.2	0.2
	P Consumer	0.5	1.0	0.5	0.7	2.5	0.4	**1.0**	**1.2**	**0.04**	1.0	33.5	0.1
	S Consumer	0.3	0.4	0.6	No consumer data			0.3	0.5	0.6	0.3	0.4	0.6
Sewage	Autotrophs	1.0	19.4	0.1	0.0	0.0	1.0	0.9	9.4	0.2	0.2	0.2	0.7
	P Consumer	0.0	0.0	1.0	0.6	1.8	0.4	0.3	0.3	0.7	0.9	9.8	0.2
	S Consumer	0.2	0.3	0.7	0.6	1.5	0.4	0.3	0.4	0.6	0.9	9.8	0.2
Agriculture	Autotrophs	0.1	0.1	0.8	0.1	0.1	0.8	1.0	0.2	0.1	0.1	0.1	0.8
	P Consumer	0.9	11.4	0.2	0.01	0.1	1.0	0.01	0.01	0.9	0.1	0.1	0.8
	S Consumer	0.4	0.7	0.6	0.4	0.8	0.5	0.1	0.1	0.8	0.6	1.7	0.4
Recreational	Autotrophs	0.7	2.9	0.3	0.7	2.9	0.3	0.9	1.2	0.2	**1.0**	**1.9**	**<0.01**
	P Consumer	0.5	0.8	0.5	0.1	0.1	0.8	0.8	2.5	0.3	**1.0**	**0.9**	**<0.01**
	S Consumer	0.01	0.01	0.9	1.0	6.2	0.1	1.0	1.6	0.1	**1.0**	**1.4**	**<0.01**

Table 5.2: Temporal relationships between diatom community matrices and environmental variables. The regression equation y = ax+ b was used to model the relationships, where x = nitrate concentration, y = biota $\delta^{15}N$, a = y intercept, and b = slope of the regression line. Relationships were considered statistically significant when $p \leq 0.05$ (indicated in bold). P = primary, S = secondary

Site	Biota type	April			July			October			January		
		R^2	F	*P*	R^2	F	*P*	R^2	F	*P*	R^2	F	*P*
Urban	Autotrophs	<0.01	<0.01	1.0	0.6	1.5	0.5	1.0	24.6	0.1	0.6	1.5	0.4
	P Consumer	<0.01	<0.01	1.0	0.4	0.7	0.6	0.0	0.0	1.0	0.1	0.1	0.8
	S Consumer	0.9	7.9	0.2	0.9	1.1	0.2	0.6	1.3	0.5	0.2	0.3	0.7
Sewage	Autotrophs	0.5	0.9	0.5	0.1	0.1	0.8	0.4	0.6	0.6	0.1	0.1	0.8
	P Consumer	0.7	2.8	0.3	0.3	0.5	0.6	1.0	25.1	0.1	1.0	30.4	0.1
	S Consumer	0.3	0.4	0.6	0.3	0.4	0.6	1.0	20.3	0.1	0.3	0.4	0.7
Agriculture	Autotrophs	0.5	0.8	0.5	0.8	0.0	0.9	0.2	0.2	0.7	0.1	0.1	0.8
	P Consumer	0.5	1.1	0.5	0.0	0.2	0.9	0.8	5.0	0.3	0.1	0.1	0.8
	S Consumer	0.1	0.1	0.8	0.6	1.2	0.5	**1.0**	**0.4**	**0.01**	0.6	1.7	0.4
Recreational	Autotrophs	0.8	0.01	1.0	0.6	2.7	0.2						
	P Consumer	0.1	0.1	0.8	1.0	2.5	0.5	No solution			No solution		
	S Consumer	0.6	1.8	0.4	0.2	0.5	0.5						

5.4 DISCUSSION

It was hypothesized that stable nitrogen isotope ($\delta^{15}N$) values of biota are higher where concentrations of ammonia and nitrate are highest, and decrease with decreases in nutrient concentrations. To test this hypothesis, biota were grouped into trophic levels (autotrophs, primary consumers, and secondary consumers) to account for the enrichment of ^{15}N through metabolic processes. The results indicated that $\delta^{15}N$ values of autotrophs were generally lower than those of consumers, and that secondary consumer $\delta^{15}N$ values were the highest. These findings were consistent with literature that the heavier nitrogen isotope is more enriched at high trophic levels in food webs (Cabana & Rasmussen 1996a; Fry 1999; di Lascio *et al.* 2013). The enrichment of the heavier isotope was in part attributed to food assimilation since consumers naturally have higher $\delta^{15}N$ values than their diets (Fry 1999), and also due to the incorporation of dissolved anthropogenic nitrogen (Robinson 2001).

5.4.1 Spatial variation in biological $\delta^{15}N$ values

There was a clear pattern of low $\delta^{15}N$ values occurring in biota upstream of the main sewage discharge point (Urban site), to high values downstream of the Sewage site in the Bloukrans River. A similar pattern was reported by Morrissey *et al.* (2013), who observed the increases in mean $\delta^{15}N$ values in invertebrates downstream of sewage treatment facilities in several rivers in Wales, United Kingdom. In Canada, Wayland and Hobson (2001) were able to differentiate between organisms who were exposed to sewage derived food sources and those who were not by simply comparing their $\delta^{15}N$ values against their local food sources. Their study revealed high $\delta^{15}N$ values in both food sources and consumers (including birds) exposed to treated sewage discharge, and lower values upstream (Wayland & Hobson 2001). In the Bloukrans River, the lower $\delta^{15}N$ values observed upstream (Urban site) were attributed to the assimilation of raw sewage by consumers. The Urban site had no instream vegetation, and the food sources were dominated by raw human and animal waste. The most dominant invertebrates at the Urban site were Chironomidae, whose species are tolerant to pollution and employ predatory and omnivorous feeding behaviors that allow them a diverse choice in prey items. Raw human and animal waste materials have relatively low $\delta^{15}N$ values since microbial decomposition is limited, and therefore invertebrates consuming raw sewage tend to reflect the low $\delta^{15}N$ values (de Bruyn & Rasmussen 2002; Anderson & Cabana 2006).

5.4.2 Tracing nitrogen in biological components of the Bloukrans River

The isotopic values of nitrogen in plants can differ by taxa (Cabana & Rasmussen 1996), but there were no significant differences in the $\delta^{15}N$ values of the representative autotrophs (i.e. epipsammon, epilithon, phytoplankton, epiphyton, filamentous algae, and course particulate organic matter) in the Bloukrans River. Similarly, there was no significant difference among the invertebrate families within the primary consumer (i.e. Baetidae, Chironomidae, Corixidae, Simuliidae, Caenidae, and Amphipoda) and secondary consumer (i.e. Potamonautidae, Aeshnidae, Lestidae, Gomphidae, and Tabanidae) groups. Other researchers reporting little to no variation in $\delta^{15}N$ values among consumers at the same tropic level (e.g. Vander Zanden & Rasmussen 2001; Anderson & Cabana 2005) suggested that fractionation by metabolism is similar among these organisms, and therefore the values may be pooled based on tropic position. In contrast, where among-species variations are significant, the pooling of data may not represent a true reflection of changes in $\delta^{15}N$ across trophic levels (McCutchan *et al.* 2003). In the Bloukrans River, differences in biota $\delta^{15}N$ values occurred spatially rather than between families occupying the same trophic position, with the lowest values occurring at the Urban site and highest at the Agriculture site. There was a significant relationship between ammonium concentrations and primary consumer $\delta^{15}N$ at the Urban site in October. This relationship contradicted the hypothesis in that primary consumer $\delta^{15}N$ was lowest (+3.0‰) at the highest NH_4^+ concentration (11.6 mg.L^{-1}). The Urban site was characterized by raw sewage and livestock dung deposition, especially during the October flooding period. Raw sewage and animal waste have $\delta^{15}N$ signatures ranging between 0-5‰ (e.g. Heaton 1986; Anderson & Cabana 2006), and hence the consumers tended to reflect these signatures.

The influence of NH_4^+ was prominent in the Bloukrans River, and $\delta^{15}N$ values of all organisms were associated with a low NH_4^+ concentration (0.1 mg.L^{-1}) at the Recreational site in January (Table 5.1), further contradicting the hypothesis that high isotopic signatures occur under high nutrient concentrations. The variation in biota $\delta^{15}N$ values across trophic levels (i.e. autotrophs +10.3‰, primary consumers +17.1‰, and secondary consumers +19.4‰) indicated the fractionation that occurred through metabolism. The mixing of nitrogen from several sources, or fractionation by chemical and ionic reactions as N from water is absorbed by autotrophs, are additional potential contributors to the enrichment of ^{15}N in biota at the Recreational site. The site had a larger N source pool, and nitrogen mixing was highly probably since local activities included spiritual cleansing (pharmaceutical effects) and agriculture (inorganic fertilizer effects). Since nitrogen entering aquatic ecosystems as inorganic nitrate or ammonium fertilizer is more depleted in ^{15}N ($\delta^{15}N$ signature ~0‰; Anderson & Cabana 2006; Diebel & Zanden 2009), the fertilizers were probably not the main source of ^{15}N enrichment at the Recreational site. The manufacturing process for South African fertilizers results in low $\delta^{15}N$ signatures ranging from -3.8‰ to +0.9‰ for NH_4^+ and NH_3, and -3.9‰ to +1.8‰ for NO_3^- (Heaton 1986), values considerably lower than those occurring in the Bloukrans River. As such, the relatively high $\delta^{15}N$ values of biota at the Recreational site probably originated from the hydrolysis of urea derived from the upstream sites that were impacted by sewage. The effects of upstream sewage disposal can often be detected some kilometers downstream from a point source (e.g. Ulseth & Hershey 2005). In the Bloukrans River, the hydrolysis of sewage derived urea produced ammonia that was easily volatilized due to the high temperatures at the site during January. Kinetic fractionation of ammonia at high temperatures favored the utilization and evaporation of ^{14}N, discriminating against ^{15}N, and eventually resulting in the enrichment of ^{15}N in the water. When the enriched nitrogen isotope reacts with oxygen in water, nitrate is produced in which the $\delta^{15}N$ values range between +10‰ to +20‰ (Heaton 1986). The assimilation of nitrate by autotrophs retains ^{15}N in their tissues, resulting in the high $\delta^{15}N$ values (+10.3‰) observed in the autotrophs of the Bloukrans River. Further fractionation occurred when primary consumers assimilated autotrophs, producing elevated $\delta^{15}N$ values (+17.1‰), and when predators assimilated the grazers (producing even higher $\delta^{15}N$ values of +19.4‰).

At the Agriculture site, nitrate concentrations were good predictors of secondary consumer $\delta^{15}N$ during October (Table 5.2). High $\delta^{15}N$ values of secondary consumers (+24.0‰) related to high nitrate concentrations (17.4 $mg.L^{-1}$) at this site, in support of the hypothesis. These results were not surprising since NO_3^- was easily transported into the river from the adjacent farms during the October floods. Studies have shown that NO_3^- is the most abundant form of dissolved nitrogen in rivers due to its low absorbance potential in the soil (Ohte 2012), and that its transportation from terrestrial to aquatic ecosystems is relatively efficient in wet seasons (Berman & Bronk 2003). In the Bloukrans River, two potential mechanisms contributed to high $\delta^{15}N$ values in secondary consumer tissues. First, bacterial activities involved in ammonium oxidation (ammonium derived from upstream sewage) resulted in ^{15}N discrimination, producing high $\delta^{15}N$ in potential food sources. Secondly, the nitrification process itself fractionates nitrogen and releases nitrate that is highly enriched in ^{15}N ($\delta^{15}N_{nitrate}$ +15‰ to +35‰; Robinson 2001), which further fractionates when assimilated by consumers. Although my results were limited by the lack of sewage effluent samples (effluent collected before and after discharge into receiving waters), variability of biological $\delta^{15}N$ above and below the sewage discharge point provided useful estimates of the effects of sewage nitrogen on the biological components of the Bloukrans River. Additionally, the lack of soil $\delta^{15}N$ from the surrounding farms limited the precise estimation of overall contributions of agriculture derived nitrogen to the river. However, the high biota $\delta^{15}N$ values observed at the agriculture impacted site suggested multiple anthropogenic nitrogen inputs including animal manure.

5.5 SUMMARY AND CONCLUSION

Sewage discharge, animal manure, and raw untreated sewage were the most influential sources of anthropogenic nitrogen in the Bloukrans River. Biota $\delta^{15}N$ values below the sewage input were higher than those upstream at the Urban site, leading to the conclusion that: (1) nitrogen in the treated sewage effluent was more enriched in ^{15}N than the water in the receiving environment, and (2) sewage effluent was the most abundant food source in the Bloukrans River such that its consumption and assimilation was reflected in organisms exposed to the effluent, but not those upstream of the effluent. When materials derived from sewage enters river ecosystems, they are usually in quantities large enough to cause noticeable changes in the isotopic signatures of receiving organisms when compared to sewage-free organisms (Cabana & Rasmussen 1996a; Cole *et al.* 2004). Although estimating anthropogenic inputs of N using stable nitrogen isotopes cannot entirely exclude additional potential sources, this method has proven useful in identifying spatial and temporal differences in contributing factors, especially fertilizer and sewage sources (Heaton 1986; Morrissey *et al.* 2013). In the Bloukrans River, the effect of treated sewage was clearly indicated by an acute increase in biological $\delta^{15}N$ values at all the downstream sites. The effects of agriculture tended to be camouflaged within the sewage, so fertilizer nitrogen was an additional important source of nitrogen.

6 SUMMARY AND RECOMMENDATIONS

6.1 MULTIPLE INDICATORS FOR WATER QUALITY ASSESSMENTS

6.1.1 Macroinvertebrates and diatoms

Macroinvertebrates and diatoms were used to assess the ecological integrity of the Bloukrans River, and the results indicated that these taxonomic groups can be useful as biological indicators of water quality and environmental degradation, although results varied between methods. Multivariate analyses revealed the most important environmental variables driving the distributional patterns of both groups. Interestingly, none of the factors affecting diatom distribution were significant for macroinvertebrates. These findings are important as they indicated that different organisms provide unique information regarding the local environmental conditions. Clearly, the use of different bioindicators provides a holistic diagnosis for current environmental issues that could have long term ecological effects on the integrity of aquatic ecosystems. However, authors have cautioned that biological indicators should not be redundant (Rooney & Bayley 2012), arguing that multiple indicators may not necessarily be needed if they are all correlated similarly with environmental conditions (Allen *et al.* 1999). Studies that employ multiple indicators are rooted in the fact that no single indicator is best suited to detect the diversity of environmental disturbances that result from human activities (Rey *et al.* 2004). Furthermore, some organisms, especially macroinvertebrates, do not emerge in winter due to reproduction requirements (Muralidharan *et al.* 2010). According to Charles (1996), diatoms have received so much attention in biomonitoring simply because of such limitations with macroinvertebrates. However, several studies have included both diatoms and macroinvertebrates as environmental indicators (e.g. Chessman 1995; Mazor *et al.* 2006; Mendes *et al.* 2014). Similarly, in the Bloukrans River, the two indicators addressed diverse ecological questions and performed differently. First, the SASS5 and ASPT water quality indices of macroinvertebrates provided useful assessments of water quality and environmental degradation. Both the indices were affected by fluctuations in channel width, water depth, and nitrate concentrations along the length of the river. Deterioration of water quality due to high ammonium and nitrate concentrations negatively affected invertebrate communities, and resulted in reduced diversity and taxa richness. Total invertebrate abundance fluctuated such that the most polluted sites had the highest abundances of pollution tolerant taxa such as Chironomidae at the expense of pollution sensitive ones. Although sensitive genera including Baetidae were observed, their relative abundance remained low throughout the study. High abundances of sensitive Baetidae species coincided with low nutrient concentrations and adequate dissolved oxygen concentrations. My study indicated that macroinvertebrate responses to pollution can provide useful information about current and potentially long term trends in human impacted rivers. Water pollution was not the only ecological concern in the Bloukrans River, as habitat degradation and structural modification of the channel were of great concern. Patterns in macroinvertebrate diversity indicated the effects of habitat modification, as diversity was altered by the removal of bed material to accommodate agricultural activities, and also by the reduction of habitat heterogeneity. The deterioration of both the chemical and physical components of the river was reflected in the macroinvertebrate communities.

Diatom communities in the Bloukrans River indicated that the entire length of the system was of generally poor quality, and that there were some shifts in their structure over time owing to seasonal changes in rainfall. During periods of increased freshwater inputs, the river was mesotrophic and nutrient dilution occurred, but in the dryer periods the river remained fairly eutrophic. The floral component of the river was not directly affected by nitrogen enrichment, but by changes in pH, temperature, flow velocity, and phosphate concentration. The lack of spatial variation in diatom community structure was indicative of a longitudinal connectivity of environmental conditions. For example, none of the influential variables differed significantly across sites, suggesting that all four parameters were equally problematic from the headwaters through to the downstream areas of the river. When compared with macroinvertebrates, the diatom community level of assessment indicated low sensitivity to environmental change. Chessman (1995) also derived different results from macroinvertebrate and diatom community data when assessing rivers in New South Wales and Victoria, Australia. Mazor *et al.* (2006) reported outputs from several biological assessment methods and found that macroinvertebrate communities were more sensitive to environmental changes compared with diatoms. The most important differences between the two taxonomic groups are that they respond differently to environmental disturbances and seasonal variations in their environment (Chessman 1995), so these must be acknowledged in any environmental study. Furthermore, additional research is needed to establish a calibration of the TDI in South Africa.

Studies incorporating both SASS5 and diatom indices are beginning to emerge from South Africa. For example, de la Rey *et al.* (2004) compared SASS5 with a diatom index called Specific Pollution Sensitivity Index (SPI) and found that the diatom based index performed better than SASS for assessing the water quality of the Mooi River in the North West province. Similar to the current study, both indices (SPI and TDI) provided valuable information about the water quality of the river (de la Rey *et al.* 2004) and indicated that the use of both diatoms and macroinvertebrates for biological monitoring in South Africa is a worthwhile exercise.

6.1.2 Incorporation of stable nitrogen isotopes in water quality assessment

Stable nitrogen ratios of organisms collected at the Bloukrans River indicated the utilization of nitrogen derived from sewage, manure, fertilizer and untreated animal and human waste. The distinction between agricultural sources and sewage was less prominent due to the overlapping nature of the $\delta^{15}N$ signatures between the two sources. Nitrogen fractionation that occurs during the manufacture of fertilizers produces low $\delta^{15}N$ signatures and resulted in similar isotopic values in the organisms, particularly the autotrophs. Since sewage nitrogen is relatively ^{15}N enriched, organisms using sewage derived nitrogen had high $\delta^{15}N$ values in their tissues. Although a number of factors including nitrogen excretion, changes in diets, and organism type can contribute significantly to ^{15}N enrichment in organisms (Ambrose & DeNiro 1986; Ponsard & Averbuch 1999; Vanderklift & Ponsard 2003), the results obtained from the Bloukrans River suggested nitrogen enrichment by sewage could not be eliminated as a contributor. The three sites located downstream of a sewage treatment plant were ^{15}N enriched throughout the study, probably as a result of nitrogen fractionation by sewage bacteria creating high $\delta^{15}N$ values in the sewage effluent. Sewage discharged into the river contained high concentrations of nitrate, and nitrates not only degraded the water quality, but they were also assimilated by living organisms. Studies that have incorporated stable nitrogen isotopes to determine water quality degradation are particularly useful for managers that must make informed decisions about managing aquatic ecosystems. One example is a study conducted by Costanzo *et al.* (2005), who used $\delta^{15}N$ values in autotrophs to determine the effectiveness of sewage upgrades in Moreton Bay (Australia) and found that sewage nitrogen inputs decreased after a treatment plant had undergone major upgrades. Their study helped to resolve disputes between several managers about which sewage treatment facility was responsible for nitrogen enrichment in the bay, as the results identified the faulty sewage facility (Costanzo *et al.* 2005).

6.2 RECOMMENDATIONS

6.2.1 The application of stable isotopes

Stable nitrogen isotopes ratios indicated that there were measurable effects of land use activities in the Bloukrans River, particularly regarding the deposition of treated sewage. Although the results could not conclusively pinpoint major sources and eliminate others, they contributed information about the quality of the water and the organisms living there. I provide below several recommendations for researchers that will utilize similar methods in the future.

1. The identification of all potential food sources, including sewage sludge, would be very useful. For example, learning how nitrogen is transformed as it leaves a sewage treatment facility, and enters the tissues of aquatic organisms, would significantly enhance the interpretation of biota $\delta^{15}N$ signatures.
2. The addition of mixing models to assess the contributions of multiple potential food sources to consumers would allow for additional conclusions about the trophic ecology of the communities. Wherever possible, gut content analysis could also provide additional evidence about the trophic ecology of some consumers.
3. Additional research on stable isotope tracers in polluted ecosystems is required to clarify and account for all possible nutrient sources. The addition of tracers such as oxygen and sulphur should complement the information derived from nitrogen isotopes. Agricultural sources of nutrient enrichment could be identified by studying the isotopic ratios of soil samples in relation to ratios of nutrients in water and in living organisms. The relative dominance of a particular nutrient source should be studied relative to all other potential sources, particularly in urbanized rivers with multiple land uses.

6.2.2 The application of biological indices

Applications of biological indices allow researchers to document and interpret ecological patterns in aquatic ecosystems. Although biological indices such as SASS and TDI have been successfully used, some limitations in their application remain. For example, researchers are cautioned to avoid high flow and flooding occasions when collecting samples intended for SASS (Dickens & Graham 2002) and TDI assessments (Kelly & Whitton 1995). However, flooding can represent an important natural event, and we need to study the effects of flooding on communities. My data on the Bloukrans River revealed that flooding diluted nutrients in the water column, and probably resulted in reductions in biodiversity and species richness. I therefore recommend that samples are collected during or after heavy rainfall in any assessment of environmental change in rivers, along with samples collected in dry seasons. Such a design will help to determine the time needed for communities to recover from flooding in addition to other factors such as sewage discharge.

The Trophic Diatom Index revealed important information about the trophic state of the Bloukrans River. However, because this index is not native to South Africa, its reliability needs to be validated through additional South African based studies. The SASS index has yielded reliable results after multiple applications and calibrations, but further work is needed, particularly in the smaller but economically important rivers such as the Bloukrans where human settlements are such a dominant feature.

6.3 SUMMARY AND CONCLUSION

My objective was to investigate the ecological impacts of pollution on the biological communities of macroinvertebrates and diatoms in the Bloukrans River. The three main questions driving the investigation were addressed, and the results showed that: (I) macroinvertebrate and diatom communities were affected by different sets of environmental parameters, which all contributed to the overall water quality status, (II) the SASS5 and TDI indices revealed that the general water quality was poor, with SASS5 scores ranging from 13 to 54 along the stretch of the river. The Urban site was the most impacted, and the last site (Recreational) had the highest scores. Although the TDI scores did not change significantly across sites, the values indicated poor water quality with signs of eutrophication, particularly in the dry seasons, and (III) stable nitrogen isotopes of organisms varied significantly across sites, with the lowest $\delta^{15}N$ values observed upstream of the Grahamstown sewage treatment plant and the highest downstream. Isotope signatures in organisms indicated the introduction of foreign nitrogen into the system, which was incorporation into consumers through their diets. The distinction of upstream from downstream

$\delta^{15}N$ signatures revealed the importance of sewage derived nitrogen in the food web, suggesting that sewage sludge was a food source for consumers in the river.

Although some of my hypotheses were not supported by the results, the ecological patterns revealed significant effects of man-mediated activities on the biological and physical components of the river. Overall, the diatom and macroinvertebrate communities told a story of environmental degradation, eutrophication, and nitrogen enrichment. Urbanization, agriculture and the Grahamstown sewage treatment facility together contributed to the ecological degradation of the Bloukrans River. This study provided evidence that (1) the river is under ecological stress due to pollution, (2) the natural and intrinsic value of the river is gradually diminishing, and (3) the only natural remedy for maintaining the chemical balance in the river is through freshwater inputs that periodically create mesotrophic conditions, although floods may also represent drivers of nutrient enrichment from non-point sources. Due to its economic importance, the river faces further major degradations and channel modifications in the future if research findings such as mine are not adequately communicated with policy makers for management purposes.

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Appendix A: Mean and standard deviation of all physical and chemical environmental parameters measured at each site from April 2013 to January 2014 in the Bloukrans River.

Period	**Environmental parameters**	**Sites**			
		B1- Urban	**B2- Sewage**	**B3- Agriculture**	**B4- Recreational**
April	Dissolved Oxygen (mg L^{-1})	0.9 ± 0.01	6.1 ± 0.01	5.9 ± 0.01	6.02 ± 0.01
	Conductivity ($mScm^{-1}$)	1.2	1.3	1.6 ± 0.01	1.2
	Total dissolved solids (ppt)	0.8 ± 0.06	0.8 ± 0.06	0.8 ± 0.06	0.8 ± 0.06
	Sodium Chloride (ppt)	0.6 ± 0.06	0.6 ± 1.5	0.6 ± 0.06	0.6 ± 0.06
	pH	7.0 ± 0.02	7.3 ± 0.02	7.3 ± 0.1	6.9 ± 0.01
	Temperature (°C)	18.6 ± 0.2	14.5 ± 0.06	15.03 ± 0.1	19.5 ± 0.06
	Water velocity ($m.s^{-1}$)	1.06 ± 0.01	0.3 ± 0.01	0.7 ± 0.01	0.8 ± 0.01
	Water Depth (cm)	39.3 ± 8.1	29.3 ± 0.6	41.3 ± 0.3	29 ± 0.3
	Channel Width (m)	3.5 ± 0.36	6.8 ± 0.2	3.8 ± 0.5	6.5 ± 0.4
	Ammonium-NH_4^+ ($mg.L^{-1}$)	9.9 ± 0.02	3.3 ± 0.01	0.8 ± 0.01	0.02 ± 0.01
	Phosphate-PO_3^{-4} ($mg.L^{-1}$)	5.2 ± 0.06	5.1 ± 0.06	4.5 ± 0.12	4.5 ± 0.2
	Nitrate-NO_3^- ($mg.L^{-1}$)	1.8	12.3 ± 0.06	8.8 ± .06	8.3 ± 0.06
July	Dissolved Oxygen (mg L^{-1})	6.2 ± 0.01	5.9 ± 0.01	8.07 ± 0.02	9.5 ± 0.02
	Conductivity ($mScm^{-1}$)	1.3	1.4	1.37	1.71
	Total dissolved solids (ppt)	0.9 ± 0.06	0.9 ± 0.06	0.9 ± 0.6	0
	Sodium Chloride (ppt)	0.6 ± 0.06	0.6 ± 0.06	0.6 ± 0.06	0.7 ± 0.06
	pH	8.1 ± 0.01	7.6 ± 0.01	7.9 ± 0.02	8.6 ± 0.01
	Temperature (°C)	15.3 ± 0.06	11.2 ± 0.1	10.7 ± 0.06	10.2 ± 0.1
	Water velocity ($m.s^{-1}$)	0.1 ± 0.01	0.07 ± 0.01	1.03 ± 0.01	0.2 ± 0.01
	Water Depth (cm)	29.1 ± 0.8	42.8 ± 0.4	38.2 ± 1.7	30.8 ± 0.2
	Channel Width (m)	3.8 ± 0.25	6.9 ± 0.4	3.9 ± 0.3	6 ± 0.5
	Ammonium-NH_4^+ ($mg.L^{-1}$)	12.9 ± 0.06	3.6 ± 0.2	0.14 ± 0.01	0.2 ± 0.01
	Phosphate-PO_3^{-4} ($mg.L^{-1}$)	4.1 ± 0.15	6.1 ± 0.1	5.2 ± 0.12	4.7 ± 0.2
	Nitrate-NO_3^- ($mg.L^{-1}$)	11.7 ± 0.1	57.7 ± 0.2	58.3 ± 0.3	6.8 ± 0.2

October	Dissolved Oxygen (mg L^{-1})	4.5 ± 0.06	6.4 ± 0.06	6.7 ± 0.02	7.7 ± 0.05
	Conductivity ($mScm^{-1}$)	1.3 ± 0.01	1.07 ± 0.01	0.8 ± 0.04	1.8 ± 0.02
	Total dissolved solids (ppt)	0.9 ± 0.6	0.8 ± 0.6	0.5 ± 0.1	0
	Sodium Chloride (ppt)	0.6 ± 0.8	0.5 ± 0.2	0.3	0.9 ± .01
	pH	8.2 ± 0.02	8.1 ± 0.02	8.2 ± 0.06	8.74 ± 0.12
	Temperature (°C)	21.2 ± 0.2	15.5 ± 0.1	15.2 ± 0.1	15.8 ± 0.06
	Water velocity ($m.s^{-1}$)	0.7 ± 0.2	1.6 ± 0.06	2.8 ± 0.02	2.21 ± 0.1
	Water Depth (cm)	42 ± 0.6	37.9 ± 1.6	49.1 ± 0.9	57 ± 0.6
	Channel Width (m)	3.3 ± 0.9	6.4 ± 0.6	3.5 ± 0.6	4.8 ± 0.2
	Ammonium-NH_4^+ ($mg.L^{-1}$)	11.6 ± 0.07	3.1 ± 0.02	1.7 ± 0.03	0.3 ± 0.1
	Phosphate-PO_3^{-4} ($mg.L^{-1}$)	3 ± 0.1	1 ± 0.1	2.7 ± 0.1	7.7 ± 0.2
	Nitrate-NO_3^- ($mg.L^{-1}$)	0.5 ± 0.1	20.6 ± 0.1	17.4 ± 0.06	0
January	Dissolved Oxygen (mg L^{-1})	6.6 ± 0.02	4.5 ± 0.01	5.07 ± 0.02	5.3 ± 0.01
	Conductivity ($mScm^{-1}$)	1.3 ± 0.01	12.3 ± 0.06	1.14	1.5 ± 0.01
	Total dissolved solids (ppt)	0.9 ± 0.6	0.9 ± 0.6	0.8 ± 0.6	0
	Sodium Chloride (ppt)	0.6 ± 0.5	0.6 ± 0.1	0.6	0.8 ± 0.6
	pH	8.4 ± 0.02	8.3 ± 0.01	8.4 ± 0.05	8.5 ± 0.02
	Temperature (°C)	25.6 ± 0.2	21.9 ± 0.1	22.4 ± 0.4	23.3 ± 0.2
	Water velocity ($m.s^{-1}$)	0.3 ± 0.01	0.08 ± 0.01	0.2 ± 0.01	0.03
	Water Depth (cm)	20.7 ± 0.3	40.4 ± 1.9	40.7 ± 1.8	26 ± 1.7
	Channel Width (m)	2.03 ± 0.4	4.8 ± 0.6	5.2 ± 0.3	3.3 ± 0.4
	Ammonium-NH_4^+ ($mg.L^{-1}$)	8.1 ± 0.01	0.4 ± 0.01	0.1 ± 0.01	0.05
	Phosphate-PO_3^{-4} ($mg.L^{-1}$)	15.6 ± 0.06	5.5 ± 0.2	3.8 ± 0.1	1.4 ± 0.1
	Nitrate-NO_3^- ($mg.L^{-1}$)	27.5 ± 0.1	23.8 ± 0.2	3.8 ± 0.01	0

Appendix B*:* Total abundance of macroinvertebrates collected at each site from April 2013 to January 2014 in the Bloukrans River. Sites: B1= Urban, B2= Sewage, B3= Agriculture, B4= Recreational.

	April				July				October				January			
Families	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**
Aeshnidae	-	-	-	8	-	-	-	8	-	-	-	-	-	-	1	3
Amphipoda	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-
Ancylidae	-	-	5	-	2	2	35	-	-	3	4	-	-	5	-	-
Anura	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Argyronetidae	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Baetidae	-	296	102	407	-	91	93	142	-	37	10	11	-	26	17	9
Belostomatidae	2	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-
Caenidae	-	-	14	56	-	-	21	-	-	4	7	17	-	-	4	-
Ceratopogonidae	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-
Chironomidae	99	35	10	10	74	75	18	8	215	78	11	-	212	13	-	-
Chlorolestidae	-	-	-	4	-	-	-	1	-	-	3	-	-	9	3	-
Coenagrionidae	2	1	19	30	-	-	-	-	-	2	6	-	-	20	5	-
Corixidae	-	-	66	182	2	-	7	-	-	-	-	-	-	-	3	-
Culicidae	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Dytiscidae	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ecnomidae	5	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
Elmidae	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	2
Gerridae	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-
Gomphidae	-	1	8	38	1	1	20	2	-	-	1	-	-	-	2	-
Gyrinidae	-	3	-	46	-	-	-	-	-	-	-	-	-	-	-	-
Hirudinea	1	3	-	-	-	14	7	9	1	8	9	-	-	3	4	-
Hydraenidae	1	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Hydropsychidae	-	12	5	24	-	-	-	145	-	-	-	4	-	6	2	77
Leptophlebiidae	-	-	-	94	-	-	-	54	-	-	-	5	-	-	-	17
Lestidae	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-
Libellulidae	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-

Lumbricidae	2	18	9	-	-	-	-	-	-	-	-	-	-	-	-	-
Notonectidae	-	-	3	-	-	-	-	-	1	-	1	-	-	2	6	-
Oligochaeta	-	-	-	-	38	7	3	4	-	9	3	-	8	1	-	-
Oxyopidae	2	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Philopotamidae	-	-	-	2-	5	-	-	9	-	-	-	-	-	-	-	-
Physidae	2	-	-	-	2	-	12	-	-	-	4	-	18	-	-	-
Platycnemidae	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
Potamonautidae	-	12	13	25	-	5	2	11	-	10	6	4	-	18	5	8
Psephenidae	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psychodidae	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Simuliidae	-	43	9	68	-	45	66	77	-	21	9	-	-	9	3	2
Syrphidae	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Tabanidae	-	-	2	7	-	-	-	9	-	-	-	1	-	-	-	-
Tetragnathidae	-	-	-	-	-	-	-	2	-	-	-	2	-	-	-	1
Tipulidae	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-

Appendix C: Total diatom abundance at each site from April 2013 to January 2014 in the Bloukrans River. Sites: B1= Urban, B2= Sewage, B3= Agriculture, B4= Recreational.

	April				July				October				January			
Diatom species	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**
Amphora veneta	18	3	-	12	16	16	6	-	14	-	5	10	20	3	2	6
Cocconeis engelbrechtii	22	15	-	41	20	48	25	35	-	37	11	18	-	-	-	-
Cocconeis sp 1	11	10	3	15	39	42	27	38	16	2	11	4	-	15	10	15
Cocconeis sp2	-	-	-	-	-	-	-	-	-	-	-	-	15	6	5	4
Craticula cuspidata	-	38	14	7	9	11	29	31	-	18	9	-	17	8	14	14
Craticula molestiformis	-	-	-	-	23	11	35	28	-	-	-	-	-	-	-	-
Cyclotella meneghiniana	12	18	-	13	23	6	34	10	24	-	2	22	-	-	-	-
Cyclotella sp 1	24	28	8	26	2	-	48	19	21	14	25	25	9	8	15	6
Cymbela sp 1	-	1	-	3	3	22	-	21	-	20	3	7	3	1	10	12
Diadesmis confervacea	-	-	-	-	-	-	-	-	14	8	15	4	-	7	5	8
Diadesmis contenta	3	1	22	7	-	1	10	17	5	28	4	-	-	16	6	14
Epithemia adnata	17	30	26	30	7	9	12	11	14	17	9	35	-	-	-	-
Epithemia sorex	1	5	18	2	31	11	18	22	13	7	14	8	20	32	17	7
Eunotia minor	7	-	11	13	-	-	-	-	24	9	12	16	-	-	-	-
Eunotia pectinalis	1	-	4	3	5	13	-	-	-	-	-	12	-	-	-	-
Eunotia sp 1	9	-	-	-	-	3	11	15	-	-	-	-	-	-	14	19
Fragilaria tenera	31	43	21	17	26	32	50	15	29	27	16	21	23	31	11	15
Fragilaria ulna	9	38	9	35	3	14	65	19	45	33	19	-	12	18	23	15
Gomphonema laticollum	15	5	11	32	-	23	17	-	6	2	4	6	-	10	7	12
Gomphonema sp 1	27	38	13	28	15	44	57	11	9	21	12	8	5	8	7	-
Gomphonema venusta	23	27	-	10	18	43	32	23	26	8	16	27	-	-	-	-
Gyrosigma attenuatum	5	-	-	-	26	28	8	23	-	-	-	-	-	-	-	-
Gyrosigma rautenbachiae	5	30	2	22	-	32	-	35	7	-	4	-	16	10	4	6
Gyrosigma Scalpioides	6	3	15	8	6	3	-	13	-	4	28	9	8	4	6	7
Melosira varians	6	-	-	-	4	-	14	-	22	12	9	15	13	14	17	16
Navicula heimansiodes	26	-	29	9	24	47	55	26	25	-	32	20	-	-	-	-

Navicula sp 1	17	40	6	20	39	35	62	52	15	22	26	-	-	-	-	-
Navicula sp 2	22	36	30	37	32	54	19	17	12	18	-	-	22	15	10	17
Navicula sp 3	15	-	30	22	39	10	28	21	7	-	4	43	-	-	-	-
Navicula vandamii	41	35	24	18	43	46	45	18	31	14	28	40	4	8	15	3
Navicula veneta	25	29	8	18	26	31	41	4	7	-	24	25	3	12	8	6
Nitzschia capitellata	22	23	26	29	36	39	22	31	38	13	33	20	6	14	-	-
Nitzschia desertorum	34	37	40	25	25	39	41	18	30	16	20	25	-	15	24	9
Nitzschia palea	5	16	39	30	14	15	29	37	20	19	16	23	18	14	8	21
Nitzschia sp 1	34	21	25	9	14	7	19	9	19	21	15	10	3	6	11	8
Nitzschia sp 2	2	14	7	22	17	43	10	3	13	-	3	18	5	7	6	6
Nitzschia umbonata	31	33	25	28	22	10	38	50	15	14	23	34	26	22	8	4
Planothidium sp 1	-	-	-	-	9	24	28	28	-	-	-	-	-	-	-	-
Pleurosigma elongatum	8	11	12	21	7	6	16	17	18	11	10	28	12	6	7	2
Rhoicosphenia abbreviata	21	10	-	4	17	15	14	20	-	9	8	11	22	17	5	4
Rhopalodia Musculus	3	1	22	20	13	6	15	14	-	6	14	11	-	-	-	-
Sellaphora seminulum	3	3	4	4	14	2	-	8	20	1	12	5	16	24	5	13
Staurosira elliptica	7	8	5	4	4	5	20	-	15	-	8	8	8	5	9	5
Surirella ovalis	21	15	37	45	26	19	22	11	2	5	3	1	-	-	-	-
Tryblionella debilis	2	3	8	4	-	9	11	6	4	6	4	8	-	-	-	-